Colorado River Aquatic Resource Investigations

Federal Aid Project F-237-R21

Dan A. Kowalski
Aquatic Research Scientist

Job Progress Report

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Andrew J. Treble, Aquatic Database Manager/Analyst, Aquatic Data Analysis Studies

Jim Guthrie, Federal Aid Coordinator
Kay Knudsen, Librarian

Prepared by: ______________________________
Dan A. Kowalski, Aquatic Research Scientist

Approved by: ______________________________
George J. Schisler, Aquatic Research Leader

Date: 8/29/14

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Project Title: Colorado River Aquatic Resources Investigations

Period Covered: July 1, 2013 through June 30, 2014

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 giant stonefly *Pteronarcys californica* in Colorado Rivers.

The giant stonefly *Pteronarcys californica* is a large 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 consistently 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 giant stonefly's biology that makes it sensitive to habitat alterations is it's 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 4-10 days. The synchronous emergence and hatching behavior allow it to be sampled in unique ways compared to other aquatic invertebrates. Giant stoneflies 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 abundant in the upper Colorado River, the abundance of giant stoneflies has declined in reaches of the river closest to 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. Knowing 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 sites for the re-introduction of *P. californica* as is being done on the Arkansas River and being considered on the upper Gunnison River.

PROGRESS

Density estimates were completed for *P. californica* at 6 sites on the Colorado River, one site on the Fraser River and 6 sites on the Gunnison River in June of 2013. Location and description of sites is presented in Table 1 and maps are in Figures 1-3. Estimates were completed by searching 30 meter (98.6 ft) sections of streambank for *P. californica* exuvia adjacent to riffle habitat. Estimates were done as soon as possible after the emergence at any one site was complete. Three to seven people intensively searched the riparian area from one to twenty meters from the water's edge. The area searched varied by site and depended on the thickness and structure of riparian vegetation. The search 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. Each area was searched two times with similar search areas, effort and personnel. A multiple pass removal model was used to estimate the total density of exuvia at each site (Zippin 1956). Methods used 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 2013 sampling is presented in Table 2.

Physical habitat surveys were completed at the 6 sites on the Colorado River in 2013. 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 conducted and 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 Figure 4 and 5. 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 and a candidate set of models will be developed to identify models which 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 will be completed on the Gunnison River and Rio Grande River (six sites each) in 2014 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 that *P.c.* represents at a single site and the potential carbon flux for which they are responsible. 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.

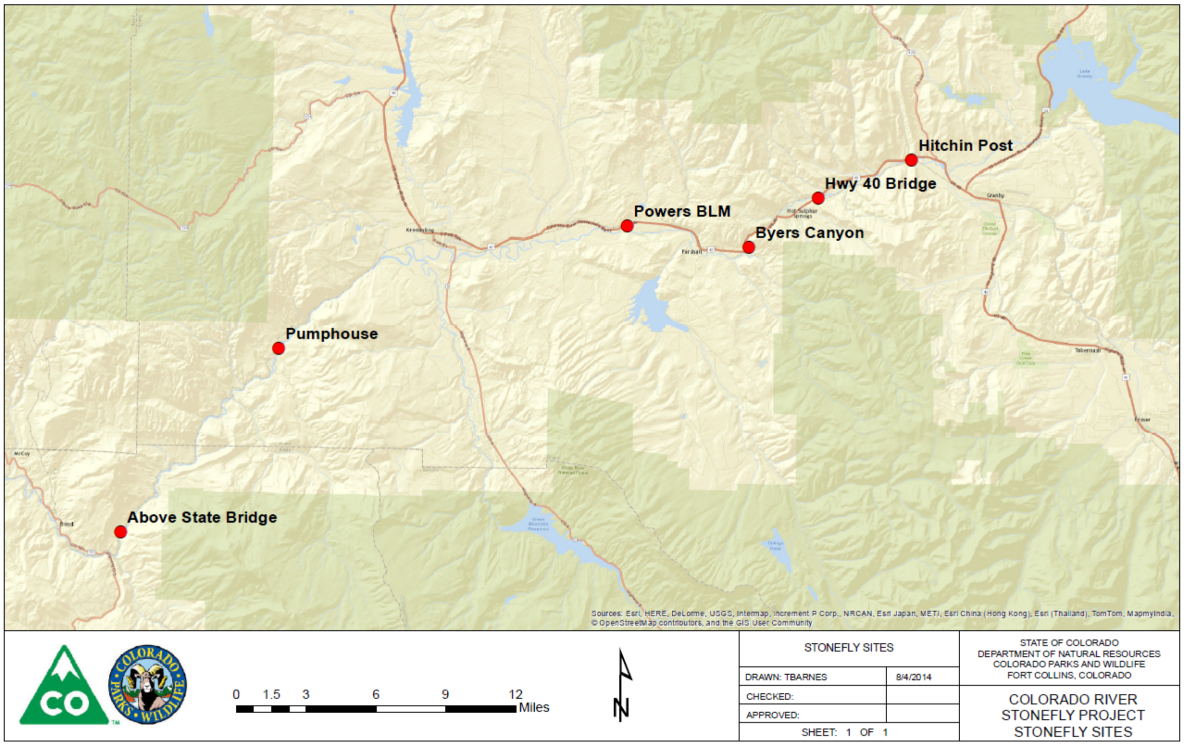

Figure 1. Map of stonefly sampling sites on the Colorado River.

Table 1. Giant Stonefly Sampling Sites

River	#	Site	Side	UTM NAD 83 (Zone 13)
Colorado	1	Above State Bridge	River Right	359889, 4414634
Colorado	2	Pumphouse BLM	River Left	370827, 4427300
Colorado	3	Powers BLM	River Right	394914, 4435762
Colorado	4	Byers Canyon	River Left	403335, 4434268
Colorado	5	Hwy 40 Bridge	River Right	408133, 4437708
Colorado	6	Hitching Post	River Left	414589, 4440304
Fraser	7	Kaibab Park in Granby	River Left	420592, 4437168
Gunnison	8	Orchard Boat Ramp	River Left	247947, 4295297
Gunnison	9	Cottonwood Campground	River Left	252129, 4295940
Gunnison	10	Goldmine	River Left	253728, 4295747
Gunnison	11	Smith Fork	River Left	253338, 4291889
Gunnison	12	Ute Park	River Left	252376, 4284894
Gunnison	13	Chukar	River Left	253421, 4278775

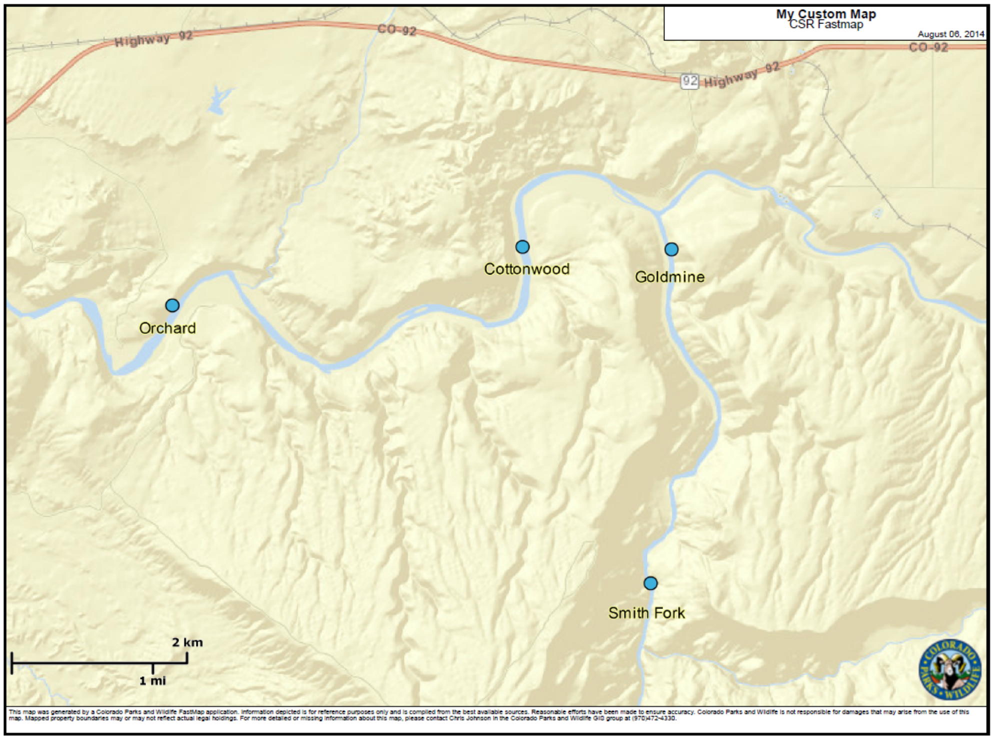

Figure 2. Map of lower stonefly sampling sites on the Gunnison River.

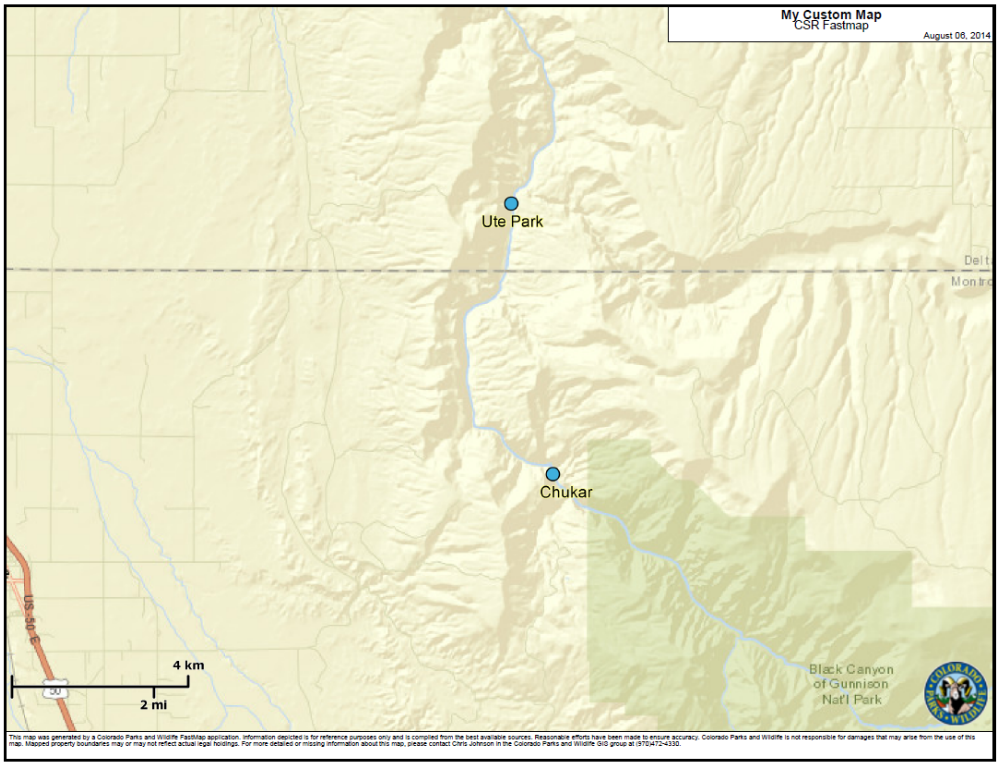

Figure 3. Map of upper stonefly sampling sites on the Gunnison River.

