

Colorado River Aquatic Resource Investigations

Federal Aid Project F-237-R20

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Job Progress Report

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Aquatic Wildlife Research Section

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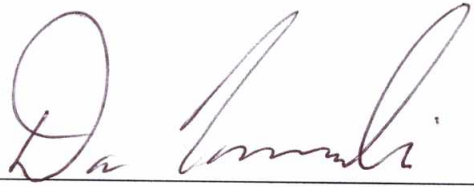
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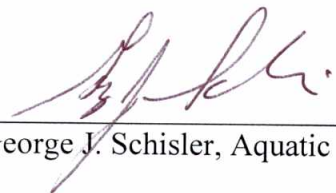
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The results of the research investigations contained in this report represent work of the authors and may or may not have been implemented as Colorado Parks & Wildlife policy by the Director or the Wildlife Commission.

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State: Colorado

Project Number: F-237-R

Project Title: Colorado River Aquatic Resources Investigations

Period Covered: July 1, 2012 through June 30, 2013

Project Objective: Document the distribution and abundance of the mottled sculpin *Cottus bairdi* and aquatic macroinvertebrates of the Colorado River in Middle Park and compare the results with historical records of the past 40 years.

Job No. 1. Colorado River Aquatic Invertebrate Investigations

Job Objective: Document the relative abundance and distribution of the aquatic invertebrate fauna of the upper Colorado River between the confluence with the Blue River and Windy Gap Dam west of Granby, Colorado.

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 decided to retract 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.

Parts of this job will continue as Job No. 1. Giant Stonefly Habitat and Ecology Studies. In May and June, 2013 density estimates of *Pteronarcys californica* stoneflies were made at six sites on the Colorado River and two sites on the Fraser River. The objective of this effort was to evaluate historic stonefly sampling sites and establish study sites for the new job. Sites were chosen to represent a range of stonefly densities and were locations where *P. californica* was historically common. Estimates were made by selecting 30 m (98.4 ft) sections of bank adjacent to riffle habitat and completing two pass removal estimates of stonefly exuvia. Data collection and analysis was ongoing at the time of this report and results will be reported in future Federal Aid Reports for project F-273-R, Job #1.

Job No. 2. Colorado River Mottled Sculpin Population Studies

Job Objective: Document the distribution and abundance of mottled sculpin *Cottus bairdi* in the upper Colorado River basin and continue to monitor historical sampling sites on the Colorado and Fraser Rivers.

PROGRESS

This field work for this job was completed in the summer of 2011 and the final report was released in September 2011. Parts of this job will continue as Job No. 2. Mottled Sculpin Habitat, Distribution and Ecology Studies. Two sites on the Colorado River and three sites on the Fraser River were sampled with two pass removal electrofishing. These sites were sampled to assist the local biologist with management efforts and to evaluate sites for future work to investigate factors that influence the distribution and abundance of mottled sculpin. Data collection and analysis was ongoing at the time of this report and results will be reported in future Federal Aid Reports for project F-273-R, Job #2.

Job No. 3. 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. Secondary objectives are to evaluate the survival, reproduction and *M. cerebralis* infection of Hofer rainbow trout in a small pond environment and evaluate the effectiveness of tiger trout (offspring of female brown trout and male brook trout) in controlling brook trout numbers.

PROGRESS

Population estimates were completed on all ponds on the Cap K Ranch in October and November, 2012 with mark recapture or multiple pass removal boat electrofishing. Results indicate that the tiger trout stocked in the ponds in May, 2008 have been successful in controlling brook trout fry in Ponds 1 and 2. Several hundred wild rainbow trout fry were observed in Pond 3 in July, 2012. One hundred of these fry (approximately) were captured by backpack electrofishing and moved into Pond 5 in July. Adipose clipped Hofer rainbow trout from the Bellvue State Fish Rearing Unit (n=150) were stocked into Ponds 1, 2, 4 and 5 June 7, 2012, while 450 were stocked into Pond 3. One thousand tiger trout fingerlings were also stocked in the ponds on the ranch in June and July 2012.

