WESTERN REGIONAL AQUACULTURE CENTER

WRAC

ANNUAL ACCOMPLISHMENT REPORT

FOR THE PERIOD SEPTEMBER 1, 2007 TO AUGUST 31, 2008

MARCH 2009

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

In cooperation with the US Department of Agriculture
Cooperative State Research, Education & Extension Services

INTRODUCTION

This Annual Accomplishment Report for the Western Regional Aquaculture Center (WRAC), covers progress made from September 1, 2007 through August 31, 2008. WRAC was designated as one of five regional aquaculture centers under USDA, for which funding would be made available to support research, development, and demonstration projects in aquaculture. WRAC comprises the 12 states in the western region—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 included in this 21st Annual Progress Report. Members of the WRAC Board of Directors, Industry Advisory Council, and Technical Committee have provided valuable input toward the Center's successful operation during the past year. We particularly appreciate the assistance of the chairs of our Board, IAC, and TC, and those serving as Technical Advisors.

We also thank the scientists and aquaculturists from across the country that 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

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

The ***Industry Advisory Council*** is composed of representatives of the industry and associated services covering multiple sectors and geographic regions within the western twelve states.

Technical Committee is 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 from 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 21 Annual Work Plans (FY'87–FY'08 funding) through USDA. This current annual report covers the activities of the WRAC Administrative Center and progress made all projects listed below with funding levels for FY'07–FY'08.

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

ANNUAL REPORTS

A. An Evaluation of the Effectiveness of Various Florfenicol Treatment Regimens to Control Mortality Caused by *Streptococcus iniae* in Cultured Hybrid Striped Bass
   2nd project year: $30,910

B. Economic Impacts of Private sector Aquaculture-Based Recreational Fishing in the Western USA
   1st project year: $98,644

C. Coldwater Disease Prevention and Control through Vaccine Development and Diagnostic Improvements
   1st project year: $81,555

D. Physiological Changes Associated with Live Haul: Maintaining Healthy Fish
   3rd project year: $86,299

E. Determining Ripeness in White Sturgeon Females to Maximize Yield and Quality of Caviar
   1st project year: $100,001

F. Development and Evaluation of Starter Diets and Culture Conditions for 3 Subspecies of Cutthroat Trout and Gila Trout
   1st project year: $91,973

G. Potential Threat of Great Lakes VHS Virus in the Western United States
   1st project year: $72,097

H. WRAC Publications
   $24,000

TERMINATION REPORTS

A. Immunological Mechanisms of Intensively-Reared Warmwater and Coolwater Finfish

B. Phosphorous Discharge

PUBLICATIONS

The WRAC Publications project (Item H above) 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 needed to produce the various printed and online publications and resources.

Administrative Support

FY'O8 FUNDING LEVEL $191,619

The Administrative Center is located in the School of Aquatic and Fishery Sciences at the University of Washington, which serves as the Host Institution. The role of the WRAC Administrative Center staff is to 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 assumed responsibility 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 21 Annual Work Plans to date (FY'87–FY'08) for the various WRAC projects. Activities of the Center and funding for its operation rely upon the annual decisions of the Board of Directors 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 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.

Administrative Bulletins are published throughout the region on an as-needed basis in order to inform the Board, IAC, TC, and project participants regarding pertinent activities related to regional and national aquaculture in general and WRAC in particular.

The Administrative Center also publishes *Waterlines*, an annual newsletter, which has been well received. *Waterlines* provides information on WRAC projects and general aquaculture news, demonstrating the importance of aquatic animal husbandry. *Waterlines* has a mailing list of more than 2,700 recipients, which include regional, state, and federal agencies; scientists; extension specialists; industry professionals; and the general public. The Winter 2009 edition of *Waterlines* was also inserted into the conference bag of each attendee at the Aquaculture America 2009 Conference held in Seattle, Washington, on February 15–18, 2009.

Other areas of support provided by WRAC Center staff during this period, include:

- Preparation of USDA grant packages and amendments
- Production of documentation and reports to the Board of Directors
- Organization of IAC and TC meetings
- Coordination of activities of the Board of Directors
- Development of research plans, budgets, and proposals
- Development of management plans and budgets
- Cooperation with the IAC & 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 (NCC), 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 of Directors and recommended nominees to the IAC and TC
- Recruitment of Administrative Center staff, as authorized by the Board of Directors
- Close communication with other fisheries and aquaculture programs to track various aquaculture activities throughout the western region

An Evaluation of the Effectiveness of Various Florfenicol Treatment Regimens to Control Mortality Caused by *Streptococcus iniae* in Cultured Hybrid Striped Bass

Reporting Period	September 1, 2007–August 31, 2008 (Year 2)		
Authors	James D. Bowker		
Funding Level	First Year Request	$29,864	
	Second Year Request	$30,910	
	Funding level to date	$60,774	
Participants	James D. Bowker*	US Fish & Wildlife Service	Montana
	Vaughn Ostland*	Kent Sea Tech Corporation	California
	Steve Harbell (*Ext. Rep.*)	Washington State University	Washington
Technical Advisor	Jerri Bartholomew	Oregon State University	Oregon

* funded participants

PROJECT OBJECTIVES

The purpose of this research project is to determine whether an alternate (i.e., higher concentration and/or longer duration) treatment regimen (other than 10 mg florfenicol/kg fish body weight administered on 10 consecutive days) is more efficacious in controlling mortality in hybrid striped bass (HSB) caused by *Streptococcus iniae*. The specific objectives for this study are listed below. Objectives that are relevant to Year 2 funding are italicized.

Objective 1.

Trial 1. Using isolates of *S. iniae,* determine which route of infection (immersion or intraperitoneally (IP) injection) of HSB will consistently yield a mean cumulative mortality of 50% in the exposed group, with the least statistical variation among replicates. Also, identify important dose-dependent variables, such as time to onset of first morbidity, time to first mortality, and total cumulative mortality.

Trial 2. Refine methodologies identified in Trial 1 to consistently yield a mean cumulative mortality of 50% in HSB of a different age exposed to isolates of *S. iniae.*

Objective 2.

Using the optimal dose and exposure route described in Objective 1 Trial 2, determine the most effective treatment dose of florfenicol to control mortality in HSB experimentally infected with S. iniae *fed a medicated feed top-coated with either 0, 10, 15, or 20 mg florfenicol/kg fish/day for 10 days. This data will identify the lowest treatment dose that results in the highest survival during the 10-day trial.*

Objective 3.

Using the lowest treatment dose that resulted in the highest survival (identified in Objective 2), determine the most effective treatment duration of florfenicol to control mortality in HSB experimentally infected with *S. iniae* fed a medicated feed for either 0, 10, 15 or 20 days. This data will identify the shortest treatment duration that results in the highest survival.

Objective 4.

Demonstrate and substantiate that the most efficacious treatment regimen identified in Objective 3 is also effective when florfenicol is administered to HSB naturally infected with *S. iniae* (i.e., controlled field trial).

ANTICIPATED BENEFITS

This project will assist and benefit the aquaculture industry by providing information so that prudent decisions can be made about therapeutic treatment regimens to control mortality in HSB caused by *S. iniae*. Currently, the treatment regimen option available to the aquaculture industry is the standard florfenicol dosage (10 mg active drug/kg fish body weight administered daily for 10 consecutive days). Results from this study will determine whether the industry standard or a higher concentration/longer treatment duration is more efficacious in controlling mortality in HSB caused by *S. iniae.* (Note that there is some evidence in the literature, and some anecdotal information, that a higher therapeutic concentration is required to control mortality caused by *S. iniae* in other warmwater finfish [e.g., tilapia].)

A second benefit to the aquaculture industry is the development of a disease challenge model to initiate an outbreak of *S. iniae* in HSB that should be suitable for testing other therapeutants and biologics.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

During the reporting period, three trials were initiated in an attempt to address Objective 2.

1. On November 27, 2007, the PIs met at the Kent SeaTech (KST) Corp. HSB farm in Mecca, California to start a trial to address Objective 2 and determine the most efficacious treatment dose of florfenicol to control mortality in HSB experimentally infected with *S. iniae*. Fish were fed medicated feed top-coated with 10, 15, or 20 mg florfenicol/kg fish/d for 10 d. Fish were randomly allocated to test tanks and treatment conditions were randomly assigned to test tanks. Day one of the 10-d treatment period began one day post-challenge. Due to low mortality, the trial was terminated on post treatment day 3.
2. On January 8, 2008, the PIs again met at the KST HSB farm to start another trial with nearly identical goals and methodologies to the previous trial. Due to low mortality, the trial was also terminated early (on post treatment day 4).
3. To help us direct future research efforts, a third trial was initiated to investigate the influence of fish age and the inclusion of sheep serum in the bacterial growth medium on the ability of *S. iniae* to induce mortality in HSB exposed to this pathogen by immersion. We also found that by using younger fish (8.7 g mean weight), we were able to improve cumulative mortality, although we were still unable to reach our target mortality of 50%. Furthermore, the addition of sheep serum to the growth medium did not increase overall mortality. Further research is planned to refine the HSB immersion challenge model to achieve our target mortality pattern of 50%.

Experimental Design and Methods

Objective 2 Trial 1

To ensure disease challenge consistency throughout this project, bacterial "working seeds" were produced and frozen for all subsequent challenges. From a single working seed, an immersion challenge inoculum was prepared and used to seed a sufficient volume of *S. iniae* broth to dose groups of fish in their respective test tanks at a pre-determined (approximate) dose. The objective of this trial was (1) to initiate a disease outbreak sufficient to evaluate the effectiveness of an antibiotic, and (2) treat fish infected with *S. iniae* with various concentrations of florfenicol (i.e., 0, 10, 15, or 20 mg florfenicol/kg fish body weight) to determine which of the tested doses resulted in the lowest mortality at the end of the study.

The *S. iniae* bacterial working seed was prepared using standard procedures. Immersion challenge inoculate was prepared and administered to naïve HSB (mean length, 22.3 cm; mean weight, 124.7 g) that had not been previously exposed to *S. iniae*.

The trial was designed to test the following null hypothesis: Ho: $\mu_{o\ mg/kg} = \mu_{10\ mg/kg} = \mu_{15\ mg/kg} = \mu_{20\ m/kg}$ (no difference in mean percent total mortality between the four treatment conditions). In addition to the four treatment conditions, an untreated negative control (fish not challenged with *S. iniae*) was included in the trial to assess incidental mortality (hence, there were five treatment conditions). Three replicates of each treatment condition were allocated among 15 tanks in a completely randomized design. Completely randomized procedures were also used to allocate 30 fish into each test tank. Treatments were administered 1 d after all fish had been challenged with *S. iniae*. In addition, all fish that died were necropsied and brain tissue streaked on blood agar plates to presumptively confirm *S. iniae* infections. Mortality, fish behavior, appetite behavior (graded on a scale of 1 = no interest in feed to 5 = all fish break surface during feeding), water temperature, and dissolved oxygen were collected/observed/measured daily during the 10-d treatment period and the proposed 14-d posttreatment period (note: due to lack of mortality and signs of morbidity, the trial was terminated on posttreatment day 3). At the end of the posttreatment period, mean mortality associated with each treatment condition was evaluated by statistically comparing mean percent total mortality between treatment groups.

Results: During the challenge phase of the study, fish were exposed to a dose of approximately 2.65 E+07 *S. iniae* colony forming units (CFUs)/mL. At the end of the trial, mean relative mortality in each treatment condition was extremely low (approximately 1%). Large numbers of *S. iniae*–like colonies were cultured from brain tissue of all fish sampled during the trial. Mean water temperature and dissolved oxygen concentration during the study was 25.9°C (±1 SD = 0.59) and 14.1 mg/L (±1 SD = 1.79), respectively. Fish behavior was characterized as normal, and feeding behavior ranged from 3.2–3.4 for all disease challenged fish and 4.5 for negative control fish.

Objective 2 Trial 2

The *S. iniae* bacterial working seed was again prepared using standard procedures. Immersion challenge inoculum was produced and administered to naïve HSB (mean length, 18.6 cm; mean weight, 59.9 g) that had not been previously exposed to *S. iniae.* The experimental design and methodologies used in Trial 2 were virtually identical to those used in Trial 1.

