WRAC

Western Regional Aquaculture Center

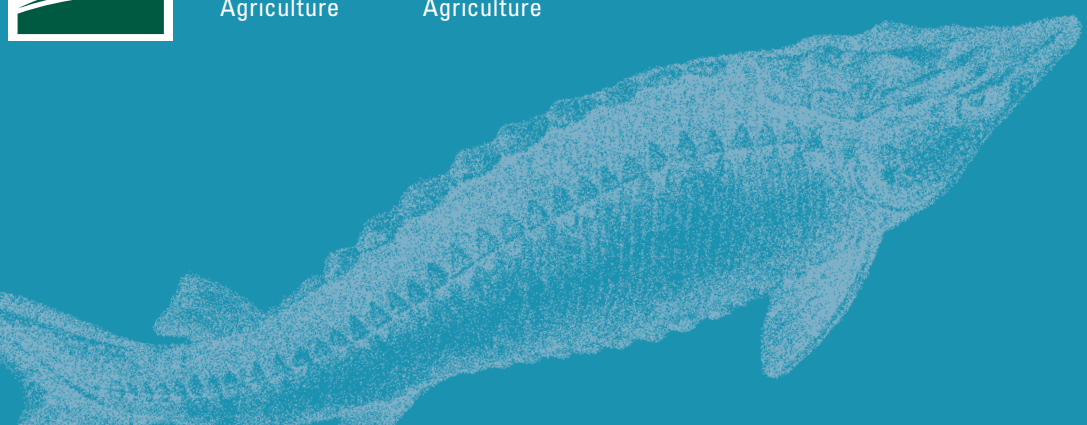
Alaska • Arizona • California • Colorado • Idaho • Montana • Nevada • New Mexico • Oregon • Utah • Washington • Wyoming

ANNUAL ACCOMPLISHMENT REPORT

SEPTEMBER 1, 2009 TO AUGUST 31, 2010

WRAC Administrative Office
School of Aquatic and Fishery Sciences
College of the Environment
University of Washington
Box 355020, Seattle, WA 98195-5020
Phone: 206-685-2479
Fax: 206-685-4674
E-mail: dgranger@u.washington.edu

MARCH 2011



USDA

United States Department of Agriculture

National Institute of Food and Agriculture

INTRODUCTION

This Annual Accomplishment Report for the Western Regional Aquaculture Center (WRAC), covers progress made from September 1, 2009 through August 31, 2010. WRAC is designated as one of five regional aquaculture centers under the United States Department of Agriculture (USDA), for which funding is made available to support research, development, and demonstration projects in aquaculture. WRAC encompasses the 12 states in the Western Region of the United States—Alaska, Arizona, California, Colorado, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington, and Wyoming.

ACKNOWLEDGMENTS

The Western Regional Aquaculture Center (WRAC) acknowledges the contributions of the Principal Investigators and Participating Scientists involved in the projects reported in this 23rd Annual Accomplishment Report. Members of the WRAC Board of Directors (Board), Industry Advisory Council (IAC), and Technical Committee (TC) have been instrumental in the successful operation of WRAC during the past year. We particularly appreciate the assistance of the chairs of our Board, the IAC, and the TC, and those serving as Technical Advisors to the Work Groups.

We also thank the scientists and aquaculturists from across the country who contributed their expertise and valuable time to review WRAC project proposals and publications. Without their help, it would be impossible to maintain the high quality of this program.

Additionally, we thank the School of Aquatic and Fishery Sciences at the University of Washington for serving as the host institution for WRAC.

ORGANIZATIONAL STRUCTURE

Board of Directors

With representation from every land-grant institution from the 12 states in WRAC, as well as one representative each from the Industry Advisory Council (IAC) and the two sub-committees of the Technical Committee (TC), the Board of Directors (Board) is WRAC's primary policy-making body. The Board reviews and appoints members to the IAC and TC. The Board also reviews recommendations from the IAC/TC and approves projects for funding and inclusion into the annual Work Plan.

Industry Advisory Council

Composed of representatives of the industry and associated services, covering multiple sectors and geographic regions within the western 12 states—Alaska, Arizona, California, Colorado, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington, and Wyoming.

Technical Committee

Composed of two sub-committees:

- The ***Research sub-committee*** includes representatives from participating research institutions, state or territorial public agencies as appropriate, as well as non-profit, private institutions.
- The ***Extension sub-committee*** includes representatives from state Extension Services.

The IAC and TC work jointly to make recommendations to the Board for new and continuing regional projects, project modifications, and project terminations.

PROGRESS REPORTS

Since the start of the regional aquaculture programs, WRAC has processed 23 Annual Work Plans (FY'87 through FY'10 funding) through USDA. This current annual report covers the activities of the WRAC Administrative Center and progress made during the 23rd year on all projects through August 31, 20010, listed below with funding levels for FY'10.

ANNUAL REPORTS

A. Coldwater Disease Prevention and Control through Vaccine Development and Diagnostic Improvements
   3rd project year: $81,637
B. Determining Ripeness in White Sturgeon Females to Maximize Yield and Quality of Caviar
   3rd project year: $100,001
C. Optimizing Dietary Protein and Energy Utilization to Improve Production Efficiency of Tilapia in the Western United States
   1st project year: $66,704
D. Cost-effective, Alternative Protein Diets for Rainbow Trout that Support Optimal Growth, health, and Product Quality
   1st project year: $119,864

TERMINATION REPORTS

A. Development and Evaluation of Starter Diets and Culture Conditions for Three Subspecies of Cutthroat Trout and Gila Trout
B. Potential Threat of VHS Virus in the Western United States
C. Economic Impacts of Private sector Aquaculture-Based Recreational Fishing in the Western USA
D. Physiological Changes Associated with Live Haul: Maintaining Healthy Fish

PROJECT REVIEW & DEVELOPMENT

Annual Budget: $58,000

All projects are reviewed for progress and accomplishment at the combined annual meeting of the Industry Advisory Council and the Technical Committee (IAC/TC) in October of each year. Support of each project is subject to satisfactory progress as determined by both groups.

PUBLICATIONS

Annual Budget: $24,000

The WRAC Publications project provides an ongoing information-sharing link among WRAC researchers, the aquaculture industry, and the public sector. Funds for this project cover actual printing costs as well as the necessary editorial and graphics expertise to produce the various publications.

ADMINISTRATIVE SUPPORT

FY'10 FUNDING LEVEL $191,619

The Administrative Center is located in the School of Aquatic and Fishery Sciences, College of the Environment at the University of Washington, which serves as the host institution. WRAC Administrative Center staff provide all necessary support services to the Board of Directors, Industry Advisory Council (IAC), Extension and Research Subcommittees of the Technical Committee (TC), and project Work Groups. As the scope of the program has expanded, the Administrative Center has become responsible for handling more detailed communications among investigators of various projects and for ensuring that the IAC and subcommittees of the TC are kept apprised of all ongoing activities.

The Administrative Center has processed 23 Annual Work Plans (FY'87 through FY'10) to date for the various WRAC projects. Activities of the Center and funding for its operation rely upon the annual decisions of the Board prior to inclusion in the work plan.

The Center assists project Work Groups with the preparation of proposals, which, upon acceptance by WRAC, are included in the funding agreement between the US Department of Agriculture (USDA) and the University of Washington's Grants & Contracts (G&C) Office. With the assistance of the G&C Office, the Center executes appropriate agreements with the subcontractors for the purpose of transferring funds to projects approved by the USDA.

Thus, the Center acts as fiscal agent in receiving and disbursing funds in accordance with the terms and provisions of its grant. Center staff monitor subcontracts to ensure proper preparation and budgetary expenditures for the funded projects.

The Administrative Center also publishes *Waterlines*, an annual newsletter that has a mailing list of more than 2,700 recipients. *Waterlines* provides information on WRAC projects and general aquaculture news in order to educate the public on the importance of aquatic animal husbandry, as well as other WRAC activities and to keep industry informed about current research projects and initiatives.

The Administrative Office has upgraded the WRAC website at *http://fish.washington.edu/wrac/*.

Other areas of Adminisirative Center support during this period, as in previous years, include:

- Preparation of USDA grant packages and amendments.
- Production of documentation and reports to the Board.
- Organization of IAC and TC meetings.
- Coordination of activities of the Board.
- Development of research plans, budgets, and proposals.
- Development of management plans and budgets.
- Cooperation with the IAC and the TC in monitoring research activities and developing annual progress reports.
- Coordination of the external review of proposals for technical and scientific merit.
- Development of liaisons with appropriate institutions, agencies, and clientele.

- Preparation of testimony, in coordination with the four other Regional Aquaculture Centers, for annual submission to the House Appropriations Subcommittee on Agriculture, Rural Development and Related Agencies in Washington, DC.
- Participation in the National Coordinating Council, which consists of the directors of the five Regional Administrative Centers and key administrators from USDA.
- Coordination of special sessions for Regional Aquaculture Centers at aquaculture meetings.
- Solicitation and coordination of appointees to the Board and recommended nominees to the IAC and TC.
- Recruitment of Administrative Center staff, as authorized by the Board.
- Close communication with other fisheries and aquaculture programs to track various aquaculture activities throughout the Western Region.

WRAC Board of Directors at Los Lunas Silvery Minnow Refugium.

Julie Maas

Coldwater Disease Prevention and Control through Vaccine Development and Diagnostic Improvements

REPORTING PERIOD

September 1, 2009–August 31, 2010 (3rd *year of project*)

AUTHOR

Ken Cain and Doug Call

FUNDING LEVEL

First Year funding	$81,555 (received February 2008)
Second Year funding	$80,043 (received March 2009)
Third Year funding	$81,637 (received April 2010)
Fourth Year Request	$81,639 (*approved 10/09*)

PARTICIPANTS

Kenneth Cain* (*Working Group Chair*)	University of Idaho	Idaho
Douglas Call*	Washington State University	Washington
Scott LaPatra*	Clear Springs Foods, Inc.	Idaho
Gary Fornshell*	University of Idaho	Idaho
Greg Weins	US Department of Agriculture	West Virginia
Rashesh Kumar	Washington State University	Washington

TECHNICAL ADVISOR

Gael Kurath	US Geological Survey	Washington

INDUSTRY ADVISOR

Jim Parsons	Troutlodge, Inc.	Washington

GRADUATE STUDENT

Amy Long	University of Idaho	Idaho
Karol Gliniewica	Washington State University	Washington

FACULTY PARTICIPANT

Devendra Shah	Washington State University	Washington

* funded participants

Fish images:US Government Printing Office Pamphlet 1996-792-501: Lake Washington Ship Canal Fish Ladder; US National Oceanic and Atmospheric Association

PROJECT OBJECTIVES

The goals of this project are to evaluate strategies that would aid in developing more effective ways of managing coldwater disease (CWD) at aquaculture facilities and to identify possible bacterial genes that may be targeted for vaccine development and testing. Presently, disease management is difficult at many facilities and there is no commercial vaccine available for *Flavobacterium psychrophilum*, the causative agent for CWD

The specific objectives for this project are to:

1. Identify potential vaccine candidates using comparative proteomic analysis of an attenuated strain of *F. psychrophilum* and determine if crude cell lysate can be used as a subunit vaccine delivery vehicle in the absence of adjuvant.
    - Candidate recombinant proteins will be tested in vaccine trials.
2. Validate quantitative diagnostic assays (ELISA and ovarian fluid filtration [FAT]).
    - Correlate assay results to risk of vertical transmission or disease susceptibility.
    - Establish threshold levels for culling broodstock and/or eggs.
3. Based on results from objective 2:
    - Develop other assays (e.g., real-time quantitative PCR) for quantification of infection in ovarian fluid.
4. Develop an integrated outreach program to meet stakeholder needs.
    - Based on results obtained and the number of deliverables made available to researchers and the aquaculture community, a number of outreach/Extension products will be developed related to prevention and control of CWD through vaccination or implementation of new disease management strategies at broodstock facilities.

ANTICIPATED BENEFITS

Coldwater disease (CWD) has become one of the most significant disease problems in commercial trout aquaculture in recent years. It is a worldwide problem, and in the Pacific Northwest, losses from CWD can range from 18%–30%, with estimated economic impacts in Idaho alone reaching approximately $10 million. In addition to the trout industry, federal, state, and tribal hatcheries that rear a variety of salmonids (steelhead and Coho salmon in particular) also suffer dramatic losses.

The ability to manage around the disease by culling eggs from heavily infected broodstock would likely provide an overall reduction of disease incidence at a facility by limiting the pathogen's ability to be vertically transmitted to progeny through the egg, or from eliminating broodstock carriers and providing an overall reduction of pathogen presence at facilities. This approach has worked well for bacterial kidney disease. In addition to benefits associated with developing improved disease management strategies, identifying antigens that may be targeted for vaccine development will be important. If effective vaccine targets are identified, the long-term goal of developing a commercial CWD vaccine would provide a tool to prevent the disease at aquaculture facilities. Currently, such preventative measures do not exist and control relies on antibiotic use.

Anticipated benefits associated with this project will include the availability of additional diagnostic tools (monoclonal antibodies and pathogen detection assays) for broodstock and/or egg culling to minimize CWD outbreaks, identification of potential vaccine candidates, and subsequent reduction of mortalities due to CWD.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Funding for this project became available in February 2008, and a PhD student (Amy Long) was recruited in May 2008, a postdoctoral fellow (Rajesh Kumar) worked on this project from July 2008 to July 2009, and a PhD student (Karol Gliniewicz) has recently joined Dr. Call's lab (2009). A program review of the project was conducted by Dr. Jerri Bartholomew in May 2010, and progress was reported to the WRAC Board. The primary Work Group members were involved in this review and it offered an opportunity to confirm upcoming plans and discuss results to date. A more formal Work Group meeting will be scheduled in November following the next round of sample collections at Troutlodge (currently scheduled for mid-October).

Objective 1: Identify potential vaccine candidates using comparative proteomic analysis of an attenuated strain of *Flavobacterium psychrophilum* and determine if crude cell lysate can be used as a subunit vaccine delivery vehicle in the absence of adjuvant.

The original project proposal included efforts to develop and screen an IVIAT library to detect *in vivo* expressed antigens. As reported last year, while we were able to detect *in vivo* expressed proteins using large culture volumes, we were unable to obtain sufficient analytic sensitivity to differentiate *in vivo*

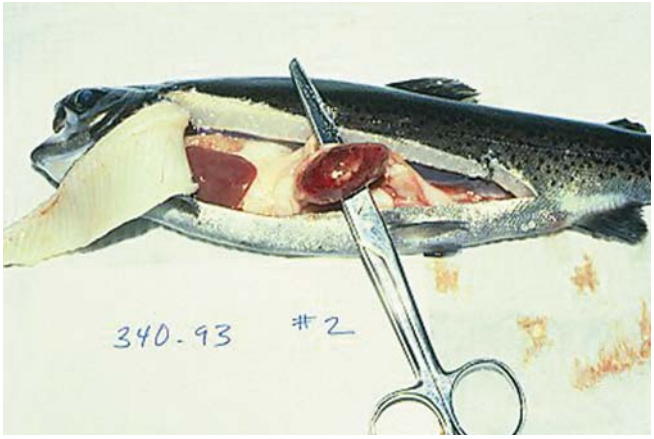
expressed proteins from background when using a high-throughput format. Lacking this higher throughput, there was no practical means to screen the library. We received approval to change our focus for this component of the project to employ comparative proteomics to identify other candidate proteins based on differential expression from a virulent strain (CSF259.93) and an attenuated strain (CSF259.93.B17; "B17"). We also proposed to determine if subunit vaccine candidates can be screened using crude lysate from our *Vibrio parahaemolyticus* expression host. The intent of this second component was to improve the efficiency of subunit testing and reduce overall cost of vaccine administration. Finally, time and resources permitting, we proposed to initiate work to determine why B17 is attenuated with the expectation that this would lead to a more rapid means to develop repeatable and stable attenuation in *F. psychrophilum* and other bacterial pathogens.

Two-dimensional gel electrophoresis experiments demonstrated that a number of proteins are differentially expressed when B17 and CSF259.93 are compared. We have now identified 12 proteins of interest and are in the process of expressing recombinant proteins and verifying their antigenicity. Based on earlier work, we rely heavily on a strain of V. *parahaemolyticus* (strain NY-4) as our host bacterium for expression of recombinant *F. psychrophilum* proteins. *V. parahaemolyticus* usually works better than *E. coli,* and we hypothesize that this is due to a closer match between codon usage for *F. psychrophilum* and *V. parahaemolyticus* compared with *E. coli. Listonella angullarum*, which is closely related to Vibrios, has been used successfully as bacterin without addition of adjuvant. Consequently, we hypothesized that we could used crude lysate from *V. parahaemolyticus* as the vehicle for delivering recombinant protein in lieu of relatively expensive purification procedures.

Our first two trial experiments indicated some support for this hypothesis, but our most recent trial indicates that delivery of recombinant protein in Vibrio lysate by itself is insufficient. Not only did we find no protective response, but a partial analysis of antisera from convalescent fish indicated that lysate-inoculated fish produced a minimal antibody response. Consequently, we have rejected our hypothesis and will continue screening subunit vaccine candidates in conjunction with FCA. Our efforts for Objective 1 will focus on completing work to identify and test immunologically reactive antigens for vaccine development. Furthermore, using data from the differentially expressed proteins, we can now proceed with efforts to determine the mechanism by which B17 is attenuated.