In November, 2012 140 adipose clipped Hofer rainbows and 12 unmarked wild rainbow trout were recaptured in Pond 5 by boat electrofishing and moved into the upstream ponds. The Hofer rainbows were released into Pond 3 and the unmarked rainbows were released into Pond 2. Fin

clips were collected from nine age 0 rainbows and three adipose clipped Hofer rainbows and submitted for genetic testing to determine their parentage. Results from previous years indicate that unmarked fry and fingerlings collected in 2009, 2010 and 2011 show genetic influence of the Hofer rainbows verifying that natural reproduction of Hofer rainbow trout was successful in the ponds in those years.

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

Rio Grande cutthroat trout and brook trout in Placer Creek were found to be heavily infected by *M. cerebralis* in 2005 and 2006. A reclamation project from 2007 to 2009 successfully removed all of the fish in the drainage above constructed fish barriers. Sentinel fish exposed in 2010 were negative for *M. cerebralis* infection through polymerase chain reaction (PCR) testing. The stream was re-stocked with cutthroat trout in 2010 and 2011. Lineage V and VI *T. tubifex* were introduced at 20 sites along a 10 km reach of main Placer Creek in 2010 and 2011. Lineages I, V and VI are resistant to infection by the *M. cerebralis* parasite while lineage III worms are highly susceptible to infection and transmit it to susceptible salmonid fishes. Approximately 37,000 worms were stocked in July, 2010 and 450,000 worms were stocked in August, 2011. In April, 2011 substrates in the vicinity of the worm introduction sites were screened for the presence of aquatic oligochaetes. Haired worms thought to be *T. tubifex* were collected at 10 of 20 collection sites and submitted for genetic testing. Lineage V DNA was detected at five of the 10 sites where worms were observed. In 2011, two of 62 fish tissue samples collected in May and June of tested positive for *M. cerebralis* indicating the parasite was still present in the stream. Lineage III *T. tubifex* DNA was present at eight of the 10 sites where oligochaete worms were found.

In 2012 sediments were screened at the 20 introduction sites for *T. tubifex* and collections of fish tissues samples were taken for *M. cerebralis* testing. Genetic screening of the oligochaete samples revealed that *T. tubifex* belonging to lineages I, V and VI were found at eight of 20 sampling sites while DNA belonging lineage III was detected at one site. A third introduction of approximately 600,000 lineage I, V and VI *T. tubifex* occurred at 20 new locations in Placer Creek in July, 2012.

Age1+ cutthroat trout (n=90) were collected from four locations on Placer Creek and the Middle Fork in 2012 and submitted for pepsin trypsin digest (PTD) testing. These fish had been in the stream for a minimum of one year. All samples were negative for *M. cerebralis*, suggesting that the parasite might no longer be present in the upper reaches of Placer Creek.

Segment Objective 3: Lower Squaretop Lake Whirling Disease Study

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

PROGRESS

This study is a continuation of work started in the Whirling Disease Investigations (Federal Aid Project F-237R) in 2009 and 2010. That study indicated that the *M. cerebralis* infection in Lower Squaretop Lake was severe enough that few of the cutthroat trout fry aerially stocked survive one year. Because the viability of *M. cerebralis* myxospores declines exponentially under ambient conditions in the aquatic environment, few if any myxospores remain viable longer than about one year. The expected life span of *T. tubifex* is two to three years. The working hypothesis for this project is that if the lakes can be kept free of fish for several years, it may be possible to eliminate the parasite from isolated portions of the drainage.

To meet the objectives of this project ongoing efforts include testing of fish and worm samples for *M. cerebralis*, screening and gill netting of the lake to keep it fishless, water filtrations to detect *M. cerebralis* triactinomyxons (TAMs), and live car exposures of trout fry to detect the parasite in the lake.

Oligochaete Sampling

Testing of *T. tubifex* has occurred from the upper lake, lower lake and Duck Lake (one mile downstream of the lower lake) between 2008 and 2012. Results indicate that the upper lake contains only lineage VI worms, the lower lake contains only lineage III and Duck Lake contains lineages I and V. Laboratory exposures of 250 worm replicates of lineage VI worms from the upper lake (50 myxospores/worm) indicated the worms were refractory to the parasite. No TAMs were detected during weekly screening of the water the worms were held in and PCR testing of these worms after seven months of exposure were negative for *M. cerebralis*. Two replicates of Lineage III worms from the lower lake exposed with the same protocols produced 5.92 million and 2.75 million TAMs. Both replicates produced TAMs at the beginning of the testing period, indicating that they were infected when collected from the lake in the fall of 2010.