Results: During the challenge phase of the study, fish were exposed to a dose of approximately 2.03 E+07 *S. iniae* CFUs/mL. At the end of the trial (the trial was terminated on posttreatment day 4), mean relative mortality in each treatment condition was extremely low (i.e., 0–1%). Large numbers of *S. iniae*–like colonies were cultured from brain and head kidney tissue of all fish sampled during the trial. Mean water temperature and dissolved oxygen concentration during the study was 26.6°C (±1 SD = 0.26) and 13.6 mg/L ± (±1 SD = 1.78), respectively. Fish behavior was characterized as normal, and feeding behavior ranged from 4.3–5.0 for all disease challenged fish fed florfenicol medicated feed, 3.4 for the positive control fish, and 5.0 for negative control fish.

Objective 2 Trial 3. Effect of Fish Size and Sheep Serum on Cumulative Mortality

The experimental design and methodologies used in Trial 3 were similar to those used in previous trials except that feeding behavior was not monitored. The main difference in this trial was that younger fish (mean weight of 8.7 g, mean length of 8.7 cm) were used and duplicate rather than triplicate tanks were used to reduce the number of animals necessary for this exploratory research. We also sought to determine whether the addition of sheep serum to the growth medium would improve bacterial growth and result in an increase in cumulative mortality.

Results: Our findings indicate that we can improve cumulative mortality of immersion exposed HSB if we use younger fish (mean cumulative mortality, 41.7%), although under the conditions employed for this trial we were still unable to produce 50% cumulative mortality. Furthermore, the addition of 5% sheep serum to the growth medium did not contribute to increased mortality (mean cumulative mortality, 38.3%), but it did improve observed growth values (expressed as OD600) of *S. iniae,* although a concomitant reduction of viable bacteria per ml was observed in the Todd-Hewitt Broth (THB) culture in the presence of sheep serum. This reduction in viable bacterial numbers *in vitro* was also evident when the challenge dose (CFU/ml tank water) was confirmed in the water 2 min after the addition of *S. iniae*.

USEFULNESS OF FINDINGS

1. Due to insufficient mortality among disease-challenged fish, findings from the second set of trials failed to address Objective 2. Usefulness of these findings are minimal. The trials will need to be repeated employing younger fish and exposing fish to a more concentrated suspension of *S. iniae*.
2. We anticipate that findings from the a trial in which sufficient mortality (~ 50% total mortality) is attained in the 0 mg/L control fish will address the question of which florfenicol dose (10, 15, or 20) is the most efficacious.

WORK PLANNED FOR NEXT YEAR

All remaining trials are planned for next year (Year 3) to evaluate the effectiveness of different dose and treatment duration regimens of florfenicol to control mortality in HSB caused by *S. iniae*.

1. The first trial will evaluate the effectiveness of 0, 10, 15, and 20 mg florfenicol/kg fish body weight to control mortality in HSB caused by *S. iniae* (experimentally induced in test fish).
2. The second trial will evaluate the effectiveness of the treatment dose identified in the previous trial as the most efficacious for 10, 15, or 20 d to control mortality in HSB caused by *S. iniae* (experimentally induced in test fish).
3. The third trial will evaluate the most effective treatment regimen (most efficacious dose and most efficacious treatment duration) to control mortality in HSB naturally infected with *S. iniae*.

Results from the remaining trials should provide us with the "most" efficacious treatment regimen to control mortality in HSB experimentally infected with *S. iniae,* and a demonstration of the treatment regimen effectiveness using production HSB naturally infected with *S. iniae*.

In addition, a study to demonstrate the safety of florfenicol in HSB is planned for Year 3. Note that this study is outside of the WRAC-funded project, but will contribute to the data required by the Center for Veterinary Medicine (CVM) to expand the use of AQUAFLOR® to allow use at a higher florfenicol dose per kg fish body weight. In this study, fish will be fed florfenicol-medicated feed at a dose of 0, 15, 45, or 75 mg florfenicol/kg fish body weight for 20 d. Resources for this study will be provided by the USFWS, Kent SeaTech Corporation, and Intervet/Schering-Plough Animal Health Corporation.

IMPACTS

Successful completion of the trial to address Objective 2 will provide HSB culturists with the option to control mortality in their fish caused by *S. iniae* with the most efficacious concentration of florfenicol.

PUBLICATIONS IN PRINT AND PAPERS PRESENTED

Publications

Bowker, J. Evaluating the effectiveness of various dosages of Aquaflor®. *Waterlines* Newsletter of the Western Regional Aquaculture Center. Autumn 2006.

Presentations

Bowker J and Ostland V. Effectiveness of Aquaflor® to control mortality in hybrid striped bass caused by *Streptococcus iniae*. Presented at Aquaculture America 2008, February 2008, Lake Buena Vista, FL.

Ostland V and Bowker J. *Streptococcus iniae* challenge model development in hybrid striped bass, Research Progress Update. Presented at the 14th Annual Aquaculture Drug Approval Coordination Workshop, July 2008, Bozeman, MT.

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		University	Industry	Other Federal	Other	Total	
1/07–8/07	29,864		12,000	24,000		36,000	$65,864
9/07–8/08	30,910		12,000	25,000		37,000	$67,910
Total	60,774		24,000	49,000		73,000	$133,774

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

Reporting Period September 10, 2007–September 9, 2008

Authors Craig Bond and Daniel Deisenroth

Funding Level $98,644 allocated to date

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
Doug Larsona[1]	Colorado State University	Colorado
Andrew Seidl* (*Outreach Coordinator*)	Colorado State University	Colorado
Collaborators		
Fred Conte	University of California, Davis	California
John Boren	New Mexico State University	New Mexico
Gary Fornshell*	University of Idaho Extension	Idaho
Amalia Davies*	Colorado State University	Colorado
Kevin Fitzsimmons	University of Arizona	Arizona
Chris Myrick	Colorado State University	Colorado
Daniel Deisenroth*	Colorado State University	Colorado

Industry Advisors

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

* funded participants

[1] no longer active on this project—will be replaced by alternate UCD faculty

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 a variety of outreach materials (including final report; and peer-reviewed, extension, and popular press articles), and disseminate information at conferences, meetings, etc.

ANTICIPATED BENEFITS

This study will benefit ASRF producers by demonstrating the direct, indirect and induced impacts of the ASRF industry on the western United States. This will be achieved by identifying the backward and forward linkages between ASRF producers and their direct customers, as well as the linkages with recreational anglers. This study will also be beneficial to the ASRF industry by demonstrating to regulatory agencies its positive economic impact and by demonstrating to the general public its positive impact on the recreational fishing industry. Finally, this study will demonstrate the substitutability between wild and hatchery-raised fish in order to inform policy decisions.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Work has progressed in accordance with the original timeline, in spite of the fact that funding did not arrive until January 2008. Principal accomplishments for each objective include:

Objective 1

1. The CSU team, with the help of Ken Cline, Rebecca Cooper, John Boren, Kevin Fitzsimmons, Fred Conte and Gary Fornshell, created a survey instrument, which was used to collect data from the ASRF industry.
2. The CSU team conducted a focus group of ASRF producers by phone.
3. The CSU team, along with Fornshell, Doug Larson, Fitzsimmons, Boren, and Conte, gathered business information necessary to collect data.
4. The CSU team conducted a pre-test of 50 randomly selected aquaculture permit holders.
5. The CSU team sent surveys to the remaining 190 aquaculture permit holders in the Western USA (many producers in Alaska were excluded because they were known to be non-profit. The pre-test round of surveying had a 52% response rate; the remainder of the surveys are still being returned.
6. We anticipate that the project will continue to stay on schedule and that data gathering from other groups will commence shortly.

Objective 2

1. Compiled relevant information regarding all active ASRF permit holders in the western USA. All information will be kept confidential as per the original agreement, and information from states with few producers will be aggregated.

Objective 3

1. One issue identified by the research team is the presence in the Northwest (primarily Washington and Alaska) of private, non-profit producers that supply recreational fish. While the key focus of the study is for-profit firms, we believe it will be appropriate to discuss the non-profits in the context of the final report. The CSU team will commence work on other components of this objective after all primary data has been collected.

Objective 4

1. Created an FAQ, which can be found at *http://dare.agsci.colostate.edu/csuagecon/wracimpact.htm*, with the help of Conte, Fornshell, Boren and Fitzsimmons, in order to better inform the public about this project. Bond and Deisenroth published two extension pieces (see Publications section of this report).

USEFULNESS OF FINDINGS

The team of researchers at CSU has compiled a list of active ASRF producers and used this list to gather information regarding basic operations, expenditures, labor, and demographics of the industry. This data will be used to demonstrate the economic impact of the ASRF industry in the Western USA. We will evaluate the direct effect of the ASRF through sales and spending, as well as the indirect and induced effects of these businesses on their local economy.

This information will directly benefit ASRF producers by serving as an educational tool for the general public and for regulatory agencies. As a result, policy decisions may be impartial and thus potentially more favorable than past legislation, which was made without the aforementioned information at hand.

Finally, while all business information gathered during this process will be kept confidential as per the original confidentiality agreement, the names and locations of the individual ASRF firms, separated from those firms who hold non-ASRF aquaculture permits, will be useful for any future research regarding this industry. Furthermore, information regarding the geographic distribution of the ASRF industry should prove useful in terms of policy decisions.

WORK PLANNED FOR NEXT YEAR

1. Create a sampling frame for the direct customers of ASRF producers, including private ponds, dude ranches, and government organizations.
2. Create a sampling frame for recreational anglers, who use privately stocked fishing outlets.
3. Obtain data from both of these groups, using a similar survey instrument to the one used with ASRF producers.
4. Integrate results from all three groups (ASRF producers, their direct customers, and recreational anglers) into input-output models such as IMPLAN in order to estimate the total economic contribution of the ASRF industry.
5. Report on and disseminate this information: final economic report, peer-review academic journals, extension publications, popular press publications, and conference and meeting presentations.

IMPACTS

The impact of this research will come in the form of greater awareness of the ASRF industry. Specifically, by demonstrating the economic impact of the industry, policy makers will be able to make more informed decisions regarding regulations, which may help to facilitate lower costs and more profitability for ASRF producers, while ensuring greater regional economic prosperity.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

Publications in Print

Bond CA and Deisenroth D. (2008a) "Colorado State University to lead effort to quantify economic contribution of recreational fish oroducers" *The Fishline, http://www.colaqua.org/articles.htm*

Bond CA and Deisenroth D. (2008b) "Estimating the economic umpacts of the aquacultural suppliers of recreational fish" *Waterlines*., Winter 2009, pp.16–17.

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						$99,624
TOTAL	$198,268						$198,268

Coldwater Disease Prevention and Control through Vaccine Development and Diagnostic Improvements

Reporting Period	September 1, 2007–August 31, 2008		
Author	Ken Cain and Doug Call		
Funding Level	First Year funding	$81,555 (received February 2008)	
	Second Year funding	$80,043	
	Third Year Request	$81,637	
	Fourth Year Request	$81,639	
Participants	Kenneth Cain* (*Working Group Chair*)	University of Idaho	Idaho
	Douglas Call*	Washington State University	Washington
	Scott LaPatra*	Clear Springs Foods, Inc.	
	Gary Fornshell*	University of Idaho	Idaho
	Greg Weins	USDA	West Virginia
	Amy Long	University of Idaho	Idaho
	Rashesh Kumar	Washington State University	Washington
Technical Advisor	Gael Kurath	USGS	Washington
Industry Advisor	Jim Parsons	Troutlodge	Washington

* funded participants

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 *in vivo*-induced antigen technology (IVIAT) followed by screening with convalescent serum from trout.
   - Test candidate recombinant proteins 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 from this project 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 of CWD and tailoring disease management 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 alone, losses from CWD can range from 18% to 30%. In addition to the trout industry, federal, state, and tribal hatcheries rearing 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. This may result from 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. These approaches have worked well for bacterial kidney disease (BKD). 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 vaccine would provide a tool to prevent CWD at aquaculture facilities. Currently, no such preventative measures exist and control relies on antibiotic use.

