COLORADO DEPARTMENT OF NATURAL RESOURCES

DIVISION OF WILDLIFE

ANNUAL PERFORMANCE REPORT
FEDERAL AID PROJECT F-83-R-21

Aquatic Animal Health Investigations & Management

July 1, 2007 – June 30, 2008

Brown Trout 9"x12" oil on board by Carolyn Gunn, DVM

ANNUAL PERFORMANCE REPORT

STATE: Colorado
PROJECT: F-83-R-21
PROJECT TITLE: Aquatic Animal Health Investigations & Management

PERIOD COVERED: July 1, 2007 – June 30, 2008

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.

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:

- **Inspections for regulated pathogens or pathogens of special concern totaled 245 in FY 07-08 (down 19 inspections from 264 in FY 06-07) and are summarized in the following table. Numbers in parentheses are the previous year's totals. Samples were collected by contracted Qualified Sample Collectors (QSC) or AAHL personnel and shipped or carried to the AAHL and/or contracted laboratories.**
- **Table 1. Total regulated pathogen inspections performed by the AAHL in FY 06-07.**

Pathogen Type	Public Fisheries			Private Fisheries		Totals
	Fish under culture	Free-ranging	Other	Fish under culture	Free-ranging	
Bacteria	21 (34)	18 (12)	0 (2)	13 (16)	1 (0)	53 (64)
Parasite	35 (45)	24 (13)	4 (6)	16 (21)	1 (0)	80 (85)
Virus*	51 (47)	39 (42)	3 (6)	18 (19)	1 (1)	112 (115)
Totals	107 (126)	81 (67)	7 (14)	47 (56)	3 (1)	245 (264)

*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. Salmonid virology inspections in FY 06-07 were analyzed by the USFWS Bozeman Fish Health Center in Bozeman, MT with lesser numbers of samples 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:

[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.

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 approximately 5,350 individual trout heads tested at the AAHL by Pepsin-trypsin Digestion (PTD) Technique for the presence of *Myxobolus cerebralis*, causative agent of Whirling Disease. 1,740 heads were tested from state fish culture sites and 793 heads from free-ranging spawning populations as part of regulated inspections. 711 individual fish were tested for the private sector. 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 1,760 salmonid heads were analyzed by PTD for agency researchers.**
- **806 individual tests for *M. cerebralis* were performed by polymerase chain reaction (PCR) at the AAHL on samples collected at state fish hatcheries. Some 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.**
- **Seven state fish hatchery lots (420 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 34 lots of salmon and trout (4,080 individual tests) sampled from state fish culture sites for regulated bacterial pathogens. An additional 13 lots (1,560 tests) were tested from free-ranging salmonids from which eggs were being collected for the state hatchery system.**
- **Thirteen private hatchery lots and one free-ranging salmonid population in private ownership (1,680 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 three, later two, QSCs[2] and four AAHL fish pathologists, for 231 fish inspections. These included >70 inspections at state fish culture sites, ~60 inspections of free-ranging salmonid populations in public waters, 18 inspections at private fish culture sites, and 1 free-ranging population in private ownership.**
- **Approximately 315 portable coolers containing sterile instruments, bacterial media, and other collection materials were prepared and shipped from the AAHL in support of QSCs for inspection sample collections.**
- **A two-day QSC training and refresher course was conducted in Denver on 14-15 July with emphasis on aquatic nuisance species (ANS) and their associated fish health problems in Colorado.**
- **No new QSCs were trained in FY 07-08.**

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 a 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.

[2] The QSC pool was reduced from four to three in December, 2006 when a DVM QSC was hired by the AAHL as a full-time fish pathologist. A new QSC had not been recruited by the end of the FY.

- **Viral Hemorrhagic Septicemia Virus inspections**

  By the end of FY 07-08, 18 viral inspections had been made for VHSV in public warm- and coolwater fisheries and stocks in 2 state hatcheries and one private facility had received VHSV inspections.