Polymerase chain reaction testing on worms collected from the lower lake in September, 2012 (11 aliquots of 50 worms) were negative.

Fish Sampling

In 1998 cutthroat trout from Lower Squaretop Lake tested positive for *M. cerebralis*. Fish sampling in September, 2008 confirmed that trout in lower lake remained positive. The catch in gill net sampling that fall was limited to four cutthroat trout and all were larger than 400 mm total length. The lack of small trout in the nets suggested poor survival of the aerial fingerling plants in 2006. In August, 2009 gill net sampling resulted in a catch of 14 cutthroat trout; 11 large fish and three that were likely recruits from the last aerial plant in 2008. All three fish were and in poor body condition and tested positive by PTD. Six of the 11 larger trout tested positive with PTD with an average myxospores burden of 179,407 (range 8,300 to 1,851,000).

In 2010, seven 125 ft. gill nets were continuously set in the lower lake for 10 days in August. The total catch was 14 cutthroat trout and eight were likely from the 2008 stocking, ranging in size from 160 to 228 mm total length. No fish were caught during the last three days of the gill netting in 2010. In August, 2010 37 cutthroats were captured in the upper lake in during a single gill net set for one night. Fifteen fish were likely recruits from the 2008 aerial plants and ranged in size from 165 to 235 mm total length. All tested negative for *M. cerebralis* by PTD. In 2011, four gill nets were fished continuously in the lower lake during July and August. Six cutthroat trout were caught and all tested negative by PTD.

In the spring of 2012, the Duck Creek outlet of the lower lake and the inlet into Duck Lake were screened to prevent upstream migration of trout into Lower Squaretop. The screens were removed over the winter months but an aluminum weir at the Duck Creek inlet to Duck Lake has been in place continuously since April, 2012. No fish have been caught or observed in Lower Squaretop in 2012 or 2013 despite having three to four gill nets set continuously from June to August.

Water Filtrations

In 2012 water samples from the lower lake were taken once a month, June through August. No *M. cerebralis* TAMs were detected during the filtration and screening of the 500 gallon samples. This suggests that prevalence of the parasite in the lake could be very low.

Live Car Exposures and M. cerebralis Testing

In August, 2011 60 rainbow trout fry (about one month post swim up) from the Mount Shavano Hatchery were placed in a floating live cage in the middle of Lower Squaretop. The cage exterior was wrapped with a double walled one millimeter mesh screen that allowed zooplankton to pass into the chambers and permitted exposure to TAMs floating in the water column. The cage had two separate chambers and floating PVC collar kept the top of cage one to two inches above the lake surface. In September, 2011 an additional 60 cutthroat fry were placed in the second chamber of the cage. Ten fry from each group were sacrificed as controls prior to stocking to ensure the fry were not exposed to the parasite prior to the exposure. All control fish tested negative for *M. cerebralis* DNA. Fry were sacrificed from the live cage every seven to eight days post exposure and tested by PCR. *Gammarus* amphipods and zooplankton collected with a plankton net were fed to the fry every seven to eight days. Results from the PCR test revealed that 20% of the exposed rainbow and cutthroat fry tested positive within seven days post exposure and both groups were 100% positive from at 30 days post exposure.

Cutthroat trout fry exposed in the floating live cage in 2011 and then held at the Parvin Lake Research Station until July 2012 were tested with PTD. Twenty-eight of 29 fish that survived to the end of the holding period were positive and the average myxospore concentration was >80,000 (range 14,767 to 200,000).

On August 1, 2012 rainbow and cutthroat fry were exposed in a floating live car in Lower Squaretop. Ten fry were sacrificed every 10 days until October 9, 2012. Testing of these fry with PCR revealed that the first detections of *M. cerebralis* DNA did not occur until 14 days post exposure. Prevalence was in the range of 50-60% after 30 days, and never reached 100% in

rainbow trout fry after 70 days. The surviving cutthroat fry were transported to the Parvin Lake Research Station in October, 2012 and were held until late June, 2013 when they were sacrificed for PTD testing.

