

**COLORADO DEPARTMENT OF NATURAL RESOURCES
DIVISION OF WILDLIFE**



**ANNUAL PERFORMANCE REPORT
FEDERAL AID PROJECT #F-83-R-22**

Aquatic Animal Health Investigations & Management

July 1, 2008 – June 30, 2009



ANNUAL PERFORMANCE REPORT

STATE: Colorado
PROJECT: F-83-R-22
PROJECT TITLE: Aquatic Animal Health Investigations & Management
PERIOD COVERED: July 1, 2008 – June 30, 2009

OBJECTIVE:

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.

APPROACH:

To accomplish the above objectives, the three main study areas listed above are further divided into 1-4 different sub-studies. Each sub-study is further divided into 1-4 specific jobs.

STUDY 1: PROVIDE AQUATIC ANIMAL HEALTH SERVICES

OBJECTIVE:

Assist in the protection, conservation, and management of Colorado's aquatic animal resources through the 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: Aquatic Animal Health Program ANS Field Technician Adam Wallerstein displays a healthy northern pike, albeit an aquatic nuisance species, that he caught in a pond in Steamboat Springs. Photograph by Joe Sapin, 2009.

Sub-Study 1-1:

Objective:

Prevent the introduction and/or spread of aquatic animal pathogens by providing regulated and non-regulated fish pathogen inspections to ensure the safe transfer of live aquatic animals and gametes between free-ranging populations and/or captive populations in hatcheries and rearing or holding facilities.

Job 1: Provide regulated and precautionary salmonid fish disease inspections conforming to state regulations, agency policies, and/or American Fisheries Society 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 salmonid 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:

- **A case-by-case record of all AAHL cases during FY 08-09 can be found in Appendix I of this report.**
- **Two hundred forty-seven (247) inspections for regulated pathogens or pathogens of special concern totaled were conducted in FY 08-09 and are summarized in the following table. Forty-three (43) virus inspections of free-ranging fish for Viral Hemorrhagic Septicemia Virus were conducted under a USDA grant. Samples were collected by contracted Qualified Sample Collectors (QSC) or AAHL personnel and shipped or carried to the AAHL and/or contracted laboratories.**

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 broodstocks for IHN Virus.

By policy or regulation, some exceptions to these sampling standards are made under certain circumstances. In situations where attribute samples of broodstocks 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.

- **Table 1. Total regulated pathogen inspections performed by the AAHL in FY 08-09.**

Pathogen Type	Public Fisheries			Private Fisheries		Totals
	Fish under culture	Free-ranging	Other	Fish under culture	Free-ranging	
Bacteria	21	16	0	18	0	55
Parasite	24	15	3	14	2	58
Virus*	35	80	1	17	1	134
Totals	80	111	4	49	3	247

*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, MT; other cases and nearly all VHSV inspections (separate grant) were processed by the Auburn University Fish Diagnostic Laboratory.

Job 2: Provide bacteriology and parasitology laboratory analysis of 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.

Work performed:

- **The collections reported for the previous job represent completion of more than 3,500 individual trout heads tested at the AAHL by Pepsin-trypsin Digestion (PTD) Technique for the presence of *Myxobolus cerebralis*, causative agent of Whirling Disease. 1,140 heads were tested from state fish culture sites and 900 heads from free-ranging spawning populations as part of regulated inspections. 840 individual fish were tested by PTD for the private sector. Once again other testing included an out-of-state private source (150 heads) that obtained bid awards for state stocking and analysis of samples to regain legal WD-negative status.**
- **An additional 1,161 salmonid heads were analyzed by PTD for agency researchers.**
- **More than 240 individual tests for *M. cerebralis* were performed by polymerase chain reaction (PCR) at a contract laboratory in Boulder. Some PCR inspections were in accordance with agency policy prior to being stocked in headwaters lakes and other critical aquatic habitat. Others were conducted to determine the loci of infection in hatcheries already contaminated.**
- **Three state fish hatchery lots (180 individual tests) were inspected by PCR at the AAHL for the presence of the microsporidian parasite *Loma salmonae* in accordance with agency policy for critical habitat stocking.**
- **Both standard culture techniques and Direct Fluorescent Antibody Technique (DFAT) were used at the AAHL to test 21 lots of salmon and trout (2,520 individual tests) sampled from state fish culture sites for regulated bacterial pathogens. An additional 16 lots (1,920 tests) were tested from free-ranging salmonids from which eggs were being collected for the state hatchery system.**