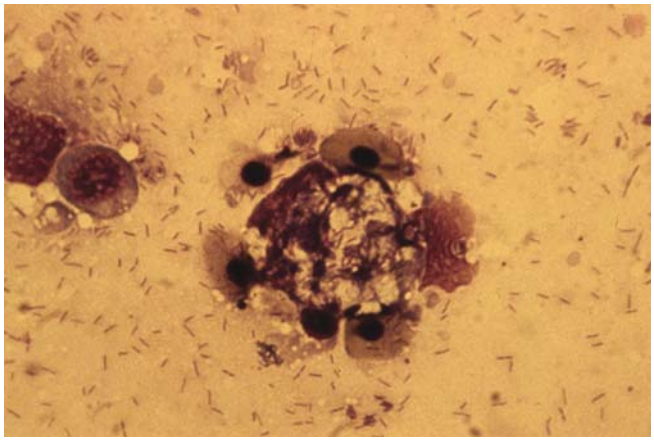
Objective 2: Validate quantitative diagnostic assays (ELISA and ovarian fluid filtration [FAT])

Optimization of the capture ELISA was completed this past year. A challenge experiment was conducted to relate infection levels to disease in fish infected with *F. psychrophilum.* Optical density (OD) values for positive samples ranged from 0.102 to 0.209 ($7.86 \times 10^4 - 2.16 \times 10^5$ CFU ml^{-1}) throughout the challenge. Interestingly, no mortality or gross clinical signs of BCWD (bacterial CWD) were observed, confirming that our



Swollen spleen from a fish infected with coldwater disease.

Courtesy of Clear Springs Foods, Inc.

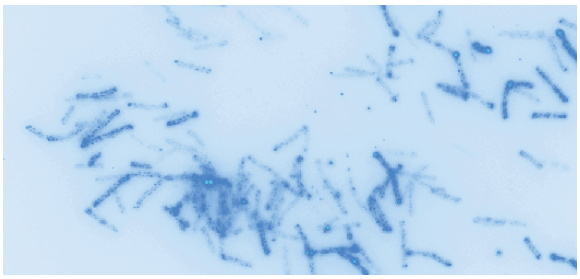


Long thin gram negative rods of F. psychrophilum in an imprint of the spleen of an infected fish.

Courtesy of Clear Springs Foods, Inc.

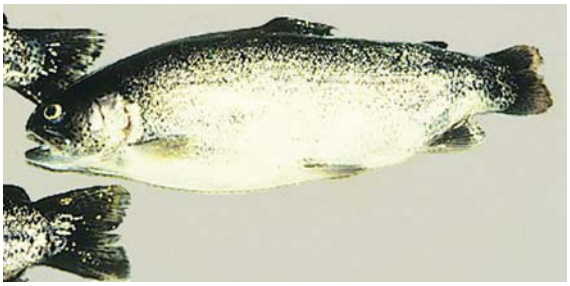
The characteristic yellow-edged lesions of the caudal peduncle caused by *F. psychrophilum*.

Benjamin LaFrentz



F. psychrophilum.

Kenneth Cain



This rainbow trout survived an epizootic of cold-water disease and exhibits spinal compression of the caudal peduncle region.

Benjamin LaFrentz

assay is capable of detecting subclinical *F. psychrophilum* infections.

In February 2010, ovarian fluid and tissue samples were collected from 60 female broodstock at Troutlodge. Samples were tested for *F. psychrophilum,* using nested PCR (nPCR), membrane filtration fluorescent antibody test (MF-FAT), culture, and capture ELISA. The results of the assays were used to select eggs from five families to use in the proposed experiments. All broodstock were infected, but infection levels appeared low when all assays were evaluated together. We selected one family positive only by MF-FAT and four families that were positive by at least two of our assays. Eyed eggs from each family were sent to the University of Idaho and reared in our wetlab facility. Progeny were sampled on a weekly basis and tested by nPCR for *F. psychrophilum.* Disinfected eyed eggs and fry tested positive for *F. psychrophilum* by nPCR. However, YPB growth on TYES-TB plates was not observed until day 36.

Once fry reached 0.5 g, controlled stress experiments were initiated in an attempt to induce a BCWD outbreak. Two different stressors were used: chronic gas supersaturation and handling. Mortalities occurred in all families, but there was no significant difference between families. Random sampling of all tanks was conducted on a weekly basis, including tissue samples used for nPCR. While a confirmed outbreak of BCWD did not occur, we did show an increase in the proportion of fish that tested positive for *F. psychrophilum* in each family. This suggests that fish had not cleared the infection prior to initiating the stress experiment and that *F. psychrophilum* infection once again increased to detectable levels.

Samples were also collected from regional hatcheries this year. Steelhead returning to Wallowa Hatchery (Enterprise, Oregon) were sampled in Spring 2010. Sixty fish were sampled over six weeks, 10 fish per sampling date. Twenty-three percent of kidney samples were positive by the capture ELISA, with the estimated CFU ml^{-1} ranging from 4.92×10^4 to 2.34×10^6. Samples collected from coho salmon spawned in Fall 2003 at two different hatcheries in Washington State were also received to compare against previous ELISA results reported by Lindstrom et al. (2009). Prevalence of infection at the two facilities was 35% and 100%, and estimated CFU ml^{-1} of these samples ranged from 3.85×10^4 to 9.09×10^5.

Objective 3: Based on results from objective 2, develop other assays (e.g., real-time quantitative PCR) for quantification of infection in ovarian fluid.

The gene encoding the antigen on the outer membrane of *F. psychrophilum* that is recognized by MAb FL43 was sequenced by the Call lab this year. Once the nucleotide sequence was known, primers for a Sybr Green assay were developed. Using a TOPO® TA cloning kit, a section of FP1493 was cloned onto a plasmid. The plasmid copy of the gene was then used to optimize qPCR conditions and determine primer efficiency.

Objective 4: Develop an integrated outreach program to meet stakeholder needs.

Outreach activities have resulted in two articles in *Waterlines*. Project deliverables include the commercialization of monoclonal antibody FL43 through Immunoprecise Antibodies, Inc. Labeled antibodies are now being sold to researchers and aquaculture facilities interested in improved diagnostics for BCWD. Protocols for ELISA and FAT assays developed in our lab were provided to Immunoprecise and are included when antibodies are sold.

USEFULNESS OF FINDINGS

Diagnostic improvements are progressing very well and should result in correlation of assays to disease risk in progeny from infected broodstock. This would increase the need for labs to purchase the commercialized antibody and use assay protocols. Vaccine work has shifted from the original objectives, but as we begin to define attenuation mechanisms, this could lead to more efficient ways to create vaccine strains for *F. psychrophilum* and possibly other fish bacterial pathogens.

WORK PLANNED FOR NEXT YEAR

Objective 1: Identify and test potential vaccine candidates based on comparative proteomic analysis of CSF259.93 and CSF259.93.B17 and determine the mechanism of B17 attenuation.

We have completed the proteomic analysis and, with the exception of some material currently in process, we do not plan to identify any additional differentially expressed proteins. At this time, we will proceed with expression of recombinant proteins and verify antigenicity by western blot, using antisera from convalescent rainbow trout. Antigenic proteins will enter our testing "pipeline," which involves immunizing trout followed by challenge with strain CSF259.93 (e.g., Sudheesh et al. 2007). Importantly, each immunization trial requires approximately three months to execute and there are usually only sufficient fish and tanks available to complete one immunization trial at a time. Thus, in the time remaining in this project, we will not be able to assess every protein identified in this study. Instead, we will focus on proteins that are immunologically reactive and are probable virulence factors, such as the Fpp2, although this will depend in part on our ability to produce a sufficient mass of the protein of interest. The expected deliverable for this segment of our project is a manuscript describing the differentially expressed proteins and, if successful, a manuscript describing identification of a vaccine candidate that induces significant protective immunity.

We will also proceed with efforts to determine why strain B17 is attenuated. Our working hypothesis is that attenuation results from altered global transcriptional regulation as evidenced by a significant change in protein expression pattern relative to the pathogenic parental strain CSF259-93. Altered expression is probably a result of a point mutation in the beta subunit (rpoB) of the RNA polymerase, and this year, we have confirmed that one of the expected mutations is present in B17. We predict that the pattern of altered protein expression is repeatable, predictive of attenuation, and can be engineered independently from rifampicin passage.

The mechanism underlying altered protein expression most likely involves a change in how the RNA polymerase binds promoter sequences or in how the RNA polymerase interacts with key transcriptional factors (sigma factors). There is insufficient time (one year) and resources remaining for this project to fully test both mechanisms. Nevertheless, we are well positioned to determine if promoter sequence binding plays a role in this process. That is, we will determine if mutations in the rpoB affect the efficiency of promoter binding using the differentially regulated genes as "bait" for this assay. This will be followed by introducing an identical mutation into a pathogenic strain of *F. psychrophilum* to recapitulate attenuation. The rationale for this series of experiments is that we will identify the specific mechanism involved in rifampicin attenuation (currently a knowledge gap), which will provide the opportunity to generate a genetically engineered and potent live-attenuated strain that will also be unlikely to revert to virulence. Knowledge of the precise mechanism of rifampicin attenuation may also offer a possibility of creating attenuated strains of other bacterial pathogens.

Objective 2: Validate quantitative diagnostic assays (ELISA and ovarian fluid filtration [FAT])

Validation of the diagnostic assays will continue this year. We anticipate sampling again at Troutlodge this fall and screening 60 female broodstock for *F. psychrophilum* using the standard

diagnostic assays. As before, five families will be selected for further experiments. In addition, we will continue to collect samples from regional hatcheries. Samples will be collected in Fall 2010 from Skookum Creek Fish Hatchery (Acme, Washington), a coho salmon spawning facility, and in Spring 2011 from Wallowa Hatchery. Not only do the samples from naturally returning fish aid in validating our assays, but we can also use the data to compare the prevalence of *F. psychrophilum* in coho salmon, rainbow trout, and steelhead.

Objective 3: Based on results from objective 2, develop other assays (e.g., real-time quantitative PCR) for quantification of infection in ovarian fluid.

Optimization of the qPCR assay will continue in the upcoming year. Sensitivity and specificity of this qPCR assay will be evaluated. Once that has been done, we will focus on DNA extraction from ovarian fluid and eliminate any possible inhibition. Ovarian fluid samples that have been collected from various hatcheries over the past two years have been stored at -80°C and will used in the qPCR assay. This will allow us to evaluate the efficiency of the assay as well as compare results to those obtained by other assays for the same samples.

In addition to the above assays, we have recently partnered with Infoscitex, a biotech company that was awarded a Phase I USDA/SBIR grant to develop new diagnostic assays for *F. psychrophilum* that use aptamers as the detection molecules. Aptamers are short sequences of DNA or RNA nucleotides that can be used in place of classical antibodies in ELISA assays. Reporter molecules can also be added to aptamers so that they can be used in qPCR. Phase 1, production of an aptamer specific to FP1493 (the protein recognized by FL43), is underway. Such assays have the potential for greater sensitivity than those currently being tested.

Objective 4: Develop an integrated outreach program to meet stakeholder needs.

Based on project deliverables, we will evaluate the need for workshops and develop a draft WRAC Extension publication. We will continue to work with Immunoprecise to inform stakeholders of the availability of improved diagnostic tools.

IMPACTS

The primary impact is the commercialization of monoclonal antibody FL43 through Immunoprecise Antibodies, Inc. This is now being sold to research labs and aquaculture companies in the un-conjugated form or conjugated to FITC or HRP. Protocols for the capture ELISA and FAT have been distributed to fish health labs in the region. Furthermore, we have provided these protocols to ImmunoPrecise to be distributed to customers when they purchase FL43.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

Refereed publications

LaFrentz BR, LaPatra SE, Call DR, Wiens GD, Cain KD. Proteomic analysis of *Flavobacterium psychrophilum* cultured *in vivo* and in iron-limited media. *Diseases of Aquatic Organisms* 87:171–182. PMID: 20099411.

Lindstrom NM, Call DR, House ML, Moffitt CM, Cain KD. A quantitative enzyme-linked immunosorbent assay (ELISA) and filtration-based fluorescent antibody test (FAT) as potential tools to screen broodstock for *Flavobacterium psychrophilum* in broodstock. *J of Aquatic Animal Health* 21:43–56. PMID: 19485125.

Plant KP, LaPatra SE, Cain KD. Vaccination of rainbow trout (*Oncorhynchus mykiss*) with recombinant and DNA vaccines produced to *Flavobacterium psychrophilum* heat shock proteins 60 and 70. *J of Fish Diseases* 32(6):521–34.

Plant KP, LaPatra SE, Call DR, Cain KD. *In review.* Immunization of rainbow trout (*Oncorhynchus mykiss*) with *Flavobacterium psychrophilum* proteins elongation factor-Tu, SufB Fe-S assembly protein and ATP synthaseb.

General articles

Cain KD. 2009. Strategies for control and prevention of coldwater disease. *Waterlines* 15(1):18–20.

Cain KD, Call DR, Snekvik KR. 2010. A tail of two diseases (coldwater disease and strawberry disease research) *Waterlines* 16(1):10–11.

Presentations

Gliniewicz K, Cain K, Snekvik K, Call D. The role of rpoB in the attenuation of *Flavobacterium psychrophilum* after passage with rifampicin. Poster presented at the 10th Annual College of Veterinary Medicine Research Symposium, October 14, 2009.

Long A, Call DR, Cain KD. Comparison of diagnostic techniques for detection of *Flavobacterium psychrophilum* in ovarian fluid. Talk presented at the 50th Western Fish Disease Workshop and AFS Fish Health Section Annual Meeting. Park City, Utah. June 7–10, 2009.

Gliniewicz K, Snekvik K, Cain K, LaPatra S, Call D. Assess-

ing the immune-protective potential of FP1493 against coldwater disease in rainbow trout. Poster presented at American Society for Microbiology general meeting, San Diego, California, May 2010.

Lanier A, Kumar R, LaPatra S, Gliniewicz K, Snekvik K, Cain K, Shah D, Call D. Production of recombinant *in vivo* induced proteins of *Flavobacterium psychrophilum* for development of a cold water disease vaccine for rainbow trout. Poster presented at the WSU Showcase, Pullman, Washington, March 2010.

Long A, Call DR, Cain KD. 2010. Use of diagnostic assays to screen rainbow trout (*Oncorhynchus mykiss*) broodstock for *Flavobacterium psychrophilum*. Talk presented at the 6th International Symposium for Aquatic Animal Health and AFS Fish Health Section Annual Meeting. Tampa, Florida, September 5–9.

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		UNIVERSITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
2008	81,555						81,555
2009	80,043						80,043
2010	81,637						81,637
2011	81,639 (approved 10/09)						81,639
TOTAL	324,874						324,874

Determining Ripeness in White Sturgeon Females to Maximize Yield and Quality of Caviar

REPORTING PERIOD

September 1, 2009–August 31, 2010

AUTHOR

Molly Webb

FUNDING LEVEL

$100,001 (first-year budget)
$100,001 (second-year budget)
$100,001 (third-year budget)

PARTICIPANTS

Molly Webb*	Montana State University	Montana
Serge Doroshov*	University of California, Davis	California
Barbara Rosco*	Washington State University	Washington
Anna Cavinato*	Eastern Oregon University	Oregon
Wendy Sealey*	Montana State University	Montana
Gary Fornshell*	University of Idaho, Extension	Idaho
Linda Lemmon	Blind Canyon Aqua Ranch	Idaho
Leo Ray	Fish Breeders of Idaho	Idaho

TECHNICAL ADVISOR

Fred Conte	University of California, Davis	California

INDUSTRY ADVISOR

Peter Struffenegger	Sterling Caviar, LLC	California

* funded participants

PROJECT OBJECTIVES

The long-term goal of this study is to develop a less invasive, faster, and better predictor of maturity than oocyte polarization index (PI) in sturgeon, and the overall objective is to correlate current predictors of maturity with instrumental and biochemical assays conducted at different stages of ovarian maturity in white sturgeon. The specific objectives are to:

1. Determine how currently utilized morphological characteristics (oocyte polarity index [PI]), ovarian follicle size, gonadosomatic index, age, and live weight) correlate with caviar quality and yield (years 1–2).
2. Determine how plasma sex steroid, total calcium, plasma protein levels, and crude chemical composition of eggs change with maturity (years 1–2).
3. Evaluate short wavelength near infrared spectroscopy (SW-NIR) and ultrasound as a non-invasive technique to predict fish maturity by taking spectra of gonads in fish (years 1–2).
4. Evaluate Fourier transform infrared spectroscopy (FT-IR) as a method to predict fish maturity from spectral measurements of blood and roe (years 1–2).
5. Using SW-NIR and plasma steroids, determine whether it is possible to detect the early signs of ovarian atresia to avoid sacrificing fish with inferior quality roe and use them during the next production cycle (year 1)
6. Conduct training and outreach programs at field sites in Idaho and California (years 3–4).

ANTICIPATED BENEFITS

Developing an accurate and less invasive predictor of maturity will allow farms to select white sturgeon during the stages of late vitellogenesis and final maturation for their optimal caviar harvest time. Females harvested at the optimal time will have the greatest yield and highest quality caviar as assessed by firmness, flavor, and shelf life. An accurate predictor of maturity will also prevent the slaughter of fish that have started ovarian follicular atresia and allow these fish to be used for caviar production after the second ovarian cycle. Harvesting caviar at the optimal time of ovarian development will also result in an increase in yield for the caviar industry in the Western Region.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

During the first year of the project, biological samples from two groups of sturgeon held at Sterling Caviar, LLC, were collected to address Objectives 1 though 5. The first group of sturgeon was sampled specifically to address whether it is possible to detect early signs of ovarian atresia to avoid sacrificing fish with inferior quality roe (Objective 5, referred to as the *Atresia Study*). This data was reported previously. The second group of females was harvested for caviar to address whether stage of maturity can be assessed less invasively using the proposed tools (SW-NIR, FT-IR, plasma steroids measured by radioimmunoassay [RIA]), and to determine if there is a correlation between oocyte PI and caviar quality and yield (Objectives 1–4; herein referred to as the *2008 Caviar Study*). In 2009, females in both California and Idaho were segregated by stage of maturity in the fall by calculating oocyte PI, and these segregated groups were harvested at different times in the spring when each group would have fully grown eggs to determine whether our alternative sampling techniques (FT-IR, SW-NIR, and plasma steroids) could accurately determine oocyte PI. This study is herein referred to as the *2009 Caviar Study.* In 2010, females in both California and Idaho were segregated into two groups (low oocyte PI and high oocyte PI) using FT-IR in the fall. The FT-IR currently appears to have the highest predictive power for determining stage of maturity. Fish were harvested in the winter and spring accordingly and comparisons were made between the actual oocyte PI and the calculated PI predicted from FT-IR. This study is herein referred to as the *2010 Caviar Study*. In 2010, we also sampled females at caviar harvest and at spawning, for additional SW-NIR studies referred to as the *Path Length Study.* This report is organized by study, with methods for each, followed by results.