Cutthroat trout from Upper Squaretop Lake have been collected in 1998, 2008, 2009 and 2010 and submitted for PTD testing. All fish have tested negative for *M. cerebralis*. All cutthroat trout collected from Duck Lake in 2010 and 2011 tested negative for *M. cerebralis* by PTD.

During the 2013 field season, three gill nets will be continuously fished in the lower lake during June, July and August. Rainbow and cutthroat trout fry will again be exposed in a floating live car to test for the presence of *M. cerebralis*. Ten fry will be sacrificed at approximately seven day intervals and screened for DNA of the parasite.

Job No. 4: Professional Publications

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

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

PROGRESS

This manuscript has been completed and published in the Journal of Aquatic Animal Health (JAAH) 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.

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 ongoing and 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 was submitted to the Journal of Aquatic Animal Health, has been reviewed and accepted for publication pending revisions.

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 on this manuscript is described in Job 1.

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.

No progress has been made on this manuscript.

Job No. 5: New Research Priorities in Coldwater Stream Ecology in Colorado

Job Objective: Examine previous research projects in coldwater stream ecology and cooperatively develop new research topics with biologists, senior biologists and other researchers to address management and research needs in the agency.

PROGRESS

Federal Aid Documents for project F-273-R as well as primary and gray literature were reviewed to provide a background in the work previously completed under this project. A multiple step process was undertaken to identify potential research topics and gather input from biologists, senior biologists, researchers and senior aquatic staff. Many formal and informal discussions occurred with various aquatic personnel to identify potential research topics. The entire aquatic biologist and aquatic researcher email list was requested by twice to submit potential research topics for their individual areas. Topics formally returned by email as well as those identified in informal discussions were compiled in a spreadsheet and evaluated by three major criteria: 1) relevance to Parks and Wildlife mission and management, 2) feasibility and 3) scientific value. A total of 17 unique ideas were evaluated with this process, a summary of those topics and ranking information is presented below.

1. Colorado River Aquatic Ecology Investigations - This would be a major project that would focus on evaluating the proposed mitigation for the Windy Gap firming project on the ecology of Colorado River. This topic was recommended by 4 biologists, received a rank of three for all three ranking factors (relevance, feasibility and scientific value) and was judged to be a productive research topic that is valuable to CPW and was recommended for inclusion in final topics.
2. Mottled Sculpin Ecology and Habitat Investigations - This project would focus on identifying habitat or biological limitations of mottled sculpin and investigate why they have been extirpated below many water diversions or projects. This topic was recommended by four biologists, received a rank of two for relevance and three for both feasibility and scientific value. It was judged a productive research topic that is valuable to CPW and was recommended for inclusion in final topics.
3. Mysis Entrainment and Ecology Investigations - Several biologists have indicated that the patterns of mysis shrimp *Mysis diluviana* entrainment and their supplementation of tailwater trout fisheries have changed over the years. This project would focus on all three major mysis tailwaters in CO (Fryingpan, Taylor and Blue Rivers) and would involve revisiting older investigations as well as using new techniques to examine how mysis supplementation affects trout fisheries. This topic was recommended by three biologists, received a rank of three for all three ranking factors and was judged to be a productive research topic but potentially time and resource consuming enough to conflict with higher priority topics. This topic was not selected as one of the initial projects but may be revisited in the future as resources allow.
4. Permethrin Impacts on Aquatic Invertebrates in the Upper Gunnison River - The widespread application of Permethrin to control mosquitoes is common in many areas of Colorado and there is anecdotal evidence that treatments are impacting invertebrate populations, especially stoneflies. This study would be a technical assistance project to assist biologist in the Gunnison area in evaluating if Permethrin is impacting aquatic invertebrates in the river. This topic was recommended by two biologists, received a rank of three for all three ranking factors, was judged to be a productive research topic that is valuable to CPW and was recommended for inclusion in final topics.
5. Phylogeny of Native Fish of the Western Slope - This project would involve sequencing DNA of mottled sculpin and speckled dace *Rhinichthys osculus* from major river basins in western Colorado to investigate relatedness of these populations. Looking at the phylogeny of small native fish that were unlikely to be moved around by anthropomorphic means could help inform the interpretation of patterns of phylogeny recently revealed in cutthroat trout. This topic was recommended by two biologists, received a rank of three for relevance, three for feasibility and one for scientific value. It was judged a productive research but potentially outside the scope of the coldwater stream ecology and may be handled better by other researchers through collaboration between biologists.

