PROJECT PERFORMANCE REPORT
FEDERAL AID PROJECT F-83-R-25

Aquatic Animal Health Investigations & Management

July 1, 2011 – June 30, 2012

STATE: **Colorado**
PROJECT: **F-83-R-25**
PROJECT TITLE: **Aquatic Animal Health Investigations & Management**

Period Covered: **July 1, 2011 – June 30, 2012**

OBJECTIVES:

The main objectives of the Aquatic Animal Health Investigations & Management project include:

- Provide aquatic animal health services
- Aquatic species protection
- Aquatic animal health technical assistance

STUDY 1: PROVIDE AQUATIC ANIMAL HEALTH SERVICES

OBJECTIVE:

Assist in the protection, conservation, and management of Colorado's aquatic animal resources through monitoring, investigation, and management of aquatic animal health in state fish hatcheries, research facilities, free-ranging public fisheries and free-ranging aquatic animal populations, as well as aquatic animal resources in the private sector by providing diagnostics, research, regulated pathogen inspections, and laboratory analysis. Maintaining or improving aquatic animal health will help insure the stability of many populations, enable the recovery of others, and improve the quality of Colorado's wildlife resources.

Cover photo: Encysted grubs of *Ornithodiplostomum ptychocheilus* (Trematoda, Digenea), in the mesenteries of a sand shiner, *Notropis ludibundus*, from the South Platte River in the northeast corner of Colorado.

Sub-Study 1-1:

Objective:

Please see Grant Narrative for detailed objectives and procedures

Job 1: Provide regulated and precautionary salmonid fish disease inspections conforming to state regulations, agency policies, U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS), American Fisheries Society, and/or the World Organization for Aquatic Animal Health (OIE) standards in public and private fish hatcheries and free-ranging fisheries.

Approach:

Fish tissue samples will be collected for analysis for regulated pathogens, generally from statistical numbers of fishes from public and private fish culture facilities and wild populations destined for translocation or from which gametes will be taken for culture.[1] These samples will either be collected by AAHL personnel or AAHL personnel will coordinate and supply inspections by contracted Qualified Sample Collectors (QSC). The samples will be transported or shipped to the AAHL and/or cooperating and contracted laboratories for analysis for viral, bacterial, and myxosporean parasite pathogens. Results of regulated inspections will be reported in the form of Fish Health Certificates.

Work performed:

See Table 1 (also see Appendix A).

Table 1. Regulated pathogen inspections performed by the AAHL in FY 10-11.

Pathogen type	Publicly owned fisheries					Privately owned fisheries				Totals
	Salmonid culture	Salmonid free-ranging	Warmwater fish culture	Warmwater free-ranging	Other	Salmonid culture	Salmonid free-ranging	Warmwater fish culture	Other	
Bacteria	24	26			6	16	1			73
Parasite	28	37			6	16	1			88
Virus[2]	36	32	7	23	8	18	1	5	1	131
Totals	88	95	7	23	20	50	3	5	1	292

[1] By agency policy, individual lots, as defined by the American Fisheries Society/Fish Health Section Blue Book, are sampled for viruses at the assumed pathogen prevalence level of 5% at the 95% level of confidence as determined by Ossiander and Wedemeyer 1973. Regulated bacterial pathogens are sampled at the same level per water supply rather than by lot. Samples for *Myxobolus cerebralis*, causative agent of Whirling Disease, are determined in the same way. However, under Colorado Wildlife Regulation Chapter 0, Appendix C, #C, 1, a, salmonids tested for *M. cerebralis* by spore concentration technique must be in a water supply at least ten months prior to testing. Agency policy recognizes only the validity of testing of lethal kidney and spleen samples for IPN Virus and VHS Virus and the testing of reproductive fluids of parental broodstock for IHN Virus.