2008 and 2009 Caviar Study Results

Regression analyses of pooled samples did not reveal a correlation of caviar yield with oocyte PI (2008: n=97, r = 0.117; 2009: n=82, r = 0.014; 2008 and 2009 pooled: n= 179, r = 0.004), suggesting that fish maturity was not a main factor affecting roe yield in this population. The lack of a relationship between the oocyte PI and caviar yield was likely due to the highly variable caviar yield in the population caused by ovarian adiposity, particularly in larger fish. Three categories of ovarian adiposity, high fat (HF), medium fat (MF) and low fat (LF) ovaries, corresponded well to the caviar yield per ovary. In general, the HF group had the highest mean body weight, smallest eggs, and the lowest caviar yields per fish, fish body weight, and ovarian weight. The best producers of roe were the females in the LF group: they had a lower mean body weight and condition factor, but produced the highest caviar yield per body weight and ovary weight.

SW-NIR 2008 and 2009 Caviar Study Results

All spectra acquired during the California and Idaho samplings conducted in 2008 and 2009 were merged to generate a larger dataset that comprises fish from both facilities. When merging spectra collected on California sturgeon with spectra collected on Idaho sturgeon, no segregation according to location was observed, indicating that spectral differences are potentially related to ovary development and not genetic or other environmental differences between fish at the two different farms.

When predicting oocyte PI using a model built on spectra collected directly on eggs, 76% tagged as having low or medium PI were correctly classified, and 70% of spectra tagged as high PI were also correctly classified. Spectra collected in the abdominal area yielded comparable results, with 74% of all spectra tagged as having low or medium PI correctly classified, and 70% tagged as high PI also correctly classified.

To optimize SIMCA predictions based on different oocyte PI cutoff values, a systematic investigation was conducted by tagging spectra as either low or high PI (merging medium and high PI into one category named "high") with cutoff values for low PI ranging from 0.12 to 0.15. An improvement in the prediction of low PI values from 74% to 81% was observed when the PI cutoff value was set at 0.13. No significant improvement was observed above 0.13. No changes in the percentage of correctly classified high PI were observed, possibly due to the limitation of assigning discrete tags to eggs that actually develop as a continuum. A modest increase (from 74% to 76%) in the number of correctly classified low PI spectra was observed for the PI cutoff at 0.13. No significant changes were observed for predictions of high PI values. A final analysis was conducted using a value less or equal to 0.18 for low oocyte PI, between 0.19 and 0.26 for medium, and greater than 0.26 for high oocyte PI. The 0.18 cutoff for low oocyte PI increased cross-validation SIMCA predictions for eggs with low oocyte PI to 84% of correctly classified spectra, but did not significantly improve predictions for spectra collected through the abdominal walls. These findings may indicate that subtle differences in oocyte PI may not be detected through the abdominal walls under the current experimental conditions.

FT-IR 2008 and 2009 Caviar Study Results and Application to 2010

Based on the previous results showing the relationship between maturity level of sturgeon (histological measurement) and the concentration of biochemical compounds in plasma (Lu et al. 2010 and Accepted; Webb et al. 2001, Linares-Casenave, et al. 2003), we continue to investigate the relationship between PI values and chemical compounds in plasma and try to sort fish according to maturity levels on the basis of plasma chemistry analysis and various chemometric models. It was validated that the oocyte PI values of 0.05, 0.1, 0.15, 0.20, and 0.25 are critical points (periods) for sturgeon maturity segregation and oocyte PI values of 0.1, 0.15, and 0.20 are most important. Many combinations of segregation models were tried during model establishment and prediction, and we confirmed that this segregation gave the best result according to fish maturity levels. Class analog analysis was performed for continuous segregation of fish on the basis of different oocyte PI ranges by supervised soft independent modeling of class analog, and a 90% correction rate of sorting based on spectral variations was achieved.

All techniques (FT-IR, SW-NIR, and plasma sex steroids) were used to predict oocyte PI and compared to the actual oocyte PI in the fall of 2009. When comparing the predictive power of each technique in the fall (predicted vs. actual oocyte PI), FT-IR appeared to be most accurate. Therefore, fish were sorted by the oocyte PIs predicted from FT-IR in the fall of 2009 for harvest in 2010. Two different types of PLSR models were established.

In Plan A PLSR, the models were constructed using spectra from fish plasma from 2007, 2008, and 2009, randomly selected with a leave-one-out cross validation to challenge the rigorousness of the calibrated model (n=10). Then, the oocyte PI values of fish sampled in 2010 were predicted based upon this validated calibration model. For Plan B PLSR, the spectra from fish plasma samples from 2007, 2008, 2009, and five fish samples from 2010 were randomly selected and used for model establishment, and leave-one-out cross validation was performed as described above (n=10). The PI values of the remaining fish (n=75) from 2010 were predicted based upon both of these validated calibration models.

Strictly speaking, only Plan A PLSR is "truly" predicting the PI values of the samples in 2010, and Plan B PLSR is only cross validating additional samples from 2010 because Plan B incorporates spectral features of the fish sampled in 2010 (n=5) into the calibration model. In general, Plan B appears to provide better predictions of oocyte PI (n=75)(93% accuracy) than Plan A (92% accuracy) due to incorporation of spectral features of fish harvested in the year of 2010 (five fish) suggesting that there may be unique features from year to year

that could affect the overall predictive power of these models. Interestingly, when all data are used to generate a global model rather than randomly selected fish from year classes, the mathematics overwhelm the model. Further work will be done to derive a global model in the following year.

Path Lengths Study Results

A study was performed to demonstrate that near infrared light in the 700–1100 nm range can penetrate through skin and muscle layers in a sturgeon and illuminate the underlying gonads. To this extent, a didymium filter, typically used as a near infrared wavelength calibrator, was employed to include characteristic spectral features that could be detected as a function of tissue thickness. Following the disappearance of these spectral features as the filter was laid under increasingly thick portions of abdominal walls, it was determined that the maximum light penetration depth for the current probe design is approximately 2 cm.

Outreach

The Work Group meeting was held in Davis, California, on August 16–17, 2010 to discuss the previous year's results, plan work for the following year, and discuss any technical challenges. At least one, possibly two, Extension publications will be drafted. One publication will address atresia, sorting females according to PI, and implementation of the predictive tool to optimize caviar harvest. The other publication will address the sturgeon reproductive cycle and the use of ultrasound to distinguish males and females. We will work to inform producers on the use and implementation of the predictive tools.

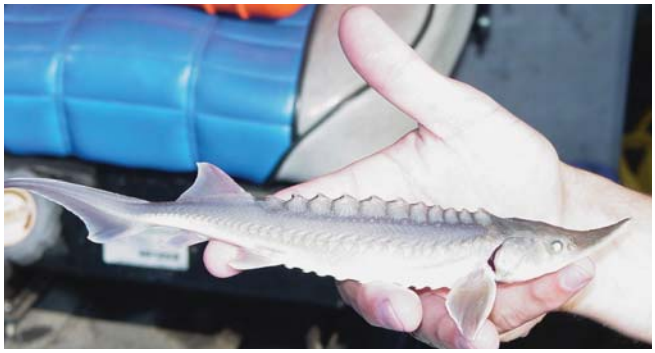
USEFULNESS OF FINDINGS

The spectral analysis of plasma by FT-IR and abdominal scans by SW-NIR were suitable for detecting maturity stages in lieu of biopsy and the oocyte PI. However, the analysis of caviar harvest during 2008 and 2009 (for 7- and 8-year-old fish, respectively) in California found that the stage of maturity (oocyte PI) does not necessarily correlate with higher caviar yields. Adiposity of the sturgeon ovary appeared to be a major factor that negatively affected caviar yield, particularly in larger, faster growing fish. It should be noted that this analysis does not invalidate the importance of detecting maturity stage by non-invasive methods because the quality of caviar is also a critical component in this proposal.

To date, FT-IR can successfully predict oocyte PI with approximately 90% accuracy, and SW-NIR can successfully

Spectra were collected directly on the ovaries in correspondence to the positions on the skin.

Courtesy of WRAC sturgeon caviar study



Juvenile white sturgeon (*Acipenser transmontanus*).

United States Geological Survey staff /life.nbii.gov

White sturgeon (*Acipenser transmontanus*).

Division of Public Affairs, US Fish and Wildlife Service, National Digital Library

predict oocyte PI with 64%–84% accuracy. Models will be refined to further determine the predictive power of these tools.

WORK PLANNED FOR NEXT YEAR

During fall 2010, we will use FT-IR to sort fish in both California and Idaho into three groups of oocyte PI. Multiple year classes of fish will be used at both facilities. At the fall sampling, blood will be collected for FT-IR and a sample of ovarian follicles will be collected for calculation of oocyte PI. The fish assigned to the low PI group will be harvested early, the fish in the medium PI group will be harvested in the middle, and the fish in the high PI group will be harvested late in the caviar harvest season. At harvest, body weight, fork length, age, ovary weight (fat included), raw egg weight (after screening, before salting), caviar yield, caviar grade, and ovarian follicles for calculation of oocyte PI will be collected. Comparisons will be made between the actual oocyte PI and the calculated PI predicted from FT-IR as well as yield among the low, medium, and high groups of oocyte PI. We will continue to refine FT-IR models to increase the sample size used to generate a model.

For SW-NIR, efforts in the next year will focus on identifying alternative classification tools and software packages that allow for more flexibility of data analysis. Possibly a chemometrician consultant will be hired to provide additional expertise in data analysis. Results from the light penetration study indicate that short wavelength near infrared light can penetrate through the abdominal walls of sturgeon up to a distance of 2 cm. We will contact fiber optics probe manufacturers to explore the possibility of designing and building a more sensitive probe with greater penetration depth for SW-NIR.

IMPACTS

The initial studies indicate that SW-NIR and FT-IR can determine stage of maturity (oocyte PI) and may be useful as an alternative method for pre-screening caviar females prior to harvest. The application of these new methods under field and commercial conditions will be further tested and refined. Some of these methods, (e.g., SW-NIR) may be applied to monitor ovarian fat accumulation (Folkestad et al., 2008) for optimizing caviar yield and selective breeding for lean ovaries.

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Lu S, Talbott M, Webb M, Van Eenennaam J, Linares-Casanave J, Doroshov S, Rasco B. Accepted. A study of morphological and immunochemical parameters associated with ovarian atresia and quality of caviar in white sturgeon (*Acipenser transmontanus*) females by Fourier transform infrared spectroscopy (FT-IR) and multivariate analysis. *Aquaculture*.

Lu X, Webb M, Talbott M, Van Eenennaam J, Palumbo A, Linares-Casenave J, Doroshov S, Struffenegger P, Rasco B. 2010. Distinguishing ovarian maturity of farmed white sturgeon (*Acipenser transmontanus*) by Fourier transform infrared spectroscopy: a potential tool for caviar production management. *J of Agricultural and Food Chemistry* 58:4056–4064.

Webb MAH, Van Eenennaam JP, Feist GW, Linares-Casenave J, Fitzpatrick MS, Schreck CB, Doroshov SI. 2001. Effects of thermal regime on ovarian maturation and plasma sex steroids in farmed white sturgeon, *Acipenser transmontanus*. *Aquaculture* 201:137–151.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

Manuscripts

Lu S, Talbott M, Webb M, Van Eenennaam J, Linares-Casanave J, Doroshov S, Rasco B. Accepted. A study of morphological and immunochemical parameters associated with ovarian atresia and quality of caviar in white sturgeon (*Acipenser transmontanus*) females by Fourier transform infrared spectroscopy (FT-IR) and multivariate analysis.

Lu X, Webb M, Talbott M, Van Eenennaam J, Palumbo A, Linares-Casenave J, Doroshov S, Struffenegger P, Rasco B. 2010. Distinguishing ovarian maturity of farmed white sturgeon (*Acipenser transmontanus*) by Fourier transform infrared spectroscopy: a potential tool for caviar production management. *J of Agricultural and Food Chemistry* 58:4056–4064.

Servid SA, Talbott MJ, Van Eenennaam JP, Doroshov SI, Struffenegger P, Webb MAH, Cavinato AG. Accepted. Rapid and non- invasive characterization of ovarian follicular atresia in cultured white surgeon by near infrared spectroscopy. *Aquaculture*.

Talbott MJ, Webb MAH, Van Eenennaam, JP, Linares-Casenave J, Doroshov SI, Guy CS, Struffenegger P. Accepted.

Plasma sex steroid concentrations during onset of ovarian atresia in farmed white sturgeon, *Acipenser transmontanus*. *Aquaculture.*

Presentations

Cavinato AG. Adapting research to meet regional needs, 237th ACS National Meeting, Salt Lake City, Utah, March 2009 (invited presentation).

Sealey W, Webb MAH, Doroshov SI, Cavinato A, Rasco B. Determining ripeness in white sturgeon females to maximize yield and quality of caviar, Idaho Aquaculture Association, Twin Falls, Idaho, June 12, 2010.

Servid SA, Cavinato AG. Rapid and noninvasive characterization of ovarian follicular atresia in cultured white sturgeon by near infrared spectroscopy, 239th American Chemical Society National Meeting, San Francisco, California, March 2010.

Talbott MJ, Van Eenennaam JP, Linares-Casenave J, Doroshov SI, Guy CS, Webb MAH. Investigating non-invasive methods to predict follicular atresia and spawning readiness in white sturgeon, AFS 139th Annual Meeting, Nashville, Tennessee, August 30–September 3, 2009.

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SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		UNIVERSITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
2007-08	100,001		18,500	79,986		98,486	198,487
2008-09	100,001	5,000	18,500	79,226		102,726	202,727
2009-10	100,001		19,000	71,023		90,023	190,024
TOTAL	300,003	5,000	56,000	230,235		291,235	591,238

Optimizing Dietary Protein and Energy Utilization to Improve Production Efficency of Tilapia in the Western United States

REPORTING PERIOD

May 1, 2010–August 31, 2010

AUTHOR

Wendy M. Sealey

FUNDING LEVEL

$66,704

PARTICIPANTS

Principal Investigators

Wendy M. Sealey*	USFWS, Bozeman Fish Technology Center	Montana
Frederic T. Barrows	USDA, ARS Trout Grains Project	Idaho
Kevin M. Fitzsimmons*	University of Arizona	Arizona
Gary Fornshell (*Outreach Corrdinator*)	University of Idaho Extension	Idaho

TECHNICAL ADVISOR

Chhorn E. Lim	USDA ARS Aquatic Animal Health Laboratory	Alabama

INDUSTRY ADVISOR

Tark Rush	Desert Springs Tilapia	Arizona

* funded participants

PROJECT OBJECTIVES

1. Identify the optimum dietary protein-to-energy ratio(s) in practical diets for two different size classes of tilapia.
2. Evaluate the ability of vitamin supplementation to improve growth performance at different protein:energy ratios.
3. Further evaluate formulations identified with potential by laboratory testing in a pilot-scale on-farm trial.
4. Develop an integrated outreach program, including at least one WRAC Extension publication to meet stakeholders' educational needs.

ANTICIPATED BENEFITS

Formulations optimized for fish grown in the high-density systems used in the Western United States will increase utilization of dietary nutrients, thus increasing production efficiency and reducing feed costs.

PRINCIPAL ACCOMPLISHMENTS

Prior to initiation of the project, PI (Wendy Sealey) relocated from University of Idaho in Hagerman, Idaho, to the USFWS, Bozeman Fish Technology Center (Bozeman Center) in Bozeman, Montana, in late August 2009. First-year funding for the project was received in May 2010. This move necessitated pursuing permitting approval for tilapia from the state of Montana prior to their importation. Exotic Species Permit (for use in research) approval was granted to Wendy Sealey and the Bozeman Fish Technology Center on April 7, 2010, pending negative disease testing of fry.

Objective 1a

In collaboration with the industry, common stocks and appropriate lines of tilapia for use in studies both in Arizona and Montana were identified. Stocks chosen were those maintained at the University of Arizona, which maintains pure stocks of both *Oreochromis niloticus* and a red hybrid of *Oreochromis mossambicus. A*s suggested by the WRAC Board, year one studies in Arizona will evaluate both tilapia species. Unseasonably cold weather delayed onset of tilapia spawning in Arizona. Upon initiation of spawning, fry were collected from several spawns and mixed until adequate numbers of fry were obtained. Permit approval from the state of Montana required disease testing of stock populations and fry prior to shipping. Disease certifications were conducted by the University of Arizona. Following submission of disease certification paperwork to the state of Montana, fry were successfully shipped to the Bozeman Center. Fry will be used in Objective 1c when fish reach adequate size to consume an extruded pellet.

Objective 1b

Experimental diets have been formulated as described. Specifically, dietary protein levels were formulated at 28%, 32%, and 36% crude protein. Diets contain minimum levels of fish meal and a constant ratio of animal-to-plant protein sources at all protein levels. Three lipid levels will then be tested at each protein level in a 3 x 3 factorial design in experiments at University of Arizona and two lipid levels tested at each protein level in a 3 x 2 design at the Bozeman Center. Supplemental lipid will be added as soybean oil and lipid-to-carbohydrate ration will be held constant at 1:3. Lipid level will be set at the minimum level to meet the essential fatty acid requirements of tilapia, 3% and two higher inclusion levels, 6% and 9% at University of Arizona and only the low and high tested at Boeman Center. Soybean oil will be the supplemental lipid source in all diets. All ingredients have been sourced and analyzed and the same supply of ingredients will be used for all project participants.

USEFULNESS OF FINDINGS

Tilapia feed prices have increased approximately 28% over the past two year due to increased commodity costs (D. Brock, Rangen, Inc., personal communication). Feed costs for tilapia growers typically comprise 50%–60% of production costs and a 28% increase in feed costs is a considerable increase for growers to absorb. Consequently, an increased precision in protein-to-energy ratio will simultaneously reduce feed costs and reduce the amount of underutilized protein excreted as nitrogenous waste and excess energy stored as fat. Many of the commercially available feeds formulated to meet the needs of tilapia cultured in a pond environment may not be optimal for meeting the needs of tilapia grown in the high-intensity culture systems used in the Western Region of the United States. Specifically, our study will identify formulations that support the rapid growth rates and immunological demands of fish cultured in these high-intensity systems

WORK PLANNED FOR NEXT YEAR

Objective 1b: Outreach and Extension: A project webpage with preliminary results will be developed.

