Colorado River Aquatic Resource Investigations

Federal Aid Project F-237-R22

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Job Progress Report

Colorado Parks & Wildlife

Aquatic Research Section

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

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State: Colorado Project Number: F-237-R21

Project Title: Colorado River Aquatic Resources Investigations

Period Covered: July 1, 2014 through June 30, 2015

Project Objective: To evaluate biological and ecological factors impacting sport fish populations in coldwater streams and rivers in Colorado.

Job No. 1. Salmonfly Habitat and Ecology Studies

Job Objective: Investigate the habitat use and emergence ecology of the Salmonflies *Pteronarcys californica* in Colorado Rivers.

The Salmonfly *Pteronarcys californica* is a large aquatic invertebrate that can reach high densities in some Colorado Rivers. They play an important ecological role as grazers in stream systems and have been documented to be extremely important to stream dwelling trout as a food resource. Nehring (1987) reported in a diet study of trout in the Colorado River that *P. californica* was the most common food item, comprising 64-75% of the mean stomach content over the four year study. Because of their high biomass and hatching behavior, they also play an important role in supplementing terrestrial food webs and riparian communities with stream derived nutrients (Baxter et al. 2005, Walters et al. 2014). While ecologically important and found in high abundance at some sites, the giant stonefly has relatively specific environmental requirements and is considered intolerant of disturbance in bioassessment protocols (Barbour et al. 1999, Fore et al. 1996, Erickson 1983).

Another aspect of the Salmonfly's biology that makes it sensitive to habitat alterations is its lifespan; it is one of the longest lived aquatic insects in the Neararctic (DeWalt and Stewart 1995). It has been reported to have a three to five year life cycle but two studies indicate it is likely to have a four year life cycle in Colorado (DeWalt and Stewart 1995, Nehring 1987). These two studies also identify *P. californica* as one of the most synchronously emerging of all species of stoneflies with emergence at any one site lasting from 5-13 days. The synchronous emergence and hatching behavior allow it to be sampled in unique ways compared to other aquatic invertebrates. Salmonflies hatch at night by crawling from the water onto riparian vegetation and other vertical structures such as rocks, cliff faces and bridge abutments where they emerge from the nymphal exuvia which is left attached to the structure. If sites are visited soon after emergence then the density of stoneflies emerging at a site can be estimated by completing multiple pass removal surveys of the exuvia. Nehring (2011) found a 0.95 correlation coefficient between post emergence exuvia density estimates and more traditional pre-emergent quantitative benthic sampling at 23 sites.

Previous work completed under Project F-237 identified that the range and density of *P. californica* have declined in the Colorado River and that these declines may be associated with flow alterations in the river (Nehring 2011). Once common in the upper Colorado River, the abundance of giant stoneflies has declined; especially downstream of Windy Gap Reservoir where flow alterations associated with trans-mountain water diversions are the greatest. The objective of this segment is to document the distribution, density and habitat use of *P. californica* in several rivers and measure environmental variables (temperature, velocity, substrate size, embeddedness, etc.) that may be limiting factors of this species in Colorado rivers. By comparing the habitat characteristics of similar sites with differing densities of stoneflies, the optimal habitat characteristics and limiting factors will be identified. Knowledge of the preferred habitat characteristics will assist in ecological restoration of sites where *P. californica* have been extirpated. Once limiting habitat features are identified, the effects of flow and sediment changes on those features will be investigated. This information will benefit management and river restoration activities as well as the evaluation of re-introduction sites for *P. californica* such as those currently being conducted on the Arkansas and upper Gunnison Rivers.

PROGRESS

Density estimates were completed for *P. californica* at six sites on the Rio Grande River, four sites on the Colorado River, one site on the Fraser River and six sites on the Gunnison River in June of 2014. Locations and description of sites are presented in Table 1 and maps are in Figures 1-4. Stonefly exuvia estimates have now been completed for at least two years at all major study sites on the Colorado, Rio Grande and Gunnison Rivers. Estimates were completed by searching 30 meter (98.6 ft) sections of stream bank for *P. californica* exuvia adjacent to riffle habitat. If possible, each site was visited 2-3 times to encompass the entire emergence. If a site was visited only once, estimates were done as soon as possible after the emergence was complete (emergence usually last from 7-13 days at our study sites). Stream flow changes and weather conditions also were taken into account when planning surveys to best estimate the total emergence at each site. Three to seven people intensively searched the riparian area from one to twenty meters from the water's edge. The search area varied by site and depended on the thickness and structure of riparian vegetation. The area was extended laterally from the water's edge until no exuvia were encountered, with the exuvia at most sites being encountered with the first 3 meters from the water. On a single sampling occasion, each area was searched two to four times with similar search areas, effort and personnel. Each exuvia on the first pass was examined to determine sex. A multiple pass removal model was used to estimate the total density of exuvia at each site (Zippin 1956). Methods were similar but not identical to previous work (Nehring 2011) and many of the sites on the Colorado and Fraser River were identical to previous work. More effort (higher number of people) were used compared to previous work resulting in higher capture probabilities that better met assumptions of the removal model and likely allowed unbiased estimates of exuvia with two depletion passes. The two pass depletion technique worked well for these estimates and many of the issues with depletion estimates encountered in fish population estimates were not a problem due to the immobile nature of the exuvia, high capture probability, and no size selective gear (Riley and Fausch 1992, Peterson et al. 2004, Saunders et al. 2011). The density estimates from the 2014 sampling are presented in Table 2. In 2014, the salmonfly exuvia estimates on the Gunnison River were compromised by high flows on the lower four sites (Orchard, Cottonwood, Goldmine and Smith Fork) which occur below the tributaries of the Smith Fork and North Fork of the Gunnison. Flows in June of 2014 on the Gunnison River below Crystal Dam exceeded bankfull flows for over 35 days and exceeded 6,000 cfs for over 24 days (Figure 8). The salmonfly emergence began at the lower four sites in early June when releases from Crystal dam were around 6,000 cfs. Flows then increased to a maximum of 9,650 cfs. Many of the stonefly exuvia were likely washed away by increasing flows so the estimates of the lower four sites likely do not represent a significant portion of the emergence. The emergence at the upper two sites (Ute Park and Chukar) occurred on a descending hydrograph and our sampling periods encompassed the majority of the emergence. The flow conditions and sampling experiences in 2014 highlight the importance of how environmental conditions affect the Salmonfly emergence as well as the ability to estimate from exuvia sampling. Because of this, multiple years of exuvia estimates will be used to evaluate annual variability in emergence and benthic samples will be collected at each site to estimate Salmonfly density across multiple year classes. On the Colorado River in 2014 flows were high but allowed for good sampling conditions because the emergence began after the peak and the majority of Salmonflies emerged on a descending hydrograph (Figure 9). The emergence began slowly on the night of June 1 and in earnest on the night of June 2. Exuvia estimates were done on June 6, 10 and 13. On both the 6th and 10th almost 5,000 exuvia were counted in two passes while only 538 were counted on the 10th. Figure 5 shows the population estimates for the three sampling occasions.

Physical habitat surveys were completed at the six sites on the Gunnison River in 2014. These surveys included pebble counts to characterize dominant substrate size (Potyondy and Hardy 1994) and two methods to measure substrate embeddedness. Embeddedness was visually estimated following the methods of Bain and Stevenson (1999) and was measured following the Weighted Burns Quantitative Method (Burns 1985, Sennatt et al. 2006). Physical surveys of each site were completed with survey-grade GPS equipment and a HydroSurveyor acoustic Doppler current profiler system (ADCP). The GPS and ADCP surveys were analyzed by CPW aquatic researcher Eric Richer. Examples of the physical habitat survey maps and bathymetric maps produced with the GPS and ADCP surveys are presented in Figures 6 and 7. The data from the physical habitat surveys will be complied to provide a list of variables that are hypothesized to explain differences in stonefly habitat quality. A candidate set of models will be developed to identify which variables best explain differences in stonefly density with the information theoretic approach (Burnham and Anderson 2002). Density estimates and habitat surveys will be completed for a total of 18 sites on all three major rivers in Colorado with large populations of salmonflies. Habitat surveys are now completed on the Colorado and Gunnison Rivers and six sites will be completed on the Rio Grande River in 2015 before data collection for this project is completed. The modeling exercise will identify habitat variables that explain differences in stonefly density and could explain their decline or extirpation from sites. This information can then be used to guide habitat improvement projects in the Upper Colorado River basin as well as inform water development decisions on how to protect in stream aquatic habitat.

In addition to the habitat use investigation, final instar nymphs of *P. californica* were collected live from the Gunnison and Colorado Rivers in a collaborative project with USGS researchers B. Zuellig and D. Walters. The objective of this project is to investigate the ecological impact of emerging stoneflies on riparian ecosystems by estimating the carbon flux they represent between streams and terrestrial areas. Density estimates for this study were the same multi-pass depletion estimates described earlier except each 30 meter site was divided into 5 meter zones delineated with surveying stakes and string and exuvia counts were kept separate for each zone. Nymphs were reared in captivity and then relationships between adult insect biomass and exuvia were made by sex and river. This information was then applied to density estimates to calculate the total biomass Salmonflies represent at a single site and the resultant potential carbon flux.

Preliminary results of this project were presented at the Society for Freshwater Science meeting in Portland Oregon (Walters et al. 2014). Abundance varied considerably within and among riffles, but this variation was small compared to among-river differences. Females were two-fold larger than males, and individual masses varied two-fold among rivers (female range = 175-300 mg AFDM). Salmonflies exported 156 g C/m shoreline/y at the most abundant site (Colorado River) in 2013, 10-fold higher than predicted for annual C flux of all insect taxa for a similarly sized river. Carbon fluxes by salmonfly emergence at other sites also commonly met or exceeded this annual prediction. This data indicates that the synchronous emergence of large, productive taxa like salmonflies is a potentially significant carbon source for riparian foodwebs, particularly in semi-arid landscapes.

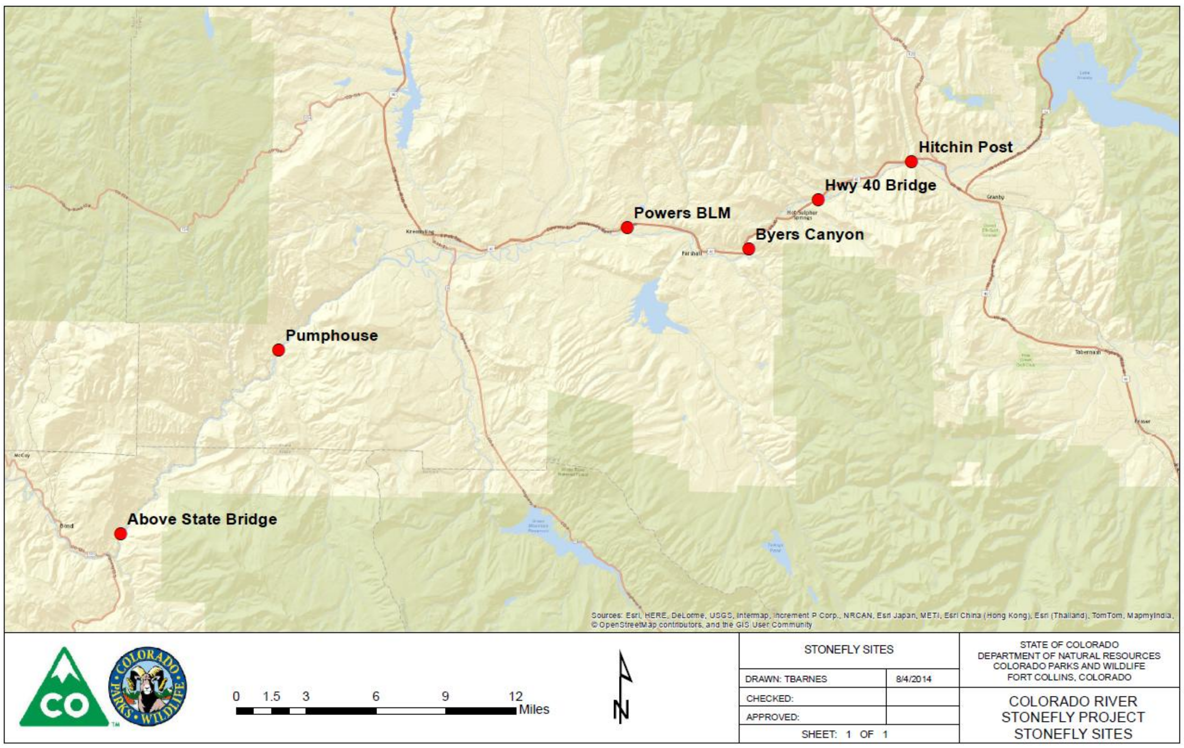

Figure 1. Map of stonefly sampling sites on the Colorado River.

Table 1. Salmonfly Sampling Sites 2014. Six sites each on the three major rivers will be included in the Salmonfly habitat study while others sites are historical sites or sampled for other projects.

River	#	Site	Side	UTM NAD 83 (Zone 13)
Gunnison	1	Orchard Boat Ramp	River Left	247947, 4295297
Gunnison	2	Cottonwood Campground	River Left	252129, 4295940
Gunnison	3	Goldmine	River Left	253728, 4295747
Gunnison	4	Smith Fork	River Left	253338, 4291889
Gunnison	5	Ute Park	River Left	252376, 4284894
Gunnison	6	Chukar	River Left	253421, 4278775
Fraser	7	Kaibab Park in Granby	River Left	420592, 4437168
Colorado	8	State Bridge	River Right	359889, 4414634
Colorado	9	Pumphouse BLM	River Left	370827, 4427300
Colorado	10	Powers BLM	River Right	394914, 4435762
Colorado	11	Byers Canyon	River Left	403335, 4434268
Colorado	12	Hwy 40 Bridge	River Right	408133, 4437708
Colorado	13	Hitching Post	River Left	414589, 4440304
Rio Grande	14	LaGarita	River Left	338264, 4182888
Rio Grande	15	Lower Wason 2	River Right	335653, 4186302
Rio Grande	16	Lower Wason 1	River Right	335353, 4187197
Rio Grande	17	Upper Wason 2	River Right	333668, 4187683
Rio Grande	18	Creede Hatchery	River Left	332145, 4187768
Rio Grande	19	Creede Boat Ramp	River Left	331362, 4187243

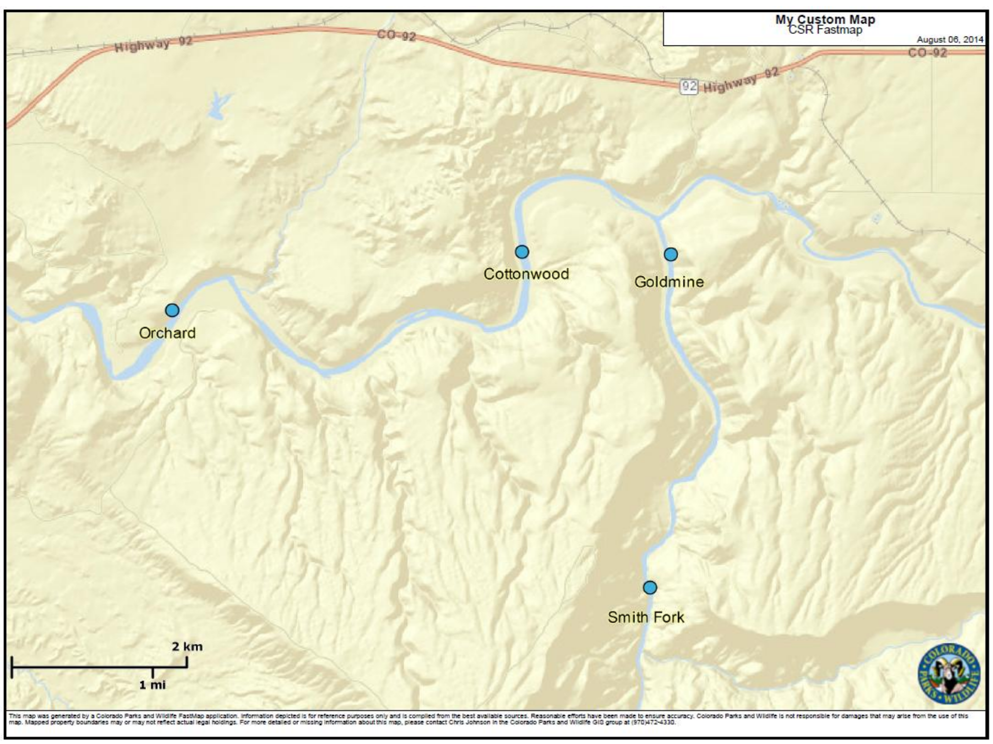

Figure 2. Map of lower stonefly sampling sites on the Gunnison River.