By policy or regulation, some exceptions to these sampling standards are made under certain circumstances. In situations where attribute samples of broodstock are not available, the sampling of all fish involved in the making of an egg lot will be acceptable. By internal policy, in the case of extremely valuable and/or critical stocks of threatened or endangered species, lethal samples may be minimized or eliminated on a case by case basis. However, such fish and/or progeny will be restricted to quarantine facilities and their fate carefully considered after weighing the risks and role of such actions in recovery efforts.

[2] The AAHL does not possess virology capability at this time. AAHL fish pathologists and Qualified Sample Collectors collect tissue samples for virology and ship them to contracted labs. Virology results are reported and certificates issued by CDOW-AAHL. Most salmonid virology inspections in FY 08-09 were analyzed by the USFWS Bozeman Fish Health Center in Bozeman, Montana. Much of the warmwater fish virology for VHSV and LMBV was performed by the Aquaculture Diagnostic Laboratory at Auburn University in Auburn, Alabama.

Job 2: Provide laboratory analysis of bacteriology and parasitology samples submitted from inspections of public and private fish culture facilities and wild populations destined for translocation or from which gametes will be taken for culture, as well as samples submitted for fishery management purposes.

Approach:

Using techniques and procedures described by Markiw and Wolf 1974, O'Grodnick 1975, and the American Fishery Society/Fish Health Section Blue Book and approved by regulations (Colorado Wildlife Regulations, Chapter 0) and agency directives and policies, analyze fish tissue samples for regulated bacterial pathogens and myxosporean parasites by biochemical, serological, and/or molecular means. Details of individual cases are presented in Appendix A.

Work performed.

See Tables 2 and 3.

Table 2. Approximate numbers of bacterial samples processed at the AAHL for regulated salmonid disease inspections in FY 11-12.

BACTERIOLOGY		
# Inspections	**# Cultures**	**# DFAT tests**
73	**4,380**	**4,380**

Table 3. Approximate numbers of salmonid heads analyzed for the presence of spores of Myxobolus cerebralis (Whirling Disease agent) by Pepsin-Trypsin Digestion in regulated inspections at the AAHL during FY 11-12.

PARASITOLOGY	
# Inspections	**# PTD tests**
87	**4,250**

Job 3: Provide coordination, training, and logistics for Qualified Sample Collectors. QSCs are private veterinarians and Certified Veterinary Technicians as authorized by the Colorado Aquaculture Advisory Board.

Approach:

Schedule fish disease inspections requested by public and private sector fish culturists and fishery biologists so as to fit the availability of QSCs and laboratories. Prepare and provide collection equipment and supplies to agency fish pathologists and contracted Qualified Sample Collectors for regulated salmonid disease inspections. Provide training for new QSC candidates as needed and annual refresher training, reporting activities regularly to the Colorado Aquaculture Advisory Board.

Work performed:

- Legal restrictions forced the discontinuance of the 15-year QSC program in the early part of the FY. As a result, one AAHL Fish Pathologist was re-assigned to collect samples full time to make field collections. With the assistance of the other AAHL fish pathologists and technicians, virtually all of the field collections representedin Table 1 were made by AAHL staff.
- Changes will be made in the next segment narrative to reflect this change.

Job 4: Conduct comprehensive fish pathogen screening on shipments of warm and cool water fishes imported by CPW fish hatcheries and fishery managers.

Approach.

By regulation attribute numbers of tissue samples are collected from all lots of nonsalmonid fishes under culture as well as from free-ranging fisheries from which fish will be transferred or eggs taken.

Work performed

- See Table 1.

Sub-Study 1-2:

Objective:

Please see Grant Narrative for detailed objectives and procedures

Job 1: Provide diagnostic services to agency fish hatcheries and installations, university and other research facilities, private sector facilities, and the public as needed.

Approach:

Investigate and diagnose fish health problems in public and private fish culture on a case-by-case basis. Depending upon circumstances, investigations may be made in the field, in the laboratory, or handled by electronic means.