Anticipated benefits include the availability of additional diagnostic tools (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; May 2008) and a Postdoctoral Fellow (Rajesh Kumar; July 2008)) have recently joined laboratories at the University of Idaho (Cain) and Washington State University (Call), respectively. Therefore, the project is just getting underway. A workgroup meeting (conference call) was held on July 23, 2008 to set priorities and organize the project for the coming year now that staff and funding are in place.

Objective 1: Identify potential vaccine candidates using* in vivo*-induced antigen technology (IVIAT) followed by screening with convalescent serum from trout.

The IVIAT library is designed to detect *in vivo*-expressed antigens and involves generating a genome-wide expression library that is screened with antibodies from convalescent rainbow trout. For this system to work, we require successful expression of genes from *F. psychrophilum* and suitable convalescent antisera. In the latter case, the antisera is "absorbed" against *F. psychrophilum* that has been grown in broth culture. This procedure "subtracts" antibodies associated with *in vitro*-grown culture, but should leave antibodies from *in vivo*-expressed proteins available for library screening.

Our immediate objective was to determine the most suitable concentration of absorbed antibody for the library screen and to determine if the absorbed antibody was still effective for detecting *in vivo*-expressed antigens. Recent work from Dr. Cain's lab identified probable *in vivo*-expressed proteins that Dr. Call's lab subsequently expressed using recombinant technology. These proteins were then used to show that the absorbed antibodies remain active against the *in vivo*-expressed proteins while not reacting to *in vitro*-expressed proteins. Thus, our absorption procedure works and we have positive controls for library screening and optimization. These experiments also showed that the concentration of antibody needed for these screens is very high and that we do not have sufficient convalescent antisera readily available to complete the library screen.

Upon hearing of our analytic sensitivity shortcomings, Dr. Scott LaPatra offered access to hyper-immunized antisera from rainbow trout. This antisera was generated by repeated challenge with viable *F. psychrophilum,* and we expect it should have both high reactivity to *in vivo*-expressed antigens and high titer. In August, Dr. LaPatra shipped 15-ml of this antisera to the Call lab where we are in the process of characterizing this material for library screening.

Objective 2: Validate quantitative diagnostic assays (ELISA and ovarian fluid filtration FAT)
Initial work associated with assay development and screening of broodstock was completed this past year. Results showed high infection rates in Coho and rainbow trout broodstock from the Lower Elwha hatchery and Troutlodge, respectively. The ability to quantify infection levels in broodstock can allow a culling program to be effective, and results from this initial work have direct relevance to the upcoming experiments planned for the WRAC project.

In the past two months, we have been working to re-optimization the enzyme-linked immunosorbent assay (ELISA) that was previously developed for quantifying infection levels in broodstock (Lindstrom et al. in press). Because initial stocks of the monoclonal antibody (MAb) FL43 previously used for assay development were depleted, new antibody has been produced by the WSU Monoclonal Antibody Center. These "batches" of new antibody have been purified from mouse ascites fluid using a thiophilic agarose method or Melon Gel IgG Purification Kit from Pierce Laboratories. A portion of these antibodies have been conjugated to horseradish peroxidase for use in the ELISA. Initial results have shown varying sensitivity according to the batches tested. To test sensitivity, a number of assays have been run using *F. psychrophilum* suspended in PBS. Thus far, the detection capabilities have been lower than expected thus far when compared to earlier results using the original FL43 stock produced in 2005. This variability is not unexpected and we will work through any issues with sensitivity in the near future. Once optimized, the new antibody stock will be stored for use in upcoming *in vivo* experiments.

Objective 3: Based on results from objective 2, develop other assays (e.g., real-time quantitative PCR) for quantification of infection in ovarian fluid.
No progress to report.

Objective 4: Develop an integrated outreach program to meet stakeholder needs.
Outreach activities have resulted in submission of an article describing this project to WRAC for publication in the upcoming issue of *Waterlines*.

USEFULNESS OF FINDINGS
None to date.

WORK PLANNED FOR NEXT YEAR
Objective 1: Identify potential vaccine candidates using in vivo-induced antigen technology (IVIAT) followed by screening with convalescent serum from trout.
Our immediate goal is to verify that the new hyperimmunized antisera is functionally relevant to our needs—that is, we should see a result very similar to material presented in the detailed report. We will also determine the minimum concentration needed for library screening. We will then migrate our existing *in vivo* protein vectors to the same *E. coli* host used to generate the IVIAT library and confirm that our positive controls work in the library screening format. Once this is done, we will be able to commence with library screening and identification of *in vivo*-expressed proteins. For each protein that we identify, clones will be recovered from the IVIAT library and sequenced. Recombinant protein vectors will be constructed and recombinant protein will be screened by western blot (see detailed report) to confirm *in vivo* expression. This same protein vector will then be used to generate protein for immunization trials. Our proposal calls for screening 15,000 clones from the library. The Call lab is currently screening a different library for a food safety project that involves 45,000 clones, and thus we have worked-out details needed for high-throughput library handling; these tools will be applied immediately to benefit the IVIAT library project.

Objective 2: Validate quantitative diagnostic assays (ELISA and ovarian fluid filtration FAT)
Once the ELISA assay is optimized, MAb FL43 will be conjugated to Alexafluor for use in the filtration FAT assay. Both assays will be used to screen broodstock. To assess the relative risk of vertical transmission it will be essential

to relate infection levels in broodstock at the time of spawning to risk of disease occurrence in progeny. To do this, we will focus on female broodstock only as it is unlikely that sperm would carry bacteria into the egg due to the size of the micropyle. Briefly, samples of kidney and ovarian fluid from up to 60 female broodstock will be collected at various times during the year. Samples will come from replacement broodstock (3 year olds) at Troutlodge in order to obtain both kidney and ovarian samples because these fish are normally culled from the population at that time. Fertilized eggs from these fish will be kept in isolated incubators available at Troutlodge and kidney/ovarian samples will be sent to the University of Idaho (UI) for analysis. During the incubation period, results from ELISA (kidney tissues), FAT (ovarian tissues) and culture (kidney and ovarian fluid) will be obtained as a means of selecting appropriate groups. Eggs from up to 10 individuals showing graded levels of *F. psychrophilum* infection (from low to high) will be selected and shipped to UI when they reach the eyed stage. Each egg group will be identified based on the parent and infection level as measured by ELISA optical density (OD) and/or FAT cell counts/ml. Progeny from egg groups will be reared separately to approximately 0.5 g in size. At that time, fish will be subdivided and stocked in triplicate into 20 L tanks (50 fish/tank). Fish will be subjected to various controlled stressors (low water (density), handling, oxygen, etc.) and monitored for 28 days in an attempt to induce a disease outbreak. All mortalities will be examined to determine cause of death. Clinical symptoms consistent with CWD and isolation of *F. psychophilum* in the absence of other known pathogens will provide strong evidence of vertical transmission. All progeny will be assayed for *F. psychrophilum* prior to stress trials and progeny from broodstock testing negative for *F. psychrophilum* will be included in all trials and serve as a negative control.

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

No work on this objective is planned for year 2.

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

Outreach objectives will begin to be addressed by increasing awareness of this project and research progress through popular press articles in aquaculture newsletters and presentations at aquaculture meetings.

IMPACTS

None to report at this time.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

Waterlines article (to be published): Coldwater disease prevention and control through vaccine development and diagnostic improvements.

Lindstrom NM, Call DR, House ML, Moffitt CM, and 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* infection. *Journal of Aquatic Animal Health* (In press).

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		University	Industry	Other Federal	Other	Total	
2007–08	81,555						81,555
Total	81,555						81,555

Physiological Changes Associated with Live Haul: Maintaining Healthy Fish

Reporting Period	September 1, 2007–August 31, 2008		
Author	John Colt		
Funding Level	2005–2006	$87,156	
	2006–2007	$81,856	
	2007–2008	$86,299	
Workgroup Chair	John Colt		
Participants	John Colt*	National Marine Fisheries Service	Washington
	Mike Rust	National Marine Fisheries Service	Washington
	Ron Johnson	National Marine Fisheries Service	Washington
	Joseph Tomasso	Clemson University	Washington
	Tracey Momoda**	Oregon State University	Oregon
	Rob Chitwood**	Oregon State University	Oregon
	Carl Schreck*	Oregon State University	Oregon
(Outreach Coordinator)	Gary Fornshell*	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

** salaried participants

PROJECT OBJECTIVES

The project objectives during 2007–2008 were to:

1. Compare crowding and different net types on bodily injury.
2. Determine effects of pre- and post-transport salt-dips on survival after transportation of tilapia.
3. Repeat 2007 assessment of parasitic and bacterial load of transported tilapia, using fish from a different grower's farm.
4. Determine the effects of fasting on ammonia levels of transport water.
5. Conduct detailed water quality monitoring during tilapia and adult chinook hauling trip.
6. Modify existing holding systems for 1–1½ lb tilapia and develop simulated hauling systems for laboratory use.
7. Assemble low-light video systems for directly observing tilapia in raceways, during crowding, and in hauling tanks.
8. Conduct outreach activities.

ANTICIPATED BENEFITS

The anticipated benefits of this research are improved fish health and survival of transported fish resulting in improved profitability for fish farmers and retailers, and improved product quality at the consumer level.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1: Compare crowding and different net types on bodily injury.

Fluorescein data from 2007 highlighted the amount of bodily injury to tilapia during the crowding and netting processes that the tilapia experience during transport. This experiment was to determine which of these experiences contribute to the majority of injury that we have observed. Fluorescein analysis of the individual, non-crowded fish indicated injury only to those sampled by the knotted net, as all individuals had a similar amount of bodily injury. Most interesting was the injury to the eye area of all fish sampled in the knotted net. Fish suspended in the fabric net did not exhibit significant injury.

Fluorescein indicated injury to all fish in the crowded treatments, whether crowded only or crowded and suspended in the fabric or knotted net. Punctures were evident, presumably a result from multiple fish in the net. Despite the injury observed, it is difficult to distinguish between crowding and net caused injury.

Objective 2: Determine effects of pre- and post-transport salt-dips on survival after transportation of tilapia based on our earlier findings suggesting positive effects of salt additives.

Tilapia were netted from a pond at a farm in Idaho. After 20 fish were counted, they were either subjected to a salt-dip or not pre-treated with salt (control) before being placed in tanks with 75.7 L (20 gal) of water. Fish were salt-dipped by placing them in water containing 3% Sea Salt (Instant Ocean) for 1 minute before loading into transport tanks. Three tanks of salt-dipped fish and three tanks of non salt-dipped fish were hauled. Each of the six tanks had 3 g/L NaCl added to the transport water and were hauled at a density of 1.2 lb/gal to replicate typical hauling conditions. Upon arrival, fish in the control treatments were moved into separate circular (400-L) tanks with 27–29°C aerated, pathogen-free well water in a flow-through system. Half of the fish in the pre-transport salt-dipped treatments were given a post-haul salt-dip treatment identical to the pre-haul salt-dip; they were then moved into separate tanks. The other half of the control fish were not subjected to a post-transport salt-dip. The three treatments for this experiment were a) control (no pre-haul or post-haul salt-dip), b) pre-haul salt-dip only, c) pre- and post-haul salt dip. Mortality was monitored along with temperature, pH, and DO of the water. Fluorescein-treated fish were photographed; these fish were sampled from the raceway pre-crowding, during post-crowding and post-loading onto truck, and upon arrival to Oregon State University.

There was no beneficial effect from the salt dips, either pre-salt only or both the pre- and post salt dips. We were surprised by these results due to our previous results from laboratory simulated hauls utilizing added salts to the hauling procedure, as well as a similar experiment with fish from a different farm. We were also surprised by the large amount of mortality that was experienced by these fish, nearly 50%. The overall pre-haul quality of the fish used for this experiment might have played a role in driving the mortality observed and thus been a factor in affecting our ability to discern treatment effects.

It should be noted that external treatments such as salt dips, permanganate baths, peroxide dips, etc. will not impact internal bacterial diseases. With the information from objective 3 and the 50% mortality, it appears that these fish may have been compromised before shipment and that shipping stress allowed pathogens such as those found below to flourish.