Table 2. Giant Stonefly Exuvia Density Estimates

#	*River*	Site	Pass 1	Pass 2	Population Estimate (30 m)	95% C.I.		#/m	95% C.I.	
1	Colorado	Orchard Boat Ramp	653	86	752.0	745.5	765.0	25.1	24.9	25.5
2	Colorado	Cottonwood Campground	1,054	218	1,328.8	1,310.1	1,356.8	44.3	43.7	45.2
3	Colorado	Goldmine	4,184	1,130	5,732.1	5,665.7	5,811.0	191.1	188.9	193.7
4	Colorado	Smith Fork	1,146	258	1,479.0	1,456.1	1,511.9	49.3	48.5	50.4
5	Colorado	Ute Park	6,433	690	7,205.9	7,186.5	7,231.3	240.2	239.5	241.0
6	Colorado	Chukar	2,585	287	2,907.8	2,895.9	2,925.9	96.9	96.5	97.5
7	Fraser	Kaibab Park	0	0	0.0	0.0	0.0	0.0	0.0	0.0
8	Gunnison	Above State Bridge	8,617	1,324	10,181.4	10,143.0	10,227.1	339.4	338.1	340.9
9	Gunnison	Pumphouse	13,364	2,869	17,017.3	16,935.1	17,109.1	567.2	564.5	570.3
10	Gunnison	Powers BLM	49	12	64.9	61.9	77.8	2.2	2.1	2.6
11	Gunnison	Byers Canyon	1,120	218	1,390.7	1,373.2	1,416.9	46.4	45.8	47.2
12	Gunnison	Hwy 40 Bridge	30	6	37.5	36.2	46.0	1.3	1.2	1.5
13	Gunnison	Hitching Post	1	0	1.0	NA	NA	0.03	NA	NA

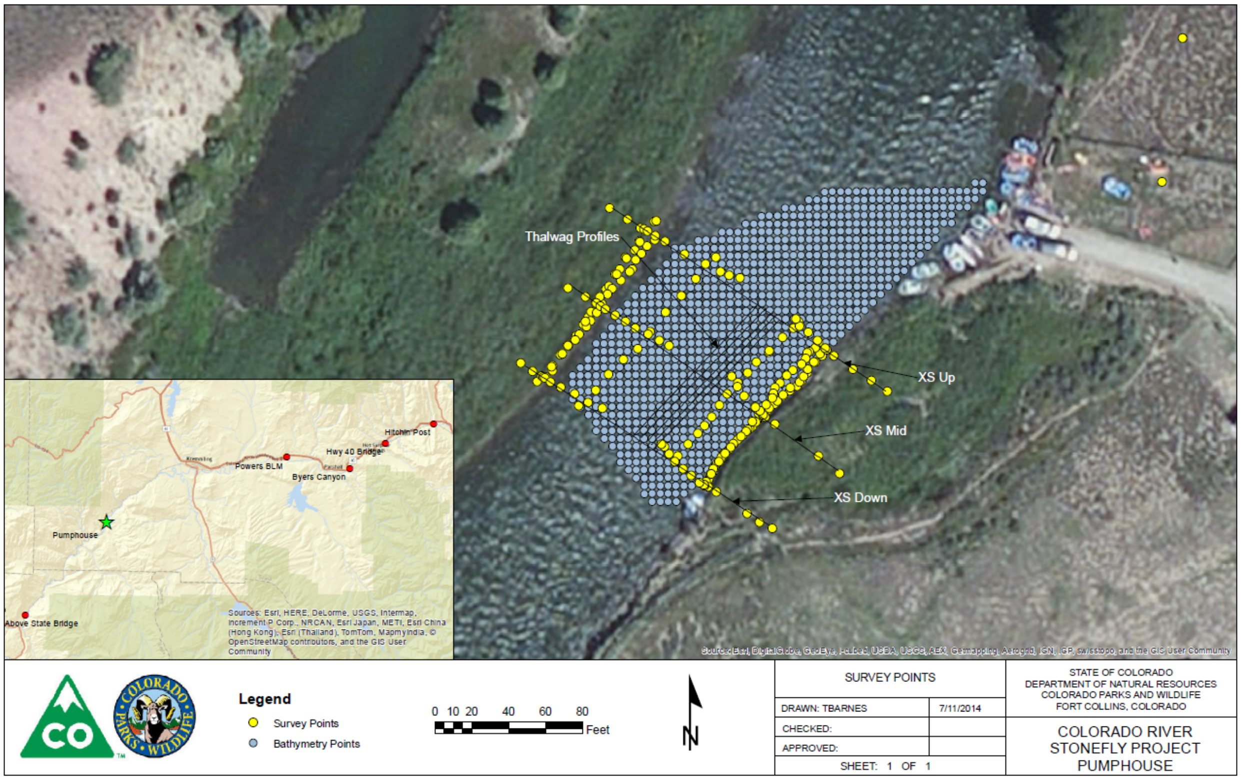

Figure 4. Survey points and bathymetry data collected with the survey-grade GPS equipment and Acoustic Doppler Current Profiler of the Pumphouse stonefly site.

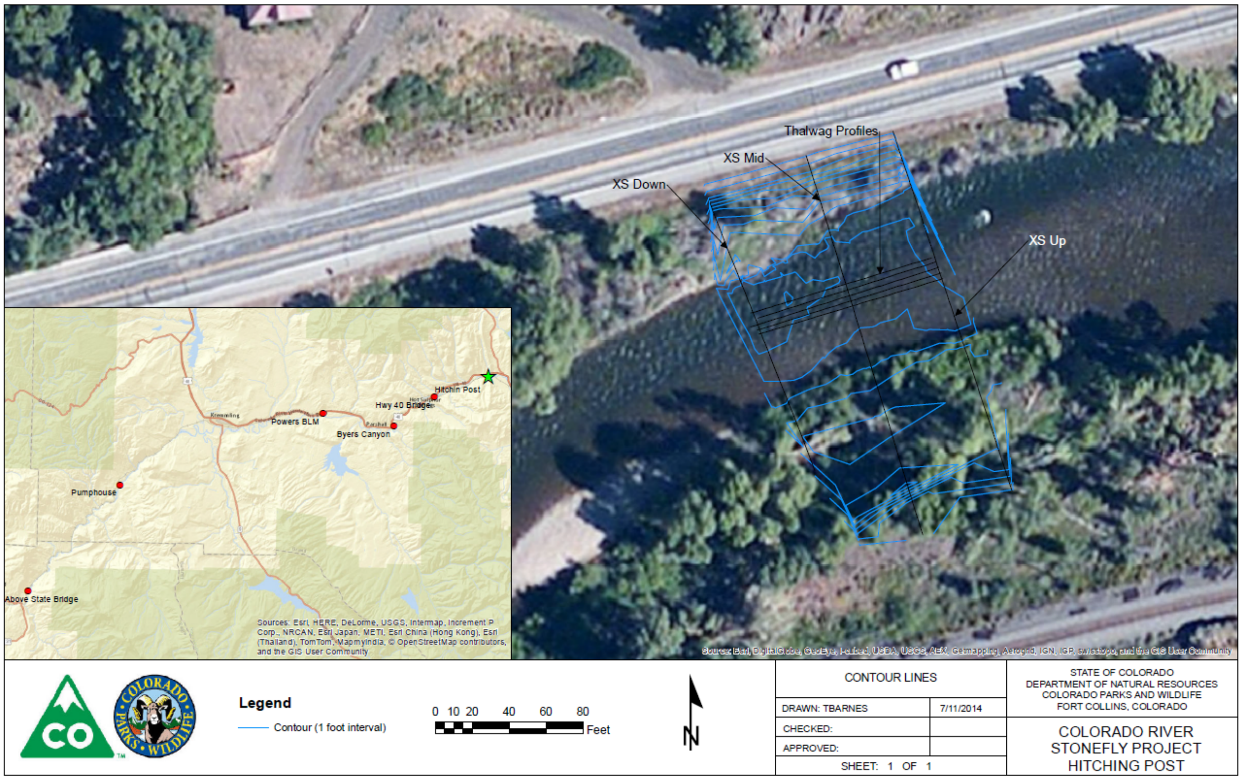

Figure 5. Bathymetric map produced by the GPS and ADCP survey used to estimate physical channel characteristics of stonefly study sites

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Job No. 2. <u>Mottled Sculpin Habitat, Distribution and Ecology Studies</u>

<u>Job Objective</u>: Investigate the distribution of mottled sculpin *Cottus bairdi* in the Colorado, Blue, Fraser and Williams Fork Rivers in Middle Park, Colorado.