Work performed:

- In FY 11-12, AAHL personnel provided hands on examinations and/or diagnostics on state fish hatcheries in a total of 63 troubleshooting cases (Case Type TS in Appendix A). 37 of these were on state fish hatcheries, 16 on free-ranging public fisheries, and 10 cases on private fish culture facilities.
- Fourteen fish health cases were handled remotely by electronic media (Case type EX in Appendix A). Cases such as these are greatly facilitated by the use of electronic photography.

Job 2: Conduct health investigations in free-ranging aquatic animal populations including fish kills as needed.

Approach:

Investigate and diagnose fish kills and aquatic animal health problems in public waters and private ponds on a case-by-case basis. Depending upon circumstances, investigations may be made in the field, in the laboratory, or handled by electronic means.

Work performed:

- Seventeen investigations (13 TS and 4 EX) of fish health problems ranging from reports of parasites to major fish kills were conducted in FY 11-12. One reason for this sharp upswing since FY 10-11 was the severe hot weather encountered in spring, 2012.
- In addition to these cases, five analyses of the City of Fort Morgan's potential drinking water were analyzed for actinomycetes to assist their efforts to discover the source of odor and off-flavor in their water. The effort was successful.

Sub-Study 1-3:

Objective:

Please see Grant Narrative for detailed objectives and procedures

Job 1: Quantitative laboratory analysis of fish heads for the myxospores of *Myxobolus cerebralis*, causative agent of Whirling Disease, by Pepsin-trypsin Digestion (PTD) Technique.

Approach:

Process individual salmonid fish heads for the isolation of myxospores of *Myxobolus cerebralis*, causative agent of Whirling Disease by sequential enzymatic digestion as described in Markiw and Wolf 1974 and enumerate the spores as outlined in O'Grodnick 1975.

Work performed:

- The AAHL processed 1,018 trout heads by quantitative PTD for Myxobolus cerebralis in fourteen submissions by whirling disease researchers. (See Appendix A)

Job 2: Analyze fish tissues, aquatic oligochaetes, and water samples for molecular evidence of *Myxobolus cerebralis*, causative agent of Whirling Disease, by polymerase chain reaction (PCR).

Approach:

Assay samples submitted by agency and university researchers for *M. cerebralis* by established or experimental single-round or nested PCR as specified by the researcher.

The demands for laboratory space and manpower imposed by aquatic nuisance species plankton analysis caused the PCR portion of the laboratory to be inactivated for the year. No PCR was

performed in FY 11-12. All PCR work (>2,000 samples) was contracted to Pisces Molecular in Boulder.

Job 3: Provide other fish pathogen analysis, expertise, and assistance to agency and university researchers.

Approach:

Analyze fish tissue samples for specific pathogens, identify aquatic wildlife, and/or provide or exchange information and expertise on fish health.

Work performed:

Two virology inspections of threatened minnow species and two troubleshooting investigations case were made on fish in the research facility at the School of Wildlife Biology at Colorado State University.

Sub-Study 1-4:

Objective:

Please see Grant Narrative for detailed objectives and procedures

Job 1: Parasite studies on West Slope sculpin species.

Approach:

A study was designed to collect and necropsy free-ranging mottled sculpins, *Cottus bairdi*, and Paiute sculpins, *C. beldingi*, to ascertain the parasites and pathogens present in these previously unstudied species. Sculpin necropsies were combined with surveys conducted by conservation biologists and fishery researchers with molecular identification (PCR and sequencing) contracted to Dr. Dennis K. Shiozawa at Brigham Young University.

Work performed:

- Continued surveys of external and internal parasites and other fish health aspects of the mottled sculpin (*Cottus bairdi*) and Paiute sculpin (*Cottus bending*) with collections on Cow Creek, Hermosa Creek and Animas River.
- Report written on results of 2010-11 sculpin surveys.
- Collected mottled and Paiute sculpin (*Cottus beldingi* or *bairdi*) samples for PCR evaluation of species.

Job 2: Digenetic trematode studies at Wray State Fish Hatchery.