6. *Pteronarcys* Stonefly Ecology and Habitat Investigations - This project would focus on identifying physical, biological and chemical limitations of *Pteronarcys* stonefly habitat and investigate why their distribution may have been altered by water diversions and other water projects. This topic was recommended by three biologists, received a rank of two for relevance, three for feasibility and three for scientific value was judged to be a productive research topic that is valuable to CPW and was recommended for inclusion in final topics.
7. Effects of Recreational Boating on Trout Movement and Habitat Use - Several biologists expressed concerns that high levels of recreational boating and tubing may negatively impact fisheries in several ways including reducing fish activity, causing fish to avoid high use areas and forcing fish to occupy suboptimal habitat. This study could be a companion study to M. Kondratieff's work on impacts of whitewater parks on fish habitat and would investigate if there are any biological impacts of high use recreational boating. This topic was recommended by three biologists, received a rank of three for relevance, two for feasibility and 3 for scientific value but it was judged resource consuming enough to conflict with higher priority topics. This topic was not selected as one of the initial projects but may be revisited in the future as resources allow.
8. Effects of Sterile Predators (Tiger Trout) on Brook Trout Populations in Streams - Using hybrid predators to affect the size structure or species compositions of lakes has been attempted in several management situations but their use in stream situations has not been evaluated. Many small mountain streams in Colorado have stunted brook trout populations that may show an improved size structure if the proper densities of predators could be maintained. This topic was recommended by two biologists, received a rank of three for relevance, three for feasibility and two for scientific value. This topic was not selected as one of the initial projects but may be revisited in the future as a technical assistance type project as resources allow.
9. Evaluation of Artificial Trout Redds for Establishing Resistant Rainbow Trout - Artificial trout redds have been used as a way of placing excess trout eggs into rivers in an attempt to supplement wild trout populations but have not been evaluated for their effectiveness. This project would most likely be a technical assistance type study evaluating artificial redds to establish WD resistant rainbow trout in rivers and would also be used to investigate a novel approach at estimating trout fry populations. This topic was recommended by two biologists, received a rank of three for relevance, three for feasibility and one for scientific value. This topic was not selected as one of the initial projects but may be revisited in the future as a technical assistance type project as resources allow.
10. Establishing Whirling Disease Resistant Rainbows in the Rio Grande River - The Rio Grande and South Fork previously supported good wild rainbow trout populations but has been impacted by whirling disease. Biologists feel that stocking resistant strain rainbows in the rivers have not yet re-established wild rainbow populations. This project would involve looking at the success of several strategies for establishing resistant fish in the river. This topic was recommended by one biologist, received a rank of three for relevance, two for feasibility and one for scientific value. Because similar work has been done in other rivers, it was decided that there was not a need for another large scale project and it could be handled better by other researchers through collaboration between

biologists.