Objective 3: Assessment of parasitic and bacterial load of transported tilapia.

Four days following the haul to Oregon State University, 12 moribund fish were sampled from the survivors for pathological data. Most individuals had: areas where scales were missing; hemorrhaging over much of the body, caudal, and dorsal fins; and, in some cases, puncture-like wounds. Various bacteria were found. It appears from our examination that these fish may have suffered trauma from the crowding, loading, and handling procedures that may have caused bacterial infections. Bacterial isolates identified from cultures taken from kidney were *Streptococcus* spp., especially *Streptococcus iniae,* and *Aeromonas* spp., especially *Aeromonas veronii* bv. sobria. Parasites identified from wet mounts from the gills of fish sampled four days post-haul were mostly Cysticercus (tapeworm larvae). Costia

infection was also observed in the gills of a few fish sampled. Histological analysis of the gill tissue also indicated some abnormalities such as fusion of the gill lamellae and hyperplasia of the respiratory epithelium.

At this point, we do not have a clear concept of what is causing the mortality of fish hauled from this particular farm. We are still looking at the role of bacteria and all aspects of water quality to understand how they may contribute to mortality.

Objective 4: Determine the effects of fasting on total ammonia concentrations in transport water.
The aim of this study is to determine the length of fasting necessary to minimize the amount of ammonia in transport water. Typically, farmers deprive fish of feed 24-48 hours before transport; however, tilapia may require up to 9 days for feed to be eliminated from their gut. Specifically, this was a range-finding study to determine total ammonia concentrations in transport water following various times of fasting in tilapia. Water samples were submitted to CH2M Hill for total ammonia analysis. As of September 8, 2008, these results are pending.

Objective 5: Conduct detailed water quality monitoring during tilapia and adult chinook hauling trip.
YSI 556 MPS systems were used to monitor barometric pressure, temperature, DO, pH, conductivity, or ORP every minute during hauling trips. Two trips were monitored: (a) tilapia from Challis, Idaho to Corvallis, Oregon and (b) adult Chinook salmon from Manchester, Washington to Salmon, Idaho. Sodium chloride was added to the tilapia hauling tanks while seawater was added to the Chinook tanks.

The initial pH of the Chinook hauling mix was higher than the pH for the tilapia hauling mix, but the drop in pH during the hauling trip was approximately –1.50 units. In comparison, the pH in the tilapia haul was initially depressed, but was very close to the initial value by the end of the trip. This difference in pH variation is likely due to difference in alkalinit and may have an important impact on un-ionized ammonia concentrations and post-haul mortality.

Objective 6: Modify existing holding systems for 1–1½ lb tilapia and develop simulated hauling systems for laboratory use.
An existing recirculation system at the Northwest Fisheries Science Center (Seattle) has been modified to hold 1,000 lb of tilapia at 20 to 28ºC. This involved purchase and installation of six 4 foot-in-diameter circular fiberglass tanks. The overall system comprises three large holding tanks plus the newly installed 4-ft tanks. This system will be used to hold market-sized fish for simulated hauling experiments. The hauling tanks used in the two Idaho hauls have been installed next to the 4-ft holding tanks.

Objective 7: Assemble low light video systems for directly observing tilapia in raceways, during crowding, and in hauling tanks.
Little is known about how tilapia respond to crowding in the raceway or in hauling tanks. We are setting up to monitor tilapia behavior during the entire hauling process. The video cameras that we intend to use have low-light capability.

Objective 8: Conduct outreach activities.
Posted workgroup PowerPoint presentation that summarizes results through late 2007 at *http://extension.ag.uidaho.edu/twinfalls/Aquaculture/Live%20Haul%20Tilapia.pdf*. The presentation emphasizes the physical damage that occurs prior to and during loading. Results to date indicate that all processes that go into loading tilapia onto trucks can cause substantial physical damage and contribute to the poor quality of the fish at the retailers.

Organized a meeting of Idaho tilapia growers and project PIs John Colt and Rob Chitwood. Growers were provided in-depth results to date and plans for next year.

Invited Rob Chitwood to speak at the Idaho Aquaculture Association meeting (June 21, 2008).

Secured a fish pump from Magic Valley Heli-Arc that will be available when PIs and a grower can schedule a test. Preliminary analysis of the cause of mortality seems to be related to the pathogenic bacteria present on fish prior to hauling. It appears that fish are being inoculated with bacteria from the spines of other fish during the crowding, and loading processes. The time-course of disease following such infection correlates well with the timing of mortality following transport. Simple changes to these processes might lessen the extent of physical injury, increase survival, and enhance the appearance of fish held in live-markets.

USEFULNESS OF FINDINGS

We have shown that the crowding process can be very injurious to fish. In addition, net type clearly can affect injury rate, with knotless nets being more benign. We also found that fish can cause puncture wounds to each other. Pre-transport fish quality appears to play a role in the ability of fish to tolerate transport well. The presence of pathogenic bacteria and poor water quality combined with the handling stressors during the hauling process all contribute to post-haul mortality. We have been successful in documenting the occurrence of bodily injury to tilapia hauled from farm to retailer during the various handling procedures using fluorescein. It appears that some fish are being inoculated with bacteria from the spines of other fish during the crowding and loading processes. The impact of alkalinity on pH depression has a critical impact on un-ionized ammonia concentrations and is likely to impact post-haul mortality and product quality.

WORK PLANNED FOR NEXT YEAR

1. Conduct further experiments to identify fasting times that produce the lowest amount of ammonia in transport water to allow proposal of an optimum pre-transport fasting period.
2. Determine the affect of ammonia exposure in transport water on survival in different recovery conditions (e.g., high vs. low pH, conditioned vs. unconditioned water) to determine the importance of the recipient water on survival of transported fish.
3. Assess prevalence of gill hyperplasia from tilapia out of rearing ponds from different farms to assess pre-transport fish fitness and the ability to tolerate transport. Sample kidneys and liver along with wet mounts for parasites.
4. Finalize work on trout transport issues. Initially, identify and assess key problems via contacts of industry cooperators and the U.S. Trout Growers with the assistance of Gary Fornshell.
5. Monitor water quality in hauling and retail stores in the San Francisco area.
6. Determine the impacts of aeration vs. pure oxygen on carbon dioxide, pH, and un-ionized ammonia under simulated hauling conditions.
7. Determine the impacts of alkalinity on mortality and quality of tilapia under simulated hauling conditions.
8. Determine the impacts of receiving water pH on transported fish.
9. Develop outreach products:
    - Two workshops, one in California and the other in Idaho, where the live-haul industry is significant within WRAC states. These will probably occur after the project is completed, all the results are analyzed, and when the Extension Publications are published. Participants will be trained in the use of the models that will be available for downloading from the WRAC website and individual presentations will cover the recommended protocols. Some or all of the co-PIs may participate in the workshops.
    - Two WRAC Extension Publications. Work on the publications will probably begin toward the end of the project as the final research results and analyses become available. The first WRAC Extension Publication will cover recommended pre-hauling handling and harvest, and hauling protocols. The second publication will include recommended protocols for final holding of fish at wholesale and retail outlets.
    - User-friendly spreadsheet models (fish stress and mortality) will be available for downloading from the WRAC website.

IMPACTS

Our research findings have highlighted the impacts of hauling mixture composition and crowding/loading protocols on post-haul survival and product quality. The commercial haulers are experimenting with our suggested hauling mix. The haulers are also interested in reducing crowding/loading damage to tilapia by use of alternative techniques. This may involve the use of different nets or the use of fish pumps.

PUBLICATIONS, MANUSCRIPTS, AND PAPERS PRESENTED

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

Colt J. Spreadsheet model to compute hauling mixture for tilapia. Available for distribution.

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

Colt J, Momoda T, Chitwood R, Feist G., Schreck C. Water quality in warmwater retail holding system in the Pacific Northwest. In preparation.

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

Colt J, Watten B, Rust M. Modeling carbon dioxide, pH, and un-ionized ammonia relationships in serial reuse systems. Submitted to *Aquacultural Engineering*, August 5, 2008.

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

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		University	Industry	Other Federal	Other	Total	
2006	87,156	3,891		37,500			$128,547
2007	81,856	3,500		30,000			$115,356
2008	86,299	4,000		20,000			$110,299
Total	255,311	11,391		87,500			$354,202

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

Reporting Period	September 1, 2007–August 31, 2008		
Author	Molly Webb		
Funding Level	$100,001 (first year budget)		
Participants	Molly Web*	Montana State University	Montana
	Serge Doroshov*	University of California, Davis	California
	Barbara Rasco*	Washington State University	Washington
	Anna Cavinato*	Eastern Oregon University	Oregon
	Wendy Sealey*	University of Idaho	Idaho
	Gary Fornshell*	University of Idaho Extension	Idaho
	Linda Lemmon	Blind Canyon Aqua Ranch	???
	Leo Ray	Fish Breeders of Idaho	Idaho
Technical Advisor	Fred Conte	University of California, Davis	California
Industry Advisor	Peter Struffenegger	Sterling Caviar, LLC	?????????

* 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 the white sturgeon caviar industry. The specific objectives of this study 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, and plasma protein levels and crude chemical composition of eggs change with maturity (years 1–2)
3. Evaluate short wavelength near infrared spectroscopy (SWNIR) 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 SWNIR 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 western region caviar industry.

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 to specifically address whether it is possible to detect the early signs of ovarian atresia to avoid sacrificing fish with inferior quality roe (Objective 5—referred to as the Atresia Study). The second group of females was harvested for caviar to address whether stage of maturity can be assessed less-invasively using the proposed tools (SWNIR, FT-IR, plasma steroids measured by radioimmunoassay (RIA) plasma calcium, and protein measured by blood chemistry analyzers, ultrasound) and to determine if there is a correlation between oocyte PI and caviar quality and yield (Objectives 1–4; herein referred to as the Caviar Study).

Fifteen late vitellogenic (2001 year class) females were initially sampled on September 10, 2007 at the Wilton warm water site of Sterling Caviar, LLC for the Atresia Study. Fish were moved the next day to the Buena Vista cold-water site (BV). They were subsequently sampled every two months through March. Two days after the March sampling, the fish were returned to the warm-water site to induce ovarian follicular atresia and sampled two more times on April 2 and April 17, 2008. At each sampling time during the Atresia Study, fish were weighed, measured, and the pit tag number recorded. Each female was given an abdominal ultrasound scan (Webb Laboratory) and SWNIR scans (Cavinato Laboratory). A blood sample was collected, and the plasma split for steroid (Webb Laboratory) and FT-IR (Rasco Laboratory) analyses. Egg samples were collected and preserved for measurement of egg size, oocyte PI, and histology (Webb Laboratory) and frozen for FT-IR analyses (Rasco Laboratory). SWNIR spectra were collected on fresh eggs (Cavinato Laboratory). At the last sampling, all females, except one, were in various stages of ovarian atresia.

For the Caviar Study, 100 (2001 year class) caviar females were sampled as described above with some modifications. Subgroups of 20 fish were sampled for blood plasma at the BV cold-water site, the day before caviar harvest, and were individually identified for caviar processing. Immediately after euthanasia, the fish were scanned with the ultrasound and SWNIR, weighed, and measured. The ovaries were removed, weighed for determining gonadosomatic index (relative weight of ovaries), and subsampled for the Webb, Rasco, and Cavinato Labs, as described above. The roe was then processed for caviar, and Sterling Caviar, LLC determined and provided data on the roe yield and grade of caviar. Sampling of 5 subgroups (n=20) was conducted on February 13, March 11, April 8, May 7, and June 3, 2008.

The morphometric measurements (PI, egg diameter [ED]), histological analysis, blood chemistry analyses (RIA, blood chemistry analyzer), and SWNIR have been completed for the Atresia Study. FT-IR spectroscopy has been completed for the September and November data for the Atresia Study. The analyses for Caviar Study are currently underway and are projected to be completed by December 2008.

