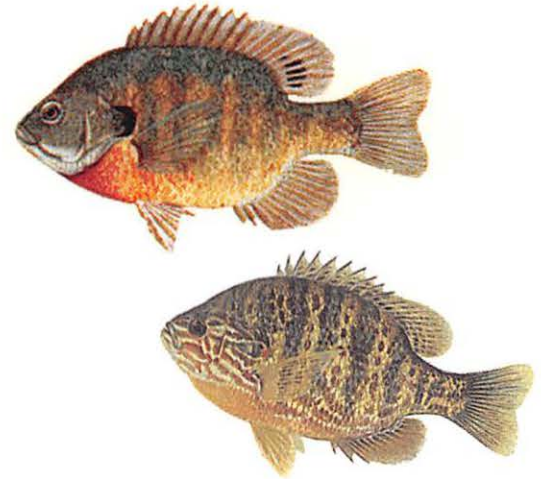


COLORADO DEPARTMENT OF NATURAL RESOURCES

DIVISION OF WILDLIFE



ANNUAL PERFORMANCE REPORT

FEDERAL AID PROJECT F-83-R-18

**Aquatic Animal Health Investigations &
Management**

July 1, 2006 – June 30, 2007

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ANNUAL PERFORMANCE REPORT

STATE: Colorado
PROJECT: F-83-R-18
PROJECT TITLE: Aquatic Animal Health Investigations & Management
PERIOD COVERED: July 1, 2006 – June 30, 2007

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 264 in FY 06-07 (up from 224 in FY 05-06) and are summarized in the following table. Samples were collected by contracted Qualified Sample Collectors (QSC) or AAHL personnel and shipped or carried to the AAHL 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	34	12	2	16		64
Parasite	45	13	6	21		85
Virus*	47	42	6	19	1	115
Totals	126	67	14	56	1	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. The 25 cases (mostly free-ranging fisheries) involving non-salmonid fisheries tested for largemouth bass virus (LMBV) were tested 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 4,100 individual trout heads tested at the AAHL by Pepsin-trypsin Digestion (PTD) Technique for the presence of *Myxobolus cerebralis*, causative agent of Whirling Disease. 1,800 heads were tested from state fish culture sites and 780 heads from free-ranging spawning populations as part of regulated inspections. 840 individual fish were tested for the private sector. Other testing included out-of-state hatcheries that obtained bid awards for state stocking and analysis of samples to regain legal WD-negative status.**
- **An additional 1,465 salmonid heads were analyzed by PTD for agency researchers.**
- **863 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.**
- **Five state fish hatchery lots (300 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 32 lots of salmon and trout (3,640 individual tests) sampled from state fish culture sites for regulated bacterial pathogens. An additional 6 lots (720 tests) were tested from free-ranging salmonids from which eggs were being collected for the state hatchery system.**
- **Eighteen private hatchery lots and three free-ranging salmonid populations in private ownership (2,520 tests) were tested at the AAHL for regulated bacterial pathogens.**

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:

- **Scheduling and logistical support was provided to the four QSCs² and AAHL fish pathologists, for 117 regulated salmonid pathogen inspections. These included >60 inspections at state fish culture sites, 42 inspections of free-ranging salmonid populations in public waters, 21 inspections at private fish culture sites, and 1 free-ranging population in private ownership.**
- **Approximately 372 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 at the AAHL on 5-6 August in Silverthorne with emphasis on fishery management and aquatic nuisance species (ANS) and their associated fish health problems in Colorado.**
- **No new QSCs were trained in FY 06-07.**

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

Approach:

At this time, local inspection of warm and cool water fishes are not routinely offered by state agencies or by most regions of the U.S. Fish and Wildlife Service. In the absence of Colorado regulations or national guidelines for uniform locally-provided health inspections of warm and cool water fishes, this work unit will initiate a program to at least evaluate the health and quality of incoming shipments of live non-salmonid fishes destined for CDOW hatcheries or for direct stocking into public lakes and streams will be screened. The agency does not possess quarantine capability. Therefore fish in these shipments will be examined "off the truck" for parasites and gross abnormalities. Laboratory samples will, by necessity, be processed at the AAHL or by contracted laboratories after delivery. However, whenever possible, free-ranging non-salmonid fish populations from which transfers into Colorado may originate will be screened prior to the date of shipment.³

Work performed:

- **A key biologist position was vacant for the first half of the fiscal year. Therefore no work was performed on this job in 2006. However, in early 2006 a related issue arose which resulted in a hastily organized study related to cool water and warm water fish importation.**
- **Large mouth Bass Virus investigations**

It was learned that LMBV is present in largemouth bass at a state fish hatchery in Texas from which CDOW obtains large numbers of hybrid striped bass ("wiper") eggs annually. Fishery managers wanted to know whether LMBV is extant in Colorado centrarchid fisheries as well as whether or not moronid fishes in close contact with infected centrarchids might potentially vector the pathogen into Colorado.

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.