Objective 1c: (Year 1) As previously described, researchers at both the Bozeman Center and University of Arizona will test

the diets produced in objective 1b in late year 1 and early year 2 for their ability to promote growth in two different size classes of tilapia. Specifically, one size class will evaluate the ability of the diets to meet the needs of small tilapia (1–10 grams) when first transitioned from starter feed to a grow-out diet, while the other size class will represent the last portion of the grow-out cycle (200–600 grams).

At the University of Arizona: Small tilapia will be stocked into 200-liter tanks and fed each of the nine experimental test diets. The tanks will be on a common recirculating system and will each receive the same water supply inside a temperature controlled greenhouse. Fifty fish will be group-weighed and randomly stocked into each of the replicate tanks (50 fish per replicate x 3 reps per diet). Initial feeding rate will be 9% of biomass per day and will be split into three feedings per day. Sample weights (10 fish per tank) will be determined every 14 days, and the feeding rate and amount will be adjusted for each treatment as appropriate. Water quality characteristics (dissolved oxygen, temperature, pH, ammonia, and nitrates, will be determined and recorded on a regular basis. For evaluation, diets produced will be fed to the triplicate replicate groups of tilapia for a minimum of three months. At the conclusion of the growth trial, nutrient retention efficiencies of each of the various diets will be determined. The feeding trial will be repeated using similar protocols with the larger size class of tilapia and stocking densities reduced accordingly to maintain water quality.

At the Bozeman Center: Small tilapia (approx. 5 g) will be stocked into 80-liter tanks and fed each of the six experimental test diets for 10 weeks. The tanks will be on a common recirculating system. Fifteen fish will be group-weighed and randomly stocked into each of the replicate tanks (15 fish per replicate x 4 reps per diet). Fish will be fed to satiation daily in two separate feedings. Fish will be counted and bulk-weighed every 14 days. Water quality characteristics (dissolved oxygen, temperature, pH, ammonia, and nitrates) will be determined and recorded weekly. At the conclusion of the growth trial, nutrient retention efficiencies for each of the various diets will be determined. The feeding trial will be repeated using similar protocols with the larger size class of tilapia and stocking densities reduced accordingly to maintain water quality.

Upon completion of both size class studies, work toward Objective 2 will commence. Those vitamins that have been reported to improve performance of tilapia in high-intensity systems will then be added to the diet formulation(s) showing the most promise from year 1 results. Vitamins to be examined include B6, niacin, C, and E, both individually and as a combined supplementation.

IMPACTS

None to date.

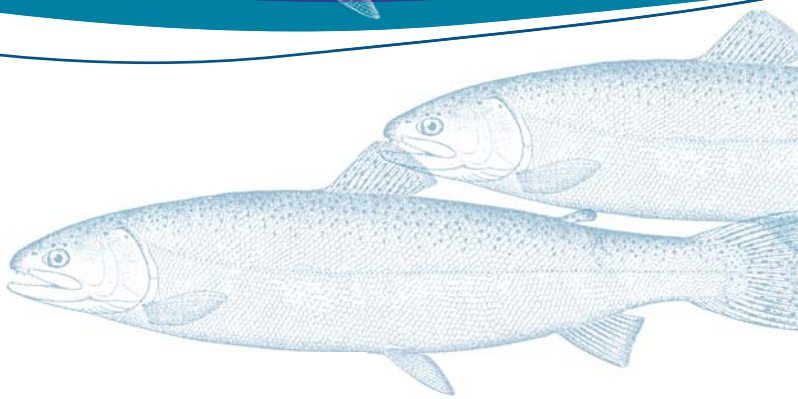
PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

None to date.

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		UNIVERSITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
2010	66,704						66,704
TOTAL	66,704						66,704

Cost-Effective, Alternative Protein Diets for Rainbow Trout that Support Optimal Growth, Health, and Product Quality



REPORTING PERIOD

April 16, 2010–August 31, 2010

AUTHOR

Wendy M. Sealey

FUNDING LEVEL

$119,864

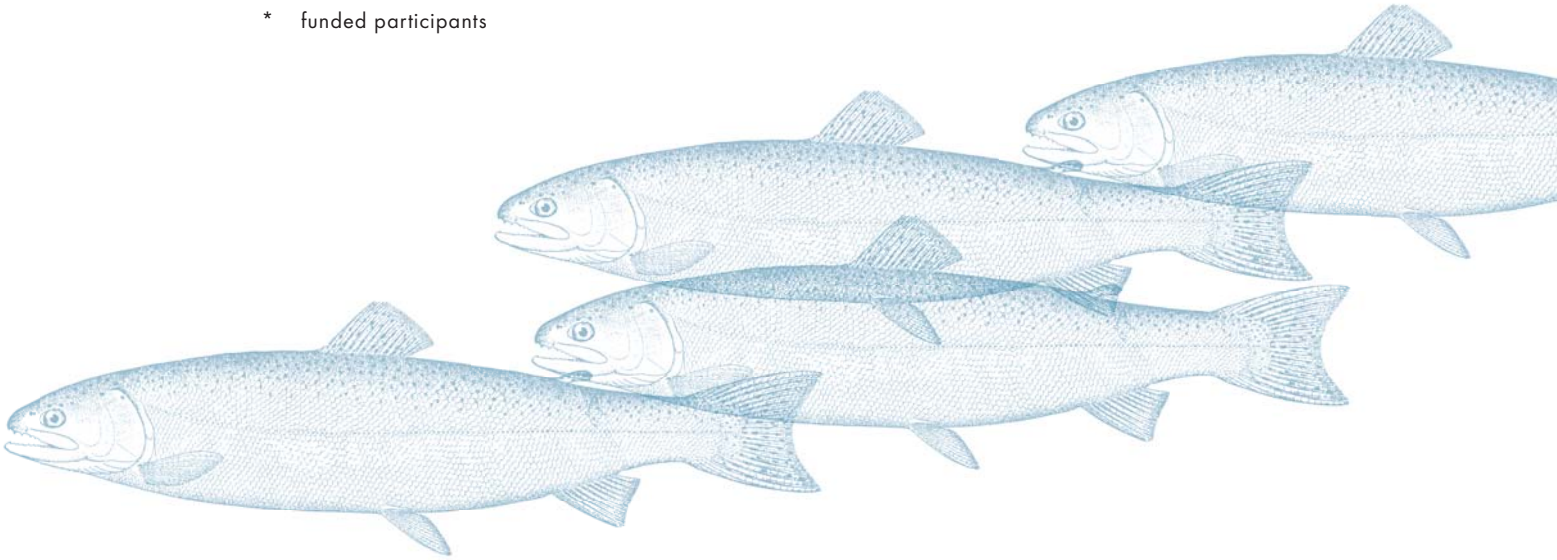
PARTICIPANTS

Wendy M. Sealey*	USFWS, Boseman Fish Technology Center	Montana
Carolyn Ross*	Washington State University	Washington
Christopher A. Myrick*	Colorado State University	Colorado
T. Gibson Gaylord*	USFWS, Boseman Fish Technology Center	Montana
Frederic T. Barrows*	USDA, ARS Trout Grains Project	Idaho
Gary Fornshell* (*Outreach Coordinator*)	University of Idaho Extension	Idaho

TECHNICAL ADVISOR

Chris Nelson	Nelson and Sons, Inc.	Utah

* funded participants



PROJECT OBJECTIVES

1. Identify commercially available alternate ingredient combinations that can meet the production needs of rainbow trout.
2. Refine alternative feedstuff blends and examine the benefits of amino acid supplementation (year 2).
3. Examine the effects of alternative feedstuffs on product quality and fish health.
4. Conduct on-farm trial of alternative feedstuff formulations at Magic Springs Farm, SeaPac of Idaho, (year 3).

5–8. Outreach (years 1–3):

5. Develop a project website on the WRAC homepage and update regularly.
6. Present research results in cooperation with field day and meetings.
7. Develop at least one WRAC Extension publication, "Alternative ingredient utilization in trout diets"
8. Visit feed manufacture plants in the Western Region to present projects results.

ANTICIPATED BENEFITS

Objective 1a and 1b. The aquaculture industry will be able to formulate trout diets using the ingredients tested on an equal digestible nutrient basis. The resulting data will provide an increased understanding of the amino acid needs of trout. The amino acid content and the availability of commercially available alternative feed ingredients will help the industry produce cost-effective feeds while maintaining fish growth, health, and product quality.

Rainbow Trout (*Oncorhynchus mykiss*).

Eric Engbretson, Eric, US Fish and Wildlife Service

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

To address year 1's objectives, the methods of Cho et al. (1982) and Bureau et al. (1999) were used to estimate apparent digestibility coefficients (ADCs). Yttrium oxide served as the inert marker. A complete reference diet that met or exceeded all known nutritional requirements of trout was blended with the test ingredients in a 70:30 ratio (dry-weight basis) to form test diets. Diets were manufactured by cooking extrusion (DNDL-44, Buhler AG, Uzwil, Switzerland) and top-coating with fish oil, using a vacuum coater (A.J. Mixing, Ontario, Canada). Digestibility trials for rainbow trout were conducted at the Bozeman Center. Each diet was fed to three different tanks of fish for 14 days prior to fecal collection. Fecal samples were obtained in three collections by manual stripping, 16–18 hour post-feeding for trout. Fecal samples for a given tank were freeze dried and stored at -20°C until chemical analyses were performed.

More than 25 commercially available and novel ingredients have been sourced. Analyses were conducted through standard procedures (AOAC 1995) to determine the proximate composition (moisture, ash, crude protein, starch, lipids, and gross energy) of the feed ingredients as well as the mineral and amino acid content. Apparent digestibilities of nutrients, and energy and amino acid availability from the ingredients in compounded, extruded diets have also been determined.

USEFULNESS OF FINDINGS

With the rapid rise in feed ingredient costs likely to continue for the foreseeable future and the finite source of fish meal, alternative aquafeed ingredients are necessary to minimize cost. An improved understanding of a wider variety of ingredients also improves formulation security and can help buffer price fluxes by providing nutritionists a variety of ingredients to choose from while still meeting nutrient demands when competition for high protein ingredient occurs or a current ingredient becomes unavailable. Limited data on the nutritive value of an ingredient, or synthetic amino acids, may be reasons that alternative ingredients have generally yielded sub-optimal performance. The data generated by these objectives can be used by researchers to improve study designs for assessing performance of alternative ingredients.

WORK PLANNED FOR NEXT YEAR

Objective 1c: (year 2) Determine the growth response to alternative ingredient blends and the effects on palatability

and potential alterations in oxygen demands, using ingredients chosen from year 1 screening for nutrient composition and digestibility.

Objective 2: Refine feedstuff blends based on results from year 1 and Objective 1c. Feeding trials will be performed at Colorado State University and the Bozeman Center to determine an optimized blend of ingredients and amino acid supplementation to maximize nutrient utilization and growth. Effects of these blends on nutrient assimilation and metabolic demands will be measured.

Objectives 5-8: (year 2) Begin working on a project website.

IMPACTS

None to date.

PUBLICATIONS, MANUSCRIPTS, AND PAPERS PRESENTED

Papers presented

Sealey WM, Gaylord TG, Barrows FT, Ross C, Myrick C, Fornshell G. Cost-effective, alternative protein diets for rainbow trout that support optimal growth, health and product quality, USTFA session of the World Aquaculture Society Meeting in San Diego, California, March 2010.

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		UNIVERSITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
2010	119,864						119,864
TOTAL	119,864						119,864

Development and Evaluation of Starter Diets and Culture Conditions for Three Subspecies of Cutthroat Trout and Gila Trout

TERMINATION REPORT

PROJECT WORK PERIOD

October 1, 2005–December 31, 2009

AUTHOR

Christopher A. Myrick

PARTICIPANTS

Christopher A. Myrick* [1]	Colorado State University	Colorado
Greg Kindschi*	US Fish and Wildlife Service (USFWS), Bozeman Fish Technology Center	Montana
Gary Fornshell (*Outreach Coordinator*)	University of Idaho	Idaho
Ken Cline [2]	Cline Trout Farms	Colorado
Chris Nelson	Nelson & Sons, Inc.	Utah
John Seals* [3]	USFWS Mora National Fish Hatchery & Technology Center	New Mexico
Molly Webb*	USFWS, Bozeman Fish Technology Center	Montana
Kevin Kappenman*	USFWS, Bozeman Fish Technology Center	Montana

REASON FOR TERMINATION

Objectives completed (with exception of those for Gila trout and ongoing outreach) and funds terminated.

* funded participants

[1] Retired from project team in 2007; replaced by Dr. Webb and K. Kappenman.

[2] Retired prior to the production trial; production trial performed by Jeremy Liley of Liley Fisheries.

[3] Left project in 2008 because of ongoing difficulties with rearing Gila trout.

PROJECT OBJECTIVES

1. Determine the effect of feed texture and formulation on survival, growth, and quality of cutthroat and Gila trout.
2. Determine the effect of water temperature on trout growth, survival, and quality when reared under laboratory conditions.
3. Determine the effect of rearing density on cutthroat trout growth, survival, and quality.
4. Conduct production-scale evaluations of the best diet × temperature × density combinations identified in the first three objectives.
5. Develop outreach products to provide fish culturists and feed manufacturers with information on optimal growth temperatures, optimal rearing densities, and diet formulations for inland cutthroat trout subspecies.

PRINCIPAL ACCOMPLISHMENTS

Objective 1. Effects of diet type on cutthroat trout survival, growth, and quality.

The two principal research institutions, the US Fish and Wildlife Service Bozeman Fish Technology Center (Bozeman Center) and Colorado State University (Colorado State) completed a set of 120-day diet trials on the effects of diet type on the survival, growth, and quality of first-feeding Snake River cutthroat trout (*Oncorhynchus clarki* subspp.), Yellowstone cutthroat trout (*O. c. bouvieri*;), and Colorado River cutthroat trout (*O. c. pleuriticus;*). The Yellowstone and Snake River cutthroat trout trials were conducted at 10.0°C, while the Colorado River cutthroat trout trial was conducted at 10.5°C. A similar study was planned for Gila trout (*O. gilae*) at the US Fish and Wildlife Service Mora National Fish Hatchery and Technology Center (Mora Center), but repeated production problems over the two years that the location received funding prevented the collection of sufficient data.

The diets used in the study were the following: Rangen Regular Trout (RRT), Rangen Soft Moist (RSM), Silver Cup Regular Trout (SCRT), Silver Cup Soft Moist (SCSM), Skretting Nutra-Plus (now Bio-Vita Fry; SNP), Skretting Nutra-Plus with 21 days of Artemia supplementation (CSU only; SNP+A), Experimental 601 (E601), and Experimental 602 (E602).

These diets were selected for inclusion in the study because they were currently available commercial diets or open-formula formulations that could be easily produced by fish feed manufacturers. It was not deemed efficient to try to produce a cutthroat trout specific diet because of the relatively small size of the potential market. All diets were fed at 4% wt/day, which turned out to be an *ad libitum* ration.

The best diet in terms of both survival and growth for Snake River cutthroat trout was SNP, with fish gaining an average of 4.78 g/fish over the 120-day trial, and survival exceeding 97%. The best diet for Yellowstone cutthroat trout was also SNP, with fish gaining 3.73 g/fish and survival of > 97%. With the Colorado River cutthroat trout, the best non-supplemented diet in terms of growth (3.89 g/fish) and survival (77.5%) was also SNP. However, when the SNP+A diet is included, the Artemia supplementation led to a 31% increase in final fish size (5.12 g/fish) and a 5.7% increase in survival. Dorsal fin index, a measure of fin condition used as a metric of fish performance, was unaffected by diet for all three subspecies tested.

One interesting side-note from the Colorado River cutthroat trout studies was that the different diets produced significant differences in fish color. When subjected to a quantitative ranking by a team of trained observers, E601 produced fish with the deepest reds, followed by SNP, SNP+A, RRT and SCSM, SCRT, and, at the yellow end of the spectrum, RSM and E602.

An additional 65-day diet trial was performed at Bozeman Center to compare the effects of different diet supplements on the growth, survival, and performance of Snake River cutthroat trout. The supplements used were: Skretting/Bio-Oregon Bio-Vita (SNP), Skretting/Bio-Oregon Bio-Vita + dry Artemia flake (SNP+DA), Skretting/Bio-Oregon Bio-Vita + freeze dried cyclopeeze (SNP+C), Silver Cup Fry Micro Pellet Salmon (SCFMP), Otohime (OTH), Otohime + freeze dried cyclopeeze (OTH+C), and Silver Cup Regular Trout (SCRT).

The results of this additional diet trial demonstrated that other starter diets (both premium and non-premium) produced the same high levels of survival (> 93%) with Snake River cutthroat trout as did SNP. Additionally, the difference in weight between the fish fed SNP and those fed SNP+A, SNP+DA, SNP+C, OTH, or OTH+C were not significant, indicating that, for Snake River cutthroat trout at least, there is no clear benefit to diet supplementation, and, more importantly, that the use of any typical premium diet provides a similar level of growth performance.

Based on these results, it is clear that the SNP diet, or a similar high-performance diet, is ideal for first-feeding of juvenile cutthroat trout, irrespective of subspecies. Additionally, supplementation of the prepared diets with live Artemia

Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*)

United States Bureau of Land Management

Snake River cutthroat trout (*Oncorhynchus clarki* subspp.)

Craig D. Young

Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri;*).

US Forest Service, US Department of Agriculture

during the first 21 days of feeding can confer a significant growth and survival advantage, particularly in cases where overall survival is lower than that seen at the Bozeman Center. Finally, if fish color is important, SNP, SNP+A, or any diet with astaxanthin supplementation (e.g., E601) can dramatically change the appearance of the fish.

Objective 2. Effects of water temperature on cutthroat trout survival, growth, and performance.