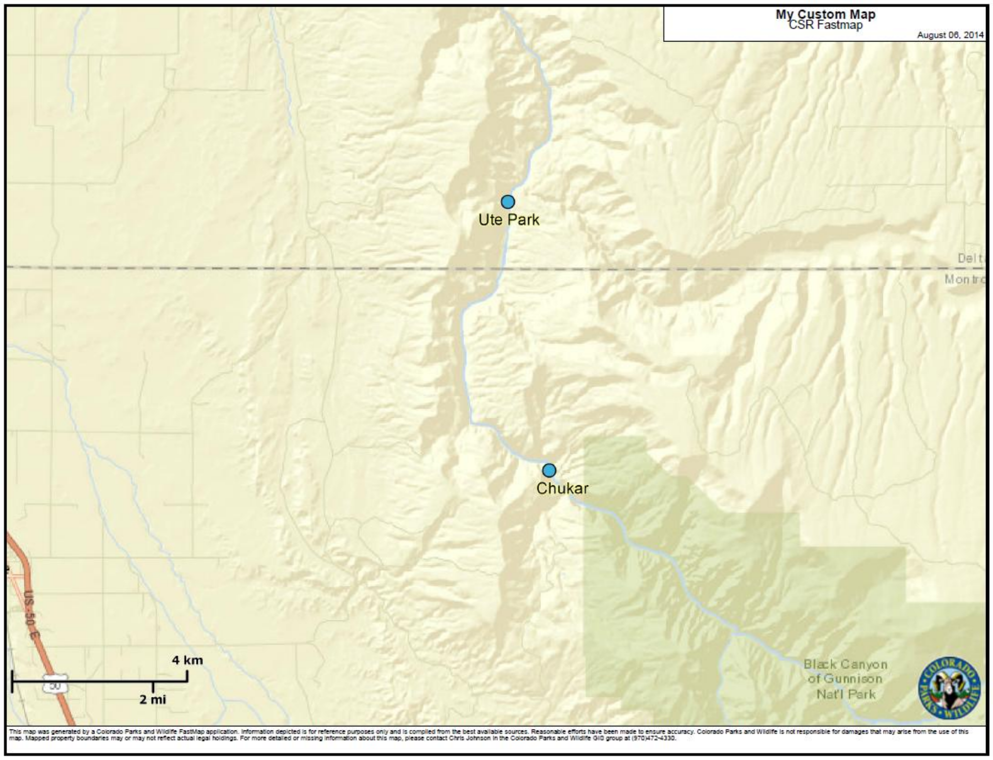

Figure 3. Map of upper stonefly sampling sites on the Gunnison River.

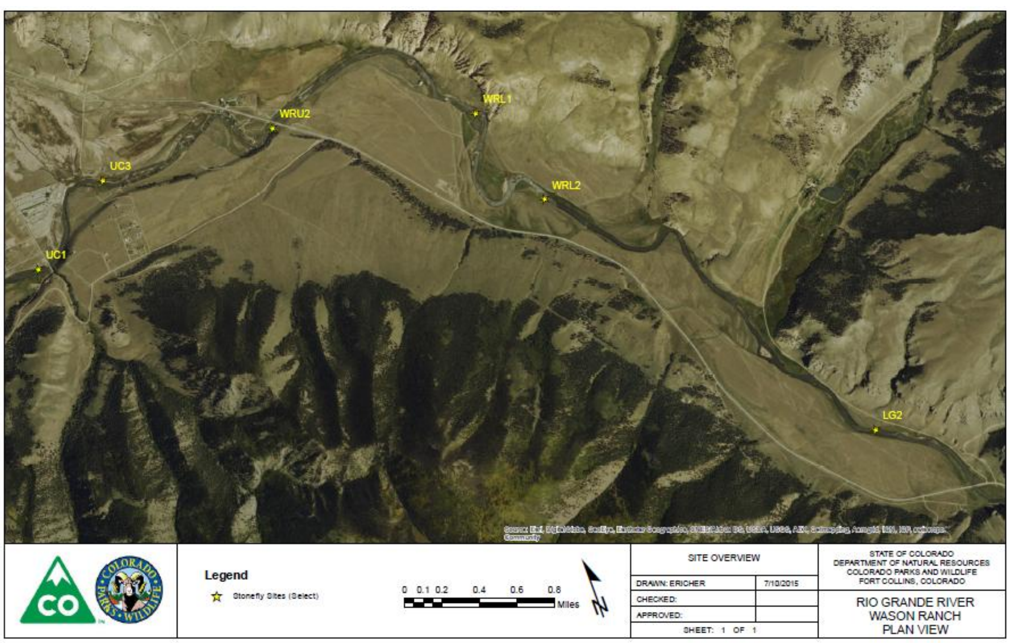

Figure 4. Map of stonefly sampling sites on the Rio Grande River. UC1 is Creede Boat Ramp, UC3 is Creede Hatchery, WRU2 is Upper Wason 2, WRL1 is Wason 1, WRL2 is Wason 2, and LG2 is LaGarita.

Table 2. Salmonfly Exuvia Estimates 2014

River	Site	Population Estimate (30 m)	95% C.I. ±	#/m	95% C.I. ±
Gunnison	Orchard Boat Ramp	175.7*	11.2	5.9	0.4
Gunnison	Cottonwood Campground	283.7*	50.1	9.5	1.7
Gunnison	Goldmine	261.0*	43.1	8.7	1.4
Gunnison	Smith Fork	530.0*	17.9	17.7	0.6
Gunnison	Ute Park	8,834.8	41.2	294.5	1.4
Gunnison	Chukar	1,267.8	27.3	42.3	0.9
Fraser	Kaibab Park	134.9	11.5	4.5	0.4
Colorado	Pumphouse #3	10,885.0	106.2	362.8	3.5
Colorado	Pumphouse #2	2,440.4	22.0	81.3	0.7
Colorado	Byers Canyon	5,216.5	42.9	173.9	1.4
Colorado	Hitching Post	13.0	62.0	0.4	2.1
Rio Grande	LaGarita	1,693.5	27.1	56.4	0.9
Rio Grande	Wason 2	214.8	8.3	7.2	0.3
Rio Grande	Wason 1	1,057.4	12.4	35.2	0.4
Rio Grande	Upper Wason 2	783.7	12.0	26.1	0.4
Rio Grande	Hatchery	12,918.5	75.4	430.6	2.5
Rio Grande	Creede Boat Ramp	777.5	13.6	25.9	0.5

*Increasing flows post emergence reduced the numbers of exuvia available during the survey and estimates at the lower four sites are likely underestimates of total emergence.

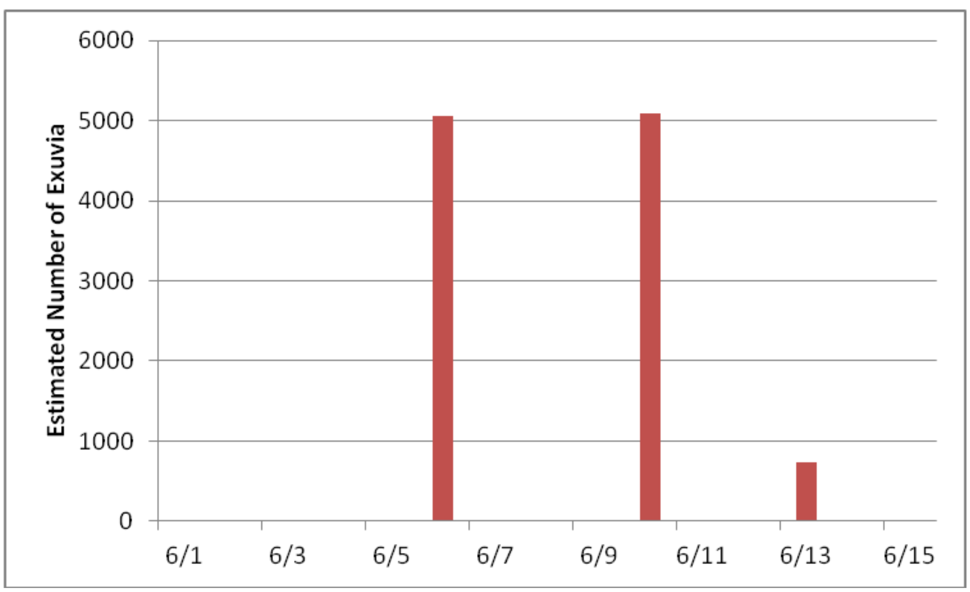

Figure 5. Salmonfly exuvia estimates at the Pumphouse site on the Colorado River on three sampling occasions in 2014. The emergence began at this site on June 1st and lasted between 12 and 14 days.

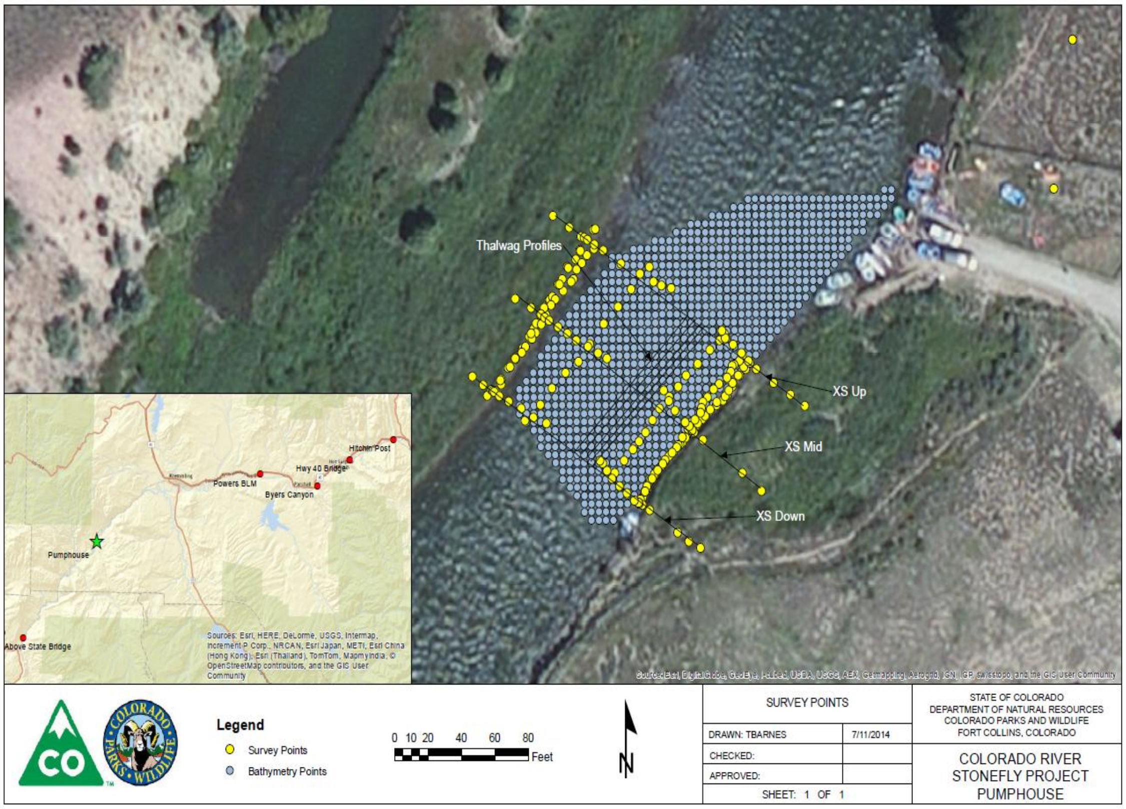

Figure 6. Survey points and bathymetry data collected with the survey-grade GPS equipment and Acoustic Doppler Current Profiler of the Pumphouse stonefly site.

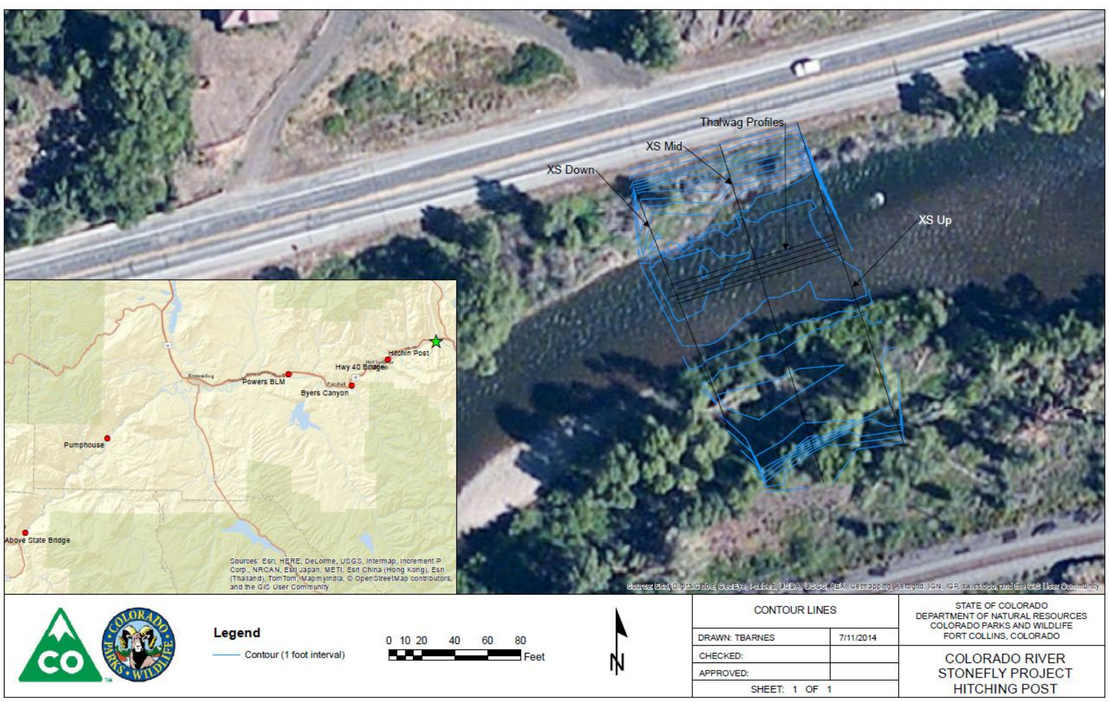

Figure 7. Bathymetric map produced by the GPS and ADCP survey used to estimate physical channel characteristics of stonefly study sites

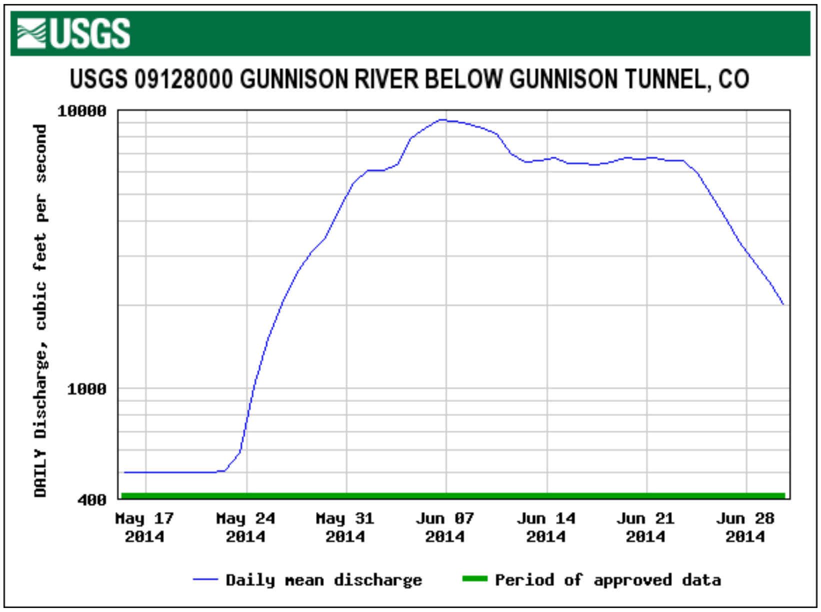

Figure 8. Flows in the Gunnison River in May and June of 2014. Salmonflies began emerging at the lower four Gunnison River sites on June 2nd before runoff peaked. Flow increases likely washed away exuvia leading to an underestimate of the emergence at these four sites. The emergence began at the Ute Park site near June 12th making a higher portion of the exuvia from the total emergence available to sample.