Segment Objective: Mottled sculpin are a bottom dwelling native fish that occupy many coldwater streams and rivers of Colorado. Their unique habitat preferences and reliance on healthy riffle habitat make them a good ecological indicator of stream health (Nehring 2011). Because the quality and function of riffle and run habitat is commonly impacted when streamflows are altered or diverted, mottled sculpin may be impacted by flow related changes before higher predators like trout. Sculpin could not only indicate ecological problems that will eventually affect sport fish like trout, but they also serve as an important food source, especially for brown trout in many Colorado rivers. Previous work has documented the relative abundance and distribution of the mottled sculpin in the upper Colorado River basin and identified sites where this native fish species has been reduced in abundance or extirpated (Erickson 1983, Nehring 2011). Sites within a single stream or river system seem to have varying densities of sculpin, and the distribution may be affected by water projects and/or flow alterations (Nehring 2011). One conclusion of that study was that the density of sculpin was much lower at sites sampled below reservoirs than above. The objective of this study is to investigate distribution of mottled sculpin in the major rivers in Middle Park, Colorado in relation to major water projects. The specific objective in 2013 was to conduct a pilot study evaluating established sampling techniques and investigate the relative abundance of sculpin in rivers around Middle Park to stimulate research hypotheses and inform future sampling designs.

PROGRESS

Because previous sampling seemed to indicate that sculpin have declined in range and numbers in areas below major reservoirs in the Upper Colorado River basin, a pilot study was completed in 2013 to look at the presence and relative density of sculpin above and below four major reservoirs; Windy Gap Reservoir, Williams Fork Reservoir, Dillon and Green Mountain Reservoir. Historic sampling records indicate sculpin were common throughout the basin before the major water projects were built. Density estimates were completed at four sites on the Colorado River, one site on the Fraser River, three sites on the Blue River and three sites on the Williams Fork River in 2013. Table 3 contains sampling site information. Several of the sites on the Fraser and Williams Fork were part of standard monitoring efforts of the local aquatic biologist while the rest of the sites sampled specifically for this project. The sampling protocols were chosen to mimic previous surveys or ongoing management efforts. Estimates were completed with multiple pass removal electrofishing using four or five Smith Root LR24 backpack electrofishers or a Smith Root 2.5 GPP bank electrofisher and 5 anodes. Two or three passes were completed at each site and estimates were made with either a simple two pass removal model or the Huggins Closed Capture model in Program Mark (Zippin 1956, Huggins 1989, White and Burnham 1999). Sampling reaches were 197-650 feet long depending on width of river and stream characteristics that would best allow for closure assumptions to be met.

Where sampling sites were concurrent with the riffles being studied in the salmonfly ecology study, the reaches were centered on the 30 meter stonefly sites. Approximated 50 feet above and below those sites were sampled for a total reach near 200 feet. On larger rivers such as the mainstem Colorado, the entire river channel could not be effectively sample with 5 anodes so half of the channel was randomly chosen and sampled with greater intensity. No block nets were used due to the size of the rivers and high flow. When sampled by electrofishing sculpin tend to become immobilized easily and if not netted quickly tend to remain in bottom substrate rather than emigrate from the sampling area. Because of their unique biology, the large sampling reaches relative to home range and the high electrofishing effort, we hoped the methods established in previous surveys would be sufficient to adequately satisfy model assumptions to generate unbiased density estimates. All sculpin capture were measure to the nearness millimeter weighed to the nearest gram and then returned to the water.

Generally it appeared that assumptions of the removal model were met acceptably to consider these data as estimates of relative density, but the methods used were probably not sufficient for rigorous population estimates. Because of sculpin's small size, cryptic behavior and the large rivers they inhabit, the overall capture probability was low and there was some evidence it varied with pass. Closure could not be guaranteed because block nets of small enough mesh size could not be used in the high velocity and volume of water in these rivers. Mottled sculpin probably allow the closure assumptions to be met without block nets better than other fish due to their limited home range, lack of swim bladder, generally immobile behavior and the large size of the sampling reaches in relation to numbers of fish and home range. Their limited length range also makes it less likely that the size selectivity of electrofishing would produce differing capture probabilities. With high amounts of effort, multiple passes (3+) and advanced modeling methods allowed in Program Mark (such as using length as a covariate and varying capture probability with pass), many of these obstacles can be overcome. However, better sampling and estimation methods should be used for future work and simple removal surveys in open populations should probably only be interpreted as presence-absence information or coarse relative density information. Future work on this project will focus on developing techniques for getting unbiased and precise estimates mottled sculpin density.

In addition to the field surveys completed in 2013, Colorado's aquatic data management system (ADAMAS) was searched for historic sampling records of mottled sculpin at or near our sampling sites and this information was compared to current distribution data to attempt to verify the patterns of sculpin distribution identified in Nehring 2011. All sampling records since 1990 at our sites and within one mile were examined.

Current and historical sampling results are presented in Table 3 and show similar pattern of sculpin distribution in Middle Park Colorado to previous work. The density of mottled sculpin varied between 0-2,092 fish/acre with the highest density of fish found in the Fraser River above Windy Gap, the Williams Fork River above Williams Fork Reservoir and the Colorado River below the Blue River confluence. Capture probabilities from the surveys varied from 0.22-0.60 with an average of 0.41. Sculpin were present and generally abundant at most sites above major water impoundments, were absent immediately below impoundments and then generally appeared again some distance downstream of impoundments below tributaries. Sculpin are present in high density above Windy Gap Reservoir in the Fraser River but are absent below. Sampling sites on the mainstem Colorado failed to find sculpin until below the Blue River confluence even though they had been reported common historically and found periodically in the last 24 years. On the Williams Fork River a similar pattern was seen with sculpin abundant above the reservoir but absent below. The Blue River is an interesting example of a similar pattern because there are two mainstem impoundments; Dillon Reservoir and Green Mountain Reservoir. Sculpin are known to be present in the Blue River above Dillon (reported in two of three surveys since 1990) and absent immediately below (never reported in 10 surveys). They appear in low numbers 12.7 miles downstream at our site on Eagles Nest SWA and increase in density just above Green Mountain Reservoir. They disappear again immediately below Green Mountain (never reported in 5 surveys since 1990) but then are present again below Deep Creek, 2.5 miles downstream (reported in both of two surveys). Below the confluence of the Blue River and the Colorado River, mottled sculpin are abundant. Dams are known to cause many major environmental and ecological changes to rivers (Vinson 2001, Ward 1998). The flow, temperature and sediment regimes below impoundments in Middle Park are no exception to this and there appears to be some patterns that indicate that habitat changes below these dams make conditions less favorable or un-inhabitable for mottled sculpin. More intensive study is needed to identify what habitat characteristics may explain these patterns and develop effective population estimation methods for these native fish.

Table 3. Mottled Sculpin Sampling Sites, Density Estimates and Historical Sampling Information Above and Below Major Impoundments in Middle Park, Colorado

#	River	Site	Reach Length (ft)	UTM NAD 83 (Zone 13)	Density (#/Acre)	95% C.I.	# Surveys Since 1990	# Surveys MTS present
1	Colorado	Pumphouse BLM	197	370827, 4427300	1,869	76-3,662	8	6
2	Colorado	Byers Canyon	199	403335, 4434268	0	0	27	1 (1994)
3	Colorado	Hwy 40 Bridge	200	408133, 4437708	0	0	6	0
4	Colorado	Hitching Post	197	414589, 4440304	0	0	23	10 (Last in 2004)
5	Fraser	Kaibab Park in Granby	650	420592, 4437168	2,092	1,345-2,839	9	7
7	Williams Fork	0.25 mile Below Dam	813	398389, 4434143	0	0	14	0
8	Williams Fork	0.1 mile Above Reservoir	210	399287, 4428503	1,157	936-1,378	3	3
9	Williams Fork	USFS Below Keyser Creek	206	406372, 4417243	647	488-806	3	3
10	Blue	0.4 mile Below Dam	238	385737, 4415658	0	0	10	0
11	Blue	Blue River SWA (0.8 mile Above Green Mtn.)	198	396087, 4409122	617	299-935	1	1
12	Blue	Eagles Nest SWA (13 miles Above Green Mtn.)	201	402931, 4399910	80	45-758	1	1

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Job No. 3. Colorado River Water Project Mitigation and Ecology Investigations

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.

Segment Objective: Previous work under Project F-237 identified some ecological impacts of stream flow alterations and a main stem reservoir on the invertebrates and fish of the upper Colorado River. 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 negotiations between the major stakeholders are ongoing and no final plan has been agreed upon. The draft Windy Gap bypass study has been released and was reviewed and discussed with other CPW personnel. The preferred alternative indentified in that report was deemed financially unfeasible by Northern Water Conservancy District and mitigation options are still being discussed. Standard monitoring sites were completed in 2013 on the Colorado and Fraser rivers to evaluate trends in native fish and aquatic invertebrates. Future work on this objective will involve disseminating knowledge from other jobs in F237R to stakeholders involved in the mitigation work and will also likely involve evaluating the effectiveness of proposed mitigation measure when they do get implemented.

Job No. 4: Gunnison River Aquatic Invertebrate and Pesticide Studies

Job Objective: Document the aquatic invertebrate communities of the Gunnison River and Colorado Rivers and investigate the potential impacts of mosquito control insecticides on invertebrates.

Segment Objective 1. Investigate the potential impact of mosquito control insecticides on aquatic invertebrates in Colorado streams by reviewing toxicity data evaluating the exposure of common treatments to rivers.

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 and 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 filed experiments have documented the toxicity of permethrin to aquatic organisms and fish. This should be expected because these chemicals are 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, 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.

While many aquatic invertebrates are extremely sensitive to permethrin at very low concentrations, fish are also very sensitive to this insecticide. 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 at or 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 late June 2013 because of concerns about impacts to aquatic invertebrates (Gunnison County 2013). Because of the decision to cancel the spraying less than two weeks before the planned application and field associated work, the planned study of the impacts of this application was impossible and had to be changed at the last minute. The study plan was modified to react to the change in applications and two separate investigations were completed to accomplish our objectives: water sampling on the Colorado River in Middle Park (Segment Objective 1) and a longitudinal study of invertebrates in the Gunnison River and Tomichi Creek (Segment Objective 2). The Colorado River near Kremmling, CO was the only location where an aerial application was known to be planned near a coldwater river containing a trout fishery. Water sampling was done on the Colorado River and Muddy Creek to determine if the aerial application of Permethin around the town of Kremmling resulted in exposure of the pesticide to aquatic invertebrates in streams and rivers near the treated area.