Snake River and Yellowstone cutthroat trout were reared at 10°, 12°, 14°, 16°, 18°, and 20°C in a 120-day growth study on first-feeding fish that were fed with *ad lib* rations (4% wt/d) of SNP. Colorado River cutthroat trout were reared for 120 days at 10°, 12.5°, 15°, 17.5°, and 20°C in a study that compared both the effects of temperature and diet (SNP+A) and Rangen Regular Trout, supplemented with live Artemia for 21 days (RRT+A). A system failure at Colorado State forced the termination of the 10°C treatment on Colorado River cutthroat trout, but otherwise the study proceeded without complications.

The results from the Snake River cutthroat trout study demonstrated the classic temperature × growth relationship, with increasing growth rates as temperature increased from 10°C to 16°C, and a decrease in growth rate between 16°C and 20°C. Regression analysis of the growth data identified the optimal temperature for growth as 14.5°C. A similar result was seen for Yellowstone cutthroat trout, with the optimal temperature for growth identified as 14.7°C. Survival of Yellowstone and Snake River cutthroat trout was unaffected by water temperature, likely because of the high-quality rearing conditions at the Boseman Center. The results for Colorado River cutthroat trout also followed the same pattern, irrespective of diet type, with optimal growth temperatures of 15.3°C for fish fed RRT+A and 16.4°C for fish fed SNP+A. A significant difference in actual and predicted growth was noted between diets, with fish fed SNP+A consistently growing larger than their counterparts fed RRT+A at the same temperature. The maximum predicted difference between the two diets at the optimal temperatures was close to 57%. Unlike the Snake River and Yellowstone cutthroat trout, however, rearing temperature did affect survival of Colorado River cutthroat trout in an inverse manner, with generally higher survival at temperatures below 15.0°C, independent of diet. Fin condition was unaffected by temperature for all three subspecies.

The objective 2 studies were able to successfully identify the optimal growth temperatures for rearing Snake River,

Yellowstone, and Colorado River cutthroat trout. They also provided information that may prove useful to resource managers trying to understand the bioenergetic responses of cutthroat trout populations in natural or hatchery settings at different temperatures.

Objective 3. Effects of density on cutthroat trout survival, growth, and performance.

Snake River and Yellowstone cutthroat trout were reared at densities of 50 to 350 fish per tank (100L tanks) to measure the effects of rearing density on survival, growth, and performance. A similar study was conducted with Colorado River cutthroat trout, but because of the results from the Snake River and Yellowstone cutthroat trout, the densities were increased to 150, 300, 400, and 600 fish/tank. Fish were reared at the optimal temperatures identified in objective 2 (14.5°C for Yellowstone and Snake River cutthroat trout; 16.4°C for Colorado River cutthroat trout) and were fed *ad lib* rations of SNP.

Growth rates of Snake River and Yellowstone cutthroat trout were slightly, but significantly, affected by rearing density, with fish reared at the 50 fish/tank weighing significantly more (20% for Snake River cutthroat trout; 15% for Yellowstone cutthroat trout) than those reared at 350 fish/tank. No differences in survival were observed between Yellowstone cutthroat trout density treatments, but Snake River cutthroat trout reared at the lowest density had slightly higher survival than those reared at 350 fish/tank (97.5% vs. 91.7%). Colorado River cutthroat trout also showed a significant density effect, with significantly lower final wet weights for fish reared at 600 fish/tank (8.1 g/fish) compare to those in the 150, 300, or 450 fish/tank treatments (final wet weights of 11.9, 12.1, and 10.4 g/fish, respectively). Interestingly, despite the significant effects of density on the growth of the three subspecies, fin condition showed no density effect.

Objective 4. Production-scale trial using Snake River cutthroat trout.

A production-scale growth trial using Snake River cutthroat trout was conducted at the Liley Fisheries facility (formerly the Cline Trout Farms Boulder Facility). First-feeding Snake River cutthroat trout were held at ambient well-water temperatures (13°C–14°C during the study) and were fed SNP at a maximum of 4% wt/d. The goal of this experiment was to see how closely the actual growth of the fish under production conditions matched the predicted growth of Snake River cutthroat trout based on the results previously found in this project.

The results of the production trial showed that the actual final wet weight of the fish (3.62 g/fish) were very close to the predicted wet weights (4.0 – 4.13 g/fish) for fish reared at 13 – 14°C. The slight difference in performance may result from the differences in culture systems, but overall, the results showed that it is possible to closely approximate production-level results from the data collected under laboratory conditions.

Objective 5. Develop outreach products on cutthroat trout production.

The primary outreach product for this project, a WRAC Extension publication on cutthroat trout production techniques, is currently being developed. During the course of the project, project personnel delivered a number of presentations at industry meetings (e.g., US Trout Farmers Association, US Aquaculture Association, Colorado Aquaculture Association) and to local, regional, and national fisheries meetings. A

RESULTS AT A GLANCE...

- First-feeding cutthroat trout have the highest growth rates and survival when fed premium trout or salmon diets.
- Additional benefits in early survival and growth may be gained by supplementing prepared diets with live Artemia for Colorado River Cutthroat trout.
- Optimal temperatures for rearing cutthroat trout are subspecies-specific, ranging from 14.5°C–14.7° for Snake River and Yellowstone cutthroat trout, to 16.4°C for Colorado RIver cutthroat trout.
- Rearing density has a slight, but significant, effect on growth, with fish reared at lower densities growing faster than those reared at higher densities.

project website was developed early on, and portions of it have recently been included in the general WRAC website.

IMPACTS

Development of culture conditions for first-feeding cutthroat trout

Relevance: In 2004, the aquaculture industry in the Western United States requested a comprehensive research project on the development of culture conditions (diet, temperature, rearing density) for first-feeding cutthroat trout of various subspecies. This request was made because past attempts to culture cutthroat trout using diets and techniques developed for rainbow trout had not been very successful.

Response: From 2005 through 2009, a multi-institution team conducted a series of experiments on the effects of diet type, water temperature, and rearing density on the survival, growth, and performance of first-feeding Snake River, Colorado River, and Yellowstone cutthroat trout. An additional production-scale experiment was conducted to verify that results from laboratory studies could be adequately transferred to production-level efforts.

Results: The study demonstrated that first-feeding cutthroat trout have the highest growth rates and survival when fed premium trout or salmon diets like Skretting Nutra Plus/Bio-Oregon Biovita Fry. Additional benefits in early survival and growth may be gained by supplementing prepared diets with live Artemia for Colorado River cutthroat trout. The optimal temperatures for rearing cutthroat trout are subspecies-specific, ranging from 14.5°C to 14.7°C for Snake River and Yellowstone cutthroat trout, to 16.4°C for Colorado River cutthroat trout. Rearing density does have a slight but significant effect on growth, with fish reared at lower densities growing faster than those reared at higher densities. The production-scale trial demonstrated that the results of the laboratory studies could be used to predict growth rates at a production-scale.

Impact: The Colorado Division of Wildlife's Glenwood Springs Fish Hatchery switched to the premium diet used in the Colorado River cutthroat trout study (e.g., Skretting Nutra Plus/Bio-Oregon Bio-vita Fry) as a direct result of this study. The use of live Artemia supplementation during the first 21-days of production has not yet been adopted on a wide scale because of the added cost. However, a state hatchery in Alaska did express interest in using the technique to try to improve early survival of first-feeding grayling.

Collaborators: Colorado State University faculty in the College of Natural Resources, University of Idaho faculty in the Cooperative Extension Service, US Fish and Wildlife Service personnel at the Bozeman Fish Technology Center, industry personnel at Cline Trout Farms, Liley Fisheries, and Nelson and Sons, Inc.

Contact: Dr. Christopher A. Myrick, Department of Fish, Wildlife, and Conservation Biology, Colorado State University, Fort Collins, CO 80523. Email: *chris.myrick@colostate.edu*.

RECOMMENDED FOLLOW-UP ACTIVITIES

Additional studies on the performance of these subspecies of cutthroat trout are probably not warranted. However, additional studies on the performance of additional cutthroat trout subspecies, particularly in regard to optimal rearing temperatures, would improve the ability of producers to predict growth rates and yields. Additionally, the western aquaculture industry should continue to work with state and federal agencies to gain greater access to cutthroat trout for production purposes.

PUBLICATIONS, MANUSCRIPTS, AND PAPERS PRESENTED

Publications in Print

Brandt MM. 2009. Optimal starter diets and culture conditions for Colorado River cutthroat trout (*Oncorhynchus clarkii pleuriticus*). Colorado State University, Fort Collins, Colorado.

Kindschi GA and coauthors. 2009. Performance of Yellowstone and Snake River cutthroat trout fry fed seven different diets. *North American Journal of Aquaculture* 71(4):325–329.

Brandt MM. 2008. Optimal culture conditions for first-feeding Colorado River cutthroat trout. *The Fishline* 20(2):1–16.

Papers Presented

Myrick CA, Brandt M, Kindschi G, and Barrows F. Getting ahead in a cutthroat world: optimizing culture conditions for Snake River, Colorado River, and Yellowstone cutthroat trout. 60th Northwest Fish Culture Conference, Redding, California, 2009.

Brandt MM, Myrick CA. Optimizing survival and growth of first-feeding Colorado River cutthroat trout—diet temperature and density implications. Western Division of the American Fisheries Society, Albuquerque, New Mexico, 2009.

Myrick CA. Fish nutrition research at Colorado State University. Department of Animal Science Seminar, Fort Collins, Colorado, 2009.

Brandt MM, Myrick CA. Optimizing survival and growth of first-feeding Colorado River cutthroat trout—diet temperature and density implications. Annual Meeting of the Colorado-Wyoming Chapter of the American Fisheries Society, Loveland, California, 2008.

Brandt MM, Myrick CA. Optimizing survival and growth of first-feeding Colorado River cutthroat trout—diet temperature and density implications. Aquaculture America, Seattle, Washington, 2009.

Brandt MM, Myrick CA. Getting ahead in a cutthroat world: optimal starter diets and rearing temperatures for Colorado River cutthroat trout. Idaho Aquaculture Association 2008 Annual Meeting, Twin Falls, ID, June 21–22, 2008.

Brandt MM, Myrick CA. Effect of diet and rearing temperature on the performance of first-feeding Colorado River cutthroat trout. Colorado-Wyoming ChapterAmerican Fisheries Society Meeting, Fort Collins, Colorado, March 3–6, 2008.

Brandt MM, Myrick CA. Effect of diet and rearing temperature on the performance of first-feeding Colorado River cutthroat trout. Aquaculture America and World Aquaculture Society Joint Meeting, Orlando, Florida, February 9–12, 2008.

Brandt MM, Myrick CA. Determination of optimal starter diets and rearing temperatures for Colorado River cutthroat trout. Colorado Aquaculture Association Annual Meeting, Mt. Princeton, Colorado, January 18–19, 2008.

Brandt MM, Myrick CA. Getting ahead in a cutthroat world —performance of Colorado River cutthroat trout fed eight starter diets. 137th Annual Meeting of the American Fisheries Society, San Francisco, California, September 2–6, 2007.

Brandt MM, Myrick CA. Getting a head start: growth of Colorado River cutthroat trout fed eight starter diets. Colorado-Wyoming Chapter American Fisheries Society Meeting, Fort Collins, Colorado, February 26–March 1, 2007.

Brandt MM, Myrick CA. Getting a head start: growth of Colorado River cutthroat trout fed eight diets. Colorado Aquaculture Association Annual Meeting, Mt. Princeton, CO, January 19–20, 2007.

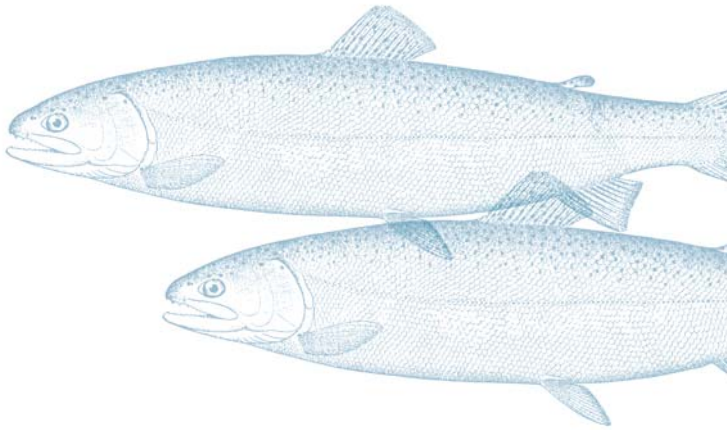
Fornshell G. Aquaculture in the West. A WRAC Perspective (and other stuff too). Colorado Aquaculture Association Annual Meeting, Mt. Princeton, Colorado, March 3–4, 2006.

Myrick CA. Development and evaluation of starter diets and culture conditions for 3 subspecies of cutthroat trout and Gila trout: an introduction to the upcoming WRAC project. Colorado Aquaculture Association Annual Meeting, Mt. Princeton, Colorado, December 10–11, 2004.

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		UNIVERSITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
2006	99,991	5,000	5,000	81,500	5,500	97,000	196,991
2007	95,677	25,829	5,000	90,000	5,500	126,329	222,006
2008	91,973	5,000	5,000	45,000		77,000	168,973
2009	35,328	8,882	20,000		1,000	29,882	65,210
TOTAL	322,969	44,711	35,000	216,500	12,000	330,211	653,180

Potential Threat of VHS Virus in the Western United States



TERMINATION REPORT

PROJECT WORK PERIOD

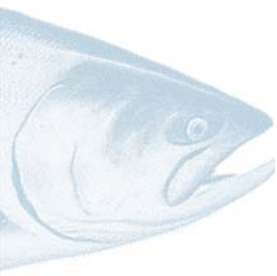
July 1, 2008–August 31, 2009
No-cost extension approved through August 31, 2010

AUTHOR

Gael Kurath

PARTICIPANTS

Gael Kurath	USGS Western Fisheries Research Center (WFRC), Seattle	Washington
Jim Winton	USGS-WFRC, Seattle	Washington
Paul Hershberger	USGS-WFRC, Marrowstone Marine Station	Washington
Carolyn Friedman*	University of Washington, Seattle	Washington
Jerri Bartholomew* (*Outreach Coordinator*)	Oregon State University	Oregon
Chang Hoon Moon (*Postdoctoral Fellow*)	University of Washington	Washington
Evi Emmenegger	USGS-WFRC, Seattle	Washington



INDUSTRY ADVISOR

Scott E. LaPatra — Clear Springs Foods, Inc. — Idaho

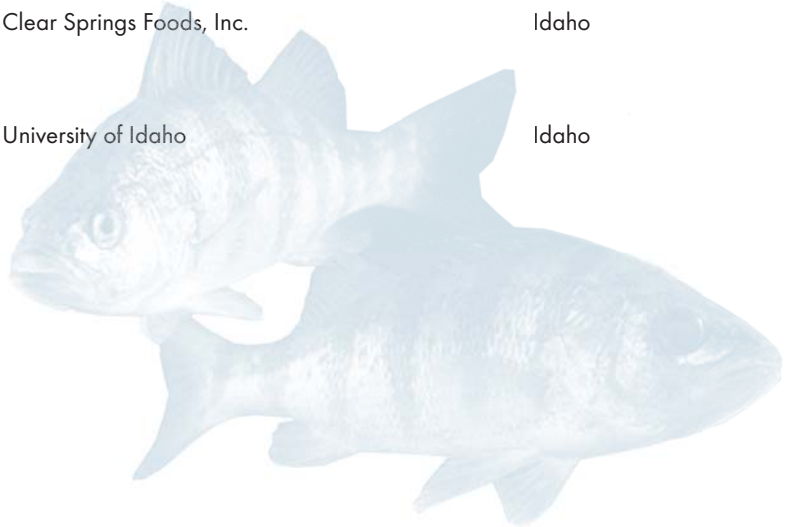
TECHNICAL ADVISOR

Kenneth Cain — University of Idaho — Idaho

REASON FOR TERMINATION

Project complete, funds expended

* funded participants



PROJECT OBJECTIVES

1. Assemble and distribute biosecurity information currently available for dealing with VHSV.
2. Develop diagnostic assays to differentiate Great Lakes VHSV IVb from endemic West Coast VHSV IVa.
3. Test susceptibility of yellow perch, rainbow trout, herring, and Chinook salmon to disease and mortality caused by Great Lakes VHSV IVb, West Coast VHSV IVa, and European VHSV I.
4. Test ability of relevant host species to act as carriers and/or reservoirs of different VHSV genotypes.
5. Develop outreach materials to communicate project results.

PRINCIPAL ACCOMPLISHMENTS

Funding for this project became available in July 2008. Dr. Chang Hoon Moon was hired as a post-doctoral fellow in August 2008, and worked full-time on the project in the Kurath laboratory at the USGS Western Fisheries Research Center (WFRC) until the funding was expended in November 2009. There have been no funded personnel on the project since November 2009.

Objective 1. Assemble and distribute biosecurity information currently available for dealing with VHSV.

Biosecurity information for the European VHSV type I and the Great Lakes VHSV type IVb strains was assembled in year 1 and a PowerPoint presentation focusing on biosecurity was prepared by the outreach coordinator, Jerri Bartholomew. The presentation has been given at a workshop for fish farmers and one for Extension agents, and also to the WRAC office early in year 2 for use on the WRAC website. This information, together with results of the research project, has been assembled into a draft "WRAC FAQs about VHSV" document that is intended to answer frequently asked questions concerning VHSV, with a focus on issues pertinent to the Western US user groups: fish farmers, anglers, and fish health diagnosticians. This FAQ sheet is currently in review by Extension personnel and will be published as a USGS fact sheet available on both the WFRC and WRAC websites. General information on VHSV from a national perspective has been assembled into fact sheets available from various other agencies, including the Northern Regional Aquacuture Center. Rather than duplicate this effort, we have provided links to these resources on our WRAC FAQs sheet. During the project, six oral presentations on VHSV that include information on biosecurity issues were given by project investigators.

Objective 2. Develop diagnostic assays to differentiate Great Lakes VHSV IVb from endemic West Coast VHSV IVa.