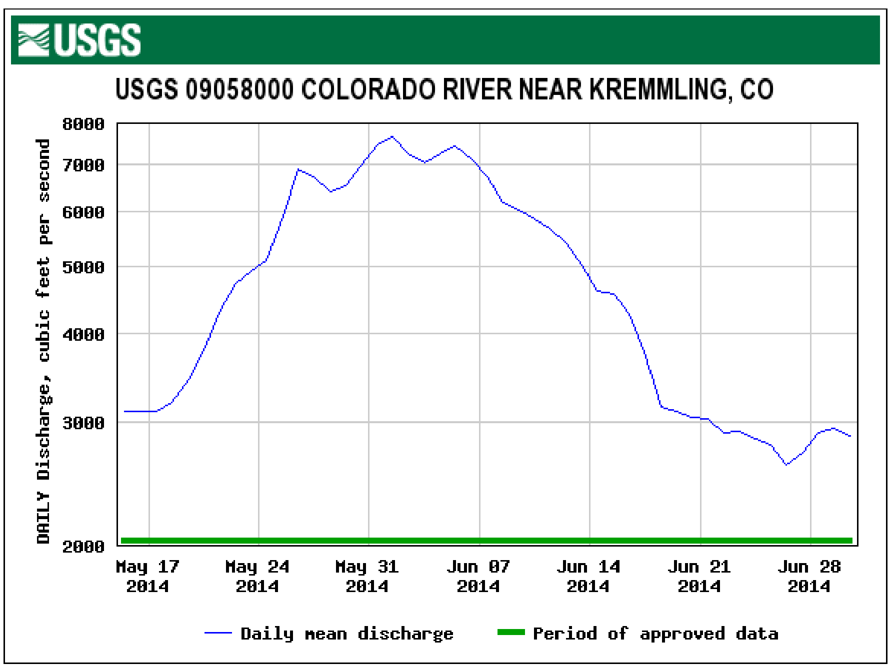

Figure 9. Flows in the Colorado River in May and June of 2014. Salmonflies began emerging at the Pumphouse sampling site on the nights of June 1st. The emergence lasted for 12-14 days at this site and occurred on descending flows, allowing for good estimates of the emergence with multiple pass removal exuvia estimates.

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Job No. 2. Impacts of Whitewater Park Development on Invertebrates, Mottled Sculpin and Trout

Job Objective: Investigate the effects of whitewater parks on invertebrates, mottled sculpin *Cottus bairdi* and trout in Colorado.

Artificial whitewater parks (WWP) are increasingly common throughout Colorado and there are concerns about how they impact fish and aquatic invertebrates (Kolden 2013, Fox 2013). Many of the rivers around the state with whitewater parks are also some of the best wild trout fisheries. The construction of whitewater parks involves replacing natural riffles with concrete or grouted rock grade control structures to produce hydraulic waves for recreational boating. Natural riffles serve many important physical and ecological roles in rivers. Ecologically, riffles serve as the most productive areas of a stream for periphyton and invertebrate production that form the foundation of the aquatic food web. Physically, riffles serve as grade control structures for streams and their location, frequency and size drive the main characteristics of stream geomorphology. Artificial pools created below WWP waves have been found to hold a lower biomass of trout than natural pools, and have more dynamic and higher magnitude flows and velocities (Kolden 2013). Whitewater parks have also been documented to cause a suppression of fish movement that is related to fish length (Fox 2013). Concerns have been raised that whitewater parks not only impact fish habitat and fish passage but could affect some aquatic invertebrates that are primary diet items for trout (Kondratieff 2012).

In addition to sportfish concerns, native non-game fish are also common at many sites of whitewater parks. Mottled sculpin are a bottom dwelling native fish that occupy many coldwater streams and rivers of Colorado. Their unique habitat preferences and reliance on quality riffle/ run habitat make them a good ecological indicator of stream health (Nehring 2011). Because the function of riffle/run habitat is commonly impacted when stream flows are altered or instream habitat is manipulated as in a whitewater park, mottled sculpin may be impacted by habitat related changes before higher predators like trout. Sculpin could not only indicate ecological problems that will eventually affect sport fish like trout, but they serve as an important food source, especially for brown trout common in many Colorado rivers. The objective of this study is to investigate the effects of building whitewater parks on mottled sculpin, aquatic invertebrates, and trout by sampling before and after construction with control sites.

PROGRESS

Two whitewater parks were constructed in western Colorado in 2014, on the Uncompahgre River in Montrose and at the Pumphouse Recreation site on the Colorado River. Before construction occurred on either river, aquatic invertebrate samples, mottle sculpin density estimates and brown trout population estimates were made.

Uncompahgre River

On the Uncompahgre River aquatic invertebrate samples were taken at five sites, one below the planned WWP, two within and two above. The WWP on the Uncompahgre River consist of six drop structures over about 0.2 miles of river. Replicate macroinvertebrate samples (n = 5) were collected at each site using a 0.086 m^2 Hess sampler with a 350 µm mesh net. Samples were collected in November of 2014, several weeks before construction of the WWP began. The replicate samples were collected from the same riffle with predominantly cobble substrate by disturbing the streambed to a depth of approximately 10 cm. Field samples were washed through a 350-µm sieve and organisms preserved in 80% ethanol. Velocity and depth were taken at each Hess sample site to ensure samples were taken from similar riffle habitat. Macroinvertebrate samples were sorted and sub-sampled in the laboratory using a standard USGS 300-count protocol, except that replicates were not composited and each one underwent the protocol (Moulton et al. 2000). All organisms, except for chironomids and non-insects, were identified to genus or species. Chironomids were identified to subfamily and non-insects (e.g., oligochaetes, amphipods) were identified to class. Each replicate sample was processed separately so an average of 1,670 individual specimens were identified at each riffle site. Many more individual specimens were identified from each site compared to standard methods to ensure rare organism were sampled and increase robustness of the comparisons between riffles sites in close proximity within the same stream (Vincent and Hawkins 1996). A preliminary summary of macroinvertebrate results is presented in Table 3 and Figure 10. Data analysis was still ongoing at the time of this report but richness was similar across all sites while density of most taxa was similar at most sites except was lower at site 2. These sites appear to be a good baseline collection for post construction comparisons.

Table 3. Summary of Macroinvertebrate Data from the Uncompahgre River in 2014.

	UNC 1	UNC 2	UNC 3	UNC 4	UNC 5
Average Density All Species (m^2)	32,198	10,240	22,380	20,295	19,395
Average Density EPT (m^2)	11,873	2,751	9,169	6,152	6,326
Average Density Ephemeroptera (m^2)	7,580	2,049	6,597	4,770	4,552
Average Density Plecoptera (m^2)	322	190	363	414	262
Average Density Trichoptera (m^2)	3,971	513	2,210	969	1,512
Average Density Coleoptera (m^2)	548	58	502	138	262
Average Density Diptera (m^2)	16,102	6,643	10,146	12,280	11,480
All Taxa Species Richness	31	26	35	28	30
EPT richness	17	16	20	14	16
Ephemeroptera richness	5	5	5	4	5
Plecoptera richness	5	5	7	5	4
Trichoptera richness	7	6	8	5	7
Coleoptera richness	2	1	2	2	2
Diptera richness	7	4	6	7	6
Non-insect richness	5	5	7	5	6
Other taxa richness	14	10	15	14	14

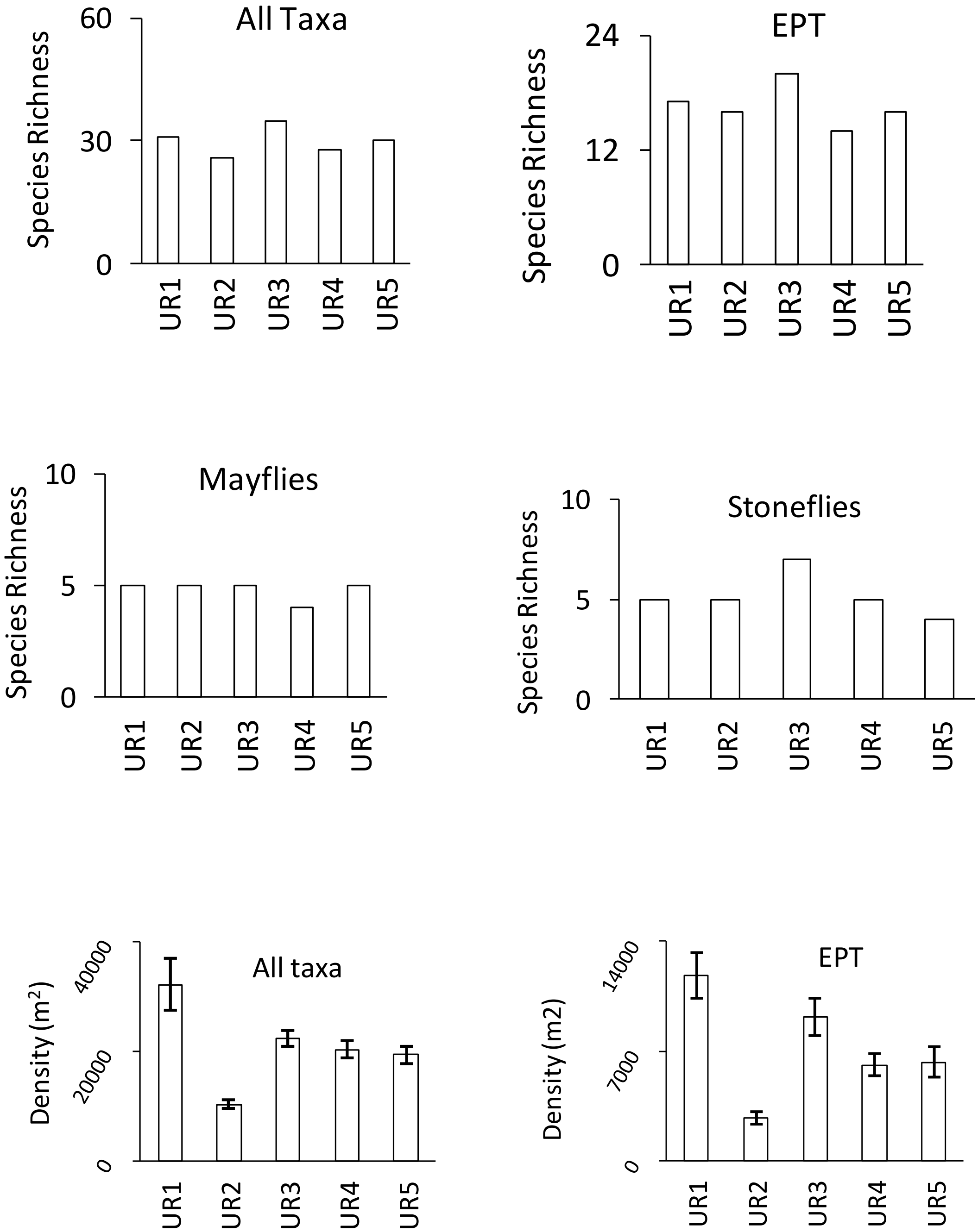

Figure 10. Species richness (total number of species in all replicates) and density (with standard errors) at sites on the Uncompahgre River. The whitewater park structure was built on top of sites 3 and 4.

To monitor mottled sculpin and brown trout, four electrofishing stations were established concurrent with the invertebrate sites, one below the WWP, one within (that encompassed two invertebrate sampling riffles) and two above. Sites 1 and 3 had habitat improvement projects done specifically for fish. The electrofishing stations averaged 704.3 ft (512-849) long. An attempt was made to use block nets, but they could not be kept in place due to high discharge and velocity. Natural stream features like shallow riffles were used as endpoints to best insure closure. Three pass removal electrofishing was completed at each site with a Smith Root VVP15 truck mounted electrofisher and five anodes. All fish were weighed, measured and population estimates were made with the Huggins Closed Capture model in Program Mark (Huggins 1989, White and Burnham 1999). To reduce the bias associated with the size selectivity of electrofishing, capture probabilities were modeled with length as a covariate similar to the approach described in Saunders et al. 2011. Four models were built for each species estimating capture probabilities by length, time, time + length, as well as a constant capture probability for all fish and all three passes. The time models allowed for different capture probabilities for the 2nd and 3rd passes compared to the first to address a common source of bias in electrofishing removal models. Model selection was done with AICc and population and parameter estimates were made by model averaging across all four models with AICc weights (Burnham and Anderson 2002). Table 4 summarizes the fish data.

Table 4. Fish Sampling Data from the Uncompahgre River in November 2014.

	Brown Trout	± 95% C.I.	Mottled Sculpin	± 95% C.I.
Site 1 (Downstream Control-Fish Habitat)	49	28.9	85.8	143.4
Site 2 (Whitewater Park)	18.4	5.8	67.9	14.9
Site 3 (Upstream Control-Fish Habitat)	43.2	102.4	165.0	950.7
Site 4 (Upstream Control)	69.8	3.6	113.9	23.3

Colorado River

On the Colorado River aquatic invertebrate samples were taken at four sites, two below the planned WWP, one within and one above. The WWP on the Colorado River consists of a single large cross channel kayak wave so fewer sites were sampled. Replicate macroinvertebrate samples (n = 5) were collected at each site using a 0.086 m^2 Hess sampler with a 350 µm mesh net. The replicate samples were collected from the same riffle with predominantly cobble substrate by disturbing the streambed to a depth of approximately 10 cm. Field samples were washed through a 350-µm sieve and organisms preserved in 80% ethanol. Velocity and depth were taken at each Hess sample site to ensure samples were taken from similar riffle habitat. Macroinvertebrate samples were sorted and sub-sampled in the laboratory using a standard USGS 300-count protocol, except that replicates were not composited and each one underwent the protocol (Moulton et al. 2000). All organisms, except for chironomids and non-insects, were identified to genus or species. Chironomids were identified to subfamily and non-insects (e.g., oligochaetes, amphipods) were identified to class. Each replicate sample was processed separately so an average of 1,379 individual specimens were identified at each riffle site. A much higher number of individual specimens were identified from each site compared to standard methods, to ensure rare organism were sampled and increase robustness of the comparisons between riffles sites in close proximity in the same stream (Vinson and Hawkins 1996). A preliminary summary of macroinvertebrate results is presented in Table 5 and Figure 11. Data analysis was ongoing at the time of this report but richness was similar across all sites while density of most taxa was lower at the middle two sites. The sites appear to be a good baseline collection for post construction comparisons.

Table 5. Summary of Macroinvertebrate Data from the Colorado River in 2014.