³ At this time there are no Colorado regulations restricting non-salmonid fish pathogens nor are there agency directives and policies for such.

The state of knowledge and questions we face are described in an internal white paper generated by the AAHL in June, 2006 (attached as Appendix I). While it seems very likely that LMBV has been introduced to Colorado repeatedly without apparent effect, in the absence of good information, the Acting Statewide Manager of Aquatic Resources made the decision to limit 2006 wiper stocking to those lakes which are neither tributary to nor contiguous with the water supplies of the three Colorado warm water hatcheries, or those lakes with substantial centrarchid fisheries (bass, crappie, bluegill, or sunfish).

In addition, biologists of the AAHL were assigned to conduct surveys of major centrarchid and moronid fisheries for the presence of LMBV.⁴ Six CDOW fishery managers and one state hatchery manager participated in the project by collecting fish, mostly by night time electroshocking from boats. AAHL biologists and technicians removed kidney, spleen and swim bladder samples from each fish and pooled them in 5-fish aliquots in Hank's Basic Salt Solution. The samples were then frozen and later shipped to the Fish Diagnostic Laboratory at Auburn University for analysis.

The results are listed in table form in Appendix II. In all, 808 fish were sampled from 16 waters in eastern Colorado. These included 619 centrarchids and 189 moronids. All tests were negative. The meaning of these results is being contemplated. No further management decisions have been made at this time.

- **Viral Hemorrhagic Septicemia Virus investigations**

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.

⁴ By coincidence, the methods and cell lines used for testing for LMBV would have demonstrated the presence of the new Viral Hemorrhagic Septicemia Virus (VHSV) now known to be spreading throughout the Laurentian Great Lakes.

In January, 2007 three AAHL fish pathologists attended a briefing on VHS hosted APHIS by in Denver. Following that event presentations have been made to agency groups and there have been numerous meetings and discussions about how to best deal with this new threat. At the close of the FY, steps are being taken to initiate a regulatory change that would require that all live fish transfers in Colorado be certified VHSV free. Other regulatory changes to safeguard against invasion by VHSV may follow.

Since the AAHL lacks sufficient manpower to maintain a virology capability, we are limited as to how much additional virology we can obtain by contract. Auburn University temporarily became unavailable in early 2007 due to the resignation of their virologist. Meanwhile the Region 6 Fish Health Laboratory at Bozeman, MT offered their services to the extent that they can be stretched.

Non-salmonid samples submitted in the first half of calendar year 2007 were selected from free-ranging populations that serve as hatchery brood stocks and Kansas fisheries from which importations were planned receiving top priority. A summary of the VHSV tests and results is presented in Appendix III.

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:

- **One hundred eight (108) diagnostics and troubleshooting cases were performed by AAHL fish pathologists in FY 06-07.**
- **Ninety-six (96) cases involved publicly-owned fish in culture.**
- **Seven (7) cases involved free-ranging public fish populations.**
- **Five (5) 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.**

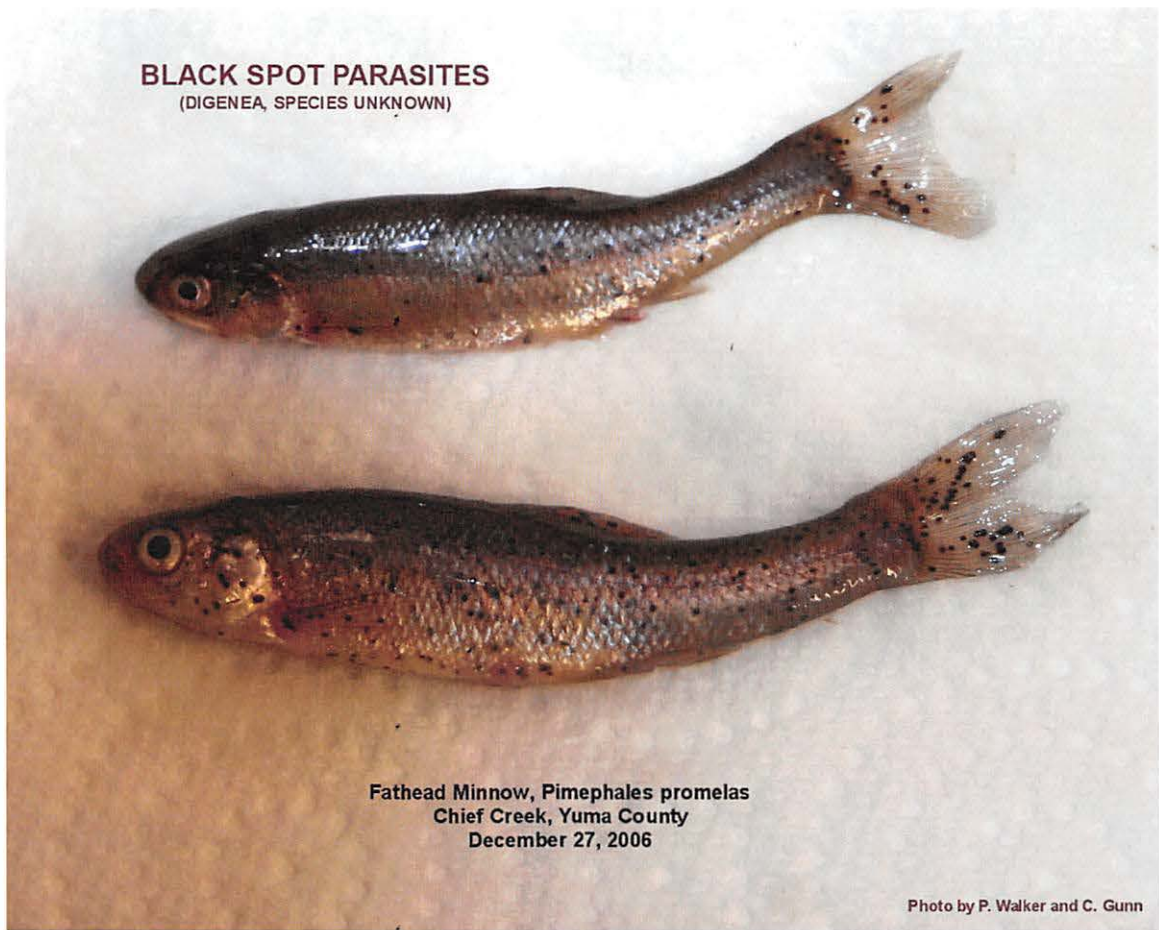
Job 2: Conduct health investigations including fish kills in free-ranging aquatic animal populations as needed.