- **Eighteen private hatchery lots in private ownership (2,160 tests) were tested at the AAHL for regulated bacterial pathogens.**

Job 3: Provide coordination, training, and logistical support 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:

- **Scheduling and logistical support was provided to the two QSCs and four AAHL fish pathologists, for 247 fish inspections. These included >70 inspections at state fish culture sites, 68 inspections of free-ranging salmonid populations in public waters (of which 43 were paid for by a separate USDA VHSV survey grant), 17 inspections at private fish culture sites, and 1 free-ranging population in private ownership.**
- **Cooler preparation procedures were modified in FY 08-09. Instead of sending out individual coolers to sample collectors and fish pathologists for each job, materials were shipped in bulk and prepared for each job by the collector. Approximately 138 individual sample coolers were received with tissue samples at the AAHL.**
- **A two-day QSC training and refresher course was conducted in Cripple Creek on 13-14 September with emphasis on bacterial fish diseases and their role in fish health in Colorado.**
- **One QSC resigned just prior to the start of the period. No new QSCs were trained in FY 08-09.**

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

Approach:

Viral Hemorrhagic Septicemia Virus (VHSV) has been known for decades as a pathogen of salmonids in northern Europe and of cod, herring and other marine fishes in both the Atlantic and Pacific Oceans. VHSV is listed as a serious salmonid pathogen by Title 50 federal regulations as well as by most states with salmonid health concerns. Colorado regulations have required inspection of salmonid fishes for VHSV for at least 25 years.

In November, 2005 a new strain of Viral Hemorrhagic Septicemia Virus (VHSV) was discovered in archived (frozen) samples from a diagnostic case involving a fish population in the

lower Great Lakes. This finding triggered extensive investigation resulting in a flood of fish health bulletins throughout 2006 and early 2007. Meanwhile the Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) declared quarantine on the 8 states and 2 provinces bordering the Great Lakes.

APHIS's action proved well founded. It soon became known that the Great Lakes strain of the virus is capable of causing mass mortalities in most species of North American game fishes as well as non-sport fishes over a broader temperature range than had previously been reported for VHS. The Great Lakes strain is most closely related serologically to the Atlantic strain, indicating its probable origin. There is now evidence in New York and Wisconsin that the pathogen has begun to move inland from the Great Lakes.

New regulations proposed by the Colorado Fish Health Board became effective in early 2008 making inspection of all fish imported, transferred, or stocked in Colorado to be inspected for Viral Hemorrhagic Septicemia Virus (VHSV). The analyses conducted during routine salmonid pathogen inspections are adequate to screen for VHSV. Thus the regulation requires the addition of warm- and coolwater inspections to AAHL duties.

- **Viral Hemorrhagic Septicemia Virus inspections**

During FY 08-09 VHSV inspections were performed on warm- and cool-water fishes in three public hatcheries and one out-of-state public hatchery whose fish were destined for Colorado waters as part of a fish trade. (Forty-three (43) free-ranging populations were inspected under a separate USDA grant.)

Sub-Study 1-2:

Objective:

Provide diagnostics and health investigations to all Colorado aquatic animal resources and free-ranging populations in both public and private ownership.