11. Cap K Ranch WD Investigations - This would be a technical assistance project to complete an ongoing project near the Frying Pan River. This topic was recommended by one biologist, received a rank of two for relevance, two for feasibility and one for scientific value. This project was judged to be a productive research topic that is valuable to CPW and was recommended for inclusion as a technical assistance project that would be finished by B. Nehring in 2013 and would then be more of a management and fish production project if this site is needed for tiger trout production.
12. Effect of Non-Native Predator removal on Warmwater Native Fish - There are large scale non-native fish control projects on several western slope rivers focused on reducing the impacts of northern pike and smallmouth bass on native fish. There has not been adequate evaluation of how effective these programs are and if they are indeed a good long term strategy to benefit native fish. There are several examples in the literature of mechanical removal being ineffective, not cost effective and even counterproductive for long term fishery goals. This topic was recommended by one biologist, received a rank of two for relevance, two for feasibility and two for scientific value. This project falls outside the scope of work of a coldwater stream ecologist and was not recommended for inclusion.
13. Placer Creek Tubifex Introductions and Whirling Disease Investigations - This would be a technical assistance project to complete an ongoing project on Placer Creek. This topic was recommended by one biologist, received a rank of two for relevance, two for feasibility and one for scientific value. This project was judged to be valuable to CPW and was recommended for inclusion as a technical assistance project that would be finished by B. Nehring in 2013.
14. Square Top Lakes Whirling Disease Investigations - This would be a technical assistance project to complete work at Square Top Lakes focused on breaking the life cycle of whirling disease through depopulating a lake. This topic was recommended by one biologist, received a rank of two for relevance, two for feasibility and one for scientific value. This project was judged to be valuable to CPW and was recommended for inclusion as a technical assistance project that would be finished by B. Nehring in 2013.
15. Blue River Aquatic Invertebrate Investigations - There are concerns that the trout fishery in the Blue River has suffered from declining or non-existent mysis shrimp supplementations. Because the flow, temperature and sediment regime of the Blue River below Dillon Reservoir are inadequate to support sufficient aquatic invertebrates, the mysis shrimp supplementation is thought to be important to the growth of trout in the river. This project would involve evaluating the limiting factors of the trout populations and evaluating ways to address those factors. This topic was recommended by one biologist, received a rank of three for relevance, one for feasibility and one for scientific value. There are likely some flow, temperature, and sediment limitations caused by water management in Dillon Reservoir that are unlikely to change. Because of the local nature of this project, the small scope, and low potential of viable alternatives, this project is not recommended for inclusion.
16. Impacts of Fire on the Cache la Poudre River Fishery - Recent large wildfires have burned portions of the lower Cache la Poudre River and it is expected to significantly impact fish habitat and the trout fishery in the river. This project would document effects

of the fire on habitat and fisheries by comparing impacted and non-impacted sights. This topic was recommended by one biologist, received a rank of three for relevance, one for feasibility and one for scientific value. Because of the local nature of this project, the small scope, and lack of pre fire data, this project is not recommended for inclusion.

17. Instream Flow Recommendations - There is a need to expand our knowledge of flow/fish habitat relationships to more sites and rivers. New cross sectional data and IFIM type studies would be useful in making management recommendations to other agencies. This topic was recommended by one biologist, received a rank of three for relevance, one for feasibility and one for scientific value. This project falls outside the scope of work of a coldwater stream ecologist and is not recommended for inclusion. Other personnel within CPW can address this need more appropriately.

In January 2013 the top seven ranked topics were presented to senior aquatic staff in Montrose. A discussion of the merits of those topics occurred and verbal comments were received from most of the senior staff. Email comments were received from the aquatic section chief and three senior biologists. After reviewing and compiling the verbal and email comments a final list of research topics was selected and presented to the aquatic research leader and formalized in the 2013-2014 federal aid segment narrative. Those research topics are presented below as Jobs 1-6 and the objective of each segment are summarized.

Priority 1. Giant Stonefly Habitat and Ecology Studies

Job Objective: Investigate the habitat use, hatching ecology and limiting factors of the Giant Stonefly *Pteronarcys californica* in Colorado Rivers.

Segment Objective: 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. 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. 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. 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 and ecological characteristics of similar sites with differing densities of stoneflies, the optimal habitat characteristics and limiting factors can be identified. Habitat preference curves will also be generated to assist in ecological restoration of sites where they have been extirpated. Once limiting habitat features are identified, the effects of flow and sediment changes on those features will be investigated. This information can then be used to inform management and restoration activities as well as evaluate sites for the re-introduction of *P. californica*. as is being done on the Arkansas River and being considered on the upper Gunnison River.

Priority 2. Mottled Sculpin Habitat, Distribution and Ecology Studies

Job Objective: Investigate the habitat use and limiting factors of mottled sculpin *Cottus bairdi* in Colorado Rivers.