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:

- **Four of the above mentioned diagnostic cases on free-ranging fish from public waters involved investigations of salmonid fish kills.**
- **Other cases involved a winter kill (oxygen deprivation under heavy and opaque ice cover) and warm water fish parasitism including two cases of parasitism investigations of free-ranging fish populations in Yuma County on the eastern plains in waters tributary to Wray State Fish Hatchery.**



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:

- **One hundred thirteen (113) research cases (a resurgence from the 50 cases during the previous FY) representing a total of 2,667 individual PTD tests (1,612 in FY 05-06) 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:

- **Five (5) cases representing 188 PCR tests for *M. cerebralis* (trout heads) 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 in FY 05-06.**
- **The AAHL assisted the Fishery Research Program by contracting more than 2,500 PCR tests for *M. cerebralis* through a private laboratory in Boulder, CO.**
- **AAHL biologists assisted the Colorado State University in three diagnostic (troubleshooting cases) involving captive red shiners (*Cyprinella lutrensis*) used in a contaminant exposure experiment at the Foothills Campus in Fort Collins.**

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 resignation of a fish pathologist during FY 05-06 precluded any work in this category through the present FY. The hiring of a new biologist will allow this project to be re-described and resumed 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 181 applications (up from 113 in FY 05-06) for Aquatic Species Importation Licenses during FY 04-05. 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**
 - **Of the seventeen (17) Whirling Disease Exemptions previously issued, eleven (11) were renewed with modifications as necessary to minimize infection levels. Of these, five are state fish culture units and six are private.**
 - **Two private salmonid fish culture sites, one federal (Leadville NFH), and one state (Pitkin SFH) fulfilled the renovations and necessary testing to regain whirling disease negative status.**
 - **One new Whirling Disease Exemption application was investigated and approved for resumption of an aquaculture license by a private trout grower.**

- **Sadly Roaring Judy SFH in Almont, after regaining negative status and operating a few years with a clean bill of health, again became contaminated in early 2007 from a source not yet identified. A new Whirling Disease Exemption was issued for this unit**

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:

- **Fifty-eight (58) stocking permits, primarily for West Slope waters, were issued during FY 06-07.**
- **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 twelve meetings of the Colorado Fish Health Board in FY06-07.**
- **Several presentations were made to the board on subjects such as ANS threats, Largemouth Bass Virus, and Viral Hemorrhagic Septicemia Virus.**
- **AAHL personnel attended the three meetings of the Colorado Aquaculture Advisory Board in FY 06-07.**

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 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:

- **AAHL biologists sampled PIT-tagged sentinel fish at one federal, three state, and two 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.**
- **One federal facility< Leadville NFH, one state facility, Pitkin SFH, and two private facilities regained negative status after renovation and a long reigime of testing sentinel fish.**
- **One state facility, Roaring Judy SFH in Gunnison County, unexpectedly tested positive during FY 06-07. Efforts were underway at the close of the FY to locate the source of contamination.**
- **One state facility, Crystal River SFH in Garfield County, and one private facility, received a total of six visits from an AAHL biologist during efforts to achieve negative status in FY 06-07. At the close of the FY, those efforts had not yet been completed.**

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.⁵ 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 number of vectors including other wildlife species, recreational vehicles and equipment, and aquaculture may aid facilitate the spread and colonization of ANS.