A total of 12 samples were collected at four sites, two on the Colorado River and two on Muddy Creek. The site information is contained in Table 4. Samples were collected from 20:30 to 23:00 on July 2, 2013. An aerial spray application took place earlier that evening. Most of the observed treatment area was directly in and around the town of Kremmling and the irrigated hay fields west of town surrounding Muddy Creek. The application was observed applying pesticide treatment directly over Muddy Creek but not directly over the Colorado River. The most upstream site on the Colorado River (Barger Gulch BLM access) was outside of the observed treated area. Samples were shipped overnight to the U.S. Geological Survey for analysis. Water, suspended sediment and bed sediment samples were collected at each site and analyzed for the presence of pesticides with gas chromatography/mass spectrometry (GC/MS) following the methods of Hladik (2009). A total of 16 pesticide compounds were tested for but only three compounds were detected in the samples; permethrin, bifenthrin and piperonyl butoxide (Table 5).

Table 4. Pesticide Sample Collection Sites near Kremmling, Colorado

Number	Name	UTM X (Zone 13)	UTM Y (Zone 13)	Time	pH	Temp C	Conductivity (µS)
CO2	Colorado River Above Blue Confluence	380769	4433460	20:58	8.5	18.6	650
CO3	Muddy Creek at Colorado River Confluence	380754	4433476	21:15	8.0	18.1	670
CO5	Colorado River Barger Creek	387212	4433405	21:54	7.5	17.2	210
CO6	Muddy Creek Hwy 40 Bridge	380706	4435413	22:49	8.0	17.5	700

Permethrin was detected in 3 of 4 water and bed sediment samples and in all 4 suspended sediment samples. Concentrations were lowest in water (0.0087 – 0.0349 micrograms per liter), higher in bed sediment (2.1 to 9.1 micrograms per kilogram), and highest in suspended sediment (299 to 1,500 micrograms per kilogram). Permethrin was not detected in water or suspended sediment at the site on the Colorado River that was upstream of Kremmling. Permethrin concentrations in water and suspended sediment were higher at the two sites on Muddy Creek than at the two sites on the Colorado River. Water concentrations were generally lower than levels reported to impact invertebrates in laboratory toxicity tests. However, permethrin levels in suspended sediment were very high in Muddy Creek and in the Colorado River downstream from Muddy Creek indicating that most of the pesticide that entered the stream was bound to suspended sediment and not dissolved in the water. This is expected because permethrin has a low solubility in water and high affinity for soil particles (Imgrund 2003). This also indicates that standard laboratory toxicity testing may not provide valid models for probability of invertebrate exposure to permethrin. Dietary routes of exposure could potentially be important especially for filter feeding invertebrates. Single species toxicity tests are known to be oversimplifications of true exposure routes and generally underestimate the concentrations of pesticides that can have ecological and community level effects. More experiments are necessary to estimate what type of risk permethrin levels that we observed in suspended sediment pose to invertebrates.

Piperonyl butoxide was detected in 3 of 4 water samples, but was not detected in any suspended or bed sediment samples. Concentrations in water ranged from 0.163 to 0.305 micrograms per liter. Piperonyl butoxide was not detected in water (or sediment) at the site on the Colorado River that was upstream of Kremmling.

Bifenthrin was detected in all 4 bed sediment samples, but was not detected in any water or suspended sediment samples. Concentrations in bed sediment ranged from 0.3 to 5.3 micrograms per kilogram.

Although this was a small scale project that involved only a single application, these results do indicate that currently used application methods are resulting in pesticide exposure to streams at levels that are expected to impact invertebrates.

Table 5. Pesticide Sample Analysis Results from Muddy Creek and the Colorado River

		Water Concentration (ppb)			**Suspended Sediment Concentrations (ppb)**			**Bed Sediment Concentrations (ppb)**		
Site Number	**Site Name**	Piperonyl Butoxide	Permethrin	Bifenthrin	Piperonyl Butoxide	Permethrin	Bifenthrin	Piperonyl Butoxide	Permethrin	Bifenthrin
CO2	Colorado River above Blue River	0.163	0.0087	nd	nd	299	nd	nd	9.09	0.87
CO3	Muddy Creek at Colorado River Confluence	0.231	0.014	nd	nd	1,500	nd	nd	6.99	5.25
CO5	Colorado River at Barger Gulch	nd	nd	nd	nd	nd	nd	nd	2.10	3.62
CO6	Muddy Creek at Hwy 40	0.305	0.035	nd	nd	1,391	nd	nd	6.19	0.34

Segment Objective 2. Document patterns in the aquatic invertebrate communities of the Gunnison River and Tomichi Creek and compare areas historically treated with mosquito control pesticides to untreated areas.

The second part of this study involved a longitudinal survey of aquatic invertebrates in the Gunnison River and Tomichi Creek. The focus was on comparing invertebrate communities outside the historically treated areas to areas that were annually treated to control mosquitoes to see if there were any community level differences in treated vs. untreated areas. A map of the treated area is presented in Figure 6. Of the nine invertebrate sampling sites, the two most upstream sites on the Gunnison (GR5 and GR4) and the one upstream site on the Tomichi (TC4) were outside of the historically sprayed area. The summary report for this study is presented in Appendix A on page 34. Results of this study show significant longitudinal patterns in the distribution and abundance of macroinvertebrates. Abundance and species richness of most groups was significantly reduced downstream, particularly at the furthest downstream sites on both the Gunnison River and Tomichi Creek. While no direct causal relationship can be inferred between the reduced abundance and richness of invertebrate communities in the historically treated areas and the mosquito control activities, this pattern does contribute some evidence for concern. With the longitudinal pattern seen here, the documented sensitivity of invertebrates to permethrin, and the direct evidence that aerial spraying in other locations exposed streams to levels of pesticides that are expected to impact invertebrates, there is cause to further investigate this topic. Future work will focus on investigating the routes of exposure of pesticides to rivers through water testing and establish the toxicity of the field formulations of permethrin and piperonyl butoxide on stonefly species present in the Gunnison River.

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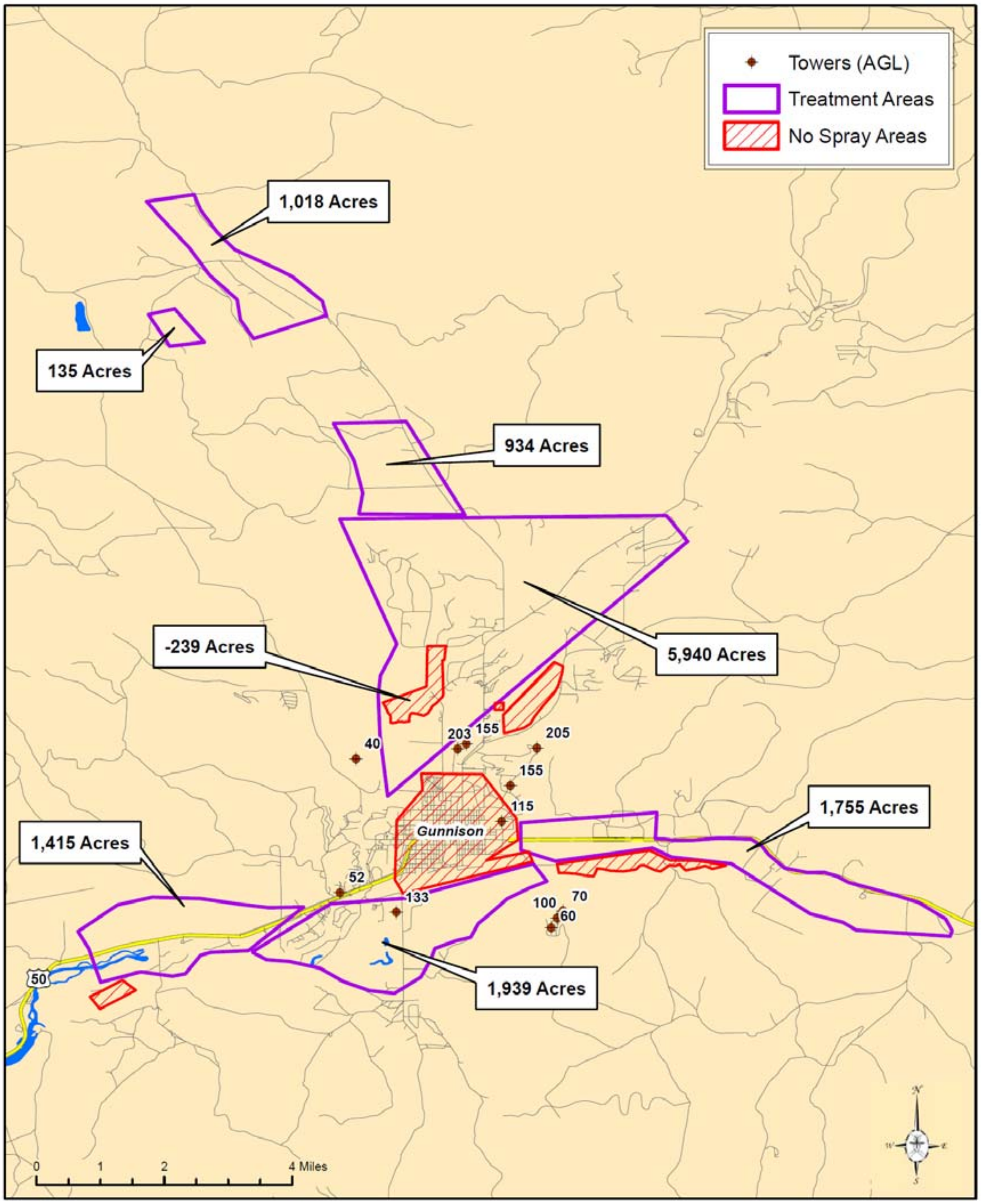

Figure 6. Mosquito control treatment areas around Gunnison, CO in 2012. Of the nine invertebrate sampling sites, the two most upstream sites on the Gunnison (GR5 and GR4) and the one upstream site on the Tomichi (TC4) were outside of the historically sprayed area.