Approach:

Try to ascertain whatever detrimental effects metacercariae of the strigeid trematode white grub, Posthodiplostomum minimum, have on young-of-the-year (YOY) bluegill at Wray State Fish Hatchery through statistical comparisons of infection levels to body weight. In addition attempt to

determine whether or not *P. m. minimum* and *P. m. centrarchi* are in fact subspecific or merely different manifestations of the same parasite in two different hosts. Epidemiological studies of the snail host will be conducted by a University of Colorado doctoral candidate in parallel with this work.

Work performed:

- It was observed that bluegill fry in grow-out ponds supplied by surface water at Wray SFH become infected with white grub almost immediately after swim up. Even 2-3 grubs in the liver of a hatchling displace a large percentage of the total mass of the liver. When the upper most pond (Pond #1) was drained and harvested, 200 YOY bluegill were collected and brought back to the lab on ice to be examined the next day for grubs per liver and incidence of infection.
- Doctoral student John Mischler (University of Colorado) is measuring chemical composition and other parameters of those same fish for correlation.

Sub-Study 1-5:

Objective:

Write an updated inventory and dichotomous key to the crayfish of Colorado.

Job 1: Collecting and preparation of information.

Approach:

Crayfish identification data from Projects F-83R and F-84R to date will be compiled by species to detail the spread or contraction of ranges by native crayfish species as well as the distribution of invasive species. Maps comparing today's ranges with those of Unger, 1978, will be made. This information along with a dichotomous key to today's crayfish species will be reported.

Work performed:

- Specimen collection continued throughout Colorado performed by both fish pathologists and 6-month field technicians.
- Species descriptions and biographies were written using current data as well as often obscure reference materials.

STUDY 2: AQUATIC SPECIES PROTECTION

OBJECTIVE:

Assist in the protection for Colorado's native aquatic animals from the introduction and spread of non-endemic fish diseases and aquatic species through regulation and proactive physical means. Maintaining the present species compositions in each drainage, compromised though they may be, will help prevent further habitat degradation and assist state and federal recovery efforts for the 23 fishes, 8 amphibians, 2 mollusks, and 1 turtle presently listed as endangered, threatened, or species of concern.

Sub-Study 2-1:

Objective:

Please see Grant Narrative for detailed objectives and procedures

Job 1. Review, approve, or deny Aquatic Species Importation Licenses.

Approach:

In cooperation and coordination with the Special License Agent, scrutinize applications for Aquatic Species Importation Licenses for compliance with regulations, policies, and directives and approve or deny based upon disease certification, species, and likelihood of contamination by aquatic nuisance species (ANS).

Work performed:

- Approximately 103 Aquatic Species Importation Licenses were processed.

Job 2. Evaluate and recommend approval or denial of CPW Whirling Disease Exemptions that allow the operation of positive facilities within salmonid habitat through stipulated best management practices (BMPs) for minimization of impact on wild resources.

Approach:

Make on-site visits before submitting written recommendations to the Statewide Manager of Aquatic Resources for signature. Study annual infection analyses of fish collected at permitted sites as well as free-ranging fish samples collected above and below facility effluents and evaluate the impact and effectiveness of BMPs. Review and evaluate existing permits on an annually for modification and renewal.

Work performed:

- Two private facilities in southern Colorado became positive for the whirling disease parasite during FY 11-12. At one facility immediate steps were taken to eradicate the parasite and begin the testing process for recertification.
- One formerly positive state hatchery regained negative status after two years of testing in FY 11-12
- At the end of the FY, 14 facilities – 3 state and ten private – remain positive and continue to exist under the terms of regulation. Data was reviewed 10 of these received exemption renewals. Two new exemption applications are expected.

Job 3. Review and comment on all Colorado Department of Agriculture Aquaculture Permit applications from the standpoint of protecting native aquatic species and aquatic animal health. Issue CPW statewide Aquatic Species Stocking Permits to those applicants who have fulfilled requirements.