Results Summary. Eleven fish survived until the end of the Atresia Study. One fish was in poor condition for the duration of the experiment, therefore data was not included in the steroid analysis. Nine of the 10 remaining females had ovaries that initiated and progressed through atresia. The first detection of atresia occurred on January 16, 2008 (cold-water site), followed by a second female on March 17, 2008 (warm-water site), then four more on April 2, 2008. Atresia was detected in the three remaining females on April 17, 2008. Histological examination identified atresia in three females before macroscopic signs of atresia were present. However, very early atresia was described macroscopically in three other females with no histological evidence present in samples from the same date.

Early atresia could be detected using both sex steroid analysis and SWNIR. Principal Component Analysis (PCA) and Soft Independent Modeling of Class Analogy (SIMCA) modeling resulted in 80% of all atretic females and 79% of all females with mature eggs to be correctly classified using eight latent variables collected from eggs. When the model was validated by leave-one-out, 60% of all atretic and 79% of all mature females were correctly classified.

USEFULNESS OF FINDINGS

These findings demonstrate the potential for using sex steroid analysis and/or near infrared diffuse reflectance spectroscopy for less invasively determining the presence or absence of atresia and classifying sturgeon according to different degrees of maturity. These techniques appear to be useful as an alternative to the more invasive ovarian biopsy for caviar production. Validation of these techniques will occur in Year 2 of the study.

WORK PLANNED FOR NEXT YEAR

While the biennial ovarian cycle in farmed sturgeon is controlled by seasonal photoperiod, there is significant individual variation of the endogenous cycle within the stock. Some fish reach full maturity (fully grown eggs) in February, while others reach full maturity in May. One way to optimize caviar yield would be to segregate the stock by maturity in the fall and harvest segregated groups at different times in the spring, when each group would potentially have fully grown eggs. To evaluate this possible management strategy, we plan to sample 160 caviar females in November 2008 at Sterling Caviar, LLC and 60 females in October 2008 at Fish Breeders of Idaho, using ovarian biopsy and ranking the marked fish by oocyte PI, a precise indicator of ovarian maturity in sturgeon. In addition, each fish will be weighed, measured, have its ovaries scanned using SWNIR, bled for FT-IR and sex steroid analysis, and its eggs will be collected for PI, diameter, and FT-IR. Previous farm observations (see proposal) show that oocyte PI in November ranges from 0.16 (most mature) to 0.37 (least mature). Subsequently, we will attempt to group females by oocyte PI into three homogenous populations (low, medium, and high oocyte PI) with approximately 30 females in each group (90 total) in California and 15 females in each group (45 total) in Idaho. The caviar processing and sampling will be conducted identical to the first year of the study with each group of females at one-month intervals, during approximately late-February, late-March and late-April in California and late-April, late-May, and June in Idaho, starting with the low PI group. A control group at each site will include randomly chosen females processed identically at each time the low, medium, and high oocyte PI groups are harvested. The yield of roe, egg size, and PI at harvest will be evaluated for each group and compared by Analysis of Variance. We anticipate the oocyte PI and roe yield to be similar in all groups. The SWNIR spectra, FT-IR spectroscopy, and plasma steroid concentrations will be further evaluated for detecting maturity stages as a means to predict oocyte PI less invasively.

IMPACTS

The initial studies indicate that the onset of ovarian atresia is marked by significant changes in plasma sex steroid and egg and plasma lipid concentrations. The SWNIR and the measurement of plasma sex steroids allow for detection of ovarian atresia and may be useful as an alternative method for pre-screening caviar females prior to harvest. The early detection of ovarian atresia can be used to prevent production of low-grade caviar and will save the fish for the next ovarian cycle. The application of these new methods under field and commercial conditions needs to be further tested and refined.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

Posters Presented

Servid SA, Twombly LR, and Cavinato AG. 2008. Non-invasive characterization of maturity status to optimize caviar yield and quality in white sturgeon. Poster presented at the 235th National Metting of the American Chemical Society, April 2008, New Orleans, LA.

Talbott MJ, Van Eenennaam JP, Linares-Casenave J, Doroshov SI, Guy CS, and Webb MAH. 2008. Determining morphological and immunochemical parameters associated with early ovarian follicular atresia in white sturgeon (*Acipenser transmontanus*) females. Poster presented at the 8th International Congress on the Biology of Fishes, July 28–August 1, 2008, Portland, OR.

Popular Press Articles

Phillips B. 2008. Sturgeon populations: Research benefits new U.S. industry. *WSU Today*. May 12, 2008.

Mills J. 2008. Domestic product challenges Russian caviar. *Lewiston Tribune*. June 23, 2008.

Webb M and Doroshov S. Submitted. Determining ripeness in white sturgeon females to maximize yield and quality of caviar. *Waterlines*.

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
Total	100,001		18,500	79,986*		98,486	$198,487

* $74,986 SeaGrant funding to UC Davis to identify cause of soft chorion/Grade C caviar at Sterling Caviar, LLC, $5,000 USFWS Bozeman Fish Technology Center funds to pay for graduate student travel from Montana to California for sample collection.

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

Reporting Period	September 1, 2007–August 31, 2008		
Author	Christopher Myrick		
Funding Level	First Year Funding Received	$99,991	
	Second Year Funding Received	$95,677	
	Third Year Funding Received	$91,973	
	Fourth year Request	$67,588[1]	
Participants	Christopher A. Myrick* (*Working Group Chair*)	Colorado State University	Colorado
	Gary Fornshell *(Extension Rep.)*	University of Idaho	Idaho
	Molly Webb* & Kevin Kappenman* *Greg Kindschi retired*	USFWS Bozeman Fish Technology Center	Montana
	Ken Cline* (*Industry Collaborator & Advisor*)	Cline Trout Farms	Colorado
	John Seals*	USFWS Mora National Fish Hatchery & Technology Center	New Mexico
Industry Advisor	Chris Nelson	Nelson & Sons, Inc.	Utah
Technical Advisor	Rick Barrows	USDA/ARS	Montana

* voting, work group members

PROJECT OBJECTIVES

The purpose of this research project is to improve the growth, quality, and survival of cutthroat trout (*Oncorhynchus clarkii* subspp.) and Gila trout (*O. gilae gilae)* with the ultimate goal of providing fish culturists and feed manufacturers with information that can be used to improve the production of these species. The specific objectives of this study are listed below. Objectives that are relevant to years 1, 2, and 3 are italicized.

1. *Determine the effect of feed texture and formulation on survival, growth, and quality of cutthroat and Gila trout.*
2. *Determine the effect and interaction of diet texture and formulation on trout growth, survival, and quality when reared at different water temperatures under laboratory conditions.*
3. *Determine the effect of rearing density on trout growth, survival, and quality.*
4. Conduct production-scale evaluations of the best diet–temperature–density combinations identified in the first three objectives. This will also allow us to test our assumption that a diet developed for 2–3 strains of cutthroat

[1] The requested funding for the 4th year of the project is lower than originally proposed ($95,147) because we are no longer requesting $27,559 for the USFWS Mora Fish Technology Center.

trout will provide superior performance for other untested cutthroat trout strains (e.g., Rio Grande cutthroat trout, *O. clarkii virginalis*, and greenback cutthroat trout, *O. clarkii stomias*) than diets developed for rainbow trout.[2]

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 and Gila trout.*

ANTICIPATED BENEFITS

This project will provide information to trout growers about optimal diet, water temperature, and rearing density for producing quality native fish for many different stocking programs for Colorado River cutthroat trout (*O. clarkii pleuriticus*), Snake River cutthroat trout (*O. clarkii behnkei*), and Yellowstone cutthroat trout (*O. clarkii bouvieri*), and Gila trout. With time, this may also apply to other subspecies of cutthroat trout, such as Rio Grande, greenback, Lahontan (*O. clarkii henshawi*), or westslope (*O. clarkii bouvieri*). Commercial feed manufacturers will be able to supply the best available customized diet to fish culturists rearing these species of fish. Recreational fishermen and outfitters will also benefit by having more fishing opportunities available for catching native fish. Indirectly, all of this benefits local and surrounding communities, providing services in the areas where these native fish are found or propagated.

Another indirect benefit is improving the recovery and restoration of these species, hopefully leading to the delisting of Gila trout from the endangered species list and preventing other strains of cutthroat trout from being listed. Gila trout were reclassified as threatened from endangered in 2006. The new classification (threatened) allows states to use Sport Fish Restoration money to propagate and stock these fish. In either case, increased survival and growth of this particular species under cultured conditions is now of greater concern because of the increased demand for progeny.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Year 2 (2007)[3]

In 2007, the Bozeman Fish Technology Center (Bozeman Center) again served as the point of distribution for the diets used in the growth trials. Based on the results from the 2006 diet trials, the Bozeman Center used Diet #5 for the Snake River cutthroat trout and Yellowstone cutthroat trout temperature and density studies. Colorado State University (CSU) requested Diets #1 and #5 because they were the two top performing diets from the 2006 diet trails. Mora National Fish Hatchery and Technology Center (Mora Center) requested Diets #3, #4, and #5, but the unfortunate loss of eyed eggs during incubation prevented Gila trout temperature trials from being conducted during 2007.

The Bozeman Center conducted comprehensive 16–week density and temperature experiments with Snake River cutthroat trout and Yellowstone cutthroat trout. The density experiment compared the performance of both subspecies of cutthroat trout when reared at densities of 50, 100, 150, 200, 250, 300, and 350 fish per tank at a fixed water temperature of 10°C. Both subspecies were reared at 10, 12, 14, 16, 18, and 20°C to determine the optimal temperature for fish growth. Both cutthroat subspecies showed significant density effects, with increasing density leading to decreased fish wet weight at the end of the 16-week growth trial. Survival of Yellowstone cutthroat trout was unaffected by density, but survival of Snake River cutthroat trout decreased significantly as density increased. The results from the temperature studies showed the classic temperature × growth rate relationship. The optimal temperatures for growth of Yellowstone cutthroat trout and Snake River cutthroat trout were 14.7°C and 14.5°C, respectively.

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[2] This was supposed to be one of the 3rd year objectives, but because Snake River cutthroat trout eggs were unavailable for the production trial, it has been moved to the final year of the project.

[3] Year 2 (Y2) results are presented because Y2 experiments were still underway when the Y2 Annual Report was prepared

CSU conducted a 120-day diet × temperature study using Diets #1 and #5 (both supplemented with live Artemia for the first three weeks) from the 2006 diet trial and temperatures of 10, 12.5, 15, 17.5, and 20°C. Diet, temperature, and the diet × temperature interaction all had significant effects on growth. For all temperatures, fish fed Diet #5 had higher growth and survival, suggesting Diet #5 was the top performing diet. Dorsal fin index was unaffected by diet, temperature, and the diet × temperature interaction. Temperature proved to be a driving force for fish growth, with the optimal temperatures for growth being 15.3°C and 16.4°C for fish fed Diets #1 and #5, respectively. Finally, there was an inverse relationship between temperature and survival, with the fish reared in 10°C water having the highest survival. Based on these results, it is recommended that Colorado River cutthroat trout be reared in 16.4°C water and fed Diet #5 to improve growth.

Several suggestions were made at the 2006 workgroup and IAC/TC meetings in regard to the Mora Center's rearing set-up and design. In place of aquaria used in 2006, 30 opaque fiberglass troughs were purchased as rearing units and plumbed. Twenty 12-h belt feeders were obtained to provide a more even feed rate over hand feeding. The system was successfully installed, but because of an unanticipated problem with the Gila trout eggs, was not put into use in 2007.

More than 30,000 Main Diamond Gila trout eggs were collected, but the hatch rate was extremely low (approximately 5%). Therefore, there were not enough Gila trout for the Mora Center to conduct the 2007 feed trial and meet their obligations to provide New Mexico with fish for ongoing recovery efforts. An attempt to take and fertilize eggs from wild fish was also unsuccessful. The Mora Center considered a recommendation to import a surrogate species such as Snake River cutthroat trout, but this was ultimately rejected because of administration concerns with having non-Gila trout in close proximity with Gila trout destined for recovery efforts.