	COR 1	COR 2	COR 3	COR 4
Average Density All Species (m^2)	20,281	9,709	13,814	24,062
Average Density EPT (m^2)	10,427	5,821	5,945	11,697
Average Density Ephemeroptera (m^2)	6,023	3,602	3,576	6,497
Average Density Plecoptera (m^2)	260	691	517	645
Average Density Trichoptera (m^2)	4,144	1,528	1,852	4,554
Average Density Coleoptera (m^2)	741	476	765	1,213
Average Density Diptera (m^2)	5,485	2,446	3,224	8,366
All Taxa Species Richness	30	32	37	34
EPT richness	18	20	21	20
Ephemeroptera richness	8	9	9	9
Plecoptera richness	4	4	4	3
Trichoptera richness	6	7	8	8
Coleoptera richness	3	4	3	3
Diptera richness	4	4	5	5
Non-insect richness	5	4	8	6
Other taxa richness	12	12	16	14

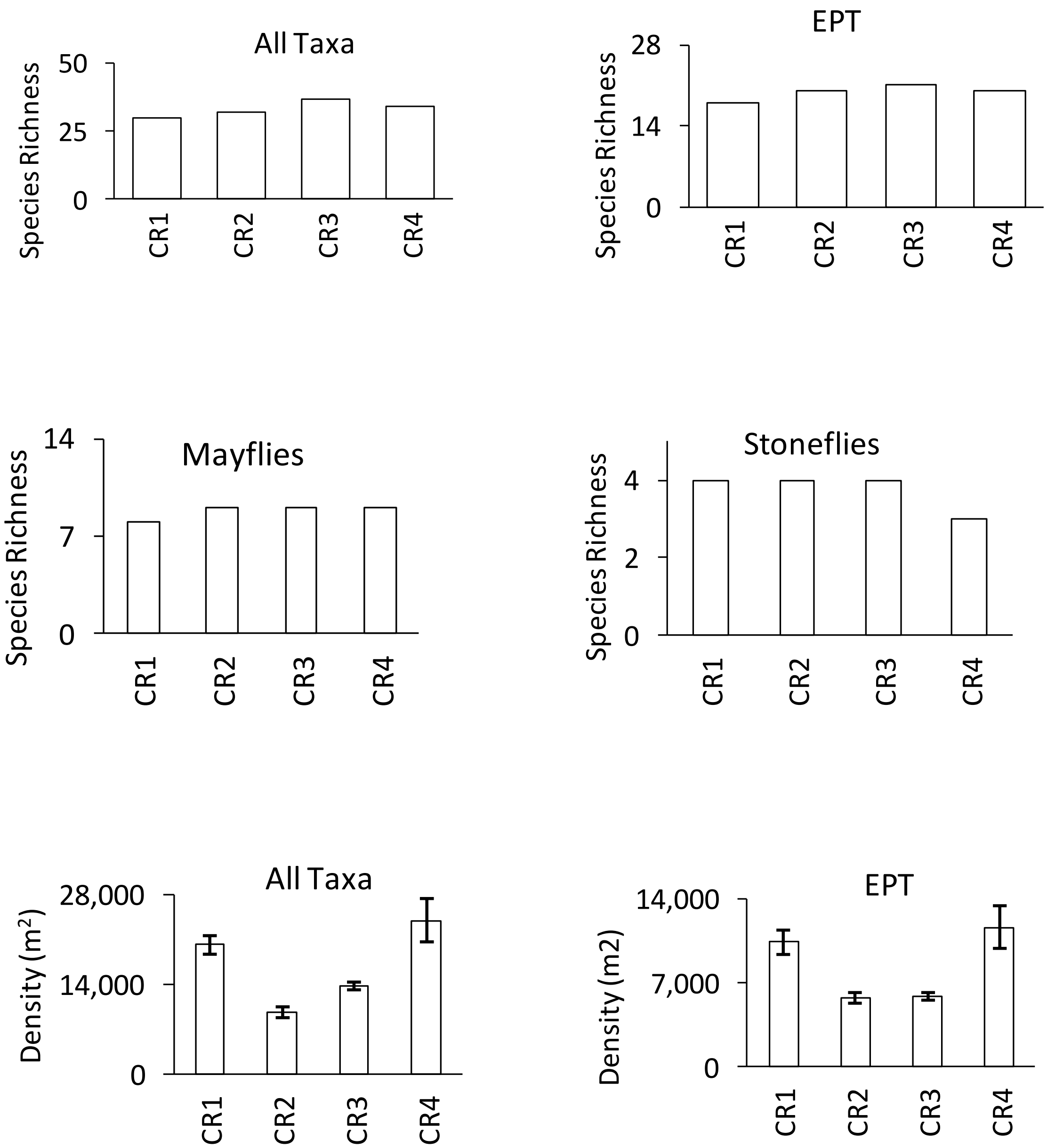

Figure 11. Species richness (total number of species in all replicates) and density (with standard errors) at sites on the Colorado River at Pumphouse. The whitewater park structure was built on top of site 3.

To monitor mottled sculpin, three electrofishing stations were established. Because the Colorado River averages 170.5 ft wide at this site, it was impossible to electrofish for mottled sculpin across the whole channel and smaller plots along the bank in run habitat were chosen. Site 1 was near BLM Boat Launch #1 above the proposed WWP, site #2 was centered on where the WWP structure would be built, and site 3 was near BLM Boat Launch #3, below the WWP. Three pass removal electrofishing was completed at each plot with three Smith Root LR24 backpack electrofishers. To evaluate the closure assumptions of the removal model and check estimated capture probabilities, mottled sculpin were captured before each site was sampled, marked with a caudal fin clip and then released inside each plot. The electrofishing sites averaged 302 feet long and 17.6 feet wide. All fish were measured to the nearest mm and population estimates were made with the Huggins Closed Capture model in Program Mark (Huggins 1989, White and Burnham 1999). To reduce the bias associated with the size selectivity of electrofishing, capture probabilities were modeled with length as a covariate similar to the approach described in Saunders et al. 2011. Four population estimation models were built modeling capture probabilities by fish length, time, time + length, as well as a constant capture probability for all fish and all three passes. The time models allowed for different capture probabilities for the 2nd and 3rd passes compared to the first to address a common source of bias in electrofishing removal models. Model selection was done with AICc and population and parameter estimates were made by model averaging across all four models with AICc weights (Burnham and Anderson 2002).

Mottled sculpin density estimates are presented in Table 6. Capture probabilities were average (0.42-0.54) and declined with subsequent passes (Figure 12). Measured capture probabilities were lower than the model averages estimates (Figure 13) indicating there was a violation of the closure assumption and/or individual heterogeneity in capture probabilities. These issues are well known with removal models with electrofishing but can be overcome in some instances (i.e. with salmonids) with high capture probabilities, modeling capture probabilities over time and by using length as a covariate to model capture probabilities (Riley and Fausch 1992, Saunders et al. 2011, Petersen et. al 2004). Because mottled sculpin are small, cryptic, lack a swim bladder and because we could not ensure closure, our density estimates are likely biased low. However, it does appear that the biases are relatively small and all in the same direction (low) so comparisons of relative density between these sites (all collected with same methods and equipment) should be valid. Petersen et al. (2004) states that, "at relatively high first-pass efficiencies (>35%) and low reduction in efficiency per pass (<1.10), the removal estimates were nearly unbiased." Riley and Fausch (1992) found that the negative bias for estimates decreased as initial capture probability increased and for three-pass estimates confidence interval coverage was actually better at low population sizes because of the larger standard deviations associated with small samples. More work is necessary to determine appropriate methods for robust population estimates for mottled sculpin in large rivers.

To monitor trout and mountain whitefish populations around the WPP, mark recapture electrofishing was done with a 16 ft aluminum jet boat and a Smith Root 2.5GPP electrofisher. The sampling reach was 7,085 ft long and averaged 170.5 ft wide and was centered on the WWP structure. Fish population estimates were made with the Huggins Closed Capture Model in Program Mark (Huggins 1989, White and Burnham 1999). Four models were built by estimating capture probabilities by length, species, species + length, as well as a constant capture probability for all fish, identical to a Lincoln Petersen model (Seber 1982). Model selection was done with AICc and population and parameter estimates were made by model averaging across all four models with AICc weights (Burnham and Anderson 2002). There were an estimated 5,146±795 brown trout, 98±41 rainbow trout, and 1,077±409 mountain whitefish in the sampling reach in October 2014 before the construction of the WWP. The study reach contained an estimated 3,908 trout per mile and exceeds the Gold Medal standard for biomass and quality fish.

Table 6. Mottled Sculpin Density Estimates from the Colorado River at Pumphouse.

	Capture Probability (SE)			Density	95% C.I.
	Pass 1	Pass 2	Pass 3	(Fish/Acre)	±
Site 1	0.473 (0.04)	0.542 (0.09)	0.542 (0.09)	2,906.0	403.8
Site 2	0.427 (0.09)	0.386 (0.14)	0.386 (0.13)	2,529.2	1,124.9
Site 3	0.424 (0.06)	0.421 (0.09)	0.421 (0.09)	1,775.2	354.7

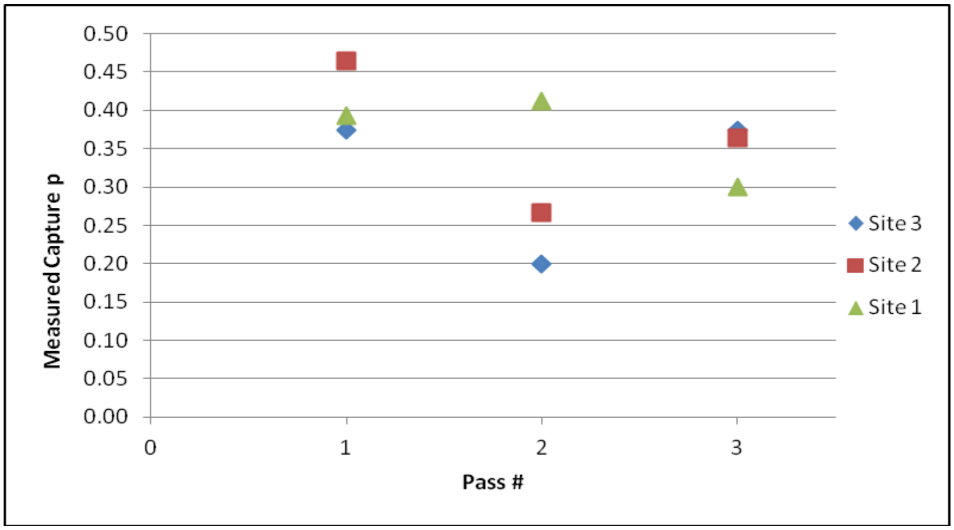

Figure 12. "Measured" capture probability across passes for mottled sculpin sites on the Colorado River. Measured capture probability was calculated by comparing the number of marked fish captured in a pass to the number available.

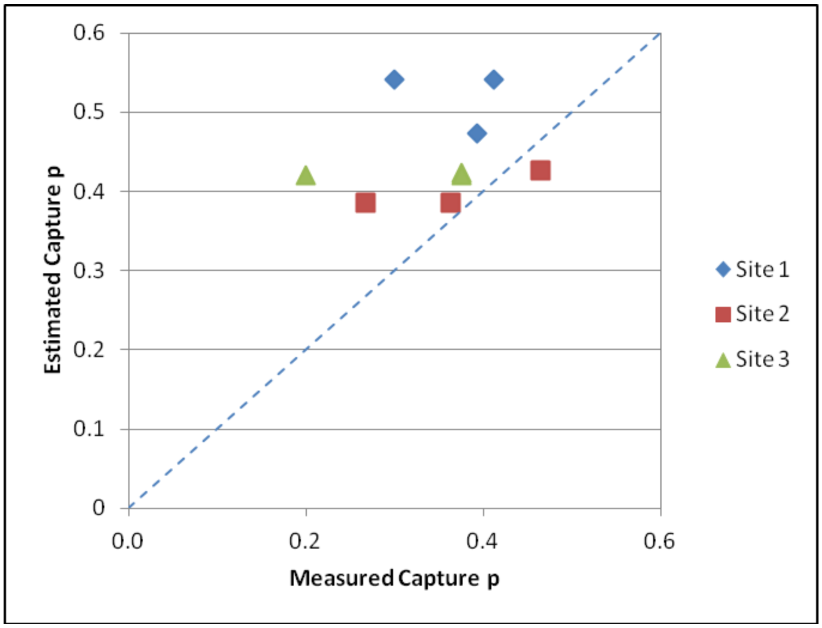

Figure 13. "Measured" capture probability compared to estimated capture probability for mottled sculpin in the Colorado River. Measured capture probability was calculated by comparing the number of marked fish captured in a pass to the number available. Estimated capture probability was from the model averaged results of the four models built in the Huggins Close Capture model in Program Mark.

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Job No. 3. **<u>Colorado River Water Project Mitigation and Ecology Investigations</u>**

Job Objective: Investigate the ecological impacts of stream flow alterations on aquatic invertebrates and fish of the Colorado River and assist in the planning and evaluation of mitigation efforts to address those impacts.

Previous work under Project F-237 identified ecological impacts of stream flow alterations and a main stem reservoir on the invertebrates and fish of the upper Colorado River (Nehring 2011). Further flow alterations and increased trans-basin water diversions are planned and there are ongoing discussions on mitigation activities to reduce the impact of the new projects. The objective of this study is to continue monitoring invertebrate and fish populations of the upper Colorado River and assist CPW staff in planning of mitigation efforts and then evaluate the effectiveness of those efforts in restoring and improving the ecological function of the Colorado River in Middle Park. The timing and specific methods used to reach this objective will depend on what types of mitigation efforts are proposed and the timeline of their completion.

PROGRESS

Mitigation planning by the major stakeholders is ongoing but no projects have yet been completed. The Windy Gap bypass study has been released and was reviewed and discussed with other CPW personnel. The preferred alternative for a bypass channel around Windy Gap has been identified but a large funding gap remains. Partners are currently working to raise the money for the preferred alternative. Progress in 2014 included making a presentation to the Upper Colorado River Learning By Doing Committee on invertebrate and fish monitoring on the Colorado River. Standard monitoring sites on the Colorado and Fraser rivers were also completed in 2014 to evaluate trends in native fish and aquatic invertebrates and are presented below in Table 7 and Figures 14. Future work on this objective will involve disseminating knowledge from other jobs in F237R to stakeholders involved in the mitigation work and conducting research to evaluate the post-implementation effectiveness of mitigation measures.

Table 7. Mottled Sculpin Monitoring Sites on the Colorado and Fraser Rivers.

#	River	Site	Density (#/Acre) 2013	Density (#/Acre) 2014
1	Colorado	Pumphouse	1,869± 1,793	2,420± 393
2	Colorado	Byers Canyon	0	0
3	Colorado	Hwy 40 Bridge	0	0
4	Colorado	Hitching Post	0	0
5	Fraser	Kaibab Park	2,092± 747	1,182± 741

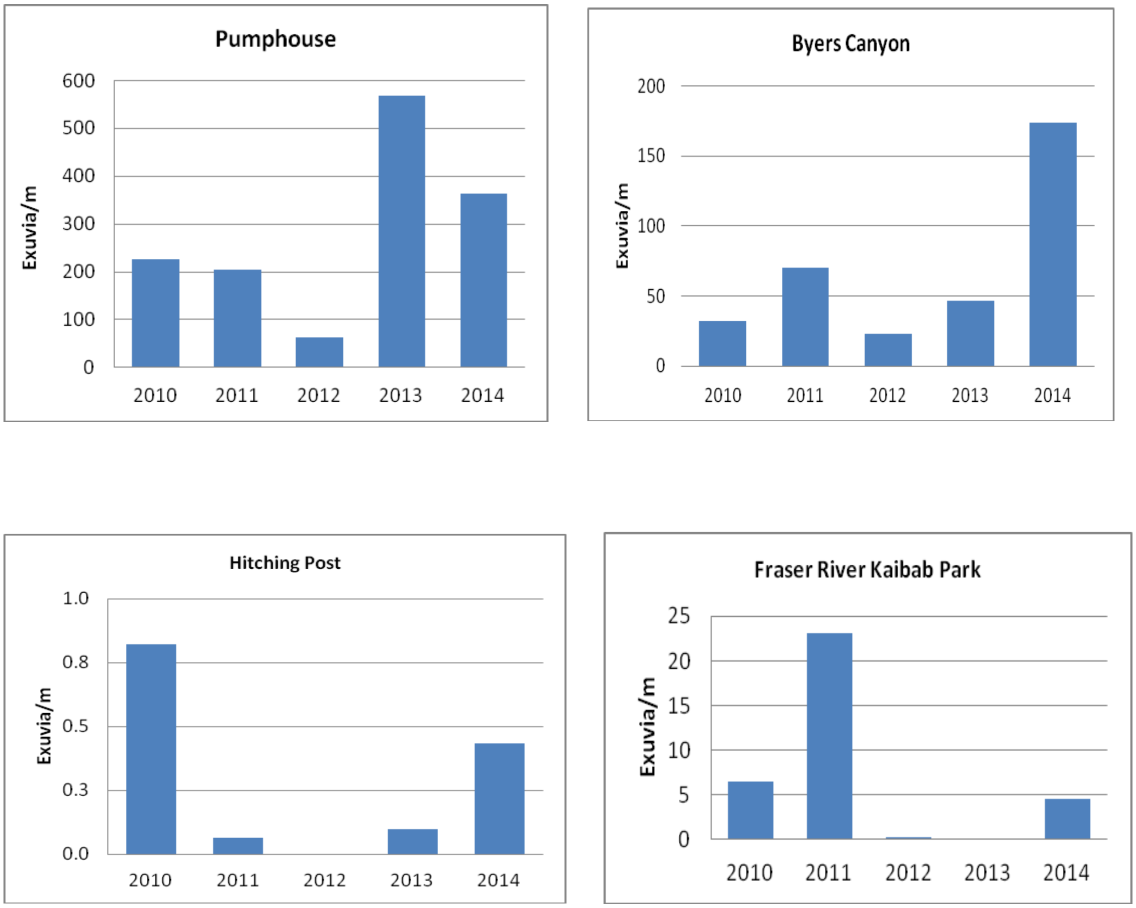

Figure 14. Salmonfly monitoring sites on the Colorado and Fraser Rivers 2010-2014.