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:

- **Ninety-five (95) diagnostics and troubleshooting cases (including extension cases) were performed by AAHL fish pathologists in FY 08-09.**
- **Seventy-five (75) cases involved publicly-owned fish in culture.**
- **Ten (10) cases involved free-ranging public fish populations.**
- **Ten (10) cases involved fish in private ownership, 3 cases of private fish culture, 5 cases of private free-ranging fish, and two involving aquaria.**

- The above cases involved all facets of fish pathology including virology, bacteriology, parasitology, epidemiology, water chemistry, limnology, feed analysis, and histopathology.
- **Table 2. Diagnoses in 83 troubleshooting cases conducted by investigators in the Aquatic Animal Health Program in FY 08-09.**

Diagnosis or nature of case	# of cases
Bacterial etiology	27
Bacterial Coldwater Disease (BCWD)	(14)
Ectoparasites	10
Hatchery water bacteriology studies	9
Nutritional deficiencies	8
Environmental problems	7
Unknown	7
Involving Rocky Mountain boreal toads, <i>Bufo boreas</i>	4
Gill lice, <i>Salmincola</i> ssp. (Copepoda)	3
Internal parasites	2
Arkansas River brown trout investigations	1
Other	5

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

- Of the ten diagnostic cases on free-ranging fish from public waters mentioned above, only two cases involved fish kills. We attribute this reduction in fish kills to greater rain and snowfall plus cooler temperatures throughout Colorado in winter and spring 2009.
- The other free-ranging fish cases in public waters mostly involved fish parasites and unusual lesions reported by the public.

Sub-Study 1-3:

Objective:

Provide aquatic animal pathogen analysis and technical assistance to agency and university researchers.

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:

- **Seventy-three (73) research cases representing a total of 1,161 individual PTD tests for the presence of *M. cerebralis* were processed or received for processing by the AAHL.**

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.

Work performed:

- No PCR was performed at the AAHL during the year. The huge increase in demand for ANS monitoring services caused manpower to be diverted to that task and all PCR was performed by an outside contract laboratory.

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:

- **AAHL fish pathologists continued to provide diverse expertise, assistance, and consultation to CDOW aquatic researchers through telephone calls and e-mail.**

- **Six (6) collections of tissue samples for disease analysis were made by AAHL fish pathologists assisting a Southeast Region fishery biologist in his investigation of the possible causes of poor growth, condition, and recruitment of wild brown trout in a stretch of the Arkansas River headwaters that was recently renovated to improve fishery habitat. No conclusions were made and monitoring continued at the start of FY 09-10.**

Sub-Study 1-4:

Objective:

Conduct original aquatic animal health research to benefit Colorado’s aquatic resources.

Job 1: Develop protocols for the efficient screening of surrogate species sharing habitat with or in proximity to populations of threatened and endangered (T&E) species and species of concern.

Approach:

Conduct literature searches and seek expert advice. Design, organize, test, and streamline a practical regime for the collection, necropsy, and tissue sampling of free-ranging fishes in ecosystems containing threatened or endangered fishes or species of concern to ascertain the parasites and pathogens present.

Work performed:

- **Work on this 2-year-dormant project was only just resuming at the end of FY 08-09.**
- **Results of the VHSV screening performed under a USDA grant during the year may be useful to this project.**

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:

Monitor aquatic animal stocking and shipping through review and approval of special aquatic licenses and permits.

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:

- **AAHL fish pathologists reviewed and commented on 231 applications for Aquatic Species Importation Licenses during FY 08-09, a 50% increase from the previous year. Approval, often with stipulations, was granted for the issuance of licenses to all applications which met Colorado's regulations.**

Job 2. Evaluate and recommend approval or denial of CDOW 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:

- **Whirling Disease Exemptions**
 - **Twelve (12) previously issued Whirling Disease Exemptions were renewed with modifications as necessary to minimize infection levels.**
 - **Six (6) were issued to state facilities including five hatcheries and one laboratory.**
 - **The other six (6) exemptions were issued to private fish culture operations.**

Job 3. Administer and issue CDOW Stocking Permits after coordinating review of applications with the three Regional Fishery Managers and staff. Maintain files and database of all permits issued for review by Fishery Managers and Wildlife Law Enforcement investigators.

Approach:

Receive applications for stocking aquatic species and review for completeness; forward to appropriate biologists or staff; then issue permits based upon staff recommendations, maintaining files and database for Fishery Managers administration and Wildlife Law Enforcement investigations.