Few organized surveys have been made in Colorado to date. Yet casual observations indicate the state has 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 (*Dreissena polymorpha*) have been 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:

Commence regular ANS monitoring of sites (number and distribution to be determined).

Work performed:

- **Approximately 10 sets of crustacean and/or mollusk samples submitted by CDOW field biologists were received at the AAHL. All specimens were identified and preserved for submission to the University of Colorado Museum. **
- **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 in FY 05-06. No positive findings were made.**
- **AAHL fish pathologists continued to make presentations and/or advise and assist administrators and the Colorado Wildlife Commission on the New Zealand mud snail crisis and other ANS issues.**

⁵ <http://www.anstaskforce.gov/>

- AAHL personnel made >100 field site searches for aquatic mollusks and crustacean. The results are list in Table 2.

Table 2. Aquatic mollusk and crustacean specimens collected during ANS searches in Colorado by CDOW field personnel in FY 06-07. (Introduced species in red.)

	Common name	Scientific name	Number of sites from which the species was collected	
Decapoda	papershell crayfish	<i>Orconectes immunis</i>	3	
	Kansas pond crayfish	<i>Orconectes nais</i>	19	
	ringed crayfish	<i>Orconectes neglectus</i>	3	
	northern crayfish	<i>Orconectes virilis</i>	8	
Amphipoda	lake scud	<i>Gammarus lacustris</i>	1	
Gastropoda	creeping ancyliid	<i>Ferrisia rivularis</i>	3	
	cloaked ancyliid	<i>Ferrisia walkeri</i>	1	
	prairie fossaria	<i>Fossaria bulimoides</i>	4	
	golden fossaria	<i>Fossaria obrussa</i>	2	
	big-eared radix	<i>Radix auricularia</i>	2	
	marsh pond snail	<i>Stagnicola elodes</i>	11	
	pygmy fossaria	<i>Lymnaea parva</i>	3	
	tadpole physa	<i>Physa acuta</i>	36	
	pouch snail	<i>Physa gyrina</i>	47	
	ash gyro	<i>Gyraulus parvus</i>	15	
	marsh ramshorn	<i>Helisoma trivolvis</i>	2	
	sharp sprite	<i>Promenetus exacuus</i>	1	
	New Zealand mudsnail	<i>Potamopyrgus antipodarum</i>	1	
	blunt ambersnail (terrestrial species)	<i>Oxyloma retusum</i>	1	
	Bivalvia	Asian clam	<i>Corbicula fluminea</i>	6
		striated fingernail clam	<i>Sphaerium striatinum</i>	1
ubiquitous pea clam		<i>Pisidium casertanum</i>	4	
ridged-back pea clam		<i>Pisidium compressum</i>	1	
shiny pea clam		<i>Pisidium nitidum</i>	1	
Other wildlife	unidentified mayfly nymphs		2	
	snail case microcaddis	<i>Helicopsyche borealis</i>	2	
	fathead minnow	<i>Pimephales promelas</i>	1	
	gizzard shad	<i>Dorosoma cepedianum</i>	1	

- No new sites of invasion by New Zealand mud snail were found. The two positive sites remain Boulder Creek in Boulder County and the South Platte River directly below Elevenmile Reservoir in Park County.

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 four times each 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 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.**

Figure 1. Comparison of non-caudal fin erosion indices in production trout under nine inches total length in June, 2007 in twelve Colorado state fish hatcheries.

Watson	19.286
Pueblo	
Chalk Cliffs	16.25
Durango	4.821
Finger Rock	
Monte Vista	28.571
Mt. Shavano	8.214
Pitkin	14.464
Poudre	23.303
Rifle Falls	10.179
Roaring Judy	16.964
Spicer Site	15.179
State Average	15.665