Approach:

Applications received by the Colorado Department of Agriculture will be forwarded to the State Fish Pathologist for review and comment.

Work schedule:

- Approximately 40 applications were reviewed and comments filed.

Sub-Study 2-2:

Objective:

Please see Grant Narrative for detailed objectives and procedures

Job 1: Serve as advisors to the CPW representative to the Colorado Fish Health Board and Colorado Aquaculture Advisory Board as well as the 5-member board as a whole in aquatic animal health, transportation, culture, and importation regulation making and related issues.

Approach:

Attend regular monthly meetings of the statutory board and assist the CPW representative and other board members by providing guidance and expertise, literature searches, informal surveys, white papers, etc. as called upon.

Work performed:

- Each monthly meeting of the Colorado Fish Health Board was attended by one or more fish pathologists.
- Advisory input and presentations were made as warranted.
- Most of these board meetings also involved Colorado Aquaculture Advisory Board meetings as well. Attendance and participation occurred as warranted.

Job 2. Advise and participate in internal policy and directive making in issues involving aquatic animal health, culture, transportation, prohibited species, ANS, and related issues.

Approach:

Represent aquatic animal health interests in internal meetings to discuss and formulate policy and directives.

Work performed:

- One or more fish pathologists participated in several meetings and conference calls with the Statewide Manager of Aquatic Resources, Chief of Hatcheries, and CPW Fish Health Board member to discuss, formulate, or modify fish health and ANS policy.
- Two fish pathologists met in the middle of the state for two days to discuss potential changes in Colorado's crayfish and bait fish regulations and draft copy for consideration by the Statewide Chief of Aquatics.

Sub-Study 2-3:

Objective:

Assist hatchery managers/owners in planning renovations to eliminate Whirling Disease and regain regulatory negative status by exposure and testing of specially tagged sentinel fish.

Job 1. Advise and assist hatchery managers and owners in regaining negative status for infection by *M. cerebralis* (Whirling Disease).

Approach:

Provide on-site evaluation and advice on renovating to establish security from Whirling Disease infection. When warranted, assist in locating point sources of contamination by testing strategically placed sentinel fish by PCR. As outlined in Colorado Wildlife Regulations, mark long-term sentinel fish with individual tags and return at 10-month and 14-month post exposure intervals to collect and analyze samples for the presence of *M. cerebralis* to re-establish negative status.

Work performed:

- During the FY, Roaring Judy State Fish Hatchery north of Gunnison.was successfully "reclaimed" from whirling disease and initial testing to obtain negative status was begun at 5H Farms near Monte Vista.

Sub-Study 2-4:

Objective

Participate in the agency's programs to prevent the invasion of injurious aquatic organisms and monitoring of waters as an early warning of such invasions.

Job 1. Monitor for dreissenid mussel invasions by sampling designated waters for dreissenid mussels (zebra and quagga) both by plankton tow for veliger stage larvae and by substrate inspection.

Approach:

Hire, assign, supervise and support temporary laboratory technicians and ANS hatchery technician funded by the State Aquatic Nuisance Species Coordinator each year who analyze samples collected by the ANS field crews are by light microscopy or Flo-Cam technology.

Work performed:

- An AAHL fish pathologist oversaw the following work in turn funded by the ANS Coordinator at the Denver headquarters: Four 6-month temporary laboratory technicians were hired in 2011 and three in 2012.
    - Plankton tow samples were delivered to the AAHL for processing.
        - Plankton samples were scanned by cross-polarization microscopy for veliger larvae.

    - Presumptive veligers were sent to Pisces Molecular for confirmation by PCR.
- All data was entered into a system wide database file.
- AAHL veterinary pathologist identified all hatchery ANS samples collected in 2011 and generated Hatchery ANS Certificates for 11 hatcheries.
- Two AAHL pathologists trained and supervised ANS hatchery technician.
- The AAHL veterinary pathologist provided input for testing of several chemical compounds to prevent spread of zebra/quagga mussel veligers during aquaculture activities with Catherine Sykes, Dexter NFH.