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 (90) diagnostics and troubleshooting cases were performed by AAHL fish pathologists in FY 06-07.**
- **Seventy (70) cases involved publicly-owned fish in culture.**
- **Nine (9) cases involved free-ranging public fish populations.**
- **Eleven (11) cases involved fish in private ownership.**
- **The above cases involved all facets of fish pathology including virology, bacteriology, parasitology, epidemiology, water chemistry, feed analysis, and histopathology.**

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:

- **Six of the above mentioned diagnostic cases on free-ranging fish from public waters involved investigations of fish kills.**
- **Other cases involved fish parasites and reports from the public of unusual lesions.**

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 (70) research cases representing a total of 1,760 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:

- **Eleven (11) cases representing 806 PCR tests for *M. cerebralis* (trout heads) and 420 tests for Loma salmonae (gills) were received and processed by the AAHL.**
- **Also, see work performed in Job 3.**

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 provided expertise, assistance, and consultation to CDOW aquatic researchers primarily through telephone calls and e-mail.**

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:

- **The training of a new fish pathologist and increasing ANS problems in Colorado precluded the resumption of this project in FY 07-08.**

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 115 applications for Aquatic Species Importation Licenses during FY 07-08. Approval, often with stipulations, was granted for the issuance of licenses to all applicants because they 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 impact and effectiveness of BMPs. Review and evaluate existing permits on an annually for modification and renewal.

Work performed:

- **Whirling Disease Exemptions**
    - **Fourteen (14) previously issued Whirling Disease Exemptions were renewed with modifications as necessary to minimize infection levels.**
    - **Seven (7) exemptions were issued to state facilities including both hatcheries and laboratories. The other seven (7) 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:

- **Approximately sixty (60) stocking permits, primarily for West Slope waters, were issued during FY 07-08.**
- **In addition, questions were answered and regulations clarified in numerous phone calls and e-mail queries from the public.**

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 two 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:

- **There was less activity on this front during FY 07-08. AAHL biologists tagged, then sampled PIT-tagged sentinel fish at one private fish hatchery for the presence of *M. cerebralis* in efforts to regain negative status. The samples were analyzed by PTD or PCR to help determine the presence or probable absence of the parasite.**

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.[3] 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

[3] 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 flumminea*) and probably New Zealand mudsnail (*Potamopyrgus antipodarum*). Zebra mussels (*Driessena 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):

Work performed:

- **An AAHL fish pathologist met twice monthly with a group representing state and federal agencies and private wildlife interest groups and played a major role in drafting a statewide Colorado Aquatic Nuisance Species Plan that was still in review at the end of FY 07-08.**
- **Submissions of crustacean and/or mollusk samples continued to come in from CDOW field biologists and AAHL personnel through November, 2007. All specimens were identified and preserved for submission to the University of Colorado Museum. No additional New Zealand mudsnail findings were made.**
- **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.**

- **After review of the data and observations made by AAHL fish pathologists, Aquatics Section Fishery Managers and Researchers, and Tom Siala, a University of Colorado graduate student, the following conclusions are made:**
  - **At this time the New Zealand mudsnail remains restricted to an approximately 4 mile stretch of Boulder Creek in Boulder County and a 5 mile stretch of the South Platte River in the tailrace of Elevenmile Dam in Park County.**
  - **Both populations are stable and not expanding.**
  - **The New Zealand mudsnail in Colorado requires flowing water that is neither warm in summer nor severely cold (<38°F) in winter.**
  - **Therefore the New Zealand mudsnail is thermally restricted from establishment in the great majority of Colorado streams and, with relatively few exceptions, does not represent a threat to Colorado's aquatic resources.**
- **One of the very last collections in November, 2007 performed by the AAHL temporary employee assigned to ANS monitoring produced a pair of tiny bivalves in an artificial substrate placed at the North Marina on Pueblo Reservoir in Pueblo County. After some difficulties in performing a new PCR for dreissenid mussels, a private molecular**

lab contracted by CDOW and a laboratory of the U.S. Bureau of Reclamation finally agreed that the samples plus veliger larvae in plankton samples were quagga mussels, *Dreissena rostriformis bugensis*. This was the first finding of a dreissenid in Colorado.

- **The positive finding in Pueblo Reservoir caused immediate alarm in state agencies all the way to the Governor's office as well as in the private sector and resulted in action at the legislative level during the early spring of 2008 that provided funds and direction for greatly increased monitoring by the AAHL as well as boat inspections and decontamination by CDOW and the Colorado Division of Parks and Outdoor Recreation (CDPOR) and other related measures.**
- **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 three ANS training workshops for CDOW, CDPOR, municipal and federal agency personnel.**

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.