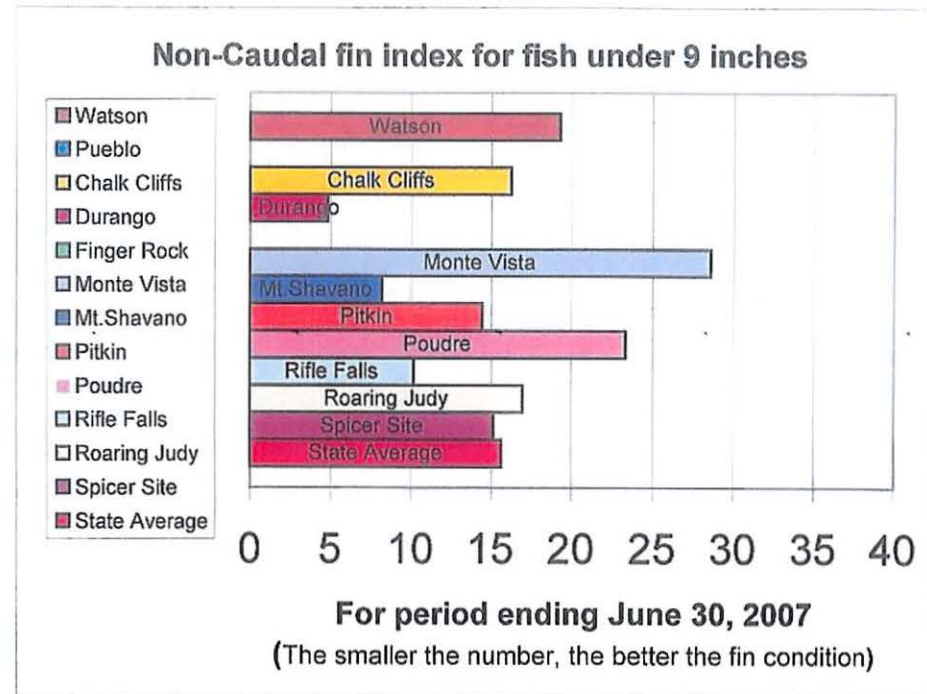
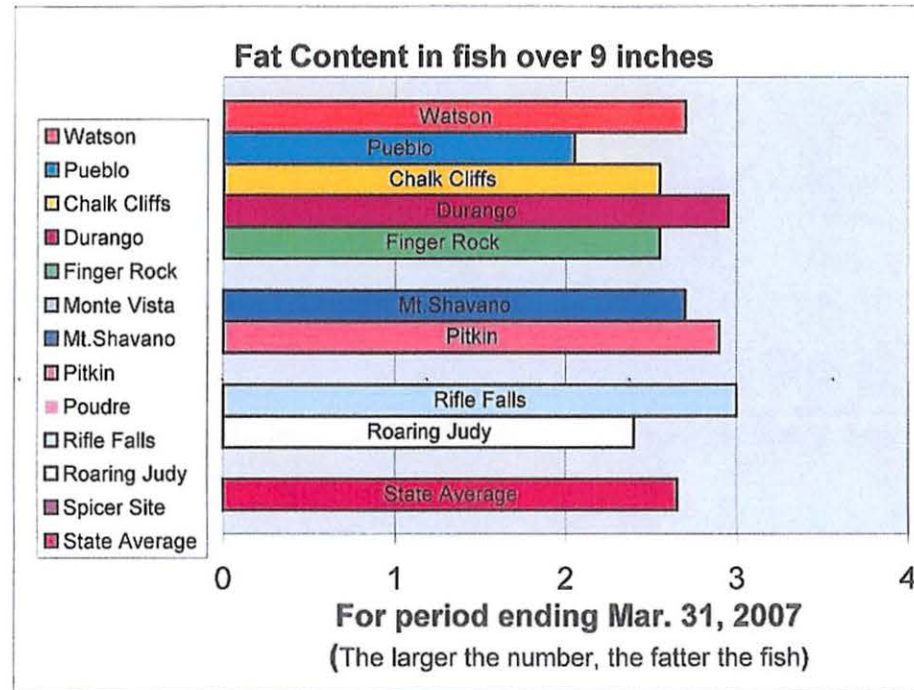


Figure 2. Comparison of visceral fat indices in trout more than 9 inches in total length at twelve state fish hatcheries in March, 2007.

Watson	2.7
Pueblo	2.05
Chalk Cliffs	2.55
Durango	2.95
Finger Rock	2.55
Monte Vista	
Mt. Shavano	2.7
Pitkin	2.9
Poudre	
Rifle Falls	3
Roaring Judy	2.4
Spicer Site	
State Average	2.656



Job 2. Serve the CDOW Hatchery Program as monitor for Investigational New Animal Drug (INAD) permits that facilitate treatment options unavailable by prescription, approving and tracking usage and providing reports and required information to the U.S. Fish and Wildlife Service (USFWS) permit holders.

Approach:

Facilitate access to treatment options unavailable by prescription through cooperative participation with the U.S. Fish & Wildlife Service in U.S. Food and Drug Administration Investigational New Animal Drug (INAD) studies, supplying all required information to USFWS and/or FDA.

Work performed:

- **Thirteen state fish hatcheries were enrolled in the US Fish and Wildlife Service's 2007 program.**
- **All appropriate paperwork and instructions were sent out.**
- **The veterinarian fish pathologist helped obtain and distribute drugs and substances used in the program, design and run trials, review and submit finished paperwork, maintain drug inventories, and develop proper dosages and intervals of dosing for special fish species, distribute MSDS, and track 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:

- **A new veterinarian fish pathologist was hired in December, 2006.**
- **The new veterinarian developed knowledge of US Food and Drug Administration and Environmental Protection Agency laws and compliance relating to use of drugs in food-producing animals.**
- **Hatchery and AAHL staffs in turn were notified of new animal drug approvals or withdrawals.**
- **The new veterinarian developed knowledge of US Food and Drug Administration and Environmental Protection Agency laws and compliance relating to use of drugs in food-producing animals and notified hatchery and AAHL staff on new animal drug approvals or withdrawals. Legal use of these drugs and substances is controlled by the Food and Drug Administration-Center for Veterinary Medicine and Food Safety and Applied Nutrition, U. S. Department of Agriculture-Animal and Plant Health Inspection Service, Animal Drug Use Clarification Act, Veterinary Feed Directive, FDA's**