Job 2. Both permanent and temporary personnel investigate streams and lakes for the presence of aquatic nuisance species as well as native mollusks and crustaceans. Collections are preserved and shipped to the AAHL to be identified and cataloged.

Approach:

Using various collecting gear such as traps, hand nets, and kick nets collect crayfish and other crustacean samples. Using nets, strainers and by hand, collect mollusks, aquatic plant samples and other potential ANS and transport or ship specimens to AAHL for identification.

Work performed:

- AAHL fish pathologists and six 6-month temporary field collectors were hired and dispatched to selected lakes and streams to collect and preserve samples of macroinvertebrates and aquatic plants.
- Labeled samples are sent to the AAHL where trained personnel examine and identify specimens and enter the results into the ANS Database
- All data was entered into a system wide database file.

STUDY 3: AQUATIC ANIMAL HEALTH TECHNICAL ASSISTANCE

Objective:

Provide aquatic animal health management expertise, education, and technical assistance to agency biologists and fish culturists and private aquaculture. Fish health management can prevent disease outbreaks, increase quality, and thus improve the product of fish culture enterprises in both the public and private sectors. Fish health education enables fish culturists to monitor and avoid potential problems or respond with treatment more rapidly than would otherwise be possible. Fish health management enables fishery managers to find ways to maintain or improve fisheries in the presence of chronic disease or environmental problems.

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Sub-Study 3-1:

Objective:

Please see Grant Narrative for detailed objectives and procedures

Job 1. Conduct regular fish quality examinations on production lots of salmonid fishes at state fish hatcheries and rearing units and compile and compare results.

Approach:

Using the same methods as described in Study 1, Sub-study 4, Job 2, at quarterly intervals conduct detailed necropsies on twenty or more randomly collected specimens from each production lot of salmonid sport fish produced at 12 state trout hatcheries and rearing units. Compile, compare, and report results to evaluate performance and highlight areas of concern.

Due to the subjectivity of several of the parameters, all evaluations are conducted by the same AAHL fish pathologist, Mike Minniear. After being weighed and measured, each specimen is examined first externally, then internally and assigned evaluative scores or codes for the following parameters: eyes, gills, pseudobranchs, thymus, mesenteries, spleen, hind gut, kidney, liver, bile (gall bladder), sex and maturity, the dorsal fin and each of the paired fins, opercles, visceral fat, and caudal fin. The data is then analyzed using a program called "AUSUM" developed by now-retired Fish Pathologist Ronald Goede of Utah Division of Wildlife Resources. Individual reports of each lot are sent to the hatchery manager and comparative data (see Figures 1 and 2) are graphed for use by the Statewide Chief of Hatcheries.

Work performed:

- Project terminated October 2011.

Job 2. An AAHL veterinarian fish pathologist will serve the CPW Hatchery Program as Monitor and facilitate access to treatment options unavailable by prescription through cooperative participation with the U.S. Fish & Wildlife Service's Aquatic Animal Drug Approval Partnership Program and the U.S. Food and Drug Administration's Investigational New Animal Drug (INAD) studies.

Approach:

Facilitate access to compassionate treatment options using investigational new animal drugs through cooperative participation with the U.S. Fish & Wildlife Service and U.S. Food and Drug Administration Investigational New Animal Drug (INAD) studies, supplying all required information to USFWS and/or FDA.

Work performed:

- The AAHL veterinary fish pathologist served as Study Monitor for 9 hatcheries enrolled in 2011 and 11 hatcheries enrolled in the 2012 INAD program; duties include enrollment of hatcheries; ensure accuracy of all paperwork and submission to INAD office; management of inventory of Chloramine-T and emamectin benzoate (SLICE™), and prescriptions for common carp pituitary. Instructed all enrollees in the new on-line reporting system.