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. 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:

- **As in FY 06-07, 12 state trout hatcheries and rearing stations were visited quarterly by an AAHL fish pathologist.**
- **The data was compiled in a database and analyzed for abnormalities, trends, and comparisons.**
- **The Statewide Chief of Hatcheries was kept apprised of the findings in this project on a regular and frequent basis as a means to monitor hatchery success or problems.**
- **Presentations of individual unit data were made to the crews at each hatchery on a quarterly basis.**
- **Comparison of averages for parameters like fin erosion, condition factor, and fat index with statewide averages during the same or previous time frames can indicate the success or failure of hatchery management decisions by their effects on fish quality.**
- **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.**
- **Figure 1 and Figure 2 follow the caudal fin indices (the lower the number, the higher the condition) for a specific facility – the San Luis Valley Pilot Project or "Spicer Site" – for a rainbow trout grow-out cycle (fingerling to catchable product) and compares these values with the statewide average.**

Figure 1.

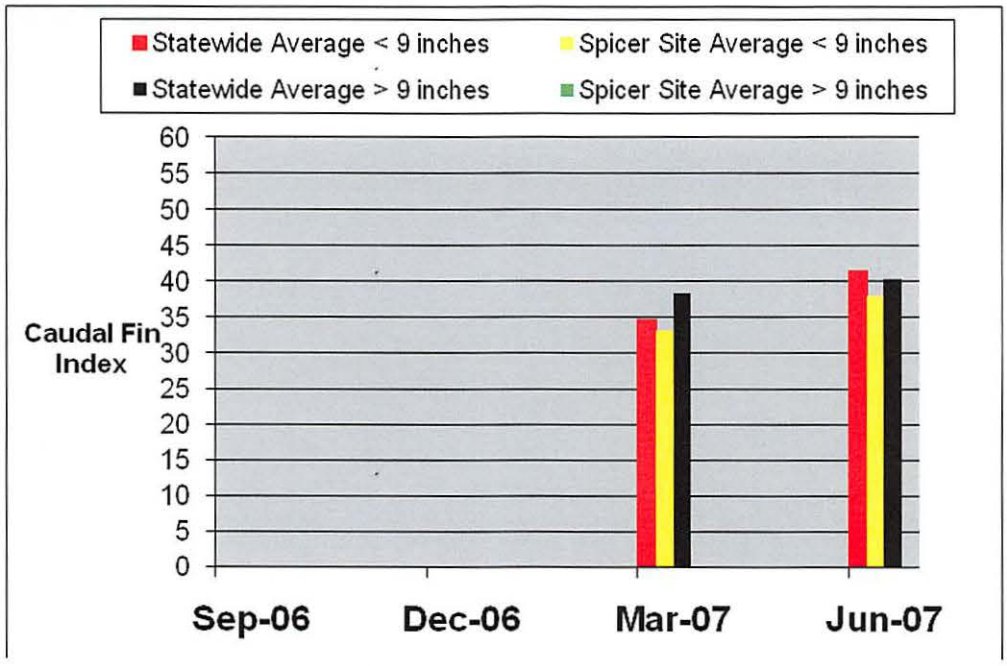
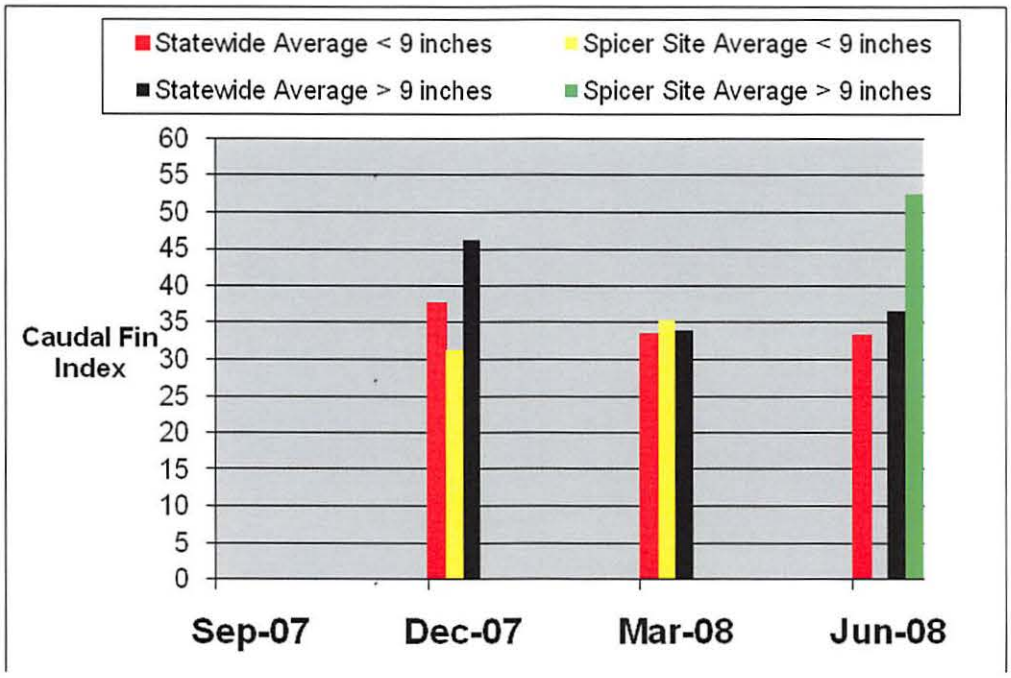


