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

Aquatic Animal Health Investigations & Management

July 1, 2014– June 30, 2015

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

Period Covered: **July 1, 2014 – June 30, 2015**

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.

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 14-15.

Pathogen type	Publicly owned fisheries				Privately owned fisheries			Totals
	Salmonid culture	Salmonid free-ranging	Warmwater fish culture	Warmwater free-ranging	Salmonid culture	Salmonid free-ranging	Warmwater fish culture	
Bacteria	31	21			11	2		62
Parasite	31	26	2		11	2		67
Virus[2]	50	30	6	14	11	2	1	110
Totals	112	77	8	14	24	6	1	242

[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 14-15.

BACTERIOLOGY		
# Inspections	**# Cultures**	**# DFAT tests**
52	**2,503**	**2,503**

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 14-15.

PARASITOLOGY	
# Inspections	**# PTD tests**
55	**1,651**

Table 4. Approximate numbers of virology samples analyzed at the AAHL for regulated viruses during FY 14-15.

VIROLOGY	
# Inspections	**# Virology tests**
98	**11,688**

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:

- AAHL Fish Pathologists perform collections at private and public sector.

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 14-15, AAHL personnel provided hands on examinations and/or diagnostics on state fish hatcheries in a total of 85 troubleshooting cases (Case Type TS in Appendix A). 80 of these were on state fish hatcheries, 2 on free-ranging public fisheries, 1 university, and 3 cases on private fish culture facilities.
- Nineteen 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:

- Two investigations of fish health problems ranging from reports of environmental problems to major fish kills were conducted in FY 14-15.

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 1,909 trout heads by quantitative PTD for *Myxobolus cerebralis* in thirty three submissions by whirling disease researchers. (See Appendix A)

Job 2: Analyze fish tissues and bacteria cultures by polymerase chain reaction (PCR).

Approach:

All positive findings of regulated pathogens must be confirmed by PCR.

The PCR laboratory at the AAHL was reactivated in June 2014.

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 85 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:

- At the end of the FY, 10 facilities – 6 state and 4 private – remain positive and continue to exist under the terms of regulation. Data was reviewed and all of these received exemption renewals.

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 62 applications were reviewed and comments filed.

Sub-Study 2-2:

Objective:

Please see Grant Narrative for detailed objectives and procedures

Job 1: Serve as advisor and acting CPW representative to the Colorado Fish Health Board and Colorado Aquaculture Advisory Board which addresses issues 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.

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 or PTD. As outlined in Colorado Wildlife Regulations, mark long-term sentinel fish with individual tags and return at post exposure intervals to collect and analyze samples for the presence of *M. cerebralis* to re-establish negative status.

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: Two 9-month temporary laboratory technicians were hired.
    - 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.
        - 564 cases were analyzed and 1557 samples processed.
- All data is entered into a system wide database file.

- AAHL staff conducted ANS inspection at 21 state hatcheries and generated Hatchery ANS Certificates.

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:

- Labeled samples are sent to the AAHL where trained personnel examine and identify specimens and enter the results into the ANS Database
- All data is 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.

Job 1. 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 (USFWS-AADAP) and the U.S. Food and Drug Administration's (FDA)Investigational New Animal Drug (INAD) studies.

Approach:

Facilitate access to compassionate treatment options using investigational new animal drugs through cooperative participation with the USFWS and FDA studies, supplying all required information to USFWS and/or FDA.

Work performed:

- The AAHL veterinary fish pathologist served as Study Monitor for 10 hatcheries and three aquatic biologists enrolled in 2013 and nine hatcheries and 1 aquatic biologist enrolled in 2014 in the INAD program. Duties include enrollment of hatcheries; ensure accuracy of all paperwork and submission to INAD office; and management of inventory of Chloramine-T, common carp pituitary, and AQUI-S 20E. Instructed all enrollees in the on-line reporting system.
- The same fish pathologist continued maintenance of hatchery monthly drug use in spreadsheet form and development of 2014 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 2. 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/Center for Veterinary Medicine, the Animal Medicinal Drug Use Clarification Act (AMDUCA), U.S. Department of Agriculture (USDA), and Environmental Protection Agency (EPA) have been met when treatment is appropriate and that the hatchery managers understand their responsibilities under federal law.

Work performed:

- Reported hatchery therapeutant use monthly to the Colorado Department of Public Health and the Environment (CDPHE) to remain in compliance with Discharge Permits for the state hatchery system.
- Prescriptions – provided 69 prescriptions and extra-label prescriptions between July 1, 2014 and June 30, 2015 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 regulatory priority status in the state hatchery system and maintained records of all drug use, including written prescriptions and, withdrawal times. Maintained required continuing education hours necessary to keep veterinary license active and in good standing in the state of Colorado. Maintained USDA/Animal and Plant Health Inspection Service (APHIS) accreditation for issuing certificates of inspection. Served as veterinarian on the Animal Care and Use Committees for George Schisler's research fish at Colorado State University, Ft. Collins.

Sub-Study 3-2:

Objective:

Please see Grant Narrative for detailed objectives and procedures

Job 1: Conduct fish health management short courses

Approach:

Provided 12-hour courses of training in fish health management including the following major topics: anatomy and physiology, the role of stress in fish health management, bacterial diseases of fishes, viral diseases, ectoparasites, metazoan parasites, and Colorado fish health regulations and biopolitics. The courses included hands-on dissection and microscopy training.

Work performed:

- The AAHL veterinary fish pathologist presented a Fish Health Course Short Course to CPW staff at Chalk Cliffs SFH on September 16-17, 2014.

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.
- Workshop on fish dissection and ANS at Trout Unlimited (TU) Youth Camp, June 12, 2014, NI Ranch, Stonewall, CO.

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:

- Attend brood stock meeting, Salida, August 12, 2014, and Carbondale, June 17, 2015.
- The AAHL veterinary fish pathologist and two fish pathologists attended CPW Hatchery Staff Meeting, October 1-2, 2014, in Breckenridge and AAHL staff gave presentation on Vaccines/Laboratory Improvements/Bacterial Sequencing.
- AAHL staff attended Hatchery Staff Meeting Salida May 5-6, 2015 give presentation on Fish Health Assessments.
- AAHL staff attended Aquatic Biologist Meeting, Cripple Creek, January 21-22.

- The AAHL hosted Rocky Plains Fish Health Meeting January 13-15, 2015 in Denver.
- Fish pathologist attended CPW Fish Biologist Meeting in Cripple Creek on January 21, 2015 and presented on AAHL topics.
- Presentation at CSU FW 402 class on the role of fish health in the CPW hatchery management, Ft. Collins, Feb. 23.
- The AAHL veterinary fish pathologist and fish pathologist wrote articles for the Colorado Aquaculture Association's quarterly newsletter on "Developing an immune response following vaccination" and "Legal and Judicious Use of Aquaculture Drugs" November 2014 and "Prevention of fish disease through fish health management" April 2015.
- The AAHL veterinary fish pathologist and two fish pathologists attended, and presented on prevention/early detection of disease and fish dissection wet lab at the Colorado Aquaculture Association meeting in Mt. Princeton, CO January 23, 2015.
- CPW AAHL in conjunction with Wyoming Game and Fish Fish Health hosted AFS Western Fish Health Section meeting in Steamboat, CO June 2-4, 2015.

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.