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Job No. 4: <u>Gunnison River Aquatic Invertebrate and Pesticide Studies</u>

<u>Job Objective</u>: Investigate the impact of mosquito control insecticides on aquatic invertebrates in Colorado streams by reviewing toxicity literature, conducting water quality sampling, collecting aquatic invertebrate samples and conducting toxicity tests.

The major impetus for this study was an incident in early July 2012 in Gunnison County, Colorado. The day after a large scale aerial application of Biomist to control adult mosquitoes in the Gunnison watershed, CPW received reports of large numbers of dead stoneflies in the Gunnison River. The incident was noted by many anglers and covered in the local news (Mensing 2012, Gunnison Trout Unlimited 2012). Parks and Wildlife personnel investigated, along with an independent inquiry by a researcher from Western State Colorado University. It was confirmed that large numbers of stoneflies (notably *Claassenia sabulosa*) died suddenly within the treated area. There were several mitigating circumstances that could have contributed to the aerial application impacting aquatic invertebrates including a new applicator with different equipment as well as record low flows in the river. There was enough evidence for a potential resource concern that a study was planned to investigate the impact of mosquito control pesticides, especially permethrin, on aquatic invertebrates in the Gunnison River and Tomichi Creek.

The primary active ingredient in Biomist and other mosquito control insecticides is permethrin. Permethrin is a broad spectrum, non-systemic pyrethroid insecticide that is commonly used to control agricultural pests and mosquitoes. The chemical name is (3-phenoxyphenyl)- methyl (+)cis-trans-3-(2,2-dicloroethenyl)-2, 2-dimethylcyclopropanecarboxylate. Common products containing permethrin include Ambush, Biomist, Dragnet, Hot Shot, Permectrin, Pounce, Raid and Unicorn. Because of environmental impacts, human health concerns and insect resistance, pyrethroid insecticides began replacing some organophosate and organochlorine insecticides in the 1970's and are commonly used today for many applications. Permethrin's chemical toxicity to insects results from disruption of the peripheral nervous system by reacting to the voltage gated sodium channels in nerves. Permethrin has been shown to be relatively selective for insects and has low toxicity to mammals and birds and does not bioaccumulate. However, pyrethroid insecticides are known to be highly toxic to aquatic invertebrates, plankton and fish (Coats et al. 1989, Haya 1989). Concerns about impacts to non-target aquatic organisms have been raised as early as the 1960's. Several literature reviews have been completed, Mian and Mulla (1992) is the most recent and thorough.

Many laboratory, microcosm and field experiments have documented the toxicity of permethrin to aquatic organisms and fish. This should be expected because it was designed to control target pests that have similar physiology and occupy the same habitats as many aquatic invertebrates. Permethrin is highly toxic to plankton and small invertebrates that are important to lake ecosystems like *Daphnia magma*, *D. pulex*, *Gammarus* amphiopods (scuds), and mysis shrimp (*Mysis diluviana*). The LC_{50} values (concentration that kills 50% of test organism in a given time period) for these invertebrates is very similar to the values for mosquito and black fly larvae, 0.02-3.0 ppb (Mian and Mulla 1992, Mulla et al. 1978, Stratton and Cork 1981). Permethrin is also highly toxic to aquatic invertebrates common to coldwater stream ecosystems like caddisflies, stoneflies and mayflies. Common caddisflies like *Brachycentrus* and *Hydropsyche* have 24-h and 96-h LC_{50} values between 0.1-0.7 ppb (Anderson 1982, Mohsen and

Mulla 1981). *Baetis*, *Ephemerella* and *Hexegenia* mayflies have 24-96h LC_{50} values from 0.17-1.1 ppb (Anderson 1982, Hill 1985, Mohsen and Mulla 1981). The stonefly *Pteronarcys dorsata* was reported to have a 72-h LC_{50} value between 0.04-1.0 ppb and sub-lethal effects were noted at concentrations as low as 0.04 ppb. In addition to laboratory toxicity studies, several field studies have documented impacts from permethrin to aquatic invertebrates. Kreutzweiser and Sibley (1991) documented massive increases in invertebrate drift (100-5,600 fold) in a boreal stream in Canada at permethrin concentration of 8.64 ppb. The treatment also resulted in significant short term (16 day post treatment) reductions in the numbers of mayflies, stoneflies and caddisflies in benthic samples immediately below the treatment areas.

Fish are also very sensitive to permethrin at low concentrations. Rainbow trout have reported 24-h LC50 of 18 ppb and 96-h LC50 values of 9.8 ppb (Imgrund 2003, Glickman et al. 1981). Atlantic salmon have a 96-h LC50 of 6.02 ppb (McLeese 1980). Rebach (1999) reported a 72-h LC50 for striped bass hybrids (*Morone saxatilis* x *Morone chrysops*) of 16.1 ppb. This test used a 1:1 mixture of permethrin and piperonyl butoxide (PBO). Piperonyl butoxide is a commonly used synergist for many pesticides including commercially available mosquito control products such as Biomist (one formulation that is used in Gunnison County). This compound is also noteworthy in that it was frequently used as a synergist with liquid rotenone formulations used for fish control applications and is known to highly toxic to aquatic invertebrates (Finlayson et al. 1999). This study concluded that rotenone treatments designed to remove unwanted fish had a lower impact to non-target invertebrates when PBO was not included in the formulation.

It is important to note how toxic permethrin is to aquatic invertebrates at extremely low concentrations. Many of the toxicity values reported here are near five parts per billion or less. One part per billion (10^{-9}) equivalent to one drop of water diluted into 250 standard drums (55 gallons). For comparison, *Culex* and *Aedes* mosquitoes have 24-h LC_{50} values between 0.7-6 ppb (Mulla et al. 1978) so application rates sufficient to control target organism are generally lethal to non-target aquatic invertebrates. Even below recommended application rates, mosquito control treatments can be expected to have serious negative impacts to aquatic invertebrates like mayflies, stoneflies and caddisflies if the application leads to target concentrations in streams and rivers. It is well established that mosquito control treatments could impact aquatic invertebrates if the pesticides are applied in a manner that results in exposure to rivers and streams. The focus of this study is to evaluate if commonly used practices actually results in exposure of the pesticide to aquatic invertebrates. The objective of this study is to investigate the potential impact of mosquito control activities on aquatic invertebrates in Colorado rivers.

PROGRESS

Aerial application of Permethrin to control mosquitoes was canceled in the Gunnison Basin in 2013 and 2014 because of concerns about impacts to aquatic invertebrates (Gunnison County 2013). A map of the areas treated prior to 2013 is in Figure 15. A water quality evaluation of an aerial spraying operation was completed in Kremmling in 2013 as well as a longitudinal survey of invertebrates in the Gunnison River and Tomichi Creek. Detailed results of that work were presented in Kowalski (2014). There was some evidence for concern about both the long term impacts of previous pesticide use as well as evidence that currently used application methods resulted in doses of pesticides in rivers above levels that would be expected to impact invertebrates. In Muddy Creek and the Colorado River below Muddy Creek, permethrin concentrations in water samples ranged from 0.0087 ppb to 0.035 ppb the night after a treatment. Concentrations in suspended sediment in the water were 299-1,500 ppb. Stream bed sediment concentrations ranged from 2.10-9.09 ppb. Clearly the aerial application of pesticide was entering the streams and rivers and adhering to suspended sediment in concentrations that are expected to impact aquatic invertebrates. In the Gunnison basin, the invertebrate study showed significant longitudinal patterns in the distribution and abundance of macroinvertebrates. Abundance and species richness of most groups was significantly reduced downstream (in the treated area), particularly at the lowest sites on the Gunnison River and Tomichi Creek. While no direct causal relationship could be inferred between the reduced abundance and richness of invertebrate communities in the historically treated areas, this pattern does contribute some evidence for concern. Historically sprayed areas had lower diversity of aquatic macroinvertebrates and were characterized by pollution tolerant species compared to areas upstream of treated reaches. The next phases of this research include an evaluation of truck mounted mosquito fogging operations as a route of exposure of pesticides to the river as well as conducting laboratory toxicity tests evaluating commonly used field formulations of mosquito control insecticides on invertebrates. Both of these projects depend on finding accurate and cost effective way of detecting pesticides in water, sediment and invertebrates samples at low concentrations. Laboratory costs for the analysis of these samples is currently restricting these projects from moving forward and new partnerships and laboratory services are currently being reviewed to find a cost effective solution.

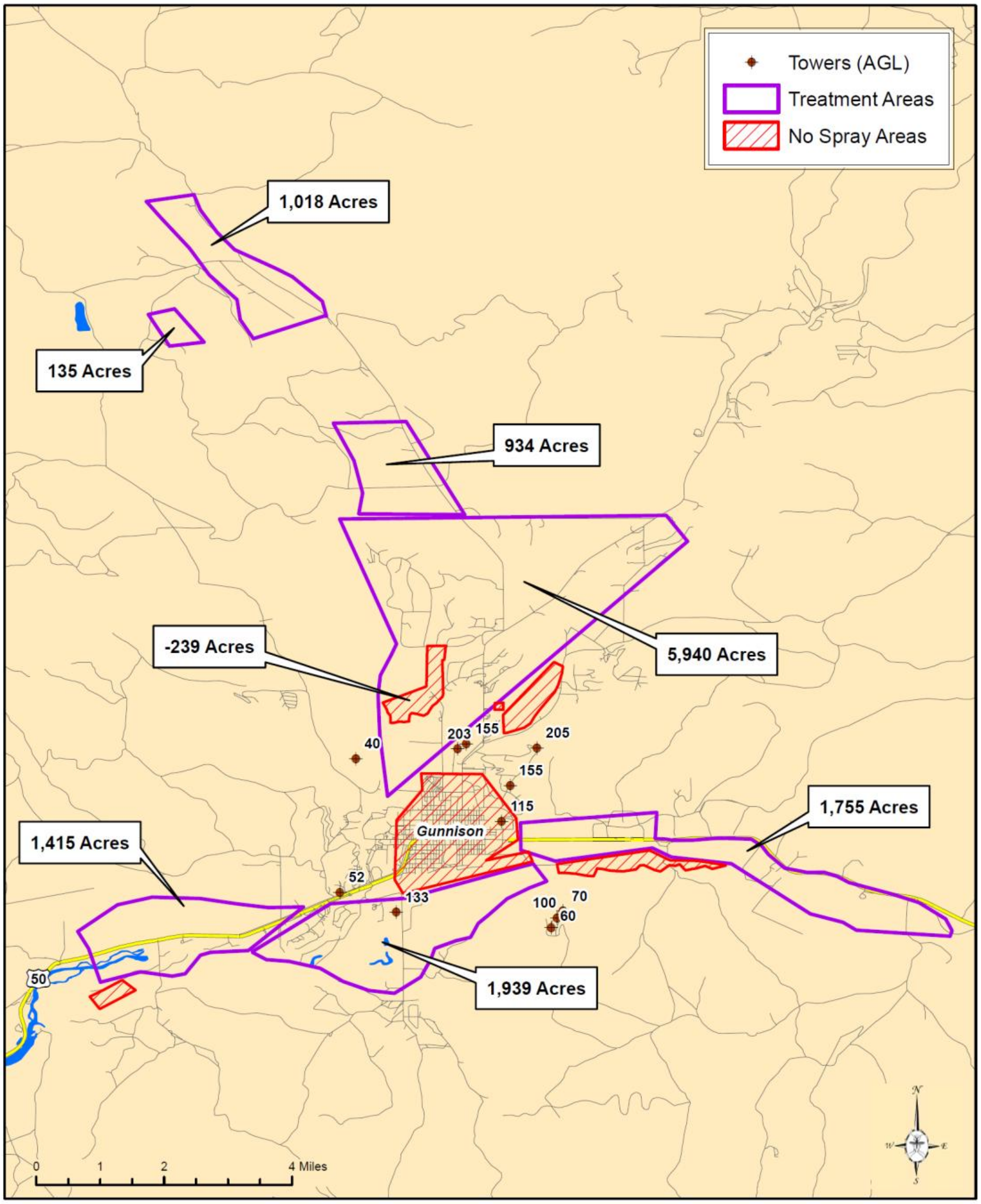

Figure 15. Mosquito control treatment areas around Gunnison, CO in 2012. Of the nine invertebrate sampling sites, the two most upstream sites on the Gunnison and the one upstream site on the Tomichi were outside of the historically sprayed area. Invertebrate samples showed a pattern of reduced diversity and increased representation of pollution tolerant species in the treated areas. Samples taken upstream of the historically treated areas had higher diversity of invertebrates including more sensitive species. Aerial spraying was discontinued after 2012 due to environmental concerns but truck mounted sprayers and fogging operations continue.

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Job No. 5. Gunnison Tunnel Electric Fish Guidance System Evaluation

Job Objective: Evaluate the effectiveness of the electric fish guidance system at the East Portal of the Gunnison Tunnel in preventing fish entrainment in the South Canal

The South Canal is an irrigation ditch in southwest Colorado that diverts an average of 360,600 acre feet of water each year, about 857 cfs average daily flow March-November, from the Gunnison River for agriculture (Bureau of Reclamation 2012). The river contains a Gold Medal trout fishery and entrainment of fish in the canal has been a documented problem for many years. The construction of a hydropower plant was expected to increase mortality of entrained fish so an electric fish guidance system (EFGS) was installed at the diversion structure in 2012. From the diversion structure and EFGS the canal travels through a 5.7 mile long tunnel before egressing approximately 0.5 miles above the power house (Figure 16). There is a total of 7.7 miles of earthen bottom canal that contains the majority of fish that are entrained from the Gunnison River. The canal diverts water from March through November each year with the amount of water depending on water supply and irrigation demand (Figure 24). During winter months the canal is generally shut off with only a very small amount of flow as a result of accretions and seepage. About twice a month it is partially opened to run approximately 100 cfs through the canal for 24-48 hours to fill a drinking water supply reservoir. Because of low and intermittent flows in the canal fish survival over winter was generally thought to be low but variable year to year depending on frequency of freezing temperatures. However, in the winter of 2012-2013, a constant flow of 20-25 cfs was run all winter long to keep water supply reservoirs full during construction of the hydropower plant. This resulted in what appeared to be a much larger number of fish in the canal in spring of 2013 due to increased survival of entrained fish.

The study reach for this project was downstream of the concrete drop below the West Portal (just below the first powerhouse) and was 0.72 miles, ending at the 2nd concrete drop structure (Figure 17, UTM NAD83 258703, 4262335). It averaged 46.1 ft wide with 20-25 cfs in March 2013 and 70.2 ft. wide at 540 cfs in October 2013. It represents 9.4% of the total earthen portion of the South Canal but is suspected of containing the highest density of entrained fish due to its proximity to the West Portal. While fish routinely pass through the high velocity concrete portions of the canal, the majority of fish reside in the lower gradient earthen portion of the canal.