The outreach component of the project continued to be coordinated by the UI. Presentations of the CSU portion of the study were delivered in 2007 at the Colorado Aquaculture Association meeting, the Colorado–Wyoming Chapter of the American Fisheries Society meeting, and the national American Fisheries Society meeting. The project website is ready for posting on the WRAC website, and the intent is to post annual progress reports and any other pertinent information as it becomes available. The URL of the website will be advertised on aquaculture association websites, newsletters including *Waterlines*, and through appropriate state and federal agencies. The website will also serve as the distribution point for the Extension publication. The workgroup held its second annual meeting on September 13, 2007, in Ft. Collins, Colorado. The 2007 trials and the full-scale production trials were discussed.

Year 3 (2008)

The Bozeman Center completed the pre-planned trials in Years 1 and 2 of the project, so we took advantage of its research facilities to plan additional studies to attempt to (1) advance the status of the Gila trout work and; (2) address some of the new questions that have been developed from the Year 1 and Year 2 research. Because of the difficulties encountered at the Mora Center, the Bozeman Center was prepared to conduct temperature and/or diet studies on Gila trout, if eggs were available. Unfortunately, Gila trout eggs were not available, so the research facilities at the Bozeman Center are being used in an additional 60-day diet study on Snake River cutthroat trout that is designed to address the question of whether the superior survival and growth seen in treatments fed Diet #5 (Skretting/Bio-Oregon Bio-Vita) supplemented with live Artemia could be matched by diets incorporating dry Artemia, dry cyclopeeze, or other premium starter diets. The study is still underway, but preliminary results show that survival is again very high (94.3 to 99.8%) across all treatments. As in Year 1 and Year 2, the Bozeman Center also served as the distribution point for experimental diets, in this case Diet #5, for CSU.

CSU used the results from the Year 1 and 2 experiments to design a 120-day density experiment that is currently underway. The original design entailed rearing fish using densities of 100, 200, 300, 400, 500, and 600 fish per tank using the optimal diet × temperature combination (Diet #5 × 16.4°C). Results from the Bozeman Center's density experiments using Snake River cutthroat trout and Yellowstone River cutthroat trout showed minor differences in

growth among treatments ranging from 50–350 fish per tank, so the workgroup decided CSU should cover a larger range of densities in the Colorado River cutthroat trout experiment. Thus, CSU decided to increase the highest density to 600 fish per tank. The first batch of Colorado River cutthroat trout eggs were received on June 13, 2008. Unfortunately, the sac-fry were lost on June 23, 2008 when the well pump supplying water to CSU's Foothills Fisheries Laboratory failed. A new pump was installed, and CSU was able to acquire more eggs on July 21, 2008, but not enough to stock fish at the densities planned. Feeling it was important to try to keep the high density treatment, CSU stocked fish at densities of 150, 300, 450, and 600 fish per tank (4 tanks/treatment). This experiment started on August 14, 2008; results are currently unavailable.

The original proposal called for a production-scale trial using Snake River cutthroat trout at Cline Trout Farms (CTF) in Boulder, Colorado. The producers worked with CSU personnel to develop a culture protocol, and were ready for the trial to start in the late spring of 2008. Unfortunately, a source of Snake River cutthroat trout eggs could not be secured, so the production trial was not initiated. Workgroup members have since entered into discussions with the US Fish and Wildlife Service (USFWS) to produce a written agreement that would allow the USFWS Jackson National Fish Hatchery to provide up to 40,000 Snake River cutthroat trout eggs to CTF in 2009 so that this critical portion of the project can be completed. We hope to have this agreement in place by the 2008 Industry Advisory Committee/Technical Committee meeting in Reno, Nevada.

The outreach component of the project continues to be coordinated by the UI. Thus far, presentations of the CSU portion of the study have been delivered in 2008 at the Colorado Aquaculture Association meeting, the Colorado–Wyoming Chapter of the American Fisheries Society meeting, the Idaho Aquaculture Association meeting, and the Aquaculture America meeting. CSU is also scheduled to present results at the Western Division of the American Fisheries Society Student Colloquium in October 2008. CSU also published an article in *The Fishline*, a publication of the Colorado Aquaculture Association.

The project website development began in late 2006 and continued into the first quarter of 2007. The website includes a description of the project, project results, and copies of IAC/TC PowerPoint presentations. Although the project website has been, and is still ready for posting on the WRAC website, a lack of resources with WRAC has delayed the posting. When available, annual progress reports and other pertinent information will be posted on the website. The URL will be advertised on aquaculture association websites, newsletters including *Waterlines*, and through appropriate state and federal agencies. The website will also serve as the distribution point for the Extension publication. The first peer-reviewed project publication, with the tentative title of "Performance of Yellowstone and Snake River cutthroat trout fry fed seven different diets" is being prepared for submission to the *North American Journal of Aquaculture*. The workgroup will hold its third annual meeting on October 8, 2008 via teleconference. The 2008 trials, possible 2009 trials, full-scale production trials, and development of the culture manual and other publications will be discussed.

USEFULNESS OF FINDINGS

Increasing our knowledge of the culture conditions and diet requirements for these unique native species will enable commercial fish farmers to supply fish to their clientele, and will ultimately allow recreational fishermen to have greater fishing opportunities on both private and public waters. In turn, this will allow for commercial trout growers and feed manufacturers to expand their marketing capabilities. Certain commercial feed manufacturers and private and public hatchery personnel now know that this study is being conducted. Consequently, workgroup investigators have been receiving inquiries as to the status of the experiments because there is much interest in knowing the outcome. Obviously, feed manufacturers have much to gain or lose pending the performance of their product. Fish culturists want to know the best available feeds, temperatures, and rearing densities to increase the performance of their fish. These recommendations will be passed on for 2009 production-scale studies.

The workgroup disclosed the identity of the diets after the diet × temperature trials were completed. This information

has now been presented at several meetings and researchers have been contacted by hatchery personnel to discuss diet performance and how to obtain the diets used. The information on the benefit of even three weeks of diet supplementation with Artemia on the survival and growth of first-feeding cutthroat trout has also been mentioned during presentations, and has generated some interest from industry and hatchery personnel. Industry and hatchery personnel have also inquired about the optimal rearing temperatures for the three subspecies of cutthroat trout.

WORK PLANNED FOR NEXT YEAR (Year 4)

1. A production-scale trial will be conducted at CTF using Snake River cutthroat trout eggs. The objective of this study is to compare the performance of the laboratory-reared Snake River cutthroat trout with that of fish reared under commercial production conditions.
2. CSU will focus on the preparation of one MS Thesis (M. M. Brandt) and two to three manuscripts based on the research conducted on Colorado River cutthroat trout. We also anticipate presenting a summary of project results to a variety of audiences, including the Colorado Aquaculture Association. CSU will also participate in the production-scale trial at CTF.
3. The Mora Center will not be requesting any further funds from WRAC for this project and will not be conducting any studies related to this project because of the ongoing difficulties in procuring enough Gila trout eggs.
4. The Bozeman Center will analyze data from the diet, temperature, and density studies to develop manuscripts for publication in journals such as the *North American Journal of Aquaculture* and *Aquaculture*. The Bozeman Center staff will also provide technical assistance for the initiation and completion of production-scale studies during 2009.
5. If analyses of the results from the 2008 studies at CSU and the Bozeman Center indicate a need for further laboratory studies, those will be undertaken, provided that the experimental systems are available.
6. Results from the first three years of the study will be presented in a variety of industry and professional association forums, including the 2009 Colorado Aquaculture Association meeting, the Colorado–Wyoming Chapter of the American Fisheries Society meeting, the Western Division of the American Fisheries Society Student Colloquium, and the US Trout Farmers Association Meeting.
7. Annual progress reports and any other pertinent information will be posted on the project website. The URL of the website will be advertised on aquaculture association websites, newsletters including Waterlines, and through appropriate state and federal agencies

IMPACTS

This project has already benefited the western aquaculture industry because it has brought a diverse group of researchers and industry representatives together. This group is working together to outline the firm goals and objectives needed to bring this project to a successful completion. Obviously, as more data are collected and analyzed, there will be more information available for distribution to the western aquaculture industry; it is then that the impact of the research will be truly quantifiable.

PUBLICATIONS, MANUSCRIPTS, AND PAPERS PRESENTED

Publications

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

Manuscripts

Kindschi GA, et al. In Preparation. Performance of Yellowstone and Snake River cutthroat trout fry fed seven different diets. *North American Journal of Aquaculture*.

Papers Presented

Brandt MM and Myrick CA. 2008. 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.

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

Brandt MM and Myrick CA. 2008. 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, FL, February 9–12.

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

Brandt MM and Myrick CA. 2007. 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, CA, September 2–6.

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

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

Myrick CA. 2004. 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 2004 Annual Meeting, Mt. Princeton, CO, December 10–11.

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
2007	91,973	5,000	5,000	45,000		77,000	$168,973
Total	287,641	40,829	15,000	216,500	11,000	300,329	$587,970

Bozeman Fish Technology Center (Bozeman Center)

Industry support comes from the technical assistance in-kind services provided in formulating feeds and supplying ingredients. "Other Federal" support is in-kind salaries, benefits, holiday pay, services, travel, gas, feed analyses, and utilities provided by the US Fish and Wildlife Service's Bozeman Center and Jackson National Fish Hatchery (Snake River cutthroat trout egg source). The "Other" support funding source above are in-kind services provided by Montana Department of Fish, Wildlife and Parks in reviewing Import Permit Applications and rearing, incubating, and delivering Yellowstone cutthroat trout eyed-eggs from the Yellowstone River State Fish Hatchery for use at the Bozeman Center.

Colorado State University (CSU)
University funding support comes from in-kind assistance in the form of one month of the principal investigator's salary, benefits, and the utility costs associated with running the diet trials at the Foothills Fisheries Laboratory. CSU also provided the administrative support necessary for hiring one MS-level graduate student and partial salary support by employing the student as a graduate teaching assistant. "Other" support was provided by Colorado Division of Wildlife Glenwood Springs Fish Hatchery for obtaining and incubating Colorado River cutthroat trout eyed-eggs for CSU, the Colorado Division of Wildlife Fish Research Hatchery for loaning experimental tanks to CSU, and the Colorado Division of Wildlife Fish Pathology Laboratory.

Mora National Fish Hatchery and Technology Center (Mora Center)
"Other Federal" support is in-kind salaries, benefits, holiday pay, services, travel, gas, feed analyses, and utilities provided by the US Fish and Wildlife Service's Mora Center.

Potential Threat of Great Lakes VHS Virus in the Western United States

Reporting Period	September 1, 2007–August 31, 2008		
Author	Gael Kurath		
Funding Level	First Year Funding	$ 72,097 (received July 2008)	
	Second Year Funding	$ 27,903	
	Total Funding	$100,000	
Participants	Gael Kurath (*Working Group Chair*)	USGS Wester Fisheries Research Center (WFRC), Seattle	Washington
	Jim Winton	USGS–WFRC, Seattle	Washington
	Paul Herschberger	USGS–WFRC, Marrowstone Marine Station	Washington
	Carolyn Friedman*	University of Washington	Washington
	Jerri Bartholomew* (*Outreach)*	Oregon State University	Oregon
	Chang Hoon Moon	University of Washington	Washington
Industry Advisor	Scott E. LaPaftra	Clear Springs Foods, Inc	Idaho.
Technical Advisor	Kenneth Cain	University of Idaho	Idaho

* voting, work group members

PROJECT OBJECTIVES

Viral hemorrhagic septicemia virus (VHSV) was first identified in the Great Lakes in 2005 as the causative agent of a large-scale die-off of freshwater drum in Lake Erie. 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 prooject's outreach and research objectives 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. The five specific objectives are to:

1. Assemble and distribute current biosecurity information 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.

ANTICIPATED BENEFITS

Knowledge of specific host susceptibility to disease or infection by the different strains of VHSV will assist aquaculture farmers to manage their operations to avoid disease, and to direct their biosecurity efforts for maximum benefit. Knowledge of biological differences between VHSV strains, including not only virulence, but also the ability to establish infections resulting in carrier or reservoir fish in different host species, will be essential for understanding how these pathogens move and persist in fish populations. In Western aquaculture facilities where Pacific coast VHSV type IVa may occur, knowledge of how this endemic VHSV genotype differs from the Great lakes VHSV

type IVb, and the ability to differentiate the two strain types, will allow the industry to detect and manage any introduction of VHSV IVb into the Western region. Finally, as an indirect benefit to aquaculture, the results of these *in vivo* studies in the Great Lakes host species, yellow perch (*Perca flavescens*), will be the first steps in the development of a laboratory model for VHSV IVb infection that can be used for more in depth studies of Great Lakes VHSV by researchers across the country.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS:

Funding for this project became available in July 2008, so at the time of this report the project has been in progress for two months. On August 11, 2008, Chang Hoon Moon was hired for the project as a post-doctoral fellow at the University of Washington, working in the Kurath laboratory at the USGS Western Fisheries Research Center (WFRC) in Seattle. Prior to the availability of funding, stocks of various fish species were obtained and reared at WFRC in anticipation of this project. Thus, despite the short time period of funding to date, progress has been made toward all objectives.