Work performed:

- **This job was eliminated from AAHL responsibility upon retirement of the Administrative Assistant in fall, 2008 and picked up by West Slope Regional Aquatics personnel.**

Sub-Study 2-2:

Objective:

Provide guidance and aquatic animal health expertise in the formulation of regulations, directives, and policies affecting the health of Colorado's aquatic resources.

Job 1: Serve as advisor to the CDOW 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 CDOW representative and other board members by providing guidance and expertise, literature searches, informal surveys, white papers, etc. as called upon.

Work performed:

- **One or more AAHL fish pathologists attended and participated in each of the approximately ten meetings of the Colorado Fish Health Board in FY07-08.**
- **Several presentations were made to the board on subjects such as ANS threats and Viral Hemorrhagic Septicemia Virus.**
- **AAHL personnel attended the four meetings of the Colorado Aquaculture Advisory Board in FY 07-08.**

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 CDOW Fish Health Board member to discuss, formulate, or modify fish health and ANS policy.**

Sub-Study 2-3:

Objective:

Assist hatchery manager/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:

- **An AAHL biologist tagged, and then sampled PIT-tagged sentinel fish at two private fish hatcheries for the presence of *M. cerebralis*. The samples were analyzed by PTD or PCR to help determine the presence or probable absence of the parasite. Both ultimately regained negative status.**

Sub-Study 2-4:

Objective:

Monitor sites in Colorado drainages for the introduction of aquatic nuisance species (ANS).

Background

Aquatic resources are suffering from a worldwide epidemic of ANS invasions due in part to the new world economy and rapid and frequent shipping technologies.² In the past decade North America has been invaded by many species of fish, mollusks, crustaceans, and aquatic macrophytes, particularly from coastal Asia via trans-Pacific shipping and Ponto-Caspian aquatic organisms carried to the Great Lakes from eastern European ports. Once established in North America, a

² <http://www.anstaskforce.gov/>

number of vectors including other wildlife species, recreational vehicles and equipment, and aquaculture may aid facilitate the spread and colonization of ANS.

Only a few organized surveys of mollusks were made in Colorado in the 20th Century. At the turn of the century, anecdotal observations indicated the state had already been invaded by several ANS including numerous fish species (Fuller et al 1999) and several mollusks such as Asian clam (*Corbicula fluminea*) and probably New Zealand mudsnail (*Potamopyrgus antipodarum*). Zebra mussels (*Dreissena polymorpha*) were reported in Kansas and Nebraska and quagga mussels (*D. bugensis*) were found in the main channel reservoirs of the lower Colorado River in Utah and Arizona during the present FY. Broader surveys (not in the purview of this program) and organized ANS monitoring are called for if the effects of ANS invasions are to be contained or minimized.

Job 2. Initiate ANS monitoring at selected sites.

Approach:

Monitor regular ANS sites selected by risk (human boating access and angler usage). In late April, 2009 monies appropriated by the Colorado legislature to fund a more rigorous ANS monitoring program were released to the AAHL. From that point forward (i.e. approximately the last ten weeks of FY 08-09) ANS monitoring came under that grant. This report covers that work accomplished prior to those grant monies becoming available.

Work performed:

- **An AAHL fish pathologist continued to represent both the program and the agency at coordination and planning meetings with state and federal agencies and private wildlife interest groups and played a major role in drafting a statewide Colorado Aquatic Nuisance Species Plan.**
- **Thirty-nine (39) submissions of crustacean and/or mollusk samples were received from CDOW field biologists and AAHL personnel. All specimens were identified and preserved for submission to the University of Colorado Museum.**
- **With the advent of watercraft inspection and disinfection stations (WID) in many localities, samples found on or scraped from the hulls of boats began to increase. Twenty (20) WID samples were examined in FY 08-09. All proved to be organisms or substances other than ANS.**
- **Qualified Sample Collectors (QSCs) continued to inspect fish culture sites, both public and private, for New Zealand mud snails as part of their annual fish health inspections. No positive findings were made.**
- **With considerable logistical assistance from Regional and Area Fishery Biologists, a schedule of plankton tow sampling and substrate monitoring was conducted on high risk waters. Presumptive testing was accomplished with cross-polarization dissecting microscopes. Confirmatory testing was performed by PCR.**
- **By late fall, 2008 seven Colorado reservoirs had been found positive for dreissenid mussels. Lake Pueblo and Grand Lake both tested positive for both zebra mussels, *D. polymorpha*, and quagga mussels, *D. rostriformis bugensis*.**