Figure 2.



- **As the trout grew, their caudal fin indices averaged a little lower than the statewide average until March, when the values began to climb due to increasing loading density as the fish grew larger. By harvest time in June densities had grown so high that the caudal fin values greatly exceeded statewide figures. Such profiles vary between stations and management regimes.**

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:

- **Enrolled fifteen state fish hatcheries in the US Fish and Wildlife Service's 2008 program.**

- **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.**
- **Fifty-nine INAD compassionate exemptions were issued during the reporting period**

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:

- **Holds 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.**
- **The new veterinarian developed knowledge of US Food and Drug Administration and Environmental Protection Agency laws and compliance, tracking federal policy and compliance regarding legal use of drugs in aquaculture and food-producing animals, new drug approvals, and change in use status for drugs and notifies hatchery and AAHL staff on new animal drug approvals or withdrawals. Legal use of these therapeutants is controlled by the Food and Drug Administration-Center for Veterinary Medicine (FDA-CVM) and Food Safety and Applied Nutrition, U. S. Department of Agriculture-Animal and Plant Health Inspection Service, Animal Medicinal Drug Use Clarification Act, Veterinary Feed Directive, FDA's Compliance Policy Guide, American Veterinary Medical Association, Aquatic Animal Drug Approval Partnership, and Food Animal Residue Avoidance Database.**
- **Designed a form in compliance with FDA regulations for tracking monthly drug treatment usage at state hatcheries, distributed it to hatcheries, and obtain copies monthly to enter into a database to track drug use at the state fish hatcheries.**
- **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. In this fiscal year, 47 prescriptions were issued.**
- **Manage prescriptions for Veterinary Feed Directive antibiotic use, and have a distributorship for holding and distributing antibiotic-medicated feeds under this directive**
- **Supervise recommendations for use of approved drugs by non-veterinary staff at the Aquatic Animal Health Lab as mandated by the FDA-CVM.**
- **Maintain a valid veterinarian-client-patient relationship with all hatchery staff as mandated by the FDA-CVM**
- **Maintain a file for two years of all prescriptions written as mandated by the FDA-CVM.**

- **Maintain 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.**
- **Worked with the FDA's Center for Veterinary Medicine and US Department of Health and Human Services to arrange for regulatory discretion and importation through US customs for use of a drug not approved in the United States (emamectin benzoate) in order to clear a valuable fish broodstock of an external parasite, and began a clinical trial for evaluation of effectiveness and safety of the drug. The results will be submitted to the INAD Coordinator and to the manufacturer of the drug. (This study started prior to July 1, 2008, but was completed in August of 2008, so I guess we can leave this in.)**

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:

- **Dreisennid mussel discoveries and the nationwide VHSV crisis once again precluded AAHL fish pathologists from conducting training courses during FY 07-08.**

Job 2: This also languished from July 1, 2007 to June 30, with the exception of points in red below. 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:

- **Developed guidelines and agreements for schools to follow to prevent dissemination of disease and to prevent inappropriate stocking**

- **Worked with a regional education specialist to develop appropriate curricula to accompany raising fish in the classroom**
- **Two presentations were made by AAHL personnel to junior high 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:

- **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.**
- **>Fifteen (15) presentations on ANS threats to Colorado fisheries were given to various public and private organizations.**

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.**

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:

- **With much assistance from a programmer with the Aquatics Research Program, the structure of the AAHL Database was completed and fine-tuned by early 2008.**

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:

- **Both new data and records were entered and checked throughout the period.**
- **QA/QC work on entered data took place throughout the period and continues with the goal of placing the database online for internal publics in the coming fiscal year.**
- **A meeting was held with agency research and IT personnel to discuss integrating the AAHL database into a greater overall program for use by Aquatics Section staff. No timetable has yet been set .**

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