Job No. 5: Professional Publications

Job Objective: Prepare manuscripts for professional publication from completed work on Project F-273-R.

Segment Objective 1. Prepare manuscripts on the role of *Tubifex tubifex* worms in reducing or eliminating the impacts of whirling disease in coldwater lake and stream ecosystems in Colorado.

PROGRESS

One manuscript has been completed and published in the Journal of Aquatic Animal Health in August of 2013. The citation is:

Nehring, R.B., B. Hancock, M. Catanese, M. Stinson, D. Winkelman, J. Wood, and J. Epp. 2013. Reduced *Myxobolus cerebralis* actinospore production in a Colorado reservoir may be linked to changes in *Tubifex tubifex* population structure. Journal of Aquatic Animal Health 25: 205-220.

A second manuscript is under review for publication in the Journal of Aquatic Animal Health. It was submitted in May 2014, minor revisions have been completed and it was re-submitted in July 2014. The citation is:

Nehring, R.B, G.J. Schisler, L. Chiaramonte, A. Horton, and B. Poole. 2014. Assessment of the long-term viability of the myxospores of *Myxobolus cerebralis* as determined by production of the actinospores by *Tubifex tubifex*.

Segment Objective 2. Prepare a manuscript on the reproductive success, growth rates and survival of Hofer rainbow trout in private ponds and compare their resistance to infection by *Myxobolus cerebralis* to brook and brown trout.

PROGRESS

Research for this job is completed but no progress has been made on manuscript.

Segment Objective 3. Prepare a manuscript assessing the relative vulnerability of populations of lineage III, IV and VI *Tubifex tubifex* worms to infection by *Myxobolus cerebralis* from major river basins across Colorado.

PROGRESS

This manuscript has been completed and published in the Journal of Aquatic Animal Health (JAAH) in March of 2014. The citation is:

Nehring, R.B., P.M. Lukacs, D.V. Baxa, M.E.T. Stinson, L. Chiaramonte, S.K. Wise, B. Poole, and A. Horton. 2014. Susceptibility to *Myxobolus cerebralis* among *Tubifex tubifex* populations from ten major drainage basins in Colorado where cutthroat trout are endemic. Journal of Aquatic Animal Health 26:19-32.

Segment Objective 4. Prepare a manuscript for publication that compares the relative abundance and distribution of aquatic macroinvertebrate fauna of the upper Colorado River basin in Grand County from 1981 to 2010.

PROGRESS

The field work for this job was completed in the summer of 2011. Drafts were reviewed by four Colorado Parks and Wildlife supervisory staff members and the final report was released in September, 2011. A manuscript titled "Changes in Benthic Macroinvertebrate Communities on the Upper Colorado River below Windy Gap Dam: 30 Years Later" has been completed by Brian D. Heinold, Justin Pomeranz, and R. Barry Nehring. It was submitted to the journal "Rivers Research and Management" in early 2013 for publication. The reviewers recommended it be considered for publication pending major revisions. The senior author retracted the manuscript from that journal and submitted it to the journal "Aquatic Insects" in July, 2013. A second manuscript on the changes in the density and abundance of the giant stonefly *Pteronarcys californica* in the Colorado River between 1980 and 2011 is currently being complete by B.D. Heinold and R. B. Nehring and is planned to be submitted for publication.

Segment Objective 5. Prepare a manuscript for publication that documents the relative abundance and longitudinal distribution of the mottled sculpin *Cottus bairdi* in the upper Colorado River basin in Grand County, Colorado.

PROGRESS

No progress has been made on this manuscript.

Job No. 6. Technical Assistance

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

Segment Objective 1: Cap K Ranch Ponds Whirling Disease Study

The objective of this study is to investigate the sources of *Myxobolus cerebralis* infection in the Fryingpan River and evaluate strategies to reduce infection in the ponds.

PROGRESS

This study is complete. The final report can be found at:
http://cpw.state.co.us/Documents/Research/Aquatic/pdf/Publications/WhirlingDiseaseInvestigationsCapK2013.pdf

Segment Objective 2: Placer Creek Whirling Disease Study

The objective of this study is to investigate strategies to reduce *M. cerebralis* infection in a cutthroat trout stream through removal of fish hosts and introduction of resistant strain *Tubifex tubifex* worms.

PROGRESS

This study is complete. The final report can be found at:
http://cpw.state.co.us/Documents/Research/Aquatic/pdf/Publications/WhirlingDiseaseInvestigationsPlacerCreek2013.pdf

Segment Objective 3: Lower Squaretop Lake Whirling Disease Study

The objective of this study is to investigate strategies to reduce *M. cerebralis* infection in an alpine lake cutthroat trout population through removal of fish hosts.

PROGRESS

This study is complete. The final report can be found at:
http://cpw.state.co.us/Documents/Research/Aquatic/pdf/Publications/WhirlingDiseaseInvestigationsSquaretop2013.pdf

Segment Objective 4: Gunnison Tunnel Electric Fish Barrier Evaluation

The objective of this study is to evaluate the effectiveness of the electric fish barrier 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, or about 857 cfs average daily flow April-October, 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 barrier was installed at the diversion structure in 2012. From the diversion structure and electric barrier the canal travels through a 5.7 mile long tunnel before day lighting approximately 0.5 miles above the power house and drop 1 (Figure 9). 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 varying amounts of water depending on water supply and irrigation demand (Figure 11). 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 an intermittent flows in the canal fish survival over winter is generally thought to be low and variable. 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 much larger numbers of fish in the canal in spring of 2013 due to increases 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 10, 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 electric fish barrier 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. The waterway at the barrier 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 barrier is believed to have operated continuously as planned throughout the entire 2013 irrigations season. Communication was lost for a brief time (6 out of over 6,000 hrs of operation) but operation of the barrier was thought to be unaffected.

The purpose of this study was to estimate fish populations in the South Canal before and after irrigation season, investigate the entrainment of fish from the Gunnison River and evaluate the effectiveness of the electric barrier in East Portal. To accomplish this fish population estimates were analyzed by 1) comparing fish population estimates before and after the barrier was built, 2) comparing fish population estimates from spring and fall, and 3) documenting any movement across the barrier with tagged fish.

METHODS

South Canal was sampled with mark-recapture electrofishing (fall 2011 and 2013) and multiple pass removal (spring 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 differing habitat and flows (20-25 cfs vs. 500-900 cfs).

On March 29th 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 as 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 (Appendix B, Figure 10). Two seine hauls were made through the stilling basin so a depletion population estimate could be made (Zippin 1956). 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 capture probability assumptions of the removal and there was no evidence in the data to indicate an unacceptable amount of bias. 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 electric fish barrier. 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.

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, efforts were made to model capture probabilities by fish species and length to account for variability. 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.

Three groups of fish were tagged and released in East Portal upstream from the Gunnison Tunnel to challenge the electric barrier. One hundred and twenty five fish (59 brown trout and 66 rainbow trout) from the March sampling of the stilling basin were moved from below the barrier to above the barrier 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) and 232 mm (range 180-392) for rainbows. Wild fish were captured by boat electrofishing on June 17 and 19, 2013, in East Portal above the barrier 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 (range 103-737) and for rainbows it was 336 mm (range 82-547). Fingerling rainbow trout from the Rifle Falls Fish Hatchery were also tagged and released into the Gunnison River in East Portal above the barrier. A total of 19,800 fish with a mean length of 68 mm were tagged with coded wire tags only on June 24-26, 2013 and stocked into the Gunnison River 0.7 m above the electric barrier on July 26. A total of 21,190 trout from 68mm to 737mm were tagged in the spring and summer of 2013 and released in the Gunnison River above the EFGS.

Fall mark recapture population estimates in the study reach were conducted in 2011 and 2013 with a 14 ft. aluminum jet boat with Smith Root 2.5 GPP electrofisher. The study reach, equipment and methods for both occasions were the same. Fish were measured to the nearest millimeter and a sample of the fish were weighed to the nearest gram. All fish were examined for adipose fin clips and checked for coded wire tags with a Norwest Marine Technology T-Wand Detector. Length-weight regressions were created by species with all fish weighed on the recapture pass ($n=644$, $r^2=0.986$) and used to model the weights of other fish to make biomass calculations. On the marking pass (conducted 10/18), all fish greater than 100 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 upstream fish barrier of the power plant further ensured geographic closure; block nets downstream were not feasible to the high volume of water in the canal. 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.

Fish population estimates were made with the Huggins Closed Capture Model in Program Mark. 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).

RESULTS

The results of the population survey are summarized in Table 5 and Figure 8 and length frequency histograms from the fall 2013 sampling are presented in Figure 7. Model selection results are summarized in Tables 6-8. 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 slightly higher flows and lower total number of fish captured. The strong length effect on capture probability resulted in a larger population estimate over a simple Lincoln-Petersen model and the lower capture probability and the length effect contributed to larger confidence interval for this estimate.

In March 2013 the top population model had 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. 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. Because of the low density of fish, capture probabilities were lower than in the stilling basin (0.62 vs. 0.77) and relatively even across size classes and the data were too sparse to support more detailed models.

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.

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. In October 2013 the study reach contained an estimated 1,764 ±279 trout over 100 mm. 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.

Two hundred and forty-six coded wired tagged stocked rainbows (plus 10 recaptures), two CWT wild brown trout (plus one recapture) and no CWT wild rainbows were captured. Of the tagged fish that were documented to have run the electric barrier, 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 of the South Canal. 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 barrier 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.