- **Table 3. The sites found positive for dreissenid mussels by the CDOW AAHL and/or the U.S. Bureau of Reclamation Laboratory in Lakewood, CO.**

Lake	County	Major watershed	Dreissenid species
Lake Pueblo	Pueblo	Arkansas River	Zebra & quagga
Grand Lake	Grand	Colorado & So Platte ³	Zebra & quagga
Shadow Mountain Res	Grand	Colorado & So Platte	quagga
Lake Granby	Grand	Colorado & So Platte	quagga
Willow Creek Reservoir	Grand	Colorado ⁴	quagga
Tarryall Reservoir	Park	Arkansas River	quagga
Jumbo Reservoir	Logan & Sedgwick	South Platte River	quagga

- **Another lake, Blue Mesa Reservoir in Gunnison County (Colorado River watershed), is suspect for both exotic dreissenids at this time, but tests have been ambiguous.**
- **AAHL personnel provided ANS prevention and management advice, comments, and counsel to CDOW administrators on a frequent basis throughout the year.**
- **Two AAHL fish pathologists assisted in conducting several ANS training workshops for CDOW, CDPOR, municipal and federal agency personnel, and the private sector.**
- **Some interesting findings in the invertebrate survey:**
 - **The tiny mud Amnicola, *Amnicola limosa*, a hydrobiid snail native to North America, was first reported from a prairie creek in eastern Colorado in 2002. This year’s ANS survey found *A. limosa* in the supply and interchange pipes at Durango State Fish Hatchery in La Plata County in the extreme southwestern part of the state.**
 - ***Didymosphenia geminata*, a native invasive diatom, coats stream bottoms with a thick growth of hair-like fibers sometimes called “rock snot.” “Didymo” was identified in one ANS case in FY 08-09.**
 - **AAHL Fish Pathologist Carolyn Gunn has become an authority on the differentiation of Paiute sculpins, *Cottus beldingi*, from the more widespread mottled sculpin, *C. bairdi*. Dr. Gunn was able to confirm the identity of several *C. beldingi* populations on the West Slope for area fishery managers.**

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

³ Grand Lake, Shadow Mountain Reservoir, and Lake Granby comprise a chain of lakes near the headwaters of the Colorado River system just west of the Continental Divide. As is the custom in the Colorado Rockies, water from the three lakes as well as from a small impoundment a few miles downstream of Lake Granby is pumped back up to Grand Lake and shunted beneath the Divide into the headwaters of the Big Thompson River near Estes Park in the South Platte River drainage. Thus the findings of dreissenid mussels in the three lakes indicates contamination of both sides of the Continental Divide.

⁴ Willow Creek Reservoir is a shallow impoundment that empties into the Colorado River just below Lake Granby. The Colorado then is joined by the Fraser River in a very shallow impoundment called Windy Gap Reservoir. Water can be pumped from Windy Gap back into Granby and eventually reach the South Platte River via the means described in the previous footnote. Thus it is conceivable that water from Willow Creek Reservoir could also contaminate the South Platte River on the opposite side of the Continental Divide.

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.

Sub-Study 3-1:

Objective:

Provide fish health management services to the CDOW Hatchery Program.

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:

- **Both "catchable" (>9") and "sub-catchable" (<9") trout slated for stocking in the following quarter were examined for health and quality parameters at ten state fish hatcheries on a quarterly basis.**
- **The data was compiled in a database and analyzed for abnormalities, trends, and comparisons. Quarterly reports and comparative graphs were issued to hatchery managers and the Statewide Chief of Hatcheries.**
- **The Statewide Chief of Hatcheries also received quarterly briefings.**
- **Comparisons of averages for parameters like fin erosion, condition factor, and fat index were made between years at the same facility and between facilities. This provides the individual managers with feedback in management techniques and decisions and allows the Statewide Manager to set goals for production on a unit by unit basis.**