Compliance Policy Guide, American Veterinary Medical Association, Aquatic Animal Drug Approval Partnership, 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.**
- **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 Coördinator and to the manufacturer of the drug.**
- **Obtained regulatory discretion for use of a substance of high regulatory priority for a species at Native Aquatic Species Restoration Facility (NASRF) in Alamosa.**

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:

- **The fish pathologist staff vacancy for the first 5 months FY 06-07 and the resulting training of a new fish pathologist precluded holding short courses this year.**

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:

- **Developed forms and enrolled several schools in aquaculture programs.**

- A total of seven presentations were presented to middle school students on fisheries conservation.
- One presentation was made to a 2nd grade class at an elementary school in Pueblo.
- One junior high school group received a tour of the AAHL and a presentation on the life cycle of the Whirling Disease organism.



AAHL Laboratory Assistant AmberLee Waller dissects a trout for a sixth grade class at Fort Morgan (CO) Middle School. May, 2007.

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 updates were made by AAHL fish pathologists at 6 Hatchery Superintendents' Meetings and 5 Aquatics Section Senior Staff Meetings during the FY.
- A lecture on aquaculture and fish transportation regulations and law enforcement was given to the 2006 CDOW District Wildlife Manager Training Class.

- **Two talks on fish health were delivered to public groups.**
- **Six (6) presentations on ANS threats to Colorado fisheries were given to various public organizations.**
- **AAHL fish pathologists provided considerable technical assistance, editing, photography, data, and other support to a joint CDOW-University of Colorado project to publish A Field Guide to the Freshwater Mollusks of Colorado by Mardy Nelson Harrold and Dr. Robert P. Guralnick. 2007. (126 pp).**

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.**
- **Seven (7) microscopes including a fluorescent antibody microscope were cleaned, adjusted, and serviced at the AAHL.**
- **One compound microscope was serviced as a courtesy to the USFWS Leadville 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:

- **Several AAHL staff met with the contracted programmers in Montrose in late 2006 to discuss final modifications and corrections to the AAHL database.**
- **At the end of the FY, the contractors had not yet delivered the promised changes.**

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:

- **Work on this job will commence as soon as the final changes are made in the program and the data already entered can be tested and QA/QCed.**

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APPENDIX I.

A REVIEW OF LARGEMOUTH BASS VIRUS WITH RESPECT TO COLORADO FISHERIES

Peter Walker, Senior Fish Pathologist
June 20, 2006

As with many subjects, there seems to be more unsubstantiated lore about Largemouth Bass Virus (LMBV) on the Internet than verifiable peer-reviewed information.

LMBV is comparatively new to science. It was first discovered during investigation of a substantial largemouth bass die-off in Santee-Cooper Reservoir, South Carolina that occurred in 1995 (Plumb et al, 1996; Plumb et al, 1999; Grizzle et al, 2000). A preliminary survey of 452 wild LMB in 1997-1998 from 78 reservoirs in southeastern states showed positive results in 6 lakes (7.7%) in 4 watersheds (Plumb et al, 1999). In the eleven years since that first finding it has been involved in a number of LMB kills in the southeastern United States and reported as present in free-ranging LMB in at least 20 states as far north as Illinois (Grizzle and Brunner, 2003).

A survey of LMB for LMBV in 15 hatcheries in 10 southeastern states found the virus present in asymptomatic broodfish at all sites but could be detected in juvenile fish in only one of the sites. The conclusion was that this virus can indeed be spread by stocking from infected hatcheries (Woodland et al, 2001). Oral transmission of LMBV was proven by force-feeding LMBV-infected guppies to LMB (Woodland et al, 2002). Transmission of LMBV occurs horizontally and by eating infected prey. Vertical transmission has not been demonstrated. (Grizzle and Brunner, 2003)

LMBV disease occurs primarily in summer in largemouth bass over 30 cm (12 inches) (Grizzle and Brunner, 2003). LMB injected with LMBV in a laboratory suffered higher mortalities at 30°C than at 25°C suggesting that outbreaks may be temperature mediated. (Grant et al, 2003)

LMB was tentatively classified as an iridovirus by Piaskoski and Plumb, 1999. More recently it has proven to be a *Ranavirus* in the family Iridoviridae and has been identified in natural infections in largemouth bass, *Micropterus salmoides*, and guppies, *Poecilia reticulata* (Plumb et al, 2000). Individual members of the emerging genus *Ranavirus* infect frogs, salamanders, and fishes. Their genomes are recorded on single pieces of DNA and they possess envelopes of host cell origin (Fauquet et al, 2005).