The EFGS was constructed in 2012 and was operational before the 2013 irrigation season. It consists of a series of vertically suspended electrodes across the east portal of the Gunnison Tunnel (Figure 25). The waterway at the EFGS is 74 ft wide, 16 ft deep and has water velocities between 0.2-0.7 m/s and conductivity of 180 µs/cm. The system is powered by three 1.5 KVA Smith Root pulsators with a max power output of 4.5 kW and is designed to operate with a frequency of 2Hz, pulse width of 0.005 s and a field strength of 1v/inch. The EFGS is believed to have operated continuously as planned throughout the entire 2013-2014 irrigations seasons. Communication has been lost for brief time periods (i.e. 6 out of over 6,000 hrs of operation in 2013) but operation of the EFGS was thought to be unaffected and it is assumed that is has functioned continuously during irrigation season the last two years.

The purpose of this study was to estimate fish populations in the South Canal before and after the EFGS and investigate the entrainment of fish from the Gunnison River. To accomplish this fish population estimates were analyzed by: 1) comparing fish population estimates before and after the guidance system was built, 2) comparing fish population estimates from spring, summer and fall, and 3) documenting any movement across the EFGS with tagged fish.

METHODS

South Canal was sampled with mark-recapture electrofishing (Oct 2011, Oct 2013, July 2014 and Oct 2014) and multiple pass removal (March 2013) to estimate fish populations of adult and juvenile trout. The study reach for all three occasions was the same but differing methods were used in the spring sampling because of the different habitat and flows when water is not being diverted (20-25 cfs vs. 500-900 cfs).

On March 29, 2013, the canal consisted of two distinct habitat types, the concrete stilling basin just below the first drop and the earthen portion of the canal below. The density of fish was much higher in the stilling basin and the physical habitat dictated that different sampling methods be used in the two locations. The reach was stratified by habitat types and two sampling reaches were chosen. The stilling basin was sampled with 50 ft bag seine that was 6 ft deep with 1/8 in. mesh. Two seine hauls were made through the stilling basin so a depletion population estimate could be made (Zippin 1956, White et. al 1982). Fish were held in a live pen and then measured for total length to nearest millimeter. Capture probability was high (estimated to be 0.74 for rainbows and 0.79 for browns) and model assumptions of closure appeared to have been met well due to the isolated and simple structure of the stilling basin. The high capture probability and lack of evidence of size selectivity of the seine is expected to help meet assumptions of the removal model and there was no evidence in the data to indicate an unacceptable amount of bias. The portion of the canal below the stilling basin consisted of shallow, slow moving channel that was 46.1 ft wide 3,528 ft long. A sampling reach was randomly chosen in this portion of the study reach that was 1,000 ft long and block nets were used to prevent escapement. Five Smith Root LR24 backpack electrofishers were used to complete a two pass removal population estimate. Fish were held in a live pen and then measured to nearest millimeter and weighed to the nearest gram, and then returned to the canal. After the March estimate, 876 fish were removed from the canal in an effort to depopulate the study reach before the first season of the operation of the EFGS. One hundred and twenty five fish from the stilling basin were tagged with coded wire tags (CWT) and adipose fin clips and transported by aerated fish truck to the Gunnison River in East Portal. They were stocked at the boat ramp approximated 0.7 miles above the East Portal and the EFGS.

Because electrofishing removal estimates are known to be biased low because of size selectivity and individual capture heterogeneity, we took several approaches to reduce this bias recommended in Riley and Fausch 1992 and Saunders et al. 2011. First efforts were made to use sufficient effort for high capture probabilities. Second, capture probabilities were modeled by fish species and length to account for heterogeneity. The data was analyzed in Program Mark with the Huggins Closed Capture Model (White and Burnham 1999, Huggins 1989). To reduce the bias associated with the size selectivity of electrofishing, capture probabilities were modeled with length as a covariate similar to the approach described in Saunders et al. 2011. Four models were built by estimating capture probabilities by length, species, species + length, as well as a constant capture probability for all fish. Model selection was done with AICc and population and parameter estimates were made by model averaging across all four models with AICc weights (Burnham and Anderson 2002). To estimate the total trout in the study reach in March 2013, the two pass removal estimate was expanded for the length of canal that contained similar habitat and added to the estimate for the stilling basin. The confidence intervals were calculated by summing the variances of each estimate (Delta Method) and multiplying by 1.96.

Four groups of fish were tagged and released in East Portal upstream from the Gunnison Tunnel to challenge the EFGS. One hundred and twenty five fish (59 brown trout and 66 rainbow trout) from the March 2013 sampling of the stilling basin were moved from below the EFGS to above and received both coded wire tags and adipose fin clips. Mean length of the tagged fish was 241 mm for brown trout (range 165-310 mm) and 232 mm for rainbows (180-392 mm). Wild fish were captured by boat electrofishing on June 17 and 19, 2013, in East Portal above the guidance system and tagged with both coded wire tags and adipose clips. A total of 1,265 fish (653 rainbow trout and 612 brown trout) were tagged, the mean length of brown trout was 281 mm (103-737 mm) and for rainbows it was 336 mm (82-547 mm). Fingerling rainbow trout from the Rifle Falls Fish Hatchery were also tagged and released into the Gunnison River in East Portal above the EFGS. A total of 19,800 fish with a mean length of 68 mm were tagged with coded wire tags on June 24-26, 2013 and stocked into the Gunnison River 0.7 m above the EFGS on July 26. Due to the results of the first study season, in 2014 the focus was on tagging larger fish and 1,841 wild fish from the Gunnison River above the EFGS were tagged with 32 mm half duplex PIT tags. The mean length was 396 mm (200-545 mm) and an estimated 21.7% of the fish larger than 200 mm in the Gunnison River above the EFGS were tagged. A total of 23,031 trout from 68mm to 737mm were tagged in the 2013-2014 and released in the Gunnison River above the EFGS.

Mark recapture population estimates in the study reach were conducted in October 2011, October 2013, July 2014 and October 2014 with a 14 ft. aluminum jet boat with Smith Root 2.5 GPP electrofisher. The study reach, equipment and methods for all occasions were the same. Fish were measured to the nearest millimeter and all fish on the recapture pass were weighed to the nearest gram. All captured fish were examined for fin clips and checked for coded wire tags with a Norwest Marine Technology T-Wand Detector and for PIT tags with an Oregon RFID handheld reader. On the marking pass all fish greater than 150 mm were marked with a caudal fin punch and held in a live pen to ensure recovery. Fish were returned by boat throughout the study reach to ensure redistribution in the population. The recapture pass was completed 72 hrs after the marking pass and generally accepted methods were followed for mark recapture studies (Curry et al. 2009). The interval between passes was chosen to maximize redistribution of marked fish throughout the population but to attempt to meet demographic and geographic closure assumptions of the model. The first power plant served as an upstream migration barrier further ensured geographic closure; block nets downstream were not feasible to the high volume of water in the canal (600-900 cfs). Model assumptions appear to have met well as marked fish were not observed to be encountered in any temporal or spatial pattern in the canal, capture probabilities were good, and the catch per unit effort of fish was similar between the passes.

A stationary PIT tag antenna was constructed above the penstock of the power plant but below the EFGS in the spring of 2014. The objective was to differentiate fish deterred by the EFGS and turbine mortality as well as increase detection of tagged fish. The antenna was operational for less than two months as the extreme velocities of the water (900 cfs in a 10.5 ft wide concrete channel) made it impossible to keep in place. No tags other than test tags were detected by the antenna.

Fish population estimates were made with the Huggins Closed Capture Model in Program Mark (Huggins 1989, White and Burnham 1999). Four models were built by estimating capture probabilities by length, species, species + length, as well as a constant capture probability for all fish, identical to a Lincoln Petersen model (Seber 1982). Model selection was done with AICc and population and parameter estimates were made by model averaging across all four models with AICc weights (Burnham and Anderson 2002).

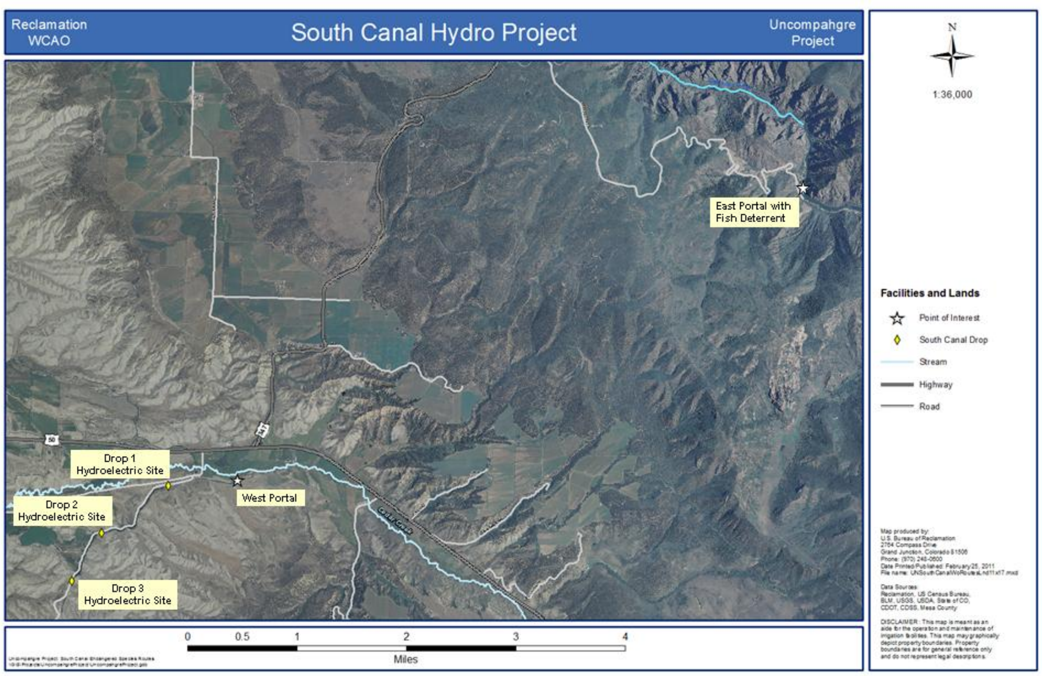

Figure 16. Area map of the Gunnison Tunnel and South Canal (Bureau of Reclamation 2012).

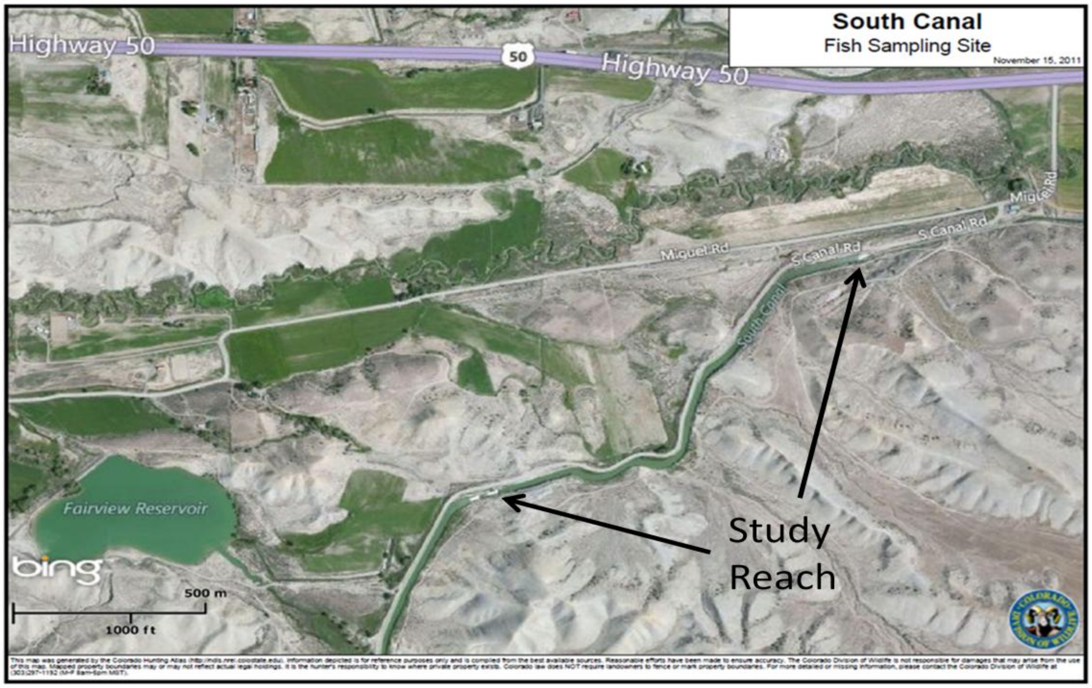

Figure 17. Fish sampling site on the South Canal. The sampling reach was 0.72 miles long (3,802 feet) and was between the first and second concrete drop structures below the West Portal.

RESULTS

The results of the population estimates are summarized in Table 8 and Figure 21 and length frequency histograms from the fall 2013 sampling are presented in Figure 20. Model selection results from are summarized in Tables 9-13.

Population modeling exercise in Program Mark gave good results and estimates appeared accurate and relatively precise except for October 2011. The expected bias of population estimates should be low due to model assumptions being met well, and the ability to reduce the size selectivity of electrofishing with fish length covariate models. The top population model for the October 2011 data contained terms that varied capture probability by length and time while the second ranked model that contained terms for species, length and time was 2.40 ΔAICc units behind. Models with a term for fish length contained 0.98% of the model weights. Capture probabilities were lower during this survey (0.10) compared to subsequent surveys due to the higher flows and lower total number of fish captured.

In March 2013, the top population model (two pass removal) for the canal and the stilling basin had a single capture probability for all fish regardless of species or length while the second ranked model contained a term for species. These two models are essentially identical to the simple Zippin two pass removal model and had 73.2% of the model weight (Zippin 1956). Although it has been shown that generally electrofishing surveys have a size related bias (large fish have a higher capture probability) this effect was not seen in these data because of how few fish were in the canal outside of the stilling basin and there was little variation in fish size compared to the fall surveys. Because of the low density of fish, moderate capture probabilities and similar probabilities across size classes, the data from the canal were too sparse to support more detailed models. There was no evidence of size selectivity in the stilling basin with the small mesh seine. The significant increase (95% level) of total fish in the canal from April 2013 to October 2013 is evidence for fish successfully running the EFGS and surviving the turbines. After the March estimate, when 876 fish were removed from the canal, the population estimate increased by 1,057 fish by October. The total number of estimated fish was significantly greater (at the 95% level) in October than in April but was not significantly different than in the October 2011. The size structure and species composition of the fish in the October 2013 also provide evidence of fish entrainment, specifically for brown trout (Figures 18 and 19).

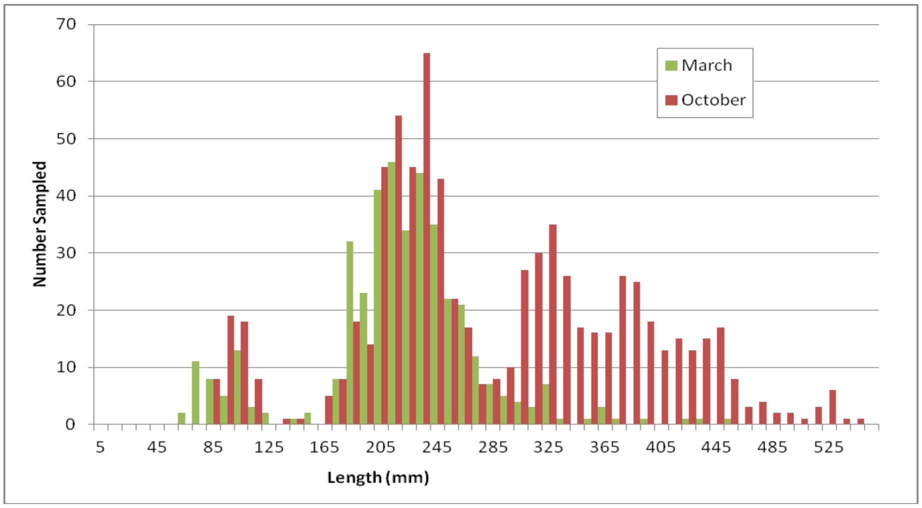

Figure 18. Length frequency histogram of brown trout captured in March and October 2013.