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 quality riffle habitat make them a good ecological indicator of stream health. Because the quality and function of riffle and run habitat is commonly impacted when stream flows 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 also serve as an important food source, especially for brown trout common 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. 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. The objective of this study is to investigate environmental, ecological and preferred habitat characteristics of mottled sculpin in Colorado rivers and identify limiting factors that could explain their extirpation from certain sites. This information can then be used to inform restoration activities and efforts to mitigate for planned ongoing stream flow impacts.

Priority 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.

Priority 4. Gunnison River Aquatic Invertebrate and Pesticide Studies

Job Objective: Document the aquatic invertebrate communities of the Gunnison River above Blue Mesa and investigate the potential impacts of the application of Permethrin on invertebrates and fish.

Segment Objective: Permethrin is a pyrethroid pesticide that is commonly used throughout Colorado for a variety of pest control applications including mosquito control. It is known to be highly toxic to aquatic invertebrates and there have been some anecdotal reports about its application in the Gunnison basin negatively impacting invertebrates, notably stoneflies like *Claassenia sabulosa*. To investigate this, a pilot study is planned on the Gunnison River and Tomichi Creek. Benthic macroinvertebrate samples will be taken at multiple sites above and below the areas treated with Permethrin, before and after the treatment. Passive water sampling will be conducted before and after the treatment to determine if the pesticide is making it into the stream systems and to estimate exposure. Trout fry sampling will occur before and after the treatment at control and treated sites to determine if there is any potential impact to trout populations. On site toxicity tests will be conducted with rainbow trout fry to determine what impacts could be expected with the ambient environmental conditions and exposure levels.

Priority 5. Professional Publications

Job Objective: Prepare manuscripts for professional publication on a variety of subjects. Previous P.I. of Project F-237 Barry Nehring will continue the publication process of several manuscripts from fiscal year 2012-2013.

Segment Objectives:

1. Prepare a manuscript for publication on the potential role of various strains or lineages of *Tubifex tubifex* worms for ameliorating or eliminating the impacts of whirling disease in coldwater lake and stream ecosystems in salmonid habitats in Colorado.
2. Prepare a manuscript for publication on the reproduction, growth and longevity of Hofer strain rainbow trout stocked into private ponds and compare their resistance to infection by *Myxobolus cerebralis* to brook and brown trout occurring in sympatry.
3. Prepare a manuscript for publication assessing the relative vulnerability of susceptible and resistant populations of *Tubifex tubifex* worms to infection by *Myxobolus cerebralis* from major river basins across Colorado.
4. Prepare a manuscript for publication that documents the relative abundance and longitudinal distribution of aquatic macro-invertebrate fauna of the upper Colorado River basin in Grand County, Colorado in 2010 compared to that observed in 1980-1981 prior to the construction and operation of Windy Gap Dam in 1983.
5. Prepare a manuscript for publication that documents the relative abundance and longitudinal occurrence of the mottled sculpin *Cottus bairdi* in the upper Colorado River basin in Grand County, Colorado in 2010 upstream and downstream of man-made impoundments in the basin.

Priority 6. Technical Assistance

Job Objective: Provide information and assistance to aquatic biologists, aquatic researchers and managers in a variety of coldwater ecology applications. Some of this work consists of ancillary research and management projects related to past F-237 projects and much of this job for the current principle investigator will be to address new requests for technical assistance.

Segment Objectives:

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 *M. cerebralis* infection in the ponds. Secondary objectives are to evaluate the survival, reproduction and *M. cerebralis* infection Hofer strain rainbow trout in a small pond environment and evaluate tiger trout's effectiveness in controlling brook trout numbers.
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.
3. Lower Squaretop Lake Whirling Disease Study: The objective of this study is to investigate strategies to reduce *M. cerebralis* infection in a high lake cutthroat trout population through removal of fish hosts.
4. Gunnison Tunnel Electric Fish Barrier Evaluation: The objective of this study is to evaluate the effectiveness of an electric barrier on the Gunnison tunnel designed to prevent fish entrainment in the South Canal.
5. Coldwater Stream Ecology Technical Assistance: Provide assistance as needed to area aquatic biologists, aquatic researchers and managers.