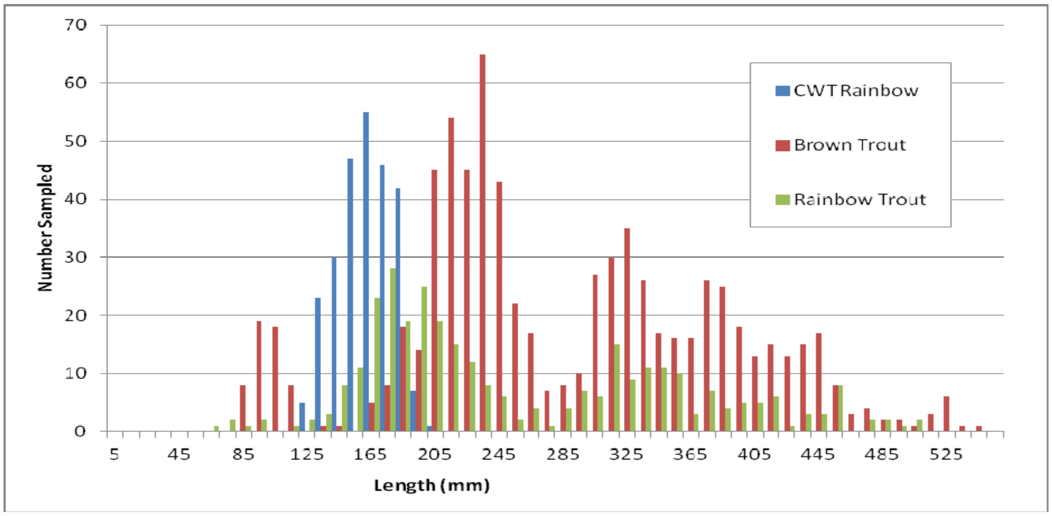

Figure 7. Length frequency histogram of trout captured in the South Canal in October 2013. A total of 246 coded wire tagged rainbows were captured that had been stocked upstream of the barrier (plus 10 recaptures). They had a mean length of 163 mm (123-204). Two other coded wire tagged fish were captured, 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.

Table 6. Summary of fish population estimates and 95% confidence intervals from the South Canal 2011-2013. These estimates are for age 1 fish and older, the stocked CWT tagged rainbows are excluded from the rainbow trout estimate. Fish biomass in the South Canal exceeds Gold Medal standards; there was a total of 214 lbs/acre of wild trout in October of 2013.

Date	Species	# Caught	Population Estimate in Study Reach
October 2011	Brown Trout	429	2,359±981
	Rainbow Trout	113	634±354
March 2013	Brown Trout	683	924±52
	Rainbow Trout	495	659±46
October 2013	Brown Trout	627	1,035±150
	Rainbow Trout	283	728±235
	Stocked CWT Rainbow	246	1,486±768
	CWT Brown	2	NA
	CWT Rainbow	0	NA

DISCUSSION

The South Canal contained high numbers of trout in the fall of 2013 (1,764 ±279). Trout numbers were higher than the spring of 2013 (1,583± 70) but lower that the fall of 2011 (2,994 ±1,043). While the fish estimates in the canal have declined since the barrier was installed, there is not a significant difference at the 95% level. A total of 248 coded wire tagged fish from 123 mm to 337 mm were documented passing through the fish barrier, with more smaller sized fish passing through the barrier than large fish. Further monitoring is necessary to document if the population of trout in the South Canal decreases over time due to the electric fish barrier reducing fish entrainment. Future investigations will focus on increased number of tagged fish of larger size, the use of passive integrated transponder tags (PIT), a stationary PIT tag antenna, and increased sampling effort in the canal to detect tagged fish.

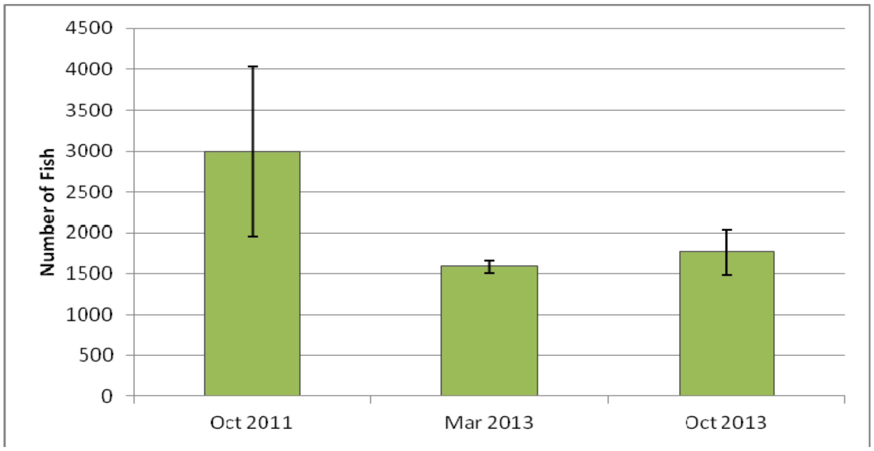

Figure 8. Estimated total number of trout age 1 and older and 95% confidence intervals in the South Canal study reach.

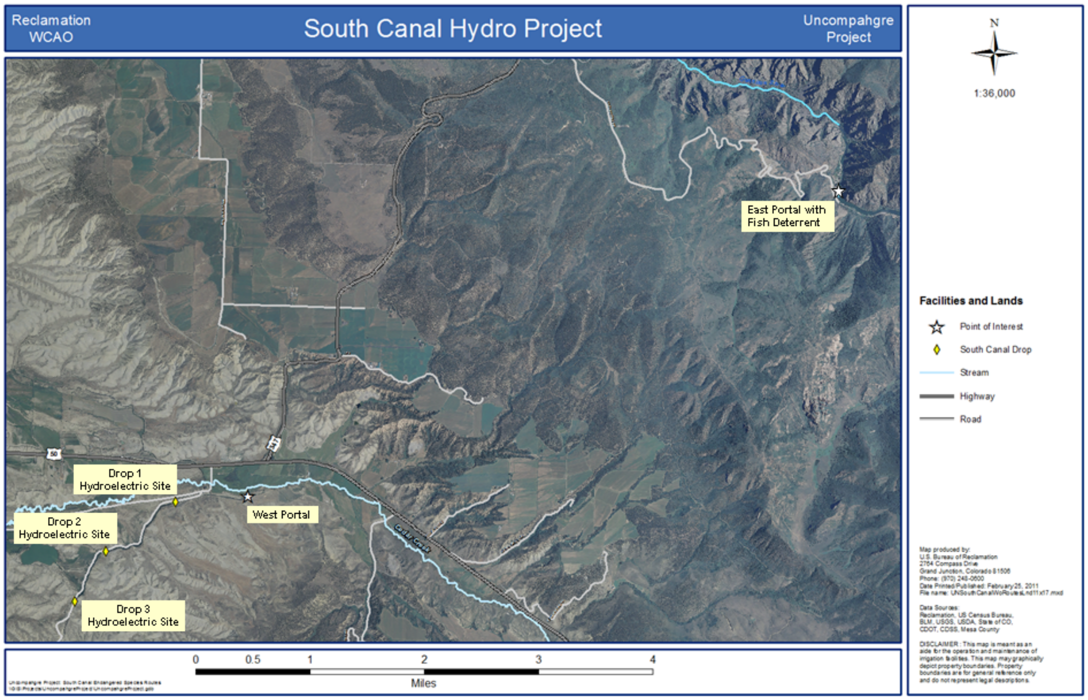

Figure 9. Area map of the Gunnison Tunnel and South Canal (Bureau of Reclamation 2012).

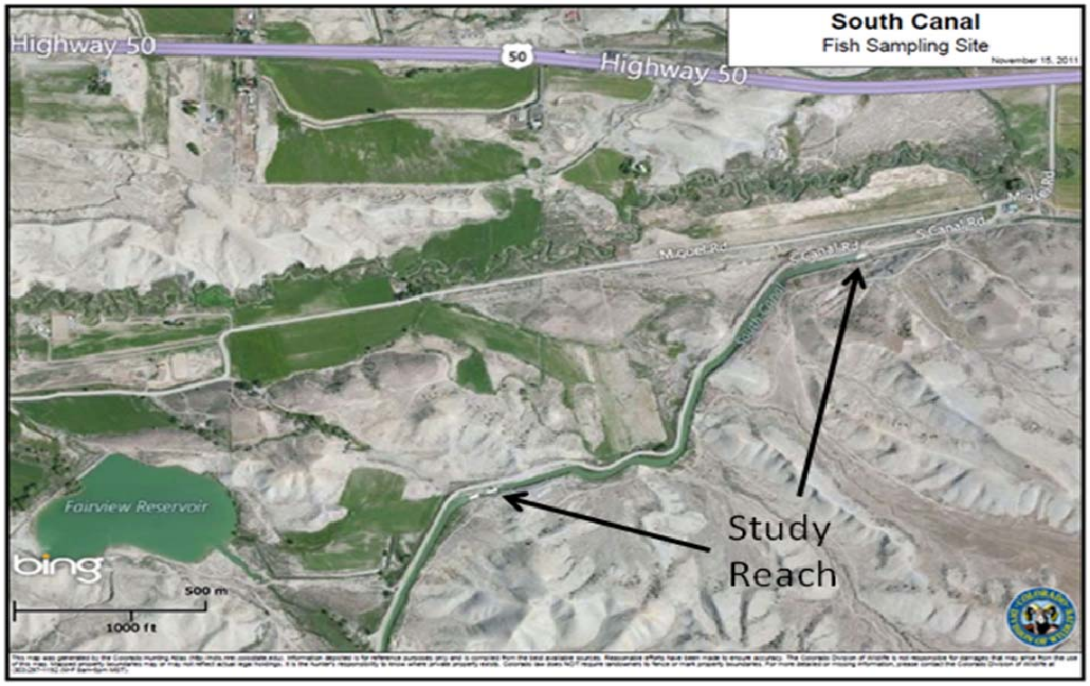

Figure 10. Fish sampling site on the South Canal. The sampling reach was 0.72 miles long (3,802 feet) and was between the first and second concrete drop structures below the West Portal.