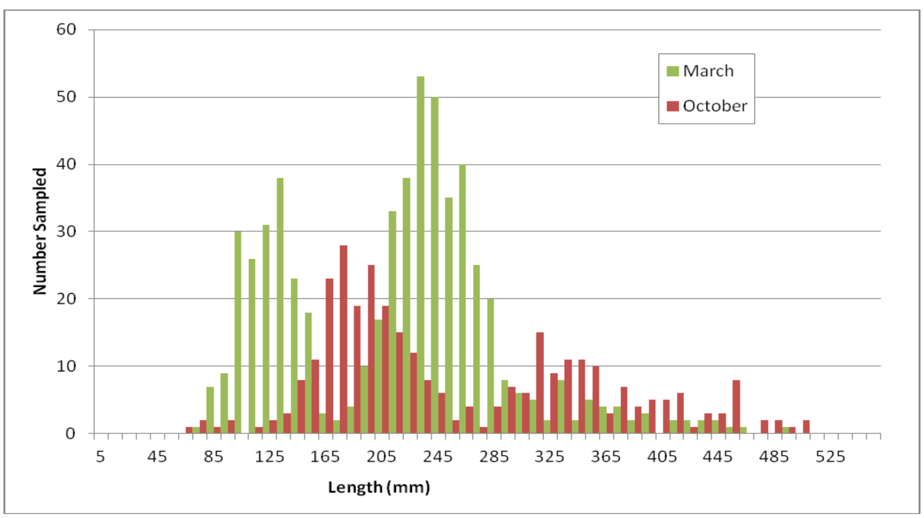

Figure 19. Length frequency histogram of rainbow trout captured in March and October 2013.

The top population model for the October 2013 data contained terms that varied capture probability by length, species and time while the second ranked model that contained terms for length and time. These two models accounted for 100% of the model weights and had much higher support than a simple Lincoln-Petersen (19-27 ΔAICc units behind). Capture probabilities were high (0.33) due to the lower flow conditions than 2011. Model selection uncertainty was taken into account in all surveys by model averaging across all four models with model weights to get parameter estimates and population estimates.

The top population models for the July and October 2014 data contained terms that varied capture probability by length, species and time. The top two models that included length accounted for 100% of the model weights. Capture probabilities were good in July (0.11-0.32) and (0.17-0.20) October. Model selection uncertainty was taken into account in all surveys by model averaging across all four models with model weights to get parameter estimates and population estimates.

The population modeling exercise for all the mark recapture data indicated that modeling capture probabilities by length was important under these conditions, which agrees with previous work on the topic (Saunders et. al 2011). Using a simple Lincoln-Petersen model under these conditions would consistently underestimate population size by overestimating the capture probability for small fish, even when using a length cutoff designed to exclude age 0 fish. See Figure 26 for an example of the estimated capture probability by length and Figure 27 for a comparison of population estimates with and without the length covariate.

In October 2011, there were an estimated 2,994±1,043 fish in the South Canal study reach. In the spring of 2013 there were an estimated 1,583±70 in the study reach, 89% in the stilling basin. Eight hundred and seventy-six of these fish were removed from the study reach leaving an estimated 707 fish when the irrigation flows first began in the spring of 2013. In October 2013 the study reach contained an estimated 1,764±279 trout. The population estimate of total fish in the study reach decreased from October 2011 to 2013 but that difference was not significant at the 95% level, mostly due to the uncertainty around the 2011 estimate caused by lower capture probability likely due to higher flows. Subsequent sampling occasions had much higher capture probabilities generally in the 20% range (0.11-0.33). In 2014 the study reach contain 1,224±239 fish in July and 1,900±379 in October.

In 2013, a total of 248 coded wire tagged fish from 123 mm to 337 mm were documented passing through the guidance system, mostly smaller stocked rainbow trout (n=246 mean length 163 mm in October). Only two larger wild brown trout were confirmed passing the EFGS (310 and 337 mm). Of the tagged fish that were documented to have run the EFGS in 2013, the stocked rainbows represent 1.24% of the fish marked in East Portal and the wild brown trout were 0.3%. Overall, 1.17% of all the tagged fish in East Portal were captured in the study reach in 2013. In the 0.72 mile study reach there was an estimated 1,486±768 coded wire tagged rainbows or 7.5% of the tagged fish in East Portal. These results do not represent an estimate of the actual rate of entrainment as only 9.4% of the total length of the canal was sampled at a single time interval; they only represent the number of entrained fish in the study area that were detected. This should be interpreted as a minimum number of fish that navigated the EFGS because fish would have to pass the through the guidance system, travel the 5.7 mile long tunnel, avoid entrainment in two small lateral canals, survive passage through the hydropower turbines and remain in the first 0.72 miles of the 7.7 mile canal to be detected.

In 2014, a total of 44 tagged fish were encountered, 40 of the hatchery rainbows (mean length 326 mm at the time of capture). Four CWT and fin clipped wild rainbow trout (296-398 mm) were found. It is unknown exactly when or what size all the tagged fish in 2014 passed the EFGS because fish lived and grew in the canal throughout the study. The 2013 data give the best idea of size of fish that ran the EFGS because they were in the canal for a maximum of seven months. The large number of CWT tagged rainbows could have passed the EFGS as small as 68 mm and then survived to be captured at a larger size.

By the end of the study 288 small or medium sized fish had been documented passing the EFGS. Only four fish >300 mm, and no fish >400 mm were documented passing the guidance system. Only 1.3% of all tagged fish were recovered in the canal study reach in two years. While turbine mortality and fish excluded from the study reach by the trash racks on the penstock cannot be differentiated from fish excluded by the EFGS, very few large fish have been observed passing these barriers.

In July 2014, 17% of the fish captured during the population estimate and 37% greater than 350 mm had been handled the previous October judging by the presence of a healed caudal punch scar. This indicates that there is fair to good over winter survival in the canal. Growth of fish that live in the study reach is also relatively high; coded wire tagged rainbows grew an average of 6.4 inches from age 1 to age 2. With the good annual survival and growth rates, the large numbers of smaller fish that do pass the EFGS and turbines maintain a relatively stable population of fish in the study reach, even though large fish do appear to be excluded from the canal.

Table 8. Fish Population Estimates and 95% Confidence Intervals from the South Canal 2011-2014. These estimates are for age 1 fish and older, the stocked CWT tagged rainbows are excluded from the rainbow trout estimates.

Date	Species	# Caught	Population Estimate in Study Reach
October 2011	Brown Trout	415	2,359±981
	Rainbow Trout	108	634±354
March 2013	Brown Trout	683	924±52
	Rainbow Trout	495	659±46
October 2013	Brown Trout	573	1,035±150
	Rainbow Trout	277	728±235
	Stocked CWT Rainbow	246	1,486±768
	CWT, Adipose Clipped Brown	2	NA
	CWT, Adipose Clipped Rainbow	0	NA
July 2014	Brown Trout	225	586±52
	Rainbow Trout	132	638±469
	Stocked CWT Rainbow	25	NA
	CWT, Adipose Clipped Brown	0	NA
	CWT, Adipose Clipped Rainbow	0	NA
Oct 2014	Brown Trout	305	964±258
	Rainbow Trout	277	936±278
	Stocked CWT Rainbow	15	NA
	CWT, Adipose Clipped Brown	0	NA
	CWT, Adipose Clipped Rainbow	4	NA

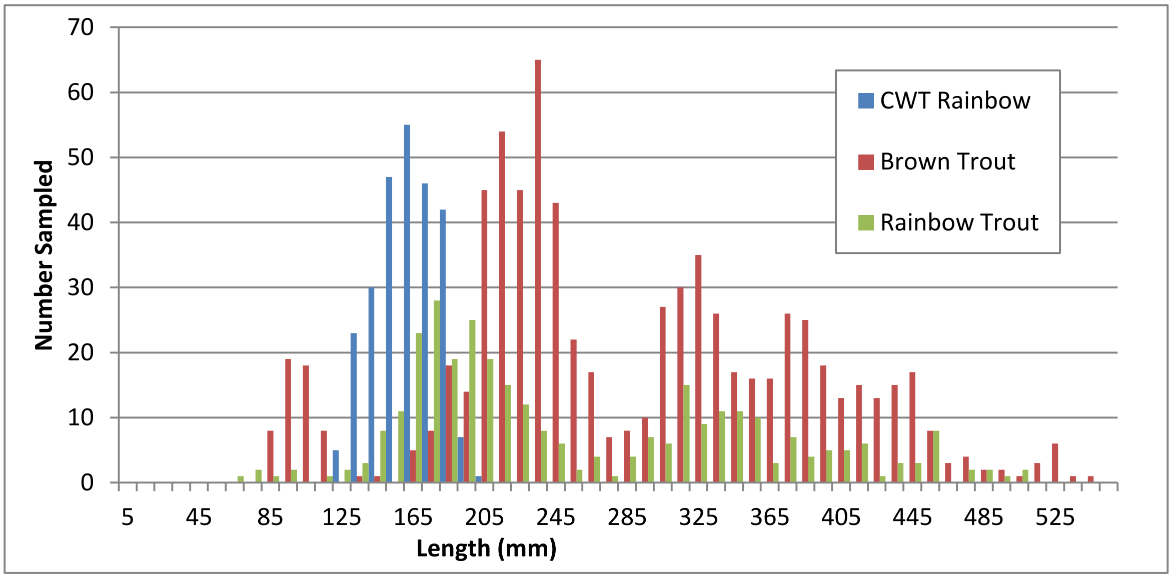

Figure 20. Length frequency histogram of trout captured in the South Canal in October 2013. A total of 246 coded wire tagged rainbows were captured that had been stocked upstream of the guidance system (plus 10 recaptures). They had a mean length of 163 mm (123-204). Two other coded wire tagged fish were captured, a 310 mm brown and 337 mm brown (the 310 mm fish was also recaptured). No tag loss was observed, all of the larger fish were double marked and no fish were observed with an adipose clip but without a CWT.

DISCUSSION

The South Canal contained approximately 1,094 fewer fish in October 2014 after the EFGS, than in October 2011. While the total fish estimates in the canal have declined since the EFGS was installed, there is not a significant difference at the 95% level mostly due to the low capture probability (0.8-0.12) and corresponding high uncertainty around the October 2011 estimate. The number of brown trout in the study reach is significantly lower at the 95% level in 2014 two years after the EFGS was installed while the number of rainbow trout has remained relatively stable (Figure 23).

Of the 23,031 tagged fish, 1.3% were recovered in the canal study reach in two years. At the end of the study, 288 small or medium sized fish had been documented passing the EFGS. Only four fish >300 mm, and no fish >400 mm, were documented passing the guidance system. This size selectivity is expected with electrically based guidance systems. Electrofishing is known to be highly size selective (Figure 26, Saunders et. al 2011). It is also likely that turbine mortality is higher on larger fish, further selecting for smaller fish to make it into the study reach. The growth and survival of fish in the canal is higher than expected as evidenced by the high proportion of recaptured fish from October 2013 to July 2014. The practice of running 100 cfs into the canal twice a month in the winter and relatively mild recent winters apparently allows for good fish survival.

Fish are clearly getting through the EFGS and surviving the turbines, but are mostly smaller fish. Their growth and survival in the canal maintains a stable fish population that is lower than before the EFGS, significantly for brown trout. The different responses of the two species is probably due to two major factors; larger size of age 0 brown trout and potential spawning of rainbow trout in the study reach. Because brown trout emerge about 8-10 weeks earlier than rainbow trout they are larger during their first summer. Because the EFGS appears to be size selective, brown trout fry can expect to be entrained at a lower rate than rainbows. The canal is first filled with water around April 1st of each year, just before rainbow trout spawn. Large numbers of age 0 rainbow trout were observed in the canal in July 2014 (they were smaller than the 150mm size cut off used in the fish population estimates). It is unknown if they were entrained fish from the Gunnison River or were spawned in the canal, both are plausible. Brown trout spawn in October in the Gunnison River and flows are generally shut off in the canal around October 31. Water flow is then stagnant or 100 cfs (twice a month for 24 hrs) in the canal in winter. There is very little spawning habitat for brown trout and it is very variable and poor quality compared to rainbow trout, which spawn when flows in the canal at higher flows that are stable or increasing.

The electric fish guidance system on the South Canal of the Gunnison River appears to effectively exclude large fish from the south canal, resulting in fewer entrained fish from the river. Fish populations in the South canal, while lower than before the EFGS, appear stable due to the number of entrained smaller fish, potential spawning of rainbow trout and better than expected growth and survival of fish in the canal.

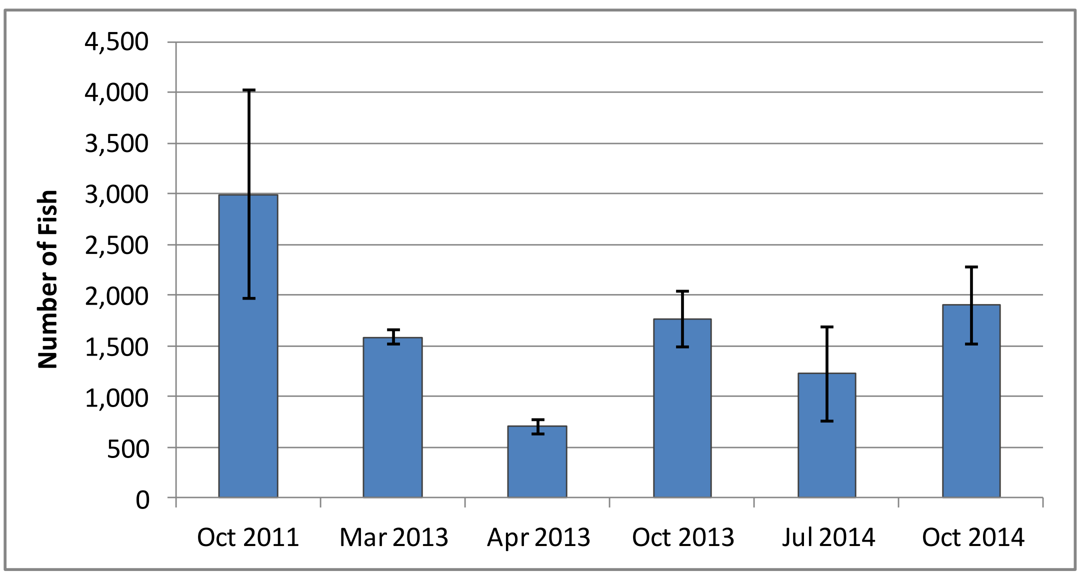

Figure 21. Estimated total number of trout age 1 and older and 95% confidence intervals in the South Canal study reach. After the March 2013 estimate, 876 fish were removed from the canal study reach and the EFGS was operational at the start of the irrigation season in April 2013. There are about 1,094 fewer fish in the study reach since the EFGS was installed but the decline is not significant at the 95% level, mostly because of the low capture probability and corresponding high uncertainty around the October 2011 estimate.

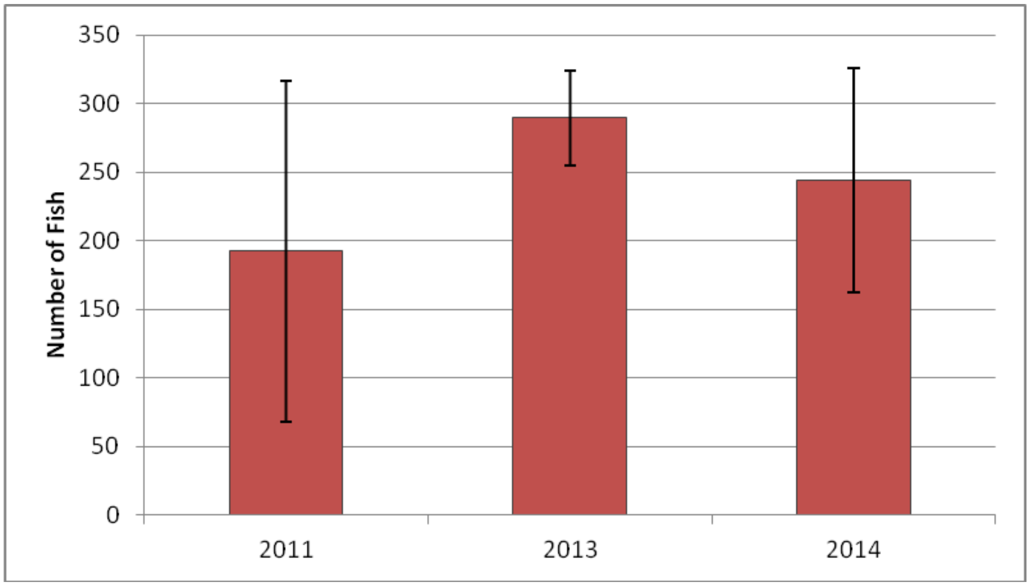

Figure 22. Estimated total number of trout greater than 350 mm in the South Canal study reach in the October sampling periods. While very few (4) fish greater than 300 mm have been documented passing the EFGS and turbines, grown and survival of smaller entrained fish supports a stable number of larger fish in the study reach.