An asymptomatic carrier state of LMBV has been documented in several centrarchid species and chain pickerel, *Esox niger* (Grizzle et al, 2003). However, white crappies, bluegills, and gizzard shad sympatric with affected LMB in a major die-off of the latter in Sardis Reservoir, Mississippi were found negative for LMBV (Hanson et al, 2001). In doses that were 100% lethal to LMB, striped bass suffered significant losses (63%) by injection and immersion (10%) in a laboratory setting. However, the virus did not persist in surviving striped bass beyond 25 days (Plumb and Zilberg, 1999).

LMBV can be cultured in both BF-2 and FHM cell lines at an optimum temperature of 30°C. A PCR test for the confirmation of suspect cell-culture isolates of LMBV has been developed at Auburn University (Grizzle et al, 2003).

External clinical signs in laboratory infected LMB juveniles include darkening, inflammation, and necrosis around the site of injection, distended abdomen, corkscrew swimming, and lateral recumbency. Internal signs include bright red spleens, reddened intestinal ceca, and pale livers. Histological changes include acute fibrinous peritonitis on the outer surfaces of all internal organs, and necrosis of the gastrointestinal mucosa (Zilberg et al, 2000). In free-ranging LMB involved in die-offs, bloated bellies and aimless wandering at the surface are indications of LMBV. Usually only large individuals are involved. A yellow, waxy substance in the swim bladder derived from blood clot residues was observed in survivors of a major LMB kill in Sardis Reservoir, Mississippi for months after the event (Hanson et al, 2001).

Here in Colorado I have investigated one possible occurrence of LMBV and I believe that Dori Lee had fish checked for it a few times as well. We have not had a positive hit for it in Colorado to date. In the case I investigated (AAHL 98-252), Charlie Bennett reported intermittent die-offs of very large hybrid striped bass in John Martin Reservoir throughout the growing season in 1998. He described the affected fish as extremely bloated and swimming listlessly at the surface as if too full of gas to submerge. Because of the distance between Brush and Lamar and the irregular nature of these die-offs that seldom involved more than half a dozen fish at any one time, we had trouble making connections and I never received a fresh specimen to sample until the very last affected fish of the year was captured on November 24, 1998. By that time the water temperature was in rapid decline. Nevertheless the specimen exhibited the distended gas bladder typical of LMBV in centrarchid bass.

Tissue samples were shipped to Dr. John Plumb at Auburn University. The test for LMBV was negative, but at the time both John and I wondered if it was just too cold and too late in the year to get a really accurate test. The fish may have been suffering from the lingering effects of an infection that had run its course by the time we looked at it. At any rate, no one else has to my knowledge reported similar observations in hybrid stripers since that time either here in Colorado or elsewhere.

Discussion

There is still a great deal we need to know about LMBV and its epidemiology before we can be completely comfortable with fishery management in its presence. We should never accept absence of evidence as evidence of absence. However, as repeated observations corroborate over time, we begin to gain a little more comfort.

Virtually all of the LMBV outbreaks observed to date have involved only largemouth bass, and then only the largest individuals present. Also, the outbreaks seem to occur in association with stress and high water temperature (~85°F). We simply don't see water temperatures that high in Colorado. It's possible that, even if present, LMBV won't be capable of causing significant die-offs here.

That LMBV is apparently not vertically transmitted is good news. However, because of the biology of largemouth bass, there is virtually no way to prevent contact between infected broodstock and offspring. While

there has so far been no indication of anything to fear in fish culture from the presence of LMBV, the potential damage from such a circumstance might be in the dissemination of the virus by stocking.

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APPENDIX II.

Samples collected from eastern Colorado waters in 2006 and tested at Auburn University for Largemouth Bass Virus (LMBV)

AAHL Case #	AU Case No.	Water	County	Water code	Biologist	Lot No.	Species	N	# tubes tested	Results
06-138	CO07-01	Martin Lake	Huerfano	79841	Melby	1	LMB	12	3	Negative
06-138	CO07-02					2	SMB	14	3	Negative
06-138	CO07-03					3	BGL	6	2	Negative
06-198	CO07-04	Aurora Reservoir	Arapahoe	56420	Winkle	1	SXW	3	1	Negative
06-198	CO07-05					2	LMB	1	1	Negative
06-204	CO07-06					3	LMB	2	1	Negative
06-204	CO07-07					4	SMB	4	1	Negative
06-204	CO07-08					5	BGL	1	1	Negative
06-130	CO07-09	Beckwith Res.	Pueblo	82026	Melby	1	LMB	60	12	Negative
06-130	CO07-10					2	BGL	8	2	Negative
06-130	CO07-11					3	SNF	3	1	Negative
06-128	CO07-12	Brush Hollow Res.	Fremont	79409	Melby	1	LMB	20	4	Negative
06-143	CO07-13	Lonetree Res.	Larimer	53152	Crockett	1	SXW	3	2	Negative
06-143	CO07-14					2	LMB	12	1	Negative
06-129	CO07-15	Trinidad Reservoir	Las Animas	81911	Melby	1	SMB	60	12	Negative
06-129	CO07-16					2	LMB	3	1	Negative
06-194	CO07-17	Union Reservoir	Weld	53570	Crockett	1	SXW	12	3	Negative
06-194	CO07-17					2	LMB	1		Negative
06-247	CO07-18	Pueblo SFH	Pueblo	N/A	Harris	1	SXW	60	12	Negative
06-406	CO07-19	North Sterling Res.	Logan	53328	Swigle	1	SXW	37	7	Negative
06-406	CO07-19					2	LMB	1		Negative
06-406	CO07-20					3	BCR	1	1	Negative
06-127	CO07-21	Pueblo Reservoir	Pueblo	81783	Melby	1	SBA	60	12	Negative
06-127	CO07-22					2	BGL	60	12	Negative
06-127	CO07-23					3	SXW	5	1	Negative
06-127	CO07-24					4	SNF	10	2	Negative
06-127	CO07-25					5	SMB	60	12	Negative