- The same fish pathologist continued maintenance of hatchery monthly drug use in spreadsheet form and development of 2011 report on drugs used and diseases treated for Chief of Hatcheries and for Chief Fish Pathologist and ensured mandatory monthly drug use reporting to CDPHE.

Job 3. Provide aquatic animal veterinary services, including prescription of therapeutants and investigation of new treatment options, to the CPW Hatchery and Aquatic Resources Sections.

Approach:

After initial diagnoses, prescribe drugs and therapeutants as warranted, keeping up with changing laws governing use in aquatic situations, demonstrating that conditions dictated by FDA/CVM, AMDUCA, USDA, and EPA have been met when treatment is appropriate and that the hatchery managers understand their responsibilities under federal law.

Work performed:

- Guidelines for hatchery chemotherapeutants/chemicals in effluents – attended planning meeting and helped comment on chemotherapeutants discharge reporting for the final draft for reissued CDPHE discharge permits for the statewide general Discharge Permits for the state hatchery system.
- Prescriptions – provided 34 prescriptions and extra-label prescriptions between July 1, 2011 and June 30, 2012 to biologists and hatchery staff for use of approved substances, VFDs, and for extra-label use of approved substances.
- Veterinary medical duties – continued monitoring compliance with Guidelines for use of Drugs in Aquaculture for the state hatchery system, monitored use of FDA-approved drugs and drugs of low priority regulation status in the state hatchery system and maintained records of drug use, including written prescriptions, withdrawal times; maintained required continuing education hours necessary to keep veterinary license in good standing; maintained USDA/APHIS accreditation for issuing certificates of inspection; Animal Care and Use Committees for George Schisler's research fish at Colorado State University, Ft. Collins.
- Published peer-review article on SLICE in the July 2011 issue of North American Journal of Aquaculture.

Sub-Study 3-2:

Objective:

Please see Grant Narrative for detailed objectives and procedures

Job 1: Conduct fish health management short courses for hatchery technicians and biologists.

Approach:

Provide 32-hour courses of training in fish health management to include the following major topics: anatomy and physiology, the role of stress in fish health management, bacterial diseases of fishes,

viral diseases, ectoparasites, metazoan parasites, Whirling Disease, and Colorado fish health regulations and biopolitics. The course will include hands-on necropsy and microscopy training.

Work performed:

- The AAHL veterinary fish pathologist presented a Fish Health Course on April 17-18, 2012, to students in the Trinidad State Junior College (Alamosa) aquaculture course.

Job 2: Assist school aquarium and aquaculture programs by providing information regarding regulatory requirements, technical information, educational materials, and guidance specific to each school's circumstances.
Approach:

Contact schools with aquarium or aquaculture programs, explain agency regulations and policies, and provide guidance, information, and assistance as warranted.

Work performed:

- The AAHL veterinary fish pathologist was the advisor to the science teachers at participating in the Trout Unlimited Trout in the Classroom project; helped instructors with fish health issues or die-offs during school year.
- Performed testing on fish prior to stocking in April 2012.
- The AAHL veterinary fish pathologist presented on fish pathology and ANS to 7th annual Trout Unlimited summer youth camp.

Job 3: Provide lectures, presentations, instruction and training in fish health, ANS, or other related subjects and technical assistance as needed.

Approach:

Per inquiry or request from agency employees, the private aquaculture industry, institutions of higher learning, angling groups, or the general public, provide lectures, training, and specific information.