During this project, we have used a general quantitative real-time polymerase chain reaction (qRT-PCR) developed by Dr. Kyle Garver of the Pacific Biological Station in Nanaimo, British Columbia. The development of genotype-specific qRTPCR assays that specifically detect type IVa only, or type IVb only, was originally a priority for this project, but during the project period several factors arose that caused us to consider this a less urgent need. Results obtained in objectives 3 and 4, dynamic changes initiated at the national level for standardization of VHSV diagnostics, and availability of existing genetic typing methods that distinguish genotypes IVa and IVb, all combined to support a decision not to pursue development of genotype specific qRT-PCR assays at this time.

Objective 3. Test susceptibility of yellow perch, rainbow trout, herring, and Chinook salmon to disease and mortality caused by Great Lakes VHSV IVb, West Coast VHSV IVa, and European VHSV I.

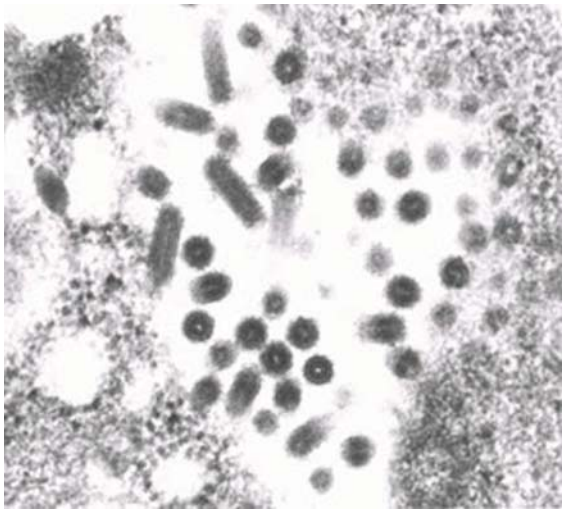
This objective has been completed and is being prepared for publication in a peer-reviewed journal by the first author, Evi Emmenegger. Susceptibility of four fish species to disease and mortality due to Great Lakes VHSV type IVb (designated VHSV IVb-GL) has been determined in controlled wet laboratory challenge studies, where virus was delivered by intraperitoneal (IP) injection at two different viral doses referred to as "low," and "high." For comparisons of different VHSV genotypes, West Coast type IVa, Great Lakes type IVb, and European type I strains were tested simultaneously. In addition, an Atlantic Coast VHSV type IVb strain from New Brunswick (IVb-NB) was included in challenge studies when possible. All infection studies were conducted on juvenile fish (2–5g) at 12°C in the WFRC Biosafety Level 3 (BSL-3) wet-lab. Mortality in mock-infected control groups was negligible.

Virulence trials by IP injection in yellow perch: Yellow perch are used in this project as a positive control host for the Great Lakes VHSV IVb, since epidemics have occurred recently in yellow perch in the Great Lakes. Challenges were conducted by IP injection with high and low doses of VHSV strains representing genotypes I, IVa, IVb-GL, and IVb-NB. In the

high-dose treatment groups, average final cumulative percent mortality (CPM) ranged from 84%–100%, and low-dose treatments groups ranged from 30%–93%. Relative virulence of the four VHSV strains, in order from highest to lowest, was IVa > IVb-GL > IVb-NB > I. In general, by the IP injection delivery route, yellow perch were highly susceptible to all strains, but the low-dose treatment indicated it is least susceptible to VHSV genotype I.

Fish (WHAT TYPE? infected with VHS virus.

Mohyammed Faisal



VHS virus.

Mohmmed Faisal

Virulence trials by IP injection in rainbow trout: Rainbow trout are used here due to their significance as a major western aquaculture species, and also as a positive control host for VHSV genotype I. In the high-dose injection group, CPM was 3%–86%, and in low dose groups, CPM was 2%–98%. Relative virulence of the four VHSV strains, in order from highest to lowest, was I >> IVa = IVb-NB > IVb-GL. In general, by IP injection, rainbow trout were highly susceptible to genotype I, but not very susceptible to genotypes IVa, IVb-GL, or IVb-NB.

Virulence trials by IP injection in Chinook salmon: Chinook salmon were also tested as an important western aquaculture species. In the high-dose treatment groups, CPM ranged from 13%–76%, and in low-dose groups, CPM was 5%–47%. Relative virulence of the four VHSV strains, in order from highest to lowest, was I >> IVb-GL=IVb-NB > IVa . In general, the susceptibility pattern of Chinook salmon was similar to that of rainbow trout, but Chinook had slightly lower mortality with genotype I, and seemed nearly resistant to the new genotype IVb-GL.

Modified virulence trials by IP immersion in herring: Pacific herring were included in this project as a positive control host species for VHSV genotype IVa, which has caused large-scale marine epidemics of herring and pilchard off the West Coast. A trial was conducted in herring using challenge by immersion rather than injection, and CPM ranged from 43%–80%. As expected, herring were very susceptible to genotype IVa, and only moderately susceptible to the other three VHSV genotypes.

Objective 4. Test ability of relevant host species to act as carriers and/or reservoirs of different VHSV genotypes.

This objective involves comparing infection levels of individual VHSV strains in different fish hosts regardless of the occurrence of disease signs or mortality. Quantification of virus in fish that died during the injection challenges described above showed that all VHSV strains replicated in all fish species, but with slightly lower titers in Chinook. Among asymptomatic fish that were randomly sampled at seven days post-challenge (dpc), yellow perch, rainbow trout, and Chinook salmon showed replication of all virus strains. In herring sampled 14 dpc, all virus strains persisted in most fish, but at 1–2 logs lower titers than in herring that died. In survivors, samples at 30 dpc, yellow perch and Chinook salmon showed all four virus strains persisted in at least some fish, but in rainbow trout

only genotypes I and IVa persisted in a small number of fish.

To compare *in vivo* growth of VHSV genotypes I, IVa, and IVb-GL, time-course experiments were conducted, measuring viral loads in rainbow trout or yellow perch challenged by immersion at 15°C. Virus replication peaked at day 3 for all three strains. In rainbow trout, the viral load of VHSV genotype I was 1–2.5 logs higher than genotypes IVa and IVb. This variation in viral growth among the three VHSV strains correlated very well with the levels of mortality in the rainbow trout virulence trials. Host immune gene response was also measured, and mirrored viral loads, being most notable in fish infected with genotype I. A similar time-course in yellow perch at 12°C showed more rapid replication of VHSV type IVa and IVb to higher viral load levels than type I, again correlating with the differences between VHSV types in virulence challenge studies. Collectively, these data indicate that the four fish species tested differ in their susceptibility to disease and infection by the various VHSV types, and they can all act as carriers and reservoirs of Great Lakes VHSV.

Objective 5. Develop outreach materials to communicate project results.

Four oral presentations and one poster focusing on data from this project have been presented by project investigators at regional and national fish health meetings. Data from the project was also included in four of the six general talks listed under objective 1. An invited presentation was made to the Idaho Aquaculture Association, and separate workshops for fish farmers and Extension personnel have been held. Educational materials that provide project results include the Powerpoint presentation and "WRAC FAQs about VHSV" fact sheer created by Jerri Bartholomew. These also include biosecurity information and are detailed in objective 1.

IMPACTS

Relevance: VHSV was first isolated from fish in the Great Lakes in 2003, and was reported in 2005 as the causative agent of a large-scale dieoff of freshwater drum in Lake Ontario. Since then, numerous epidemics in multiple host species have occurred in the Great Lakes region, resulting in an extreme level of concern and severe restrictions on aquaculture activities. This project involved outreach and research objectives that address specific needs of fish farmers in the Western Region of the United States, and contribute to the national response to the emergence of VHSV in the Great Lakes.

Response: The Kurath lab conducted research to address concerns of western aquaculture producers about the threat of the Great Lakes strain of VHSV for western aquaculture species. Four strains of VHSV, representing genetic types from the Great Lakes, the Pacific Coast, the Atlantic Coast, and Europe were tested for virulence and persistence in four fish species. The fish selected for study were relevant to western aquaculture or to VHS epidemics in the Great Lakes and the Pacific Ocean. Funds of $100,000 supported a 15-month research and outreach project.

WHAT WE'VE LEARNED ABOUT VHS VIRUS THAT CAN HELP TO CONTROL ITS SPREAD…

- VHSV can infect a wide variety of fish and survivors may be lifelong carriers—*importing any species represents a risk.*
- Signs of disease can be variable and many fish die with no signs—*difficult to detect without culture/PCR.*
- Transmission is horizontal—*importation of properly disinfected eggs a low risk.*
- Virus persists days-weeks in water, longer in sediment—*need to disinfect equipment.*
- Disease is most likely to occur in spring as temperatures fluctuate—*reduce stress during this period.*
- Drying and heat are effective in inactivating the virus.
- Freezing significantly reduces virus infectivity—*Live bait represent a higher risk than frozen; however, freezing does not eliminate risk.*

Results: Variations in virulence and persistence in different fish hosts were identified for different strains of VHSV. No biological difference was demonstrated between VHSV strains from the Great Lakes and those currently endemic in Pacific marine fish species. Rainbow trout and Chinook salmon had low susceptibility to North American VHSV strains but high susceptibility to European VHSV. In contrast, yellow perch and Pacific herring were more susceptible to the Great Lakes strain than the European strain.

Impact: Two workshops were held to communicate these results along with biosecurity information to fish farmers and Western Region Extension agents. Biosecurity measures to prevent introduction of any VHSV into aquaculture facilities will reduce costs to producers, both in terms of disease impacts and regulatory compliance. Several general VHSV talks and talks on the project results were presented to various interest groups, and a document titled "WRAC FAQs about VHSV" has been prepared as a fact sheet.

RECOMMENDED FOLLOW-UP ACTIVITIES

The results obtained in this project have succeeded in answering the most urgent questions pertinent to salmonid aquaculture in the Western United States. by finding low susceptibility, but carrier potential, in rainbow trout and Chinook salmon. Follow-up activities might include:

- Testing virulence and carrier potential in Atlantic salmon, hybrid striped bass, and/or sturgeon.
- Testing virus delivered by immersion in fish species where mortality was seen by injection.
- Conducting susceptibility studies with wild/free-ranging fish species present in the West that is already on the list of 28 host species in the Great Lakes. VHSV IVb has been isolated from these species, but many have not been tested for level of susceptibility to disease, which would assess the risk they pose as potential reservoir species if VHSV IVb is introduced in the West.
- If VHSV IVb is introduced and is detected in western aquaculture facilities, this would elevate the priority of developing genotype specific qRT-PCR assays for VHSV IVa and IVb.

RECOMMENDATIONS...

- Improve pathogen detection.
- Enhance surveillance.
- Rigorous biosecurity in culture facilities.
- Eliminate fish transfers between waters or require testing.
- Improve practices of bait industry and restrict risky uses of baitfish.
- Require emptying live wells and bilges when leaving boat launches in positive areas.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

Publications Online

Bartholomew J, Problems presented by emerging pathogens: Potential threat of Great Lakes VHS virus in the Western United States. PowerPoint provided for addition to WRAC website.

Manuscripts in Preparation

Emmenegger EJ, Moon CH, Kurath G. Variable susceptibility of salmonid, cyprinid, clupeid, and percid fish species to VHSV genotypes from freshwater, brackish water, and marine environments. In preparation for *Diseases of Aquatic Organisms*.

Bartholomew J, Kurath G. WRAC FAQs about VHSV. In review for publication as a USGS fact sheet.

Papers Presented

Kurath G. VHSV: The history and basics of the virus and the disease. 33rd Eastern Fish Health Workshop, VHSV Continuing Education Session, Atlantic Beach, North Carolina, April 4, 2008.

Emmenegger EJ, Kurath G, Wargo A, Binkowski F, Goetz R. Yellow perch (*Perca flavescens*) susceptibility to viral hemorrhagic septicemia virus isolates from Europe and North America. Western Fish Disease Workshop, Ocean Shores, Washington, June 2008.

Kurath G, Emmenegger EJ, Wargo A, Binkowski F, Goetz R. Susceptibility of yellow perch to VHS virus strains from the Great Lakes, Pacific coast, and Europe. AFS-Fish Health Section Annual meeting, Charlottetown, Prince Edward Island, Canada, July 2008.

Kurath G, Emmenegger EJ, Moon CH. Susceptibility of Pacific salmonids, yellow perch, and koi to viral hemorrhagic septicemia virus strains from the Great Lakes, Pacific and Atlantic coasts of Canada, and Europe. Aquaculture America, Invited talk for session on RAC research on VHSV. Seattle, Washington, February 17, 2009.

Kurath G. How we got here: A review of VHS in the US . Invited talk for special "Producers session" on VHSV at Aquaculture America, Seattle, Washington, February 17, 2009.

Kurath G. Fish RNA viruses: VHSV epidemiology and evolution. Invited guest lecture in Fish Disease course at Oregon State University, Corvallis, Oregon, February 23, 2009.

Kurath G. History and current affairs of VHS virus. Invited talk in special VHSV session at the International Conference on Aquatic Invasive Species, Montreal, Canada, April 23, 2009.

Emmenegger EJ, Moon CH, Kurath G. Susceptibility of western aquaculture species to different strains of VHS virus, including Great Lakes VHSV. Idaho Aquaculture Association, Twin Falls, Idaho, June 2009.

Moon CH, Emmenegger EJ, and Kurath G. Susceptibility of Pacific salmonids, yellow perch, and koi to four strains of VHS virus, including Great Lakes VHSV. Poster presentation at AFS Fish Health Section Annual meeting, Park City, Utah, June 10, 2009.

Kurath G. Epidemiology of fish rhabdoviruses part 2: VHSV. Invited guest lecture in Oregon State University Salmon Disease Workshop, Corvallis, Oregon, July 21, 2009.

Bartholomew J. Problems presented by emerging pathogens: Potential threat of Great Lakes VHS virus in the Western United States. Workshop for fish farmers at Hagerman Fish Culture Experiment Station, Hagerman, Idaho, August 14, 2009.

Bartholomew J. Problems presented by emerging pathogens: Potential threat of Great Lakes VHS virus in the Western United States. Workshop for Extension agents, presented in conjunction with WRAC meeting, Spokane, Washington, October 8, 2009.

Kurath G. Viral hemorrhagic septicemia virus: and old virus with new tricks. Plenary talk, 6th International Symposium on Aquatic Animal Health, Tampa, Florida, September 9, 2009.

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		UNIVERSITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
2008 & 2009**	72,097						72,097
2010	27,903						27,903
TOTAL	100,000						100,000

* Substantial in-kind support provided throughout this project included donated time of G. Kurath, E. Emmenegger, P. Hershberger, and J. Winton at the USGS-WFRC lab, and donated pathogen-free fish stocks from P. Hershberger (USGS-WFRC Marrowstone Marine Station), S. LaPatra (Clear Springs Foods, Inc.), and F. Goetz and F. Binkowski (UWM, Great Lakes WATER Institute).

** The funds for this project were emergency funds awarded outside the standard timing of the WRAC funding cycle. Funds became available initially in July of 2008, so that in FY 2008 there were only 2 months of activity on the project. Therefore, FY2008 and FY2009 have been combined together in the table above as "Year 1" of the project, which was funded at $72,097. FY 2010—the funds remaining for year 2 were $27,903, which supported approximately 3 months of work.

Economic Impacts of Private Sector Aquaculture-Based Recreational Fishing in the Western USA

TERMINATION REPORT

PROJECT WORK PERIOD

September 1, 2007–August 31, 2010

AUTHORS

Craig Bond and Daniel Deisenroth

PARTICIPANTS

Principal Investigator

Craig A. Bond*	Colorado State University	Colorado

Co-Principal Investigators

Steve Davies*	Colorado State University	Colorado
John Loomis*	Colorado State University	Colorado
Y. Hossein Farzin	University of California, Davis	California
Andrew Seidl* (*Outreach Coordinator*)	Colorado State University	Colorado

Collaborators

Fred Conte	University of California, Davis	California
Jon Boren	New Mexico State University	New Mexico
Gary Fornshell	University of Idaho Extension	Idaho
Kevin Fitzsimmons	University of Arizona	Arizona
Chris Myrick	Colorado State University	Colorado
Daniel Deisenroth*	Colorado State University	Colorado
Timothy Rakitan*	University of California, Davis	California
Jay Griebling*	Colorado State University	Colorado

INDUSTRY ADVISORS

Kenneth Cline	Cline Trout Farms, Inc.	Colorado
Rebecca Cooper	Cline Trout Farms, Inc.	Colorado

TECHNICAL ADVISOR

Gunnar Knapp	University of Alaska, Anchorage.	Alaska

REASON FOR TERMINATION

Objectives completed.

* funded participants

Background image: US National Oceanic and Atmospheric Association

PROJECT OBJECTIVES

1. Collect primary data from three distinct subpopulations: aquacultural suppliers of recreational fish (ASRF), their direct customers, and recreational anglers, and prepare an economic report quantifying the magnitude and value of the economic contributions of the ASRF industry.
2. Provide an appropriate sampling frame for tracking and documenting trends over time in the ASRF industry for use in subsequent economic analyses.
3. Generate primary research about the impacts of the regulator and competitive environment on the aquaculture industry, including the relationships between private and public hatcheries, interstate trade regulations, and Native American reservation policies.
4. Develop outreach materials (including final report and peer-reviewed, Extension, and popular press articles), and disseminate information at conferences, meetings, etc.

PRINCIPAL ACCOMPLISHMENTS

Objective 1. Collect primary data from three distinct subpopulations: aquacultural suppliers of recreational fish (ASRF), their direct customers, and recreational anglers, and prepare an economic report quantifying the magnitude and value of the economic contributions of the ASRF industry.

1a. Data Collection

Surveys were administered to ASRF producers, ASRF direct customers, and recreational anglers. All surveys were administered according to the well-respected and widely used Dillman Tailored Design Method (Dillman, 2000). Of the 418 permit holders that were identified, 245 indicated that they were not in business, leaving 173 potentially active ASRF producers. Of these, 52 responded (30% response rate).

The second survey, of ASRF customers, was administered between November 2009 and January 2010. Of the 686 surveys originally mailed, 74 respondents' addresses were undeliverable and 20 responded that they were no longer operating a fishery of any type and had not stocked fish recently. Of the remaining 592 potential respondents, 260 mailed their survey back (44% response rate).