06-141	CO07-26	Valco Pond No. 2	Pueblo	81412	Melby	1	LMB	57	12	Negative
06-141	CO07-27					3	SNF	5	5	Negative
06-141	CO07-28					2	BGL	60	12	Negative
06-142	CO07-29	Horsetooth Res.	Larimer	55168	Kehmeier	1	SMB	62	13	Negative
06-139	CO07-30	Horseshoe Res.	Huerfano	79803	Melby	1	SMB	60	12	Negative
06-139	CO07-31					2	LMB	28	6	Negative
06-144	CO07-32	Nee Gronde Res.	Kiowa	79613	Ramsay	1	MIX: SXW + WBA	60	12	Negative
06-144	CO07-33					2	SMB	10	2	Negative
06-140	CO07-34	Valco Pond No. 1	Pueblo	81410	Melby	1	LMB	45	9	Negative
06-140	CO07-35					2	SMB	8	2	Negative
06-140	CO07-36					3	BGL	58	12	Negative
06-140	CO07-37					4	SNF	5	1	Negative
06-155	CO07-38	Jackson Reservoir	Morgan	53037	Swigle	1	SXW	9	3	Negative
06-155	CO07-39					2	BGL	1	2	Negative
06-155	CO07-39					3	BCR	1	1	Negative

*Kidney, spleen, and swim bladder samples were pooled in =5-fish aliquots and frozen in Hank's Basic Salt Solution, then shipped to the Fish Diagnostic Lab at Auburn University for analysis under the supervision of Research Assistant Briana Keafer.

Species codes:	
BCR	black crappie
BGL	bluegill
LMB	largemouth bass
SMB	smallmouth bass
SBA	spotted bass
SNF	green sunfish
SXW	"wiper"
WBA	white bass

APPENDIX III. Non-salmonid samples tested for Viral Hemorrhagic Septicemia Virus (VHSV) collected from Colorado sites or Kansas sources for Colorado importation in 2007 and tested at the USFWS Region 6 Fish Health Laboratory in Bozeman, MT.

AAHL Case #	Water	County	Water Code	Biologist	Lot #	Species	# fish	# tubes tested	Results
07-045	Pueblo Reservoir	Pueblo	81783	Melby	1	WAL	22	5	negative
07-046	Pratt SFH Kansas ⁶	Pratt	n/a	Kumberg	1	CCF	60	12	negative
07-050	Farlington SFH Kansas	Crawford	n/a	Nelson	1	CCF	60	12	negative
07-055	Cherry Creek Reservoir	Arapahoe	52580	Winkle	1	WAL	60	12	negative
07-057	Chatfield Reservoir	Jefferson	54306	Winkle	1	WAL	60	12	negative
07-060	Carter Lake	Larimer	54255	Crockett	1	WAL	60	12	negative
07-074	Catamount Lake	Routt	65721	Atkinson	1	NPK	60	12	negative
07-099	Salt Fork of Arkansas River, Kansas ⁷	Barber	n/a	Aberson	1	SMN	10	2	negative
07-106	Wray SFH	Yuma	n/a	Lee	1	SMB	5	1	negative
07-112	Pawnee Power Plant Lake	Morgan	61250	Swigle	1	SMB	5	1	negative
					2	SXW	5	1	negative
07-127	Magic, Inc ⁸	n/a	n/a	n/a	1	EMS	20	4	No RNA
					2	GSD	20	4	No RNA
07-136	Chartier's Pond	Morgan	52578	Swigle	1	BCR	17	4	negative

Species Codes	
BCR	black crappie
CCF	channel catfish
EMS	emerald shiner
GSD	gizzard shad
NPK	northern pike
SMB	smallmouth bass
SMN	suckermouth minnow
SXW	"wiper"
WAL	walleye

⁶ Tests arranged by CDOW prior to importation to public waters from these sources.

⁷ Free-ranging fish tested prior to importation to Colorado Native Aquatic Species Restoration Facility in Alamosa.

⁸ Pickled bait fish of Great Lakes origin marketed in sporting goods stores in Colorado. Tested at Pisces Molecular in Boulder for viable RNA, but none survived the pickling process.