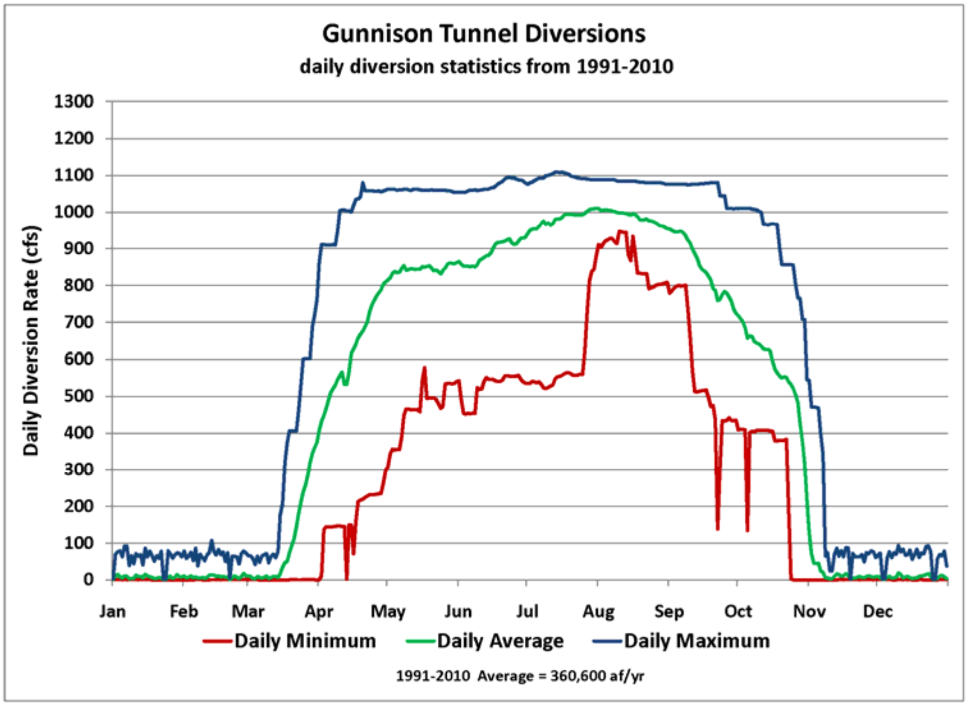

Figure 11. Water diversion records for the south canal below the Gunnison Tunnel 1991-2010.

Model Selection Results

Table 7. 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	0.00	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 8. Model selection results for the two pass electrofishing removal 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	0.00	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 9. 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	0.00	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

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Appendix

Gunnison River Aquatic Invertebrate and Pesticide Studies
(Job No. 4, Segment Objective 2)

Spatial Variation of Macroinvertebrate Communities in the Gunnison River Basin, CO

William H. Clements
Department of Fish, Wildlife and Conservation Biology
Colorado State University
Fort Collins, CO 80523

Dan Kowalski
Colorado Parks and Wildlife
Montrose, CO 81401

Background

The primary objective of this study was to investigate the potential effects of mosquito spraying operations on stream communities in the Gunnison River and Tomichi Creek. To test the hypothesis that benthic communities varied spatially, macroinvertebrates were sampled along a longitudinal gradient in both streams on 25-26 June, 2013.

Study Site and Methods

Study sites were located along a longitudinal gradient on the mainstem of the Gunnison River (5 sites) and on Tomichi Creek (4 sites), a tributary to the Gunnison River (Table 2). Replicate macroinvertebrate samples ($n = 5$) were collected using a 0.1 m^2 Hess sampler fitted with a 350 µm mesh net. Samples were collected from riffle habitats with predominantly cobble substrate by disturbing the substrate to a depth of approximately 10 cm. Substrate was removed from the sampler and all organisms were washed into the collection net.

In the field samples were washed through a 350-µm sieve and organisms were preserved in 80% ethanol. Macroinvertebrate samples were sorted and sub-sampled in the laboratory using a standard USGS 300-count 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. Routine water quality characteristics (conductivity, pH, temperature) were measured at each station (Table 1).

We report patterns in spatial abundance for major macroinvertebrate groups (Ephemeroptera, Plecoptera, Trichoptera, Diptera, Coleoptera and non-insects), dominant taxa and several common macroinvertebrate metrics (EPT, EPT abundance, total species richness and total abundance).

Differences in abundance and species richness of macroinvertebrate groups among stations within each stream were analyzed using one-way ANOVA (PROC ANOVA in SAS). If the overall F-statistic was significant ($p < 0.10$), Duncan's Multiple Range Test was used to identify differences among individual stations within each stream. Abundance data were log-transformed to satisfy assumptions of parametric statistics. Canonical discriminant analysis, a multivariate statistical technique, was used to identify separation and overlap of stations within each stream based on abundance of the dominant taxa (13 in Tomichi Creek and 14 in the Gunnison River). These dominant taxa accounted for > 95% of all organisms collected in each stream.

Results

Abundance and species richness of macroinvertebrate communities varied significantly among stations in the Gunnison River and in Tomichi Creek (Figs. 1 & 2). Although the responses differed among individual taxa and among macroinvertebrate groups, the most consistent pattern across both streams was a general decrease in abundance and richness from upstream sites to downstream sites. This trend was especially obvious in Tomichi Creek where abundance and richness of most groups was significantly reduced at TC1, the furthest downstream station. In addition to the longitudinal trend in the Gunnison River, abundances of mayflies (Ephemeroptera) and dipterans were significantly reduced at station GR3 compared to other stations. The reduced abundance of mayflies is of importance because these organisms are generally considered highly sensitive to anthropogenic stressors.

Results of canonical discriminant analysis also showed large variation in benthic macroinvertebrate communiites among stations. Based on abundance of the 14 dominant taxa in the Gunnison River (*Acentrella*, *Baetis*, *Diphetor*, *Ephemerella*, *Brachycentrus*, *Lepidostoma*, Chironomini, Tanytarsini, Orthocladini, Tanypodini, Diamesinae, *Simulium*, Elmidae, and Oligochaetes), stations GR3 and GR1 were distinctly separated from all other sites (Fig. 3). The greatest separation among stations was observed along canonical axis 1, which explained 76.4% of the variation.

Benthic communities at station TC1 in Tomichi Creek were also quite different from those at other sites in the watershed (Fig. 3). Canonical discriminant analysis of benthic communities from Tomichi Creek was based on abundance of the 13 dominant taxa (*Acentrella*, *Baetis*, *Tricorythodes*, *Antenella*, Hydropsychidae, *Oecetis*, *Helicopsyche*, Chironomini, Orthocladini, Tanypodini, *Simulium*, Elmidae, and Oligochaetes). Although there was some separation among the 3 upstream sites (TC2, TC3 and TC4) along canonical variable 2, this variation was relatively small compared to separation of TC1 from all other stations.

Another consistent pattern observed in these data was the relatively low abundance and species richness of stoneflies (Plecoptera) in both streams. Although stonefly density did not vary significantly among sites in the Gunnison River, abundances were relatively low compared to other Colorado streams. Results of a spatially extensive survey of 73 streams in Colorado’s Southern Rocky Mountain ecoregion reported an average of 35 individuals and 5 species per 0.1 m^2 from reference streams (Clements et al. 2000). Similar patterns were observed in a more extensive survey of > 150 central Colorado streams (Schmidt et al. 2010). In contrast to these results, average density and species richness of stoneflies in the current study were < 4 individuals and 1-2 species per $0.1m^2$.

Conclusions

Results of this study show highly significant longitudinal patterns in the distribution and abundance of macroinvertebrates. Abundance and species richness of most groups was significantly reduced downstream, particularly at the furthest downstream sites (GR1 & TC1). The low density of Plecoptera in the Gunnison River relative to other Colorado streams is especially noteworthy because some stoneflies are considered to be highly susceptible to pesticides and other anthropogenic stressors. Although these patterns are generally consistent with the hypothesis that pesticides associated with mosquito spraying operations affected benthic communities in the Gunnison River and Tomichi Creek, additional laboratory and field experiments would be required to provide evidence for a causal relationship.

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Table 1. Water quality and physical characteristics measured at Gunnison River and Tomichi Creek stations, June 25-26, 2013.

Station	UTM (NAD83 Zone 13)	Elevation (masl)	pH	Temperature (^{o}C)	Conductivity (µS/cm)	Current velocity (m/s)
GR5	338653, 4280294	2433	8.14	9.7	169	0.70
GR4	337617, 4278891	2421	8.18	10.2	173	0.70
GR3	332512, 4271993	2366	8.20	13.7	223	1.25
GR2	330670, 4270311	2348	8.14	14.1	241	0.82
GR1	324071, 4264856	2300	8.17	18.4	266	0.97
TC4	343445, 4265439	2380	8.28	15.0	378	0.38
TC3	340878, 4265628	2370	8.31	13.3	390	0.38
TC2	333917, 4267199	2341	8.16	14.6	393	0.60
TC1	328817, 4264920	2320	8.21	18.8	182	0.48