The third survey collected data from recreational anglers. We surveyed 1841 anglers at 53 private and public fisheries in California and Colorado in order to obtain the most representative sample possible. There were 1070 surveys returned, for a response rate of 58%.

1b. Economic Contributions of Each Surveyed Group

Using sales and expenditure data from the three surveys, two new sectors were constructed in IMPLAN input-output software, one for ASRF producers and another for ASRF customers. The production functions for these sectors map a dollar of sales of a particular product into a set of expenditures on supplies, equipment, and personnel, collectively referred to as "backward linkages." Results are often reported in the form of economic multipliers, which indicate the magnitude of the "ripple effect" that is generated in a local or regional economy from the economic activity of one industry. For example, the output multiplier for the ASRF industry is $1.85, which means that for every $1.00 of fish sold, $1.85 of total sales or "output value" is generated in the local or regional economy.

ASRF Producers

As stated above, for every dollar spent on ASRF products, $1.85 of total sales or "output value" is generated in the Western Economy. This includes the "direct effect" of ASRF producer sales (valued by definition at $1), the "indirect effect" of $.35 of sales of suppliers of inputs to ASRF producers, and the "induced effect" of $.50 of spending by employees and proprietors of ASRF firms and their suppliers. In addition, every million dollars of ASRF sales results in 21.61 full-time jobs in the Western economy. There are 173 ASRF producers in the Western United States, and these businesses do $53.2 million in direct recreational fish sales annually.

ASRF Customers

The average ASRF customer purchases $2,656 in ASRF products and attributes $13,593 of annual sales to the purchase of these products. An estimated 20,053 ASRF customers exist in the Western United States, purchasing $53.2 million of ASRF products and selling $272.6 million worth of recreational-fish related products to anglers. Using IMPLAN software to construct a new ASRF customer industry sector, model results indicate that every dollar of ASRF customer sales results in an additional $.79 in indirect and induced economic activity in the Western Region. In addition, every million dollars sold supports 41 full-time jobs.

Recreational Anglers

California anglers spend an average of $180 on a typical fishing day for items such as airfare and gasoline, while Colorado anglers spend $135. The average sampled angler spends $150 per day within the Western United States. Using reported average angler expenditure at ASRF customer sites, along with estimated aggregate annual ASRF customer sales, ASRF

industry-induced angler days total $6.99 million annually. Total direct ASRF-induced angler expenditures are estimated to be $1.04 billion annually in the Western Region. IMPLAN software is used to estimate that every dollar of angler expenditures leads to an additional $.83 of economic activity in the region and every million dollars of angler expenditures support 36 full-time jobs.

Forward Linkages and Total Economic Contribution

Accounting for the multiplier effect of ASRF-induced angler expenditures yields a total of $1.913 billion in annual expenditures in the Western Region. The multiplier effect of ASRF-induced angler expenditures results in 26,229 full-time jobs in the Western United States. These economic contributions are rooted in the $53.2 million in ASRF direct sales, implying that every dollar of ASRF producer sales leads to $35.92 in annual within-region output. By the same logic, every million dollars of ASRF sales supports 493 full-time jobs. With most of the producers concentrated in California, Colorado, Oregon, Utah, and Washington, the geographic distribution of the ASRF industry's impact is not uniform. Nearly half of the total economic contribution of the ASRF industry accrues within California.

Although Alaskan hatcheries are excluded from the analysis of the for-profit recreation-based aquaculture industry, secondary data is used to estimate the economic contribution of the not-for profit recreation-based aquaculture industry in that state. The heavily regulated Alaskan salmon enhancement program contributed roughly 345,564 additional fish to the sport fishery harvest in 2008, resulting in an economic contribution of $184 million of output and 1814 jobs in the Alaskan economy during that year.

Objective 2: Provide an appropriate sampling frame for tracking and documenting trends over time in the ASRF Industry for use in subsequent economic analyses.

- In order to track and document trends over time in the ASRF industry, it is necessary to collect data from three distinct subgroups: ASRF producers, their direct customers, and recreational anglers.
- The Colorado State University team compiled relevant information regarding all active ASRF permit holders in the Western United States. There are currently no more than 173 potential ASRF producers identified in the Western United States.
- A list of 686 direct customers to the ASRF industry was identified in Colorado. These customers encompass all potential types of ASRF customers, including municipalities, private ranches and clubs, homeowners' associations, and other private property.
- A sampling frame with 53 policy relevant recreational angling sites was created, and 1841 recreational anglers in both California and Colorado were surveyed.

Objective 3: Generate primary research about the impacts of the regulator and competitive environment on the aquaculture industry, including the relationships between private and public hatcheries, interstate trade regulations, and Native American reservation policies.

One identified area of research is the nature of the regulatory structure of the ASRF industry, especially as the regulators tend to be production competitors. From an economic standpoint, this is an extremely unusual structure, which likely leads to incentives that may not be in line with maximization of social welfare. The team intends to explore this relationship in a theoretical paper that will document the various incentives that result from this structure. A second identified area of research is the potential to account for substitution patterns among anglers that would occur in the absence of the ASRF industry. Current state-of-the-art models of economic activity fail to account for close substitutes in consumption among end-users of a particular product. Researchers at Colorado State University will continue to develop models to capture this substitution over the next year.

A third identified area of research is the potential to account for bioeconomic feedback loops in recreational fish stocking. Fish stocking augments fish population and fishery quality, thereby encouraging angler visitation. However, this angler visitation leads to more fish harvest and lower fishery quality. Accounting for these feedback loops will help future researchers evaluate the benefits and costs of recreational fish stocking. Research along this thread has already been presented at the Agricultural and Applied Economics Association annual meetings in Denver, Colorado in July 2010, and will continue to be developed throughout the next year.

Objective 4: Develop a variety of outreach materials (including a final report and peer-reviewed, Extension, and popular press articles, and disseminate information at conferences, meetings, etc.

In order to inform producers, their customers, and recreational anglers about the nature of the project and the reasons for collecting the data, three FAQ websites were created, one for each

surveyed group. In addition to these websites, 10 presentations about the economic contribution of the ASRF industry have been given to various associations in the West. Furthermore, 10 Extension articles and one economic development report have been authored in order to broaden the audience that is exposed to the results of this study. Finally, researchers at Colorado State University are finalizing an economic study for submission to the *Journal of Aquaculture Economics*. Research over the next year will address angler substitution patterns and fishery bioeconomic feedback loops, and additional manuscripts will be prepared for submission to various peer-reviewed journals. The Extension efforts will continue with completion of the Final Economic Report, preparation and distribution of a multi-page summary of results from the study, and presentations.

IMPACTS

Relevance: While most people are aware of federal and state fish stocking agencies, such as the USFWS or state-level fish and game departments, few are aware of the private aquaculture businesses that grow fish used for stocking in both private and public fisheries. These businesses grow and sell fish for stocking thousands of bodies of water in the Western United States, including municipal, county, and state public waters; private fishing clubs and dude ranches; fee fishing ponds; and private land. Fisheries stocked with ASRF-produced fish supplement fishing opportunities offered by state and federal fisheries. The stocking of fish in public and private waters undoubtedly encourages tourism, which in turn stimulates the economies of the rural communities adjacent to these waters.

Response: In 2006, with producer support, WRAC sponsored a project to assess the economic contribution of the aquacultural suppliers of recreational fish (ASRF). The objectives were to: develop a sampling frame for the industry, its direct customers, and anglers; document the economic contribution of that industry; and develop a set of outreach materials to educate the public about this topic.

Results: Throughout 2008–2009, surveys were distributed to all ASRF producers, 686 of their direct customers, and 1841 recreational anglers in the Western United States. Using IMPLAN input-output models, the economic contribution of the ASRF industry, taking into account both forward and backward linkages, is estimated to be $1.91 billion and to support 26,229 jobs annually in the Western United States. Every dollar of ASRF sales results in a multiplier effect of $35.92 generated in the region, and every million dollars of ASRF sales results in 493 full-time jobs.

Impact: This information will ultimately benefit ASRF producers by acting as an educational tool for the general public and for regulatory agencies. Having this information will help policy makers make informed decisions that potentially could be more favorable to the aquaculture industry.

Collaborators: Faculty at Colorado State University; University of Arizona; University of California, Davis; University of Idaho; and New Mexico State University, along with Cline Trout Farms, Liley Fisheriesm and E & J Fish Farms.

Contact: Dr. Craig A. Bond, Department of Agricultural and Resource Economics, Colorado State University, Fort Collins, CO 80523-1172, *Craig.Bond@Colostate.edu*, (970) 491-6951.

RECOMMENDED FOLLOW-UP ACTIVITIES

There are three primary areas of research that could prove valuable to ASRF producers:

STUDY RESULTS INDICATE...

- The economic contribution of the aquacultural suppliers of recreational fish (ASRF) industry, taking into account both forward and backward linkages, is estimated to be $1.91 billion dollars and to support 26,229 jobs annually in the Western United States.
- Every dollar of ASRF sales results in a multiplier effect of $35.92 generated in the region, and every million dollars of ASRF sales results in 493 full-time jobs.
- This information should inform policy decisions in the future.

1. Waters that are privately stocked by homeowners' associations (HOA) are likely to increase the home value of HOA members. Research documenting the magnitude of this value has not been undertaken, and the estimates in this study do not capture this additional value to homeowners. A primary concern of WRAC stakeholders should be to address this gap in the literature.
2. Another interesting thread of research incorporates net economic value, rather than the economic activity supported by the ASRF industry. Net economic value is the difference between what an angler would be willing to pay for a fishing trip minus the amount that he or she actually pays. As most recreational fishing studies have evaluated net economic value, comparison of net economic value between private and public fisheries in a similar region may lend insight into the formation of policies that could affect the ASRF industry in a positive manner.
3. The Alaskan non-profit salmon enhancement program is primarily intended to augment commercial fish harvests. However, stocked fish that are not harvested by commercial fishermen in fact augment sport fishing catch rates, thereby encouraging tourism and promoting economic activity in Alaska. While this study uses secondary analysis to estimate the economic contribution of hatchery-reared, sport-harvested fishing in Alaska, a more thorough investigation of the linkages between the aquaculture industry and recreation-based economic contributions would prove valuable to any policymaker interested in the health of the Alaskan economy.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

Publications in Print

Deisenroth DB, Bond CA. 2010b. A brief look at the customers of the aquacultural suppliers of recreational fish. *The Fishline* 22,3.

Deisenroth DB, Bond CA. 2010c. Progress report: The economic contribution of the private recreation-based aquaculture undustry in the Western United States. *The Fishline* 22,1.

Deisenroth DB, Bond CA. 2010d. The aquacultural suppliers of recreational fish (ASRF): A look at the freshwater recreational fish industry in the Western United States. Department of Agricultural and Resource Economics Economic Development Report, 3-1, pp.1–11 *http://dare.colostate.edu/pubs/edr10-01.pdf.*

Deisenroth DB, Bond CA. 2010e. The total economic contribution of the private, recreation-based aquaculture industry in the Western United States. *The Fishline* 22,4.

Deisenroth DB, Bond CA. 2009a. Combining information from private aquaculture facilities, private fisheries, and anglers to estimate the economic contribution of the aquacultural suppliers of recreational fish. *The Fishline* 21,4.

Deisenroth DB, Bond CA. 2009b. Moving forward with the economic contribution of the aquacultural suppliers of recreational fish. *The Fishline* 21,3

Deisenroth DB, Bond CA. 2009c. Update: Estimating the economic contribution of the aquacultural suppliers of recreational fish (ASRF) in the Western U.S. *The Fishline* 21,2

Deisenroth DB, Bond CA. 2009d. Update: Estimating the economic contribution of the aquacultural suppliers of recreational fish (ASRF) in the Western United States. *The Fishline* 21,1

Bond CA, Deisenroth DB. 2008a. Estimating the economic impacts of the aquacultural suppliers of recreational fish. *Waterlines http://www.fish.washington.edu/wrac/.*

Bond CA, Deisenroth DB. 2008b. Phase one of Colorado State University study on the economic impacts of the aquacultural suppliers of recreational fish nearing completion. *The Fishline* 20,4.

Bond CA, Deisenroth DB. 2008c. The economic impacts of the aquacultural suppliers of recreational fish phase one nearing completion. *The Fishline* 20,3.

Bond CA, Deisenroth DB. 2007. Colorado State University to lead effort to quantify economic contribution of recreational fish producers. *The Fishline* 19,4.

Websites

Deisenroth DB. Bond CA. 2009. Angler survey frequently asked questions. *http://dare.agsci.colostate.edu/csuagecon/anglersurvey*.

Deisenroth DB, Bond CA. 2009. Privately stocked fishery survey frequently asked questions. *http://dare.agsci.colostate.edu/csuagecon/privatefisheryimpact.aspx*.

Bond CA, Deisenroth CB. 2008. The economic contributions of the suppliers of recreational fish: Frequently asked questions. *http://dare.agsci.colostate.edu/csuagecon/wracimpact.htm*.

Manuscripts

Deisenroth DB. In Preparation. 2010. Incorporating complex spatial substitution patterns and bioeconomic feedback loops into the valuation of a renewable natural resource: The case of a recreational fishery. PhD Dissertation.

Deisenroth DB, Bond CA. In Preparation. 2010a. Accounting for backward and forward linkages to estimate the economic contribution of the private recreation-based aquaculture industry in the Western United States.

Deisenroth DB, Bond CA, Loomis JB. In Preparation. 2010. Combining information from the random utility model with input output models in order to account for substitution effects: The case of a recreational fishery.

Deisenroth DB, Bond CA. In Preparation. 2010b. The economic significance of bioeconomic feedback loops: Incorporating complex spatial substitution patterns into the optimal management of a recreational fishery.

Papers Presented

Deisenroth, D. Combining information from the random utility model with input-output models in order to account for substitution effects: The case of a recreational fishery. North American Regional Science Council Annual Meeting, Denver, Colorado, November 2010.

Deisenroth, D. The economic contribution of the private, recreation-based aquaculture industry in the Western United States. United States Trout Farmers' Association and National Association of State Aquaculture Coordinators joint annual meeting, Branson, Missouri, September 2010.

Deisenroth D. The economic significance of bioeconomic feedback loops: The case of a recreational fishery. Department of Agricultural and Resource Economics Lunch Seminar Series, Fort Collins, Colorado, April 2010.

Deisenroth D. The economic significance of bioeconomic feedback loops: The case of a recreational fishery. Poster presentation, Agricultural and Applied Economics Association annual meetings, Denver, Colorado July 2010.

Bond C, Deisenroth D. Aquaculture and stocking recreational water in the West: A socioeconomic assessment. California Aquaculture Association Special Session, San Diego, California, March 2010.

Deisenroth D. A bioeconomic approach to capturing the economic value and economic contribution of fish stocking in Colorado waters. Departments of Agricultural and Resource Economics and Economics Graduate Student Symposium, Fort Collins, Colorado, February, 2010.

Deisenroth D. The economic contribution of private sector aquaculture-based recreational fishing in the Western USA —Research project update. Colorado Aquaculture Association annual meetings, Mount Princeton, Colorado, January 2010.

Bond C. Economic impacts of private sector aquaculture-based recreational fishing in the Western USA. Western Regional Aquaculture Center IAC/TC annual meeting, Spokane, Washington, October 2009.

Deisenroth D. The economic contribution of the aquacultural suppliers of recreational fish in the Western United States. Western Agricultural Economics Association annual meetings, Lihue, Hawaii, June, 2009.

Deisenroth D. The economic contribution of the aquacultural suppliers of recreational fish in the Western United States. Western Division of the American Fisheries Society Student Colloquium, Fort Collins, Colorado, October 2009.

Deisenroth D. A preliminary look at the aquacultural suppliers of recreational fish in the Western United States. Colorado Aquaculture Association annual meetings, Mount Princeton, Colorado, January 2009.

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		UNIVERSITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
2007-08	98,644						98,644
2008-09	99,624			60,000	15,000	75,000	174,624
TOTAL	198,268			60,000	15,000	75,000	273,268

Physiological Changes Associated with Live Haul: Maintaining Healthy Fish

TERMINATION REPORT

PROJECT WORK PERIOD

September 1, 2006–August 31, 2009

No-cost extension approved through August 31, 2010

AUTHORS

John Colt, Carl Schreck, and Gary Fornshell

PARTICIPANTS

John Colt	National Marine Fisheries Service	Washington
Eric Kroeger	National Marine Fisheries Service	Washington
Mike Rust	National Marine Fisheries Service	Washington
Ron Johnson	National Marine Fisheries Service	Washington
Joseph Tomasso	Clemson University	Washington
Grant Feist*	Oregon State University	Oregon
Tracey Momoda*	Oregon State University	Oregon
Rob Chitwood*	Oregon State University	Oregon
Carl Schreck	Oregon State University	Oregon
Gary Fornshell* (*Outreach Coordinator*)	University of Idaho	Idaho
Leo Ray	Fish Breeders of Idaho	Idaho
Jim Parson	Troutlodge	Washington
Ken Beer	The Fisheries	California
Mark Francis	Aquaneering, Inc.	California

* funded participants

REASON FOR TERMINATION

Project completed.

PROJECT OBJECTIVES

The overall objective of this program was to improve the health and survival of transported fish. Sub-objectives for tilapia and rainbow trout included:

1. Document current holding and hauling protocols and transport systems for long-haul conditions.
2. Evaluate the impact of hauling conditions on fish appearance, tissue quality, and overall customer acceptance.
3. Develop simple computer models to predict water quality as a function of time, density, and other important operational parameters.
4. Construct experimental transport systems to simulate long-haul conditions. Develop "standardized" hauling conditions and compare with observed data.
5. Evaluate the impacts of chemical addition and temperature modification on fish quality and post-haul mortalities using an experimental transport system.
6. Develop a mortality model to allow prediction of post-haul mortality based on the hauling conditions and quality of fish at the end of haul.
7. Develop hauling criteria and protocols. Make recommendations for modification of existing transport systems to industrial cooperators.
8. Develop outreach products that can be used by live-haulers to make informed decisions. This will consist of WRAC Extension publications and a workshop.