Virus stocks: VHSV isolates representing genotypes IVb, IVa, and I (from the Great Lakes, Pacific Coast, and Europe, respectively) have been amplified and quantified at WFRC to prepare standardized virus stocks for all experiments. In addition, a VHSV strain IVb isolate from New Brunswick, representing Atlantic Coast virus (considered to be the likely source of introduction of VHSV into the Great Lakes), has been obtained and amplified in the same manner.

Fish Stocks: Stocks of pathogen-free yellow perch and Chinook salmon sufficient for all experiments in the first year of the project are now available at WFRC. Pathogen-free herring are available at the WFRC Marrowstone Marine station, and pathogen-free rainbow trout are available from Dr. S. LaPatra at Clear Springs Foods, Inc.

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

Funding arrived in late August at Oregon State University (OSU), yet progress has been made on several outreach components. Currently available biosecurity information for the European VHSV I and the Great Lakes VHSV IVb strains has been collected for use in preparing fact sheets that will be relevant to the region and to each of the user groups: fish farmers, anglers, and fish health professionals. Several powerpoint presentations have been made available by project PIs and collaborators.

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

A general quantitative real-time polymerase chain reaction (qRT-PCR) assay that detects both VHSV types IVa and IVb has been developed by Dr. Kyle Garver of the Pacific Biological Station in Nanaimo, British Columbia. We have tested this assay and it works well for our VHSV strains, so it is used in objectives 3 and 4 below for general detection of VHSV. Development of genotype-specific qRT-PCR assays that specifically detect type IVa only, or type IVb only, has not yet begun.

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. Susceptibility of four fish species to disease and mortality due to Great Lakes VHSV type IVb will be determined in controlled wet laboratory challenge studies. West Coast VHSV type IVa and European VHSV type I will be tested simultaneously for comparisons of virulence in these hosts. As an addition to the study design of the proposal, we will also include an Atlantic Coast VHSV type IVb strain from New Brunswick in these comparisons whenever possible. All fish infection studies will be conducted in the WFRC Biosafety Level 3 (BSL-3) wetlab for containment of the VHSV types I and IVb that are not endemic to the Western region.

Pilot studies in yellow perch. Yellow perch are used in this project as a host that should be susceptible to Great lakes VHSV IVb, since epidemics have occurred in yellow perch in the Great Lakes. In preparation for this project, two pilot studies were conducted. Using small group sizes of 5 fish with three VHSV strains (gt I, gtIVa, and gtIVb-Great Lakes), we first tested a high challenge dose of 10e7 plaque forming units (PFU) of virus per fish, injected intraperitoneally (IP). Mock injected fish that received no virus survived for 28 days, and the majority of fish injected with any virus strain died within 5–8 days. Several fish had disease signs typical of VHS, including external hemorrhage, exophthalmia, and accumulation of ascites. A second pilot scale test at the lower dose of 10e3 PFU/fish yielded similar results. Thus, we confirmed survival of negative control fish under conditions in the BSL-3 wetlab and confirmed ability to generate VHS disease in experimental challenges of yellow perch. These preliminary results indicated no difference in virulence between the three VHSV strains tested, and challenge doses of 10e6 and 10e3 PFU/fish were selected as high and low doses for use in subsequent full-scale challenges.

Pilot studies in herring. Pathogen-free herring are available at the WFRC Marrowstone marine station approximately 3 hours drive from Seattle. We have brought herring from Marrowstone to WFRC twice, but to date we have had trouble maintaining them due to high mortality after transport and during holding in the BSL-3, likely due to difficulties in maintaining cold temperatures while providing static seawater.

Comparative virulence test in Chinook salmon. The full-scale virulence test of four VHSV strains in Chinook salmon has been completed. Chinook salmon fry reared from eggs in the WFRC wetlab were transferred into the BSL-3 wetlab and triplicate groups of 20 fry were challenged by IP injection at both high (10e6 PFU/fish) and low (10e3 PFU/fish) doses of virus. Control fish were injected with media that had no virus. Fish were then held for 28 days, with daily monitoring for mortality and removal of any fish that died. Control fish had no mortality, and groups that had virus experienced mortality ranging from 3–45% in the low-dose treatments, and 13–78% in the high-dose treatments. In Chinook, the European type I VHSV had the highest virulence and Pacific Coast type IVa was lowest. Both Great Lakes type IVb and New Brunswick type IVb were intermediate in virulence. Some fish with each virus strain had clinical signs of hemorrhage and exophthalmia typical of VHS. This is the first comparison of virulence of Great Lakes VHSV with other VHSV strains in any host, and to our knowledge it is the first demonstration that European VHSV is virulent in Chinook.

Concomitant with the virulence test above, a fourth group of 20 Chinook fry were injected with the high dose of each virus as a preliminary test for viral infection levels and shedding. Water samples 7d post-challenge had only extremely low levels of virus near the detection threshold in a portion of the samples. No virus was detected in water samples 28d post-challenge.

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 over time after immersion challenge, and ultimately testing co-infection with both VHSV types IVb and IVa. To date we have conducted one infection time-courses in yellow perch and one in Chinook salmon. In both time courses only a small proportion of fish had detectable virus, indicating that a higher challenge dose will be required to generate this data.

Objective 5. Develop outreach materials to communicate project results.

Two oral presentations on the preliminary studies in yellow perch have been presented at regional and national fish health meetings.

USEFULNESS OF FINDINGS

None to date.

WORK PLANNED FOR NEXT YEAR

1. Assemble and distribute current biosecurity information for dealing with VHSV: Information that has been gathered will be compiled and updated into fact sheets that will be relevant to the region and to each of the user groups: fish farmers, anglers, and fish health professionals. Powerpoint presentations from PIs will be compiled to make an annotated presentation for the website.
2. Develop diagnostic assays to differentiate Great Lakes VHSV IVb from endemic West Coast VHSV IVa. All available sequences from VHSV IVa and IVb isolates will be used to design genotype-specific qRT-PCR assays that will subsequently be tested for efficiency, sensitivity, and specificity.
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. Full-scale virulence trials will be conducted in yellow perch and rainbow trout, using the same low- and high-challenge doses (10e3 and 10e6 pfu/fish) as used in the completed Chinook experiment. We plan to test herring again, using smaller fish during the winter, when it will be easier to maintain cold water temperatures in the outdoor seawater tank.
4. Test ability of relevant host species to act as carriers and/or reservoirs of different VHSV genotypes. Dose tests will be conducted in Chinook and rainbow trout to identify an optimal immersion challenge dose that reliably generates infection in at least 90% of the fish. Time courses comparing infection levels of different VHSV strains will then be conducted using this challenge dose in Chinook, yellow perch, and rainbow trout.
5. Develop outreach materials to communicate project results. Data from Objectives 3 and 4 will be compiled into a table and used to update the fact sheets on the WRAC website. A workshop will be organized in conjunction with the annual IAC/TC meeting in year 2 for training extension personnel who will contact their target audience. Fish producers of all species in the western region will receive information on objectives 3 and 4 through the website and extension personnel that receive VHSV training at the workshop. Dr. Kurath will present results of this project at the VHSV session that will be held at Aquaculture American meeting in February 2009 in Seattle, and additional presentations will be made at other conference venues.

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IMPACTS

None to report at this time.

PUBLICATIONS, MANUSCRIPTS, AND PAPERS PRESENTED

Presentations

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

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 E, Wargo A, Binkowski F, Goetz R. Presentor: Gael Kurath, USGS-WFRC .

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		University	Industry	Other Federal	Other	Total	
2008	72,097						$72,097
Total	72,097						$72,097

Substantial in-kind support for this project includes 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).

Immunological Mechanisms of Intensively Reared Warmwater and Coolwater Finfish

Termination Report

Project Work Period November 1, 2003–November 1, 2007

Author Vaughn Ostland

Participants			
	James Winton	Western Fisheries Research Center	Washington
	Carolyn Friedman*	University of Washington	Washington
	Vaughn Ostland*	Kent SeaTech Corporation	California
	Scott LaPatra*	Clear Springs Foods, Inc.	Idaho
	Steve Harbell*	Washington State University Cooperative Extension	Washington

Reason for Termination Funding ended

PROJECT OBJECTIVES

1. Develop a suite of reagents and assays that can be used to quantify the specific humoral and cellular immune responses of warmwater and coolwater finfish that could be used as indicators of healthy finfish immune systems and assess the efficacy of novel vaccines and methods of immunization.
2. Determine the immune response kinetics of hybrid striped bass and rainbow trout to *S. iniae* and *A. hydrophila*.
3. Determine the effects of hatchery practices on immune functions of hybrid striped bass and rainbow trout.
4. Assess the immune response and cytokine gene expression of hybrid striped bass and rainbow trout to *S. iniae* following delivery by a novel method for mass immunization.
5. Transfer the tools and research findings from this project to industry.

PRINCIPAL ACCOMPLISHMENTS

Objective 1. Develop a suite of reagents and assays that can be used to quantify the specific humoral and cellular immune responses of warmwater and coolwater finfish that could be used as indicators of healthy finfish immune systems and assess the efficacy of novel vaccines and methods of immunization.

This project has enabled the successful development of both humoral and cellular immune assays for assessing immune function of two economically important finfish species—hybrid striped bass (HSB) and rainbow trout (RBT). The serum immunoglobulin assays (enzyme-linked immunosorbent assay or ELISA) developed for this project allowed us to study the serum immunoglobulin response following exposure to live Gram-negative and Gram-positive bacterial pathogens. Furthermore, we developed three macrophage-based cellular assays (migration, phagocytosis, chemiluminescence) in HSB and RBT and all have utility for studying how bacterial pathogens, various vaccine preparations, and methods of immunization can affect cellular immune function.

To augment the humoral and cellular assay development efforts, quantitative PCR (qPCR) assays to assess expression of RBT cytokines were developed previously at the Western Fisheries Research Center and used to identify which HSB inflammatory response genes were modulated after experimental exposure to *S. iniae*. A qPCR assay for HSB hepcidin gene was chosen initially since this gene had been sequenced and published previously and was used to design authentic hepcidin primers. Using these reagents, a greater than 1000-fold increase in hepcidin gene expression was seen in the infected fish. This assay will allow biologists to study the impact of aquaculture on healthy finfish immune systems.

***Objective 2. Determine the immune response kinetics of hybrid striped bass and rainbow trout to* S. iniae *and* A. hydrophila.**

Standardized challenge models were developed for experimental infection of HSB and RBT with *S. iniae* and *A. hydrophila*. A standard injection challenge model was developed for *S. iniae* infection in HSB. The LD50 of *S. iniae* for HSB was estimated to range from 2.95 x 105 to 3.93 x 105 (mean 3.44 x 105) CFU in a 0.1 ml injection volume. A standard challenge model for *A. hydrophila* in rainbow trout and HSB was developed. The LD50 for *A. hydrophila* in HSB was estimated to range from 5.53 x 106 to 4.15 x 106 (mean 4.84 x 106) CFU in a 0.1 ml injection volume. The LD50 for A. hydrophila in rainbow trout has been estimated at 3.37 x 107 CFU.