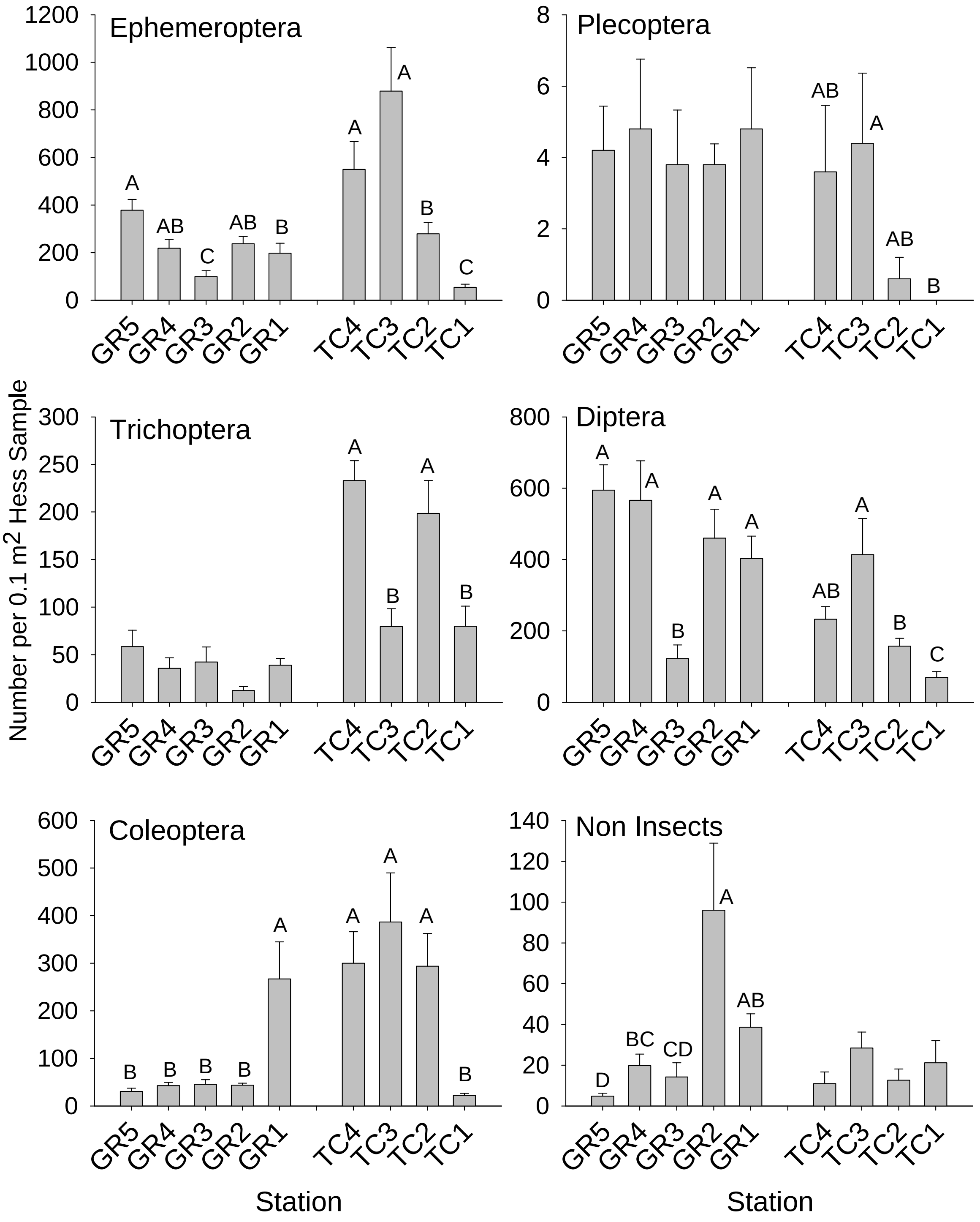

Figure 1. Spatial variation in abundance of major macroinvertebrate groups in the Gunnison River and Tomichi Creek, June 2013. Stations with the same letter were not significantly different based on Duncan's multiple range test.

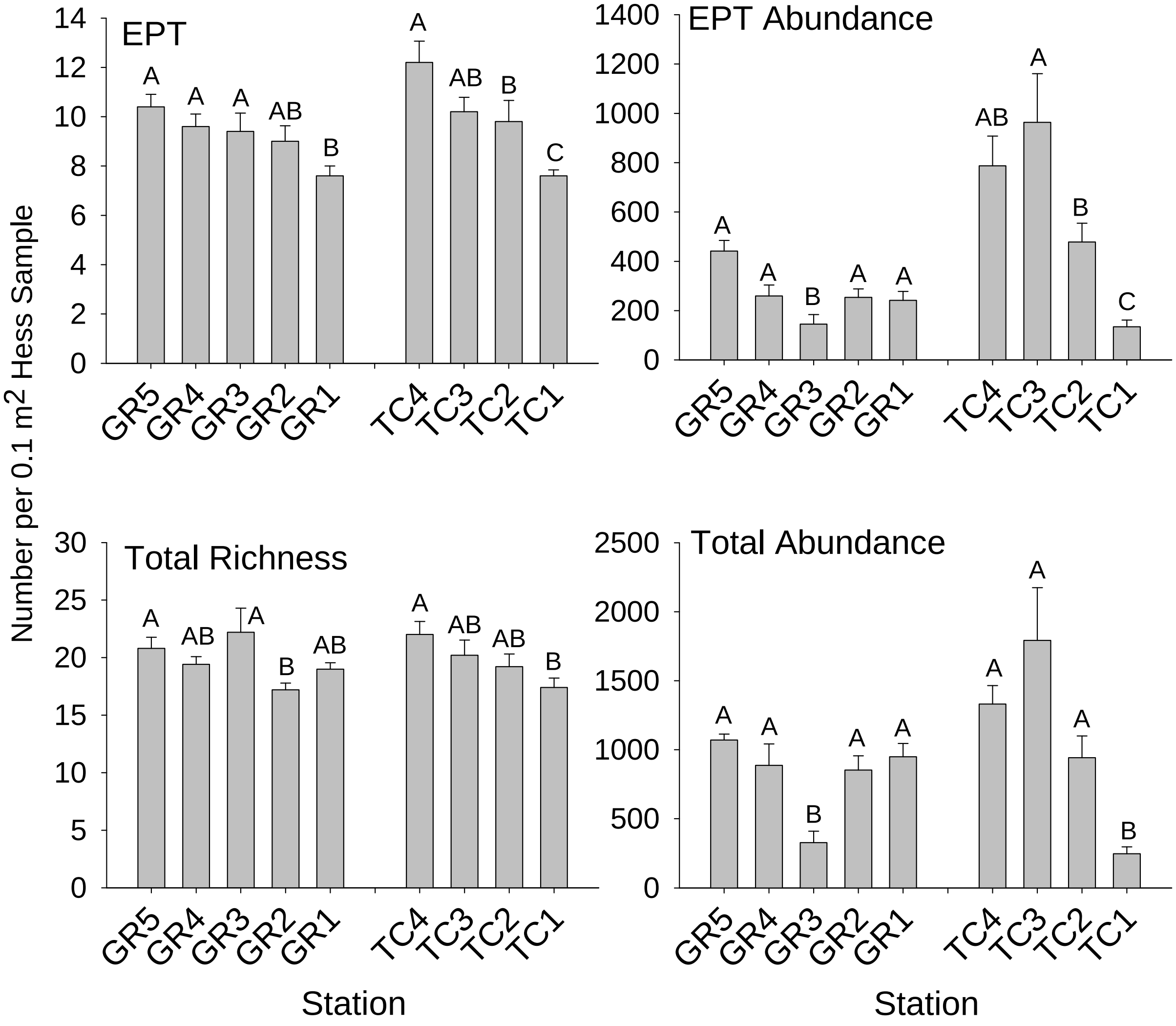

Figure 2. Spatial variation in macroinvertebrate abundance and richness in the Gunnison River and Tomichi Creek, June 2013. Stations with the same letter were not significantly different based on Duncan's multiple range test.

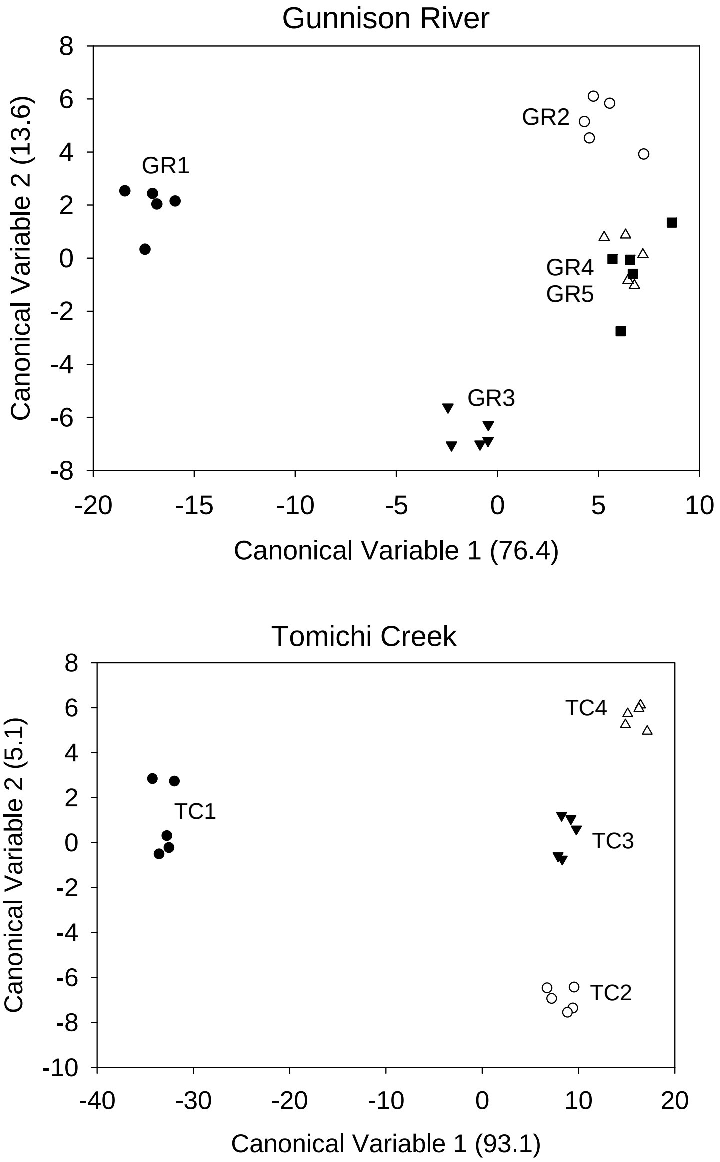

Figure 3. Results of canonical discriminant analyses showing separation and overlap of benthic communities based on abundance of dominant taxa in the Gunnison River (upper panel) and Tomichi Creek (lower panel). Values in parentheses show the amount of variation explained by each canonical axis.

Table 2. Locations and site descriptions at the Gunnison River and Tomichi Creek sampling stations, June 25-26, 2013.

Site	Description	Location
GR5	Almont USFS Campsite	Upstream end of USFS campground, 0.8 miles below East and Taylor confluence
GR4	Lost Canyon Resort	Downstream of bridge and private boat ramp
GR3	North Bridge	Downstream of footbridge, river right
GR2	Van Tuyl Unit Gunnison SWA	Below Ohio Creek confluence, river left
GR1	Neversink	Northeastern most NPS access at Neversink
TC4	Clark Ranch	Upstream of Butch (Ralph) Clark's house, 0.6 miles below Cochetopa Creek
TC3	Lost Miner Ranch	Upstream of irrigation diversion just east of CR42 bridge
TC2	Tomichi SWA	Downstream of bridge and irrigation diversion near parking lot
TC1	Dos Rios	South of cul-de-sac on Tomichi Trail Rd.