PRINCIPAL ACCOMPLISHMENTS

Tilapia

Objective 1: Documentation of current holding and hauling protocols and transport systems for long-haul conditions.

Water quality information was collected in rearing raceways, hauling systems, and in retail holding systems for tilapia. Additional information was collected on the design and operation of hauling systems and retail holding systems.

Water quality at tilapia farms is typical of low-temperature geothermal waters in Idaho. Dissolved oxygen (DO) concentrations in the hauling tanks were in excess of 19 mg/L at the end of the haul. Over the duration of the haul, the pH dropped by 1.0–1.7 units. Carbon dioxide concentrations ranged from 40–80 mg/L. TAN concentrations ranged from 17–29 mg/L, but the un-ionized ammonia concentration ranged from 20–70 µg/L.

There was a wide variation in the water quality parameters at the retail stores. The pH varied from 4.2 to 7.4 and the TAN ranged from 0.0 to 65 mg/L. Based on published environmental requirements, the most important parameters in retail holding systems are temperature, pH, and un-ionized ammonia. Dissolved oxygen and carbon dioxide were not limiting in retail holding.

Objective 2: Evaluate the impact of hauling conditions on fish appearance, tissue quality, and overall customer acceptance.

Physical Damage. A fluorescein dye method was used to assess physical injury incurred by tilapia during transport. Fluorescein analysis revealed puncture wounds and abrasions in all tilapia sampled directly from the raceway before crowding. However, these fish were apparently crowded and moved the day before sampling. Fish sampled after crowding and loading into the truck showed a similar amount of puncture wounds as well as many more abrasions to the cranial and ventral sides of the body and eye injury. After arrival at Richmond, British Columbia, tilapia sampled directly from the truck had similar bodily injury as pre-loading with more abrasions to the caudal and pectoral fins noted. Tilapia 24-hour and 48-hour post-holding at the retailers did not look much different after fluorescein exposure than the fish sampled at unloading. However, at this point, lesions and hemorrhaging on skin were apparent to the naked eye.

Comparison of (1) knotted net and (2) smooth, large-mesh rubber net showed greater bodily injury from the use of knotted nets. Injury to the eye area was also more common in the fish sampled in the knotted nets. Because of the observed serious damage from conventional loading techniques, an alternative loading system was evaluated. Tilapia (*Oreochromis mossambicus*) were sampled from Pacific Aqua Farms (Niland, California) where they use a fish pump. Very little injury was found throughout our sampling at any stage of the loading process at this farm.

Gill histology. After an experimental haul from one farm in Idaho, high post-haul mortality occurred. Histological analysis of the gill tissue from these fish indicated some abnormalities, such as fusion of the gill lamellae and hyperplasia of the respiratory epithelium, which likely resulted in severely restricted intake of oxygen.

To assess the potential impact of gill damage on transport mortality, gill samples were collected from five tilapia farms

in Idaho. No differences were observed among the farms and overall, all the gills appeared healthy. Of the 20 fish sampled, only three had signs of hyperplasia of the respiratory epithelium, and the three fish were from different farms. All 20 fish showed signs of mild chronic inflammation in the gills. However, this level of inflammation can be considered background inflammation that is not indicative of diseased fish. Additional sampling and research are needed to document the development and impact of gill damage on transported tilapia.

Objective 3: Develop simple computer models to predict water quality as a function of time, density, and other important operational parameters.

A simulation program was developed to improve our understanding of water quality changes that occur during hauling. This program estimates oxygen consumption, carbon dioxide excretion, and ammonia excretion as a function of temperature and activity. The program predicts the rapid decrease in pH at the start of hauling, followed by an increase in pH over the haul, as was observed in the experimental hauls from Idaho. The accuracy of this model strongly depends on the values of metabolic production factors used and the specific geometry of the hauling tanks and liquid oxygen (LOX) distribution system.

Objective 4: Construct experimental transport systems to simulate long-haul conditions.

Because of the difficulty of riding with commercial drivers and the distances involved, it was necessary to build a number of experimental systems for hauling work: a portable hauling system and a prototype transport system. Two systems were developed to evaluate the use of video systems in determining fish distribution and behavior within hauling tanks. The video systems work well for small fish at low densities, but are unable to determine fish distribution at production hauling densities because of overlapping fish and increased turbidity.

Objective 5: Evaluate the impacts of chemical addition and temperature modification on fish quality and post-haul mortalities using an experimental transport system.

Tilapia are typically hauled in a simple salt (sodium chloride) solution of approximately 2–5 g/kg, depending on the individual hauler. An "isotonic" hauling solution was developed using an Excel spreadsheet. Two trials were conducted to compare pretreatment with 30 ppt Instant Ocean for one minute before loading into transport tanks. In the first haul, the pre-haul salt treatment significantly reduced posthaul mortality. In the second haul from a different farm, there was no beneficial effect of the salt dips, but many of the fish arrived in a moribund condition. The overall pre-haul quality of the fish used for this experiment might have played a role in driving the mortality observed, and thus have been a factor in affecting our ability to discern treatment effects.

Objective 6: Develop a mortality model and hauling constant.

Based on published literature, a "safe" hauling density depends on length (or weight) of the fish, duration of the haul, and temperature:

$$\text{Density(g/kg)} = K_{species}\left[\frac{\text{Fish Length}^{a}}{\text{Time}^{b} \times \text{Temperature}^{c}}\right] \quad (1)$$

Higher densities can be used for shorter times, lower temperatures, and larger fish. For this equation to be dimensionally homogeneous, the right side of the equation must have the same dimensions. The Buckingham ϖ theorem provides a method for computing these dimensionless parameters (a, b, and c) from the given variables. Work is ongoing to define the dimensionless groups that are important in hauling. When this is complete, experimental verification of the hauling constant approach will be conducted with tilapia.

Objective 7: Develop hauling criteria and protocols.

Formal recommendation for hauling criteria and protocols will be developed in the outreach products and will include recommendations for the following major items:

- Operation of pure oxygen system.
- Transition from the distribution tanks to the retail tanks.
- Crowding and netting techniques.
- Operation of retail holding systems.
- Pre-haul fasting.
- Pre-haul salt treatment.
- Ability of the fish to cope with haul-related stressors.

Objective 8: Develop outreach products.

The following outreach products will be prepared:

1. A workshop in Idaho to present project results to commercial haulers.
2. Two WRAC Extension publications.
3. User-friendly spreadsheet models (fish stress and mortality) available for download from the WRAC website.

Rainbow Trout

This section presents work with rainbow trout. (Objectives 3, 4, 6, and 8 for tilapia apply to rainbow trout as well.)

Objective 2: Evaluate the impact of hauling conditions on fish appearance, tissue quality, and overall customer acceptance.

Increased Density. Trout of mixed diploid/triploid stock from the Oregon Hatchery Research Center (Oregon Hatchery Center) in Alsea, Oregon, were hauled to Oregon State University Fish Performance and Genetics Laboratory (Oregon State Lab) in Corvallis, at varying densities: 1x (1lb/gal), 2x (2 lb/gal), and 3x (3lb/gal). The fish were catchable-sized rainbow trout loaded onto the truck following typical raceway crowding, netting, weighing in a basket, and stocking into respective tanks. The haul from Oregon Hatchery Center to Oregon State Lab took approximately one hour, and then the fish were left in the haul tanks for another 16.5 hours. Two receiving water quality conditions were tested:

"Good" water temperature = 13.5°C and DO = 9.5 mg/L
"Bad" water temperature = 20°C and DO = 6.0 mg/L

No mortality was observed in the 1x or 3x treatment, immediately following the haul. All fish from the 3x treatment placed in the "Good" holding condition survived; however, mortality was observed in the 3x treatment placed in the "Bad" holding condition. This suggests that 3x (3 lb/gal) is an achievable hauling density, and survival is dependent on the receiving conditions. Additionally, we are interested in determining whether additives to the haul medium can further increase the densities at which fish can be transported.

Bodily Injury during Crowding and Loading. Bodily injury was assessed using the fluorescein technique. Overall, very little bodily injury was detected. The only noteworthy injury was the 3x haul treatment, in which three out of the five fish sampled had eye injuries. No fish transported at 1x and only one from the 2x treatment had eye injury. This increased eye injury may be a consequence of the increased density in the 3x treatment.

top to bottom:
Dipnetting tilapia prior to transport; Visually checking quality of tilapia; Live haul transport truck.

Courtesy of Study Work Group participants.

Objective 7: Develope hauling criteria and protocols.

Formal recommendation for hauling criteria and protocols were developed in the outreach products and included the following:

- Rainbow trout can be transported at 2 and 3 lb/gallon with reasonable mortality.
- Loading protocols and diffuser layout may be more important at higher density.
- Little bodily injury was detected with the fluorescein technique.
- Eye injury may be more prevalent at 3 lb/gallon and require smoother interior surfaces.
- Chemical additions may be useful at higher densities.

Other Accomplishments

The characteristics of oxygen flow through ceramic fine bubble diffusers depends strongly on the type of diffuser and prior history of its use. This observation was examined and the results disseminated. Basically, the mass transfer rates and gas flow rates are much lower when the diffuser is saturated with water before the gas flow starts. This means that this type of fine bubble diffuser performance can change drastically depending upon whether the gas flow is temporarily interrupted—such as by changing gas supplies or other actions or by wetting the diffusers before turning on the gas flow.

RESULTS AT A GLANCE...

- Increased survival and product quality of transported fish as a result of adopting the recommended protocols and using of the models.
- While the main impacts will occur after the outreach workshops and publications have been completed, the following changes have already occurred:
  - Following a presentation of our results to participating haulers in Idaho, an open dialog among haulers was established.
  - After this meeting, one hauler reduced his hauling density.
  - Based on documentation of net damage, another hauler replaced all its harvest basket netting material to softer knotless mesh to reduce physical damage.

IMPACTS

Relevance: In certain markets, live fish can be sold for substantially higher prices than fresh dressed fish. A significant live-haul industry has developed in the United States and fish are commonly hauled 1,500–2,000 miles (25–30 hours) to market. Increased feed and transportation costs have reduced profits in the live-haul industry. Because of the economic importance of the live fish market, improved systems and protocols are needed to allow the live-haul industry to expand and prosper. One major constraint to improving overall fish health in transport systems is that very little information has been published on the chemical and physical conditions during long-distance transport, and this limited data may not be representative of current commercial systems.

Response: Water quality data was collected from the farms, hauling tanks, and retail stores. Physical damage to the fish from crowding and loading was documented using a fluorescein dye technique. The characteristics of oxygen flow through ceramic plate and carbon diffusers were studied. This research was directed toward hauler and retail store owners. Based on this work, changes in the following tasks or processes were recommended to minimize physical and physiological damage to transported fish:

- The use of knotted nets should be avoided.
- The use of a fish pump may result in less physical damage to tilapia than conventional loading techniques.
- In retail holding systems, potential water quality problems include suboptimal water temperature, high un-ionized ammonia concentrations, and high gas supersaturation levels.
- The transfer of fish from the distribution tanks to retail holding is a very stressful event that may result in serious physiological and endocrinological impacts.
- Rainbow trout can be transported at 2 and 3 lb/gallon with reasonable mortality.

- The characteristics of oxygen flow through fine bubble diffusers depend strongly on the type of diffuser and prior history of its use.

Results: The impact of this project will be increased survival and product quality of transported fish as a result of adopting the recommended protocols and utilization of the models. A survey comparing pre- and post-project live-haul success, using the recommended protocols should provide the information necessary to evaluate project impact. The survey may be conducted by phone or mail, and will take place one to two years after project completion to allow for protocol adoption.

While the main impacts will occur after the outreach workshops and publications have been completed, the following changes have already occurred:

- Following a presentation of our results to haulers in Idaho, an open dialog among haulers was established.
- After this meeting, one hauler reduced his hauling density.
- Based on documentation of net damage, another hauler replaced all its harvest basket netting material to softer knotless mesh to reduce physical damage.

Collaborators: Significant roles were played by fish farmers, distributors, and manufacturers: Leo Ray, Fish Breeders of Idaho; Mark Lupher, Epicenter Enterprises; John Lambregts, Falls Services; Brian Tadlock, Pristine Springs; Bob Williams; Blundell Seafoods Ltd.; Jim Parson, Troutlodge; Ken Beer, The Fisheries; Mark Francis, Aquaneering, Inc.

Contact: John Colt, 2725 Montlake Blvd. East, Seattle, Washington 98112, *john.colt@noaa.gov*; Carl Schreck, Oregon State University, Department of Fish and Wildlife, 104 Nash Hall, Corvallis, Oregon 97331, *carl.schreck@oregonstate*.edu; Gary Fornshell, University of Idaho Extension, Twin Falls County, 246 3rdAvenue East, Twin Falls, ID 83301, *gafornsh@uidaho.edu.*

RECOMMENDED FOLLOW-UP ACTIVITIES

The impact of hauling on tilapia was found to depend strongly on the origin of the fish. Additional work is needed to define the nutritional, physiological, and disease status of commercially reared tilapia and the impacts of these parameters on survival and product quality of transported fish.

Rainbow trout can be transported at 2–3 times normal density with acceptable mortality. The use of chemical additives to increase hauling density needs to be documented. This might include: buffers, ammonia lock, and anaesthetics.

PUBLICATIONS, MANUSCRIPTS, AND PAPERS PRESENTED:

Publications in Print and Manuscripts

National Marine Fisheries Service, Oregon State University, University of Idaho. 2006. Working Paper No. 1: Environmental requirements of Nile tilapia (*Oreochromis niloticus*).

National Marine Fisheries Service, Oregon State University, University of Idaho. 2006. Working Paper No. 2: Water quality in farm, hauling, and retail holding systems.

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Colt J. 2009. Draft recommendations for the computation of hauling mixtures, Excel spreadsheet, 2 pages.

Colt J, Schuur A, Cryer E, Miles T. 2009. Modeling of multiple stocks and programs for master planning and feasibility studies. *Aquacultural Engineering.* 41:176–187.

Colt J, Watten B, Rust M. 2009. Modeling carbon dioxide, pH, and un-ionized ammonia relationships in serial reuse systems. *Aquacultural Engineering* 40:28–44.

Colt J. 2010. Computation of Dissolved Gas Concentration in Water as Functions of Temperature, Salinity and Pressure, Second Edition, Draft, 325 pages.

Colt J, Kroeger E , Rust M.2010. Characteristics of oxygen flow through fine bubble diffusers used in aquaculture hauling applications. *Aquacultural Engineering*. In press, 9 pages, doi:10.1016/j.aquaeng.2010.06.001.

Colt J, Watten B, Pfeiffer T. 2010. Carbon dioxide stripping in aquaculture—Terminology, reporting, and modeling. Accepted for publication, September 6, 2010, *Aquacultural Engineering*.

Colt J, Momoda T, Chitwood R, Fornshell G, Schreck C. 2010. Water quality in tilapia transport: from the farm to the retail store. Internal NOAA review, To be submitted to *Aquaculture*, 44 pages.

Colt J. Impact of alkalinity and respiratory carbon dioxide on the pH, un-ionized ammonia, and free carbon dioxide in fish transport systems. In Preparation.

Momoda T, Chitwood R, Colt J, Schreck CB. Effects of stress and injury associated with transporting live tilapia. In Preparation.

Papers presented

Chitwood R, Feist G, Momoda TS, Schreck CB, Colt J. Stress effects of transporting tilapia to the live fish market and recommendations to enhance health and survival. World Aquaculture Society. San Antonio, Texas, 2007.

Chitwood R, Momoda TS, Feist G, Colt J, Schreck CB. Mortality and effects associated with stress and handling in transporting live tilapia. 2008. Idaho Aquaculture Association. Twin Falls, Idaho, June 21, 2008.

Colt J, Chitwood R, Momoda T, Feist G, Schreck C. Water quality in retail tilapia holding systems. Aquaculture American 2008, Orlando, Florida, February 9–12, 2008.

Colt J, Rust M. 2008. Modeling of water quality in warmwater transport systems. Aquaculture American 2008, Orlando, Florida, February 9-12, 2008.

Momoda T, Chitwood R, Feist G, Colt J, Schreck C. Stress and injury associated with transporting tilapia to the live fish market affects pathology-related survival. Aquaculture American 2008, Orlando, Florida, February 9–12, 2008.

Schreck C, Momoda T, Chitwood R, Feist G, Kent M, Holt R, Colt J. Effects of injury and stress associated with transport of live tilapia: Do they inoculate each other with pathogens? International Congress on the Biology of Fish. Portland, Oregon, July 2008.

Colt J, Watten B, Rust M. Impact of attached algae, suspended bacteria, and re-aeration on oxygen and carbon dioxide balances in series raceways. Aquaculture America 2009, Seattle, Washington, February 15–18, 2009.

Colt J, Kroeger E, Rust M. Impacts of alkalinity and carbon removal on the mortality and product quality of tilapia. Annual meeting, World Aquaculture Society, San Diego, California, March 1–5, 2010.

Colt J, Ray L. Hauling channel catfish fingerling from Arkansas to Idaho: an adventure with Leo Ray. Annual meeting, World Aquaculture Society, San Diego, California, March 1–5, 2010.

Kroeger E, Colt J, Rust M. Fine bubble diffusers: impacts of pressure, water, and handling on mass flowrates and transfer efficiency. Annual meeting, World Aquaculture Society, San Diego, California, March 1–5, 2010.

Schreck C, Momoda T. Effects of stress and injury associated with transporting live tilapia. Annual meeting, World Aquaculture Society, San Diego, California, March 1–5, 2010.

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		UNIVERSITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
2006	89,456	3,891[a]		37,500[b]		41,391	130,847
2007	81,856	3,500[a]		30,000[b]		33,500	115,356
2008	86,299	4,000[a]		20,000[b]		24,000	110,299
2009	96,789	4,000[a]		30,000[b]		34,000	130,789
2010				35,000[b]		35,000	35,000
TOTAL	354,400	15,391		152,500		167,891	522,291

[a]Salary for Carl Schreck, OSU/USGS
[b]Salary for John Colt and Eric Kroeger, NOAA