- **The system is proving to be a very sensitive indicator of crowding and helps the Statewide Hatchery Manager to determine the maximum loading capacities of each of the units with the ultimate goal of providing the angler with fish of maximum and uniform quality.**
- **Examples of comparative fish quality graphs can be reviewed in Appendix II at the end of this document.**

Job 2. Serve the CDOW Hatchery Program as monitor for the National Investigational New Animal Drug (INAD) Program that allows for participation in a drug research program that is focused on the generation of efficacy and target animal safety data to support new animal drug approvals, approving and tracking usage and providing reports and required information to the U.S. Fish and Wildlife Service (USFWS) Aquatic Animal Drug Approval Partnership Program.

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:

- **AAHL Fish Pathologist Carolyn Gunn continued as Study Monitor for 12 hatcheries enrolled in 2008 and eight hatcheries enrolled in the 2009 INAD program; duties include enrollment of hatcheries, review of accuracy of all paperwork, management of inventory and prescriptions for CCP, and management of target effluent discharge levels.**
- **The veterinarian fish pathologist ensures that established approved protocols are followed, obtains and distributes drugs and substances used in the program, designs and runs trials, reviews and submits finished paperwork, maintains drug inventories, , distributes MSDS, ensures allowable discharges of substances into public waters, enforces withdrawal times, and tracks drug expirations.**

Job 3. Provide aquatic animal veterinary services, including prescription of therapeutants and investigation of new treatment options, to the CDOW 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:

- Held a current valid state and national license in good standing to practice veterinary medicine in the state of Colorado, including accrual of 16 hours of continuing education every two years to maintain the license.
- Provided veterinary prescriptions for FDA approved drugs and extra-label prescriptions for approved drugs used in an extra-label manner, which, under federal law is the only legal mechanism by which certain drugs and substances can be used to treat food fish.
- Managed prescriptions for Veterinary Feed Directive antibiotic use, and have a distributorship for holding and distributing antibiotic-medicated feeds under this directive
- Supervised recommendations for use of approved drugs by non-veterinary staff at the Aquatic Animal Health Lab as mandated by the FDA-CVM.
- Maintained a valid veterinarian-client-patient relationship with all hatchery staff as mandated by the FDA-CVM
- Maintained a file for two years of all prescriptions written as mandated by the FDA-CVM.
- Maintained a valid USDA-APHIS-Colorado Department of Agriculture Veterinary Accreditation necessary for writing Certificates of Veterinary Inspection and provide such certificates for interstate shipment animals under the **Standards for Accredited Veterinarian Duties in Title 9, Code of Federal Regulations, Part 161.**
- Developed and set in place guidelines outlining legal use of chemotherapeutants in the state hatchery system including a presentation to CDOW administrators and other fish pathologists; and presented those guidelines at Hatchery Staff Meeting on January 13-14, 2009 in Colorado Springs where they were adopted by the state fish hatchery system.
- Provided 41 prescriptions between July 1, 2008 and June 30, 2009 to biologists and hatchery staff for use of approved substances and for extra-label use of approved substances.
- Developed *Guidelines for use of Drugs in Aquaculture* for the state hatchery system and 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 (34 to date), withdrawal times.
- Maintained required continuing education hours necessary to keep veterinary license in good standing.
- Other veterinary duties and accomplishments
 - Maintained USDA/APHIS accreditation for issuing certificates of inspection.
 - Animal Care and Use Committees for the tilapia operation at Trinidad State Junior College, Alamosa.
 - George Schisler's research fish at Colorado State University, Ft. Collins.
 - Obtained regulatory discretion from the US Department of Health and Human Services for use of emamectin benzoate (SLICE™) at Poudre State Fish Hatchery.

Sub-Study 3-2:

Objective: Provide training and technical assistance to CDOW fish hatchery personnel, biologists, and private fish growers and consultants.