Work performed:

- The AAHL veterinary fish pathologist reviewed Hofer culture/disease in the state system presented to the September, 2011 Hatchery Staff Meeting in Glenwood Springs, CO.
- The AAHL veterinary fish pathologist presented on updates to January, 2012 hatchery staff meeting in Glenwood Springs, CO.
- The AAHL veterinary fish pathologist made a presentation on BGD/BCWD at Colorado Aquaculture Association on February 3, 2012.
- The AAHL veterinary fish pathologist presented AAHL updates at the Aquatic Biologist annual meeting on January, 2012 in Fort Collins, CO.
- The AAHL veterinary fish pathologist presented on CPW Reference Library and how to use it for the May 2012 Hatchery Staff meeting.
- The deputy fish pathologist presented on AAHL updates to the May 2012 Hatchery Staff meeting attendees.
- The AAHL veterinary fish pathologist wrote articles on fish parasites for the August, 2011 Fishline; article on VHS surveillance and changes in AAHL disease collection program for

January, 2012 Fishline; article on fish nutrition for April 2012 Fishline; and article on genetically engineered fish for July 2012 Fishline.
- The AAHL veterinary fish pathologist reviewed/updated all hatchery and aquatic biologist Hazard Analysis and Critical Control Point Plans (HACCP), including writing several new plans.
- An AAHL fish pathologist made an instructional presentation at the National Aquaculture Association meeting in Denver in October, 2011.
- An AAHL fish pathologist made an instructional presentation at the Colorado Aquaculture Association meeting in Nathrop in February, 2012.
- An AAHL fish pathologist gave a lecture on the burgeoning aquatic nuisance species invasion to attendees of a pet dealers workshop coordinated by the Colorado Department of Agriculture at the Denver Animal Shelter in May, 2012.
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Job 4: Service, adjust, and repair microscopes at CDOW fish hatcheries to maintain on-site diagnostic and parasite screening capabilities.

Approach:

Dismantle, clean, repair, reassemble and adjust as needed.
Work performed:

- An AAHL Fish Pathologist cleaned and serviced all microscopes in the CPW Hatchery system, all microscopes in the AAH program as well as courtesy servicing of microscopes at the two National Fish Hatcheries in Colorado and the training microscopes in the Fish culture lab at Trinidad State Junior College.

Sub-Study 3-3:

Objective:

Please see Grant Narrative for detailed objectives and procedures

Job 1. Maintain laboratory database.

Procedures:

Maintain AAHL database, enter laboratory data from new case accessions and laboratory results as completed. Insure laboratory database is continuously running and available from a CDOW server.

Approach:

Enter data from existing case files current and historical into MS Excel files for use in Microsoft Access database management program constructed by contract and open access to selected publics.

Work performed:

- Data from AAHL was entered into T-6 as warranted

PROJECT COSTS

Estimated cost of the proposed project is $ ------. Of this amount $ ------
(25% of the total project costs) will come from state funds. Federal funds are requested for the remaining $ (75% of the total project costs). A more detailed budget is attached.

PROGRAM INCOME:

No program income is expected from this project.

PERSONNEL:

Vicki Milano	CPW, Managing Fish Pathologist	970-842-6308
Paula Nicholas	CPW Federal Aid Coordinator	303-291-7244

SCHEDULE:

MAINTENANCE:

No maintenance costs are associated with this project.

LAND CONTROL:

No land control issues are associated with this project.

RELATION TO OTHER FEDERAL PROJECTS:

This project will have no known or identifiable impacts on any other federal projects.

PRIME/UNIQUE FARMLANDS:

This project will have no impact on prime or unique farmlands.

FLOODPLAINS/WETLANDS:

This project will have no impact on any floodplains or wetlands.

ENDANGERED SPECIES:

This project should have positive impacts on the recovery of state and/or federally listed endangered species.

ENVIRONMENTAL ASSESSMENT:

This project is covered by a categorical exclusion under 516 DM 6, Appendix 1. See attached NEPA Compliance Checklist for more detail.

ENVIRONMENTAL JUSTICE (Executive Order 12898):

This project will not have disproportionately high and adverse human health or environmental effects on low-income populations, minority populations or Indian tribes.

INVASIVE SPECIES (Executive Order 13122):

The proposed activities of this project will not result in the introduction of any invasive species not impact the status of an existing invasive species.

HISTORICAL/CULTURAL RESOURCES:

The proposed activities of the project will have no impact on historical or cultural resources.