The development of the RBT/*A. hydrophila* challenge model has led to research designed to develop an injectable adjuvanted vaccine against *A. hydrophila*. Our first experiments demonstrated that RBT could be passively immunized against *A. hydrophila* and that survivors post infection where virtually completely resistant to re-infection. These results led to injection vaccination experiments that were quite encouraging because relative percent survival of RBT post challenge ranged from 69-100 depending on the challenge dose. It appears that this type of vaccine may be feasible and that an immersion delivered vaccine may also be attainable.

Objective 3. Determine the effects of hatchery practices on immune functions of hybrid striped bass and rainbow trout.

An experiment was conducted to study the effect of loading density on the humoral immunoglobulin response in HSB and RBT following injection vaccination with bovine serum albumin. Serum immunoglobulin levels of both RBT and HSB were measured after maintaining fish at low, medium, and high densities up to 10 weeks post vaccination. Both HSB and RBT held at low density displayed a slight but positive increase of serum immunoglobulin over the course of the six-week experiment. When held at normal density, serum immunoglobulin levels increased over the duration of the trial, but in the high-density group, HSB serum immunoglobulin levels increased slightly from 1 to 3 weeks post vaccination but almost doubled this level by 6 weeks post immunization. A similar trend was observed in RBT; the highest levels of serum antibody were detected in the high-density group at 6, 8, and 10 weeks post-immunization. These results seem counter-intuitive in that one might hypothesize that loading density would be immunosuppressive.

Objective 4. Assess the immune response and cytokine gene expression of hybrid striped bass and rainbow trout to* S. iniae *following delivery by a novel method for mass immunization.

Northwest Marine Technologies (NMT), the world leader in the design and manufacture of equipment for the automated mass coded wire tagging of salmonids, developed proprietary technology for mass vaccination of salmonids without the need for anesthesia. The WRAC Immunology group assisted in the first proof of principle studies for the safety and efficacy of this technology for salmonids under laboratory conditions. We demonstrated that the NMT AutoFish SV vaccination platform successfully delivered the vaccine and thus provided effective protection of coho salmon against a *V. anguillarum* challenge. This effort is expected to lead to the availability of a novel mass vaccine delivery technology that will significantly improve the health and disease resistance of fish reared at, or released from, federal, state, tribal, and private sector facilities. This will provide a significant new benefit to fish stocks in aquaculture, resource enhancement, and threatened and endangered captive broodstock programs.

Objective 5. Transfer the tools and research findings from this project to industry.

To date two extension publications, "Measurement of the Innate Cellular Immune Responses of Hybrid Striped Bass and Rainbow Trout" and "Measurement of rainbow trout and hybrid striped bass antibody using an enzyme-linked immunosorbent assay (ELISA)," have been written, submitted, and reviewed by our extension specialist. Based on feedback from outside sources, these findings are suitable for publication and are currently in the final editorial stages prior to printing. A third extension publication, "Testing of novel mass delivery methods for fish vaccines,"

has been written and is in final stages of preparation. A fourth product, "Assessment of the feasibility of developing a vaccine against Aeromonas hydrophila in rainbow trout," is in the planning stage.

IMPACTS

Objective 1. The HSB serum immunoglobulin ELISA developed for this project is being tested to determine whether it could serve as a predictor of infection or previous exposure to an infectious agent(s). Also HSB serum immunoglobulin levels may correlate with a dose-dependent response to vaccination and serve as a predictor of vaccine efficacy in clinical trials.

Objective 2. The development of an efficacious vaccine to reduce *A. hydrophila* mortality associated with commercially reared rainbow trout will have a direct impact on survival and cost of production for this cultured fish species.

Objective 3. Our results suggest that the elevated stocking densities typically encountered during intensive aquaculture practices do not seem to negatively impact humoral immunoglobulin production. This finding needs to be repeated to confirm our initial findings. It appears that domestication may select for animals that can survive and respond under similar conditions.

Objective 4. Mass vaccination technology is desperately needed to ensure reliable, safe, and effective delivery of vaccines (and other biologics) to finfish. Our efforts with NMT to prove their technology were highly successful. At this time however, it is felt that the economics of introducing mass injection vaccination technology appear to greatly exceed the economics that aquaculture is willing to pay to have relatively low-priced animals injection vaccinated.

Objective 5. The fish immunology-related extension products currently submitted for publication will assist fish health researchers, biologists, and aquaculturists with management decisions regarding the effect of intensive culture conditions on fish immune function.

RECOMMENDED FOLLOW-UP ACTIVITIES

Investigate in greater detail the role that hatchery practices (for example, stocking density) play in modulation of fish immune function. Given the tools that have been developed here, further insights into how healthy HSB and RBT immune systems function in response to intensive culture practices could have significant practical implications for animal husbandry and animal welfare issues in the western US.

There is a desperate need for affordable mass vaccination technology in US aquaculture. The importance of vaccination in aquatic animal health has been realized and is generally necessary to successfully rear economically important fish species. There is now a need for ways of economically delivering immunologically relevant (i.e., protective) antigens to finfish. This achievement should be considered the cornerstone to the future economic viability of US aquaculture.

PUBLICATIONS, MANUSCRIPTS, AND PAPERS PRESENTED

Publications in print

None

Manuscripts

Outreach products

1. Measurement of the innate cellular immune responses of hybrid striped bass and rainbow trout. Authors: Alcorn S, Ostland V, LaPatra S, Friedman C, and Winton J. Final manuscript submitted and in production.
2. Measurement of rainbow trout and hybrid striped bass antibody using an enzyme-linked immunosorbent assay (ELISA). Authors: Ostland V, Alcorn S, LaPatra S, Friedman C, and Winton J. Final manuscript submitted and in production.

3. Testing of novel mass delivery methods for fish vaccines. Authors: Winton J, Alcorn S, Ostland V, LaPatra S, and Friedman C. Final version in preparation.
4. Assessment of the feasibility of developing a vaccine against *Aeromonas hydrophila* in rainbow trout. Authors: LaPatra S, Alcorn S, Ostland V, Friedman C, and Winton J. Final version in preparation.

Peer-reviewed manuscripts

1. Development of assays to measure the cell-mediated innate immune response of hybrid striped bass and rainbow trout. Alcorn et al. *Journal of Fish Diseases*. Manuscript in preparation.
2. Measurement of hybrid striped bass antibody using a novel enzyme-linked immunosorbent assay. Ostland et al. *Fish and Shellfish Immunology*. Manuscript in preparation.
3. The adaptive humoral response of hybrid striped bass and rainbow trout is density dependent. LaPatra et al. Manuscript in preparation.

Papers presented

None

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		University	Industry	Other Federal	Other	Total	
2003	100,000	7,000[a]	25,530[b]	25,000[c]		57,530	$157,530
2004	100,000	7,000[a]	25,530[b]	25,000[c]	1,000[d]	58,530	$158,530
2005	100,000	7,000[a]	25,530[b]	25,000[c]		57,530	$157,530
2006	100,000	7,000[a]	25,530[b]	25,000[c]	2,000[d]	59,530	$159,539
Total	400,000	28,000	102,120	100,000	3,000	233,120	$633,120

a in-kind salary for Friedman (0.1 FTE)
b in-kind support from Kent SeaTech, Clear Springs Foods
c in-kind salary, ancillary laboratory equipment, facilities, reagents for Winton
d in-kind support from Northwest Marine Technologies
e in-kind contribution (reagents, salary) from Aquatic Diagnostics Limited, Stirling Scotland

Phosphorous Discharge

Termination Report			
Project Work Period	April 1, 2003–June 30, 2007; no cost extension approved through 6/30/08		
Author	Gary Fornshell		
Funding Level	$8,000		
Participants	Gary Fornshell*	University of Idaho	Idaho
Reason for Termination	Funds terminated		

PROJECT OBJECTIVES

To develop outreach products related to Best Management Practices for flow-through systems based upon the "Reducing phosphorus discharge from high density, flow-through aquaculture facilities" WRAC project (March 2000 through April 2004).

PRINCIPAL ACCOMPLISHMENTS

Developed an array of outreach products to inform and educate stakeholders on Best Management Practices to enable them to meet NDPES discharge compliance limits and the national EPA aquaculture effluent rule, requiring BMPs for solids control and management.

IMPACTS

EPA approved the national aquaculture effluent rule in 2004. In December 2007, EPA issued new NPDES permits for Idaho, incorporating waste load allocations for phosphorus and total suspended solids, making these permits the most stringent to date for Idaho aquaculture producers. With few exceptions, producers are meeting the compliance limits.

RECOMMENDED FOLLOW-UP ACTIVITIES

The "reducing phosphorus discharge from high density, flow-through aquaculture facilities" project had an objective to evaluate BMPs on commercial facilities, which was never realized for a variety of reasons. In the future, it will be critical for the success of any WRAC project that has a field component to complete that objective.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

Publications

Fornshell G and Hinshaw JM. 2008. Best Management Practices for Flow-through Aquaculture, *in* Environmental Best Management Practices for Aquaculture. C.S. Tucker and J.A. Hargreaves (editors) Blackwell Publishing, Ames, Iowa.

Chen S and Fornshell G. Reducing phosphorous discharge from aquaculture systems. *Aquaculture Magazine* May/June 2005 Vol. 31 No.3

Chen S and Fornshell G. Reducing phosphorous discharge from aquaculture systems.*Waterlines,* Winter 2005

Fornshell G. 2004. Aquaculture Effluent Task Force. University of Idaho Extension Impact Statement.

Tucker, C, Belle S, Boyd C, Fornshell G, Hargreaves J, LaPatra S, Summerfelt S and Zajicek P. 2003. Best Management Practices for Flow-Through, Net-Pen, Recirculating, and Pond Aquaculture Systems. 98 pg. This document was prepared as part of an Interagency Agreement (IAG 12939368) between the United States Environmental Protection Agency (EPA) and the United States Department of Agriculture Cooperative State Research, Educa-

tion, and Extension Service (CSREES), and a Cooperative Agreement (CSREES-01-OA-2205-218) between CSREES and Mississippi State University.

Abstracts

Fornshell G. 2007. Serial Reuse Systems Used for Coldwater and Warmwater Fish in Idaho. World Aquaculture Society, Book of Abstracts, p. 309.

Fornshell G. 2004. Solids Management Techniques in Flow-through Systems to Reduce Phosphorus Discharge. World Aquaculture Society, Book of Abstracts. p. 200.

Presentations

Fornshell G. Serial Reuse Systems Used for Coldwater and Warmwater Fish in Idaho. The International Triennial Conference of the World Aquaculture Society, San Antonio, Texas, February 28, 2007.

Fornshell G and Hinshaw J. 2005. A National Effluent Rule for 245 Facilities. Aquaculture America' 05, New Orleans, Louisiana, January 17, 2005.

Fornshell G and Hinshaw J. 2004. A National Effluent Rule for 245 Facilities. US Trout Farmers Conference, Twin Falls, Idaho, September 17, 2004.

Fornshell G. Solids Management Techniques in Flow-through Systems to Reduce Phosphorus Discharge. Aquaculture'04, The International Triennial Conference of the World Aquaculture Society, Honolulu, Hawaii, March 2, 2004.

Fornshell G. Lessons Learned: Mid-Snake TMDLs, WLAs, and NPDES Permit. Aquaculture'04, The International Triennial Conference of the World Aquaculture Society, Honolulu, Hawaii, March 2, 2004.

Fornshell G. BMPs in Aquaculture. US Trout Farmers Association Conference, Shepherdstown, West Virginia, October 17, 2003.

Fornshell G. Options under a No Net Increase for Raceway Culture. Aquaculture America'03, Louisville, Kentucky, February 20, 2003.

Workshops

BMPs for new NPDES discharge permit, Hagerman, ID, November 27, 2007.

BMPs and Treatment Options for Raceways, Hagerman, ID, June 22, 2004.

Tours (waste management and BMP tours of facilities) (July 08, November 06, June 03, and February 03)

One-On-One Consultations

Dozens of individual consultations either by farm visits or telephone.

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		University	Industry	Other Federal	Other	Total	
2003–2008	8,000						$8,000
Total	8,000						$8,000