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:

- **Conducted the first of four, 2-1/2 day fish health management courses to aquaculture students and CDOW hatchery personnel at Trinidad State Junior College in Alamosa covering the use of microscopes, fish anatomy and physiology, necropsy procedures, parasites through ciliates, and a wet lab for dissection.**
- **Conducted the 2nd of four scheduled courses for 10 state fish hatchery employees at Chalk Cliffs SFH June 23-24, 2009.**

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:

- **Worked with CDOW Regional Education Specialists to develop guidelines for the SW Region's *Chubs in the Classroom* programs to ensure that guidelines were followed.**
- **Visited schools to troubleshoot equipment and fish problems.**
- **Provided documentation to administrators including the agency Director concerning fish disease and stocking concerns, costs, and guidelines and agreement forms for a pilot Trout Unlimited Trout in the Classroom program for Colorado.**
- **Two presentations were made by AAHL personnel to middle school classes during the fiscal year.**

Job 3: Provide other 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 veterinarian fish pathologist made a presentation on the use of Emamectin benzoate in the control of *Salmincola californiensis* at the 14th Annual Drug Approval Coordination Workshop, July 29-31, 2008, Bozeman, MT.
- A Whirling Disease update was presented at the Colorado Wildlife Society annual meeting, February 5, Grand Junction, CO.
- A presentation of the VHS statewide survey results for the Colorado Aquaculture Society annual meeting, January 23, Mt. Princeton Hot Springs, CO.
- Three AAHL fish pathologists each made presentations at the ANS Workshop, May 5, 2009, Jeffco County Fairgrounds.
- An overview of VHS and ANS surveys in Colorado was made to the USFS/San Juan National Forest Leadership Team, May 12, Durango Public Lands Office.
- A 3-hour lecture on fish culture and fish transportation law enforcement was made at a Wildlife Law Enforcement class at Front Range Community College in Fort Collins.
- Fish health presentations and reports were made by AAHL fish pathologists at 3 Hatchery Superintendents' Meetings and 4 Aquatics Section Senior Staff Meetings during the FY.
- A lecture on aquaculture and fish transportation regulations and law enforcement was given to the 2007 CDOW District Wildlife Manager Training Class.
- >Ten (10) presentations on ANS threats to Colorado fisheries were given to various public and private organizations by AAHL personnel.
- An AAHL fish pathologist attended the annual meeting of the Aquaculture Program at Trinidad State Junior College.
- Publications during FY 08-09
 - October 2008. Gunn, Carolyn. Update of VHS statewide surveillance and the new finding of *Aminicola limosa* in *The Fishline*, newsletter of the Colorado Aquaculture Association.
 - January 2008. Gunn, Carolyn. Article on the prevention of spreading dreissenid mussels in *The Fishline*.
 - Schisler, George J., Nicole K.M. Vieira, and Peter G. Walker. 2008. Application of household disinfectants to control New Zealand mudsnails. *North American Journal of Fisheries Management*; 28:1172-1176. (See abstract in Appendix III.)

Job 4: Service, adjust, and repair microscopes at CDOW fish hatcheries to maintain on-site diagnostic and parasite screening capabilities.

Approach:

Dismantle, clean, adjust, and repair as needed.

Work performed:

- **Approximately thirty-six (36) compound microscopes and dissection scopes were cleaned, adjusted, and serviced at CDOW hatcheries and Parvin Lake Research Station.**
- **Seven (7) microscopes including a fluorescent antibody microscope were cleaned, adjusted, and serviced at the AAHL.**
- **Two compound microscopes were serviced as a courtesy to the USFWS Leadville National Fish Hatchery and Hotchkiss National Fish Hatchery.**
- **Ten compound microscopes were serviced as a courtesy to the aquaculture program at Trinidad State Junior College.**

Sub-Study 3-3:

Objective:

Disseminate fish health information electronically to administrators, researchers, fishery managers, hatchery managers, and the public.

Job 1. Create a laboratory data base to streamline internal data entry on one end and be available on-line to agency and external publics on the other.

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:

- **Job completed.**

Job 2. Maintain laboratory database.

Approach:

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. Work schedule (July 1, 2005 – June 30, 2009):

Work performed:

- **Entry of both new data and records were entered and checked intermittently throughout the period.**

PERSONNEL:

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