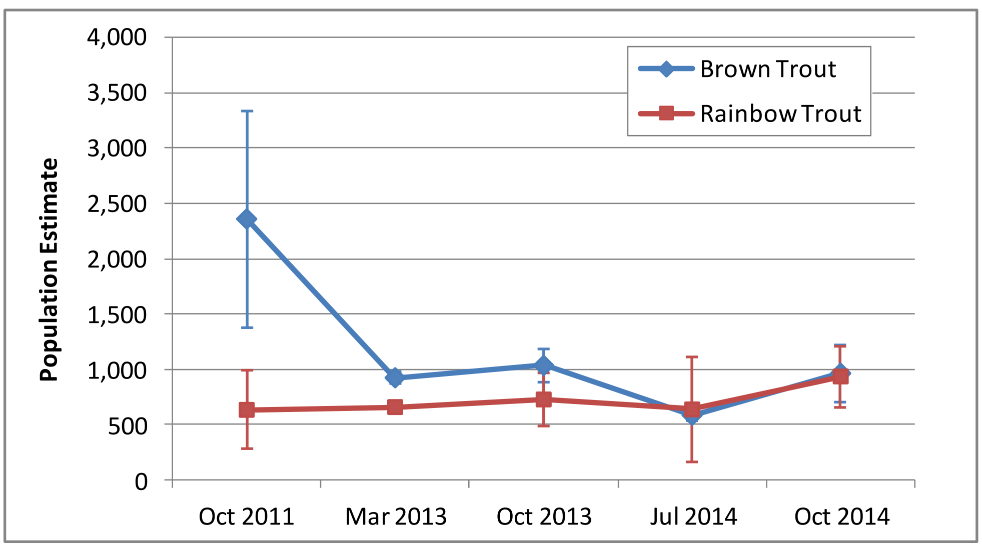

Figure 23. Population estimates of rainbow and brown trout and 95% confidence intervals for the South Canal Study reach 2011-2014. There were signifcantly fewer brown trout in 2014, two years after the installation of EFGS. Rainbow trout numbers have raimained relatively stable.

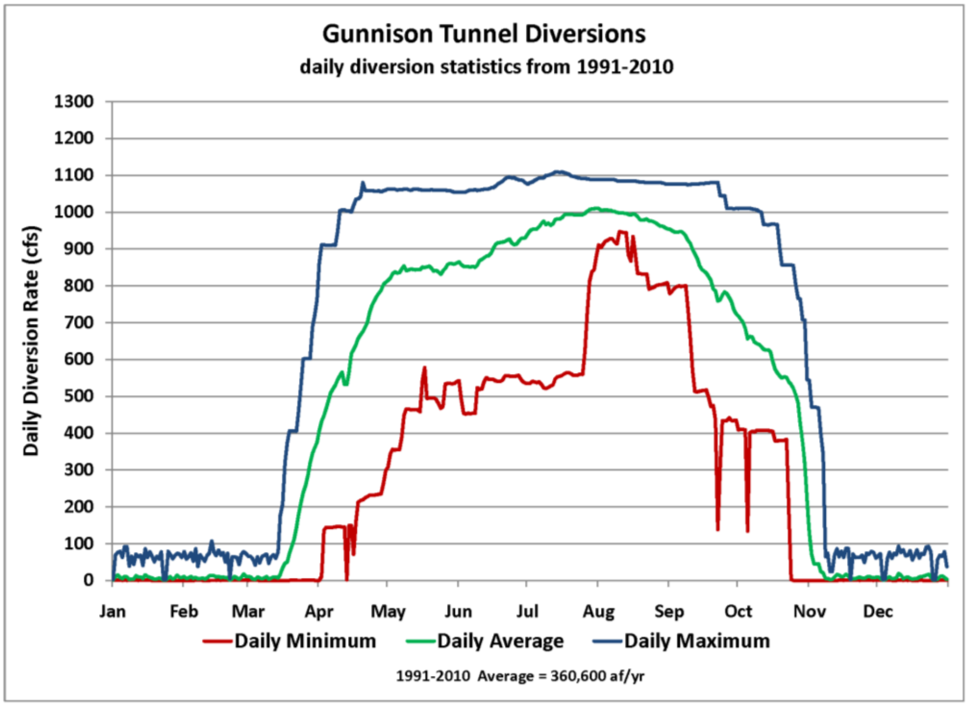

Figure 24. Water diversion records for the south canal below the Gunnison Tunnel 1991-2010.

Figure 25. The electric fish guidance system on the east portal of the South Canal.

Table 9. Model Selection Results for the Mark Recapture Electrofishing in October 2011. Population estimates and capture probabilities were calculated by model averaging across all four models using model weights. The "Time" and "Time+Species" models are identical to the standard Lincoln

Petersen model.

Model	AICc	Number of Parameters	Delta AICc	AICc Weights	Model Likelihood
Time+Length	893.0038	3	0	0.75	1.00
Time+Species+Length	895.4048	5	2.40	0.23	0.30
Time	900.3091	2	7.31	0.02	0.03
Time+Species	902.7106	4	9.71	0.01	0.01

Table 10. Model Selection results for the Two Pass Removal Electrofishing in March 2013. Population estimates and capture probabilities were calculated by model averaging across all four models using model weights.

Model	AICc	Number of Parameters	Delta AICc	AICc Weights	Model Likelihood
Constant p	117.40	1	0	0.528	1.00
Species	119.30	2	1.90	0.204	0.39
Length	119.39	2	2.00	0.195	0.37
Length+Species	121.34	3	3.94	0.073	0.14

Table 11. Model Selection Results for the Mark Recapture Electrofishing in October 2013. Population estimates and capture probabilities were calculated by model averaging across all four models using model weights. The “Time” and “Time+Species” models are identical to the standard Lincoln Petersen model.

Model	AICc	Number of Parameters	Delta AICc	AICc Weights	Model Likelihood
Time+Species+Length	1760.461	5	0	0.77	1.00
Time+Length	1762.837	3	2.38	0.23	0.30
Time+Species	1779.036	4	18.58	0.00	0.00
Time	1787.185	2	26.72	0.00	0.00

Table 12. Model Selection Results for the Mark Recapture Electrofishing in July 2014. Population estimates and capture probabilities were calculated by model averaging across all four models using model weights. The "Time" and "Time+Species" models are identical to the standard Lincoln Petersen model.

Model	AICc	Number of Parameters	Delta AICc	AICc Weights	Model Likelihood
Time+Species+Length	663.08	5	0	0.802	1
Time+Length	665.88	3	2.80	0.198	0.2469
Time+Species	685.91	4	22.83	0.000	0
Time	693.13	2	30.05	0.000	0

Table 13. Model Selection Results for the Mark Recapture Electrofishing in October 2014. Population estimates and capture probabilities were calculated by model averaging across all four models using model weights. The "Time" and "Time+Species" models are identical to the standard Lincoln Petersen model.

Model	AICc	Number of Parameters	Delta AICc	AICc Weights	Model Likelihood
Time+Length	1106.96	3	0	0.876	1.000
Time+Species+Length	1110.87	5	3.9097	0.124	0.142
Time	1122.71	2	15.7451	0.000	0.000
Time+Species	1126.72	4	19.7574	0.000	0.000

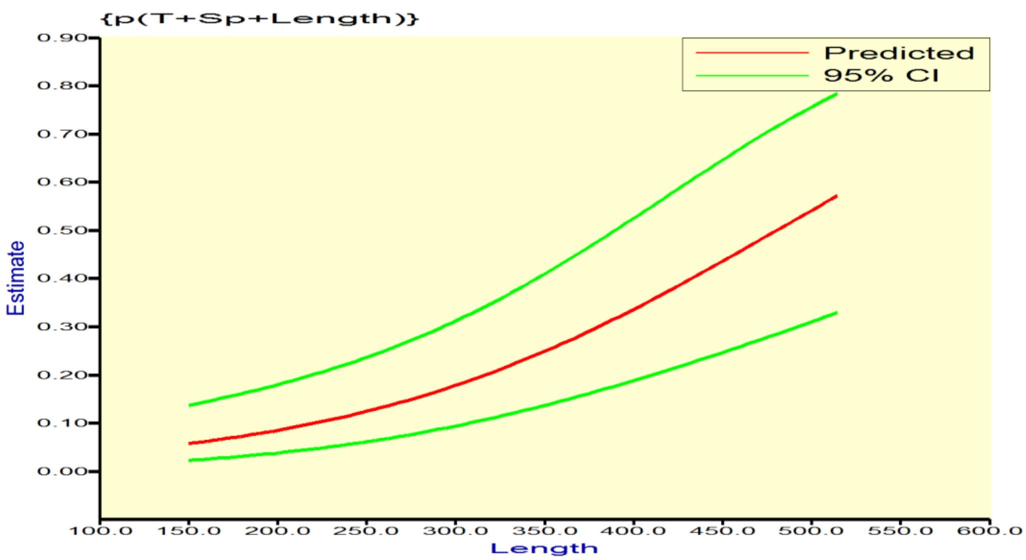

Figure 26. Estimated capture probability by length and 95% confidence interval for trout in the South Canal in July 2014.

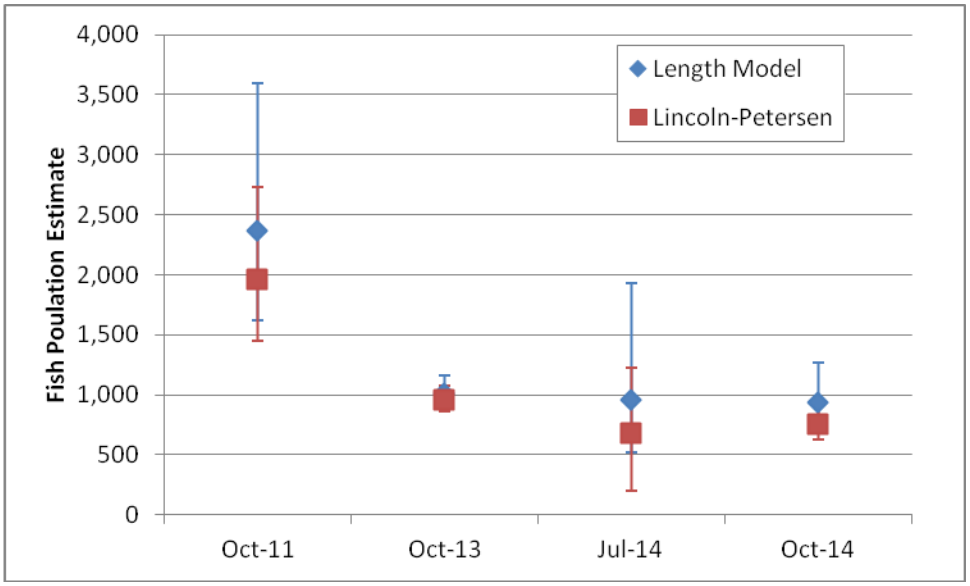

Figure 27. Brown trout population estimates from the Huggins Closed Capture model in Program Mark comparing models with a fish length covariate to a standard Lincoln-Petersen. The estimates that used length to model capture probabilities were on aver 23% higher (6-41%) than the LP. Models containing length as a covariate had between 98-100% of the model weight across all mark recapture sampling occasions.

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Job No. 6. Technical Assistance

Job Objective: Provide information and assistance to aquatic biologists, researchers and managers.

Segment Objective 1: Arkansas River Aquatic Invertebrate Investigations

Salmonflies (*Pteronarcys californica*) were reintroduced in the Arkansas River near Salida from 2012-2014. The objective of this study is to evaluate the success of the *Pteronarcys californica* reintroduction and investigate its effects on the invertebrate community of the Arkansas River. Aquatic invertebrate samples were taken at six sites on the Arkansas River, two upstream of the reintroduction area, two within and two downstream (Table 14). Replicate macroinvertebrate samples (n = 5) were collected at each site using a 0.086 m^2 Hess sampler with a 350 µm mesh net. Samples were collected in September 2014. The replicate samples were collected from the same riffle with predominantly cobble substrate by disturbing the substrate to a depth of approximately 10 cm. Field samples were washed through a 350-µm sieve and organisms preserved in 80% ethanol. Velocity and depth were taken at each Hess sample site to ensure samples were taken from similar riffle habitat. Macroinvertebrate samples were sorted and sub-sampled in the laboratory using a standard USGS 300-count protocol, except that replicates were not composited and each one underwent the protocol (Moulton et al. 2000). All organisms, except for chironomids and non-insects, were identified to genus or species. Chironomids were identified to subfamily and non-insects (e.g., oligochaetes, amphipods) were identified to class. Each replicate sample was processed separately so an average of 1,889 individual specimens were identified at each riffle site. More individual specimens were identified from each site compared to standard methods to ensure rare organism were sampled and increase robustness of the comparisons between riffles sites in close proximity in the same stream (Vinson and Hawkins 1996).

Table 15 contains a summary of the results from the macroinvertebrate data. No *P. californica* were sampled at any site. They were known to occur at several of these sites earlier that year due to the presence of emerging adults in the spring of 2014. However, densities appear low enough at all sites that none were sampled with our protocols. A larger sampling effort (number of replicates) and/or a quantitative sampler with a larger sampling area may better detect

Salmonflies at very low densities. This is not a unique phenomenon, sampling rare insects is a known problem (Vinson and Hawkins 1996) and Salmonflies tend to occupy larger substrate in cobble bottomed streams and may be under sampled by standard Hess samplers (Nehring 2011 and Kowalski unpublished data). Data analysis was ongoing at the time of this report and future progress reports will contain more data and analysis. All sites were relatively similar in invertebrate richness. The density of invertebrates was similar for all sites except ARK4 Salida East and ARK6 Rincon had lower densities. The lower densities in several metrics at ARK4 were mostly due to fewer Trichoptera at that site, although it had the lowest Plecoptera density as well. These sites appear a good baseline collection for this project.

Table 14. Arkansas River Aquatic Invertebrate Investigations Sites

#	Site Name	UTM (NAD83 Z13)
ARK1	Big Bend	405999, 4270216
ARK2	Richardson's	407360, 4269777
ARK3	Hatchery	410633, 4267056
ARK4	Salida East	416011, 4262808
ARK5	Wellsville	419984, 4260760
ARK6	Rincon	4244447, 425643

Table 15. Summary of Macroinvertebrate Data from the Arkansas River in 2014.

	ARK 1	ARK 2	ARK 3	ARK 4	ARK 5	ARK 6
Average Density All Species (m^2)	31,657	30,169	22,322	7,772	22,099	15,238
Average Density EPT (m^2)	26,337	24,314	17,145	2,032	16,331	8,033
Average Density Ephemeroptera (m^2)	1,140	680	638	671	924	1,062
Average Density Plecoptera (m^2)	256	302	442	139	241	145
Average Density Trichoptera (m^2)	24,942	23,331	16,066	1,222	15,166	6,826
Average Density Coleoptera (m^2)	727	1,192	1,041	355	531	358
AverageDensity Diptera (m^2)	3,483	3,913	2,944	4,592	3,679	5,233
Average Density Non-insect (m^2)	1,110	750	1,192	780	1,534	1,614
Average Density Other Taxa (m^2)	5,320	5,855	5,176	5,740	5,767	7,205
All Taxa Species Richness	34	32	38	33	33	33
EPT richness	19	18	20	17	18	16
Ephemeroptera richness	6	7	6	5	6	6
Plecoptera richness	4	2	5	5	3	3
Trichoptera richness	9	9	9	7	9	7
Coleoptera richness	2	3	3	3	4	2
Diptera richness	9	8	10	7	7	10
Non-insect richness	4	3	5	5	4	5
Lepidoptera	0	0	0	1	1	0
Other taxa richness	15	14	18	16	15	17

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