

WESTERN REGIONAL AQUACULTURE CENTER



## ANNUAL ACCOMPLISHMENT REPORT

FOR THE PERIOD SEPTEMBER 1, 2008 TO AUGUST 31, 2009

MAY 2010

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# INTRODUCTION

This Annual Accomplishment Report for the Western Regional Aquaculture Center (WRAC), covers progress made from Sept 1, 2008 through Aug. 31, 2009. 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 encompasses the twelve states in the western region of the United States—Alaska, Arizona, California, Colorado, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington, and Wyoming.

## ACKNOWLEDGMENTS

The Western Regional Aquaculture Center (WRAC) acknowledges the contributions of the Principal Investigators and Participating Scientists involved in the projects reported in this Twenty-second Annual Progress Report. Members of the WRAC Board of Directors, Industry Advisory Council, and Technical Committee have provided valuable inputs to the successful operation of WRAC 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

**Board of Directors:** With representation from every land-grant institution from the twelve states in WRAC as well as one representative each from the Industry Advisory Council (IAC) and the two sub-committees of the Technical Committee (TC), the Board of Directors is the primary policy-making body for WRAC. The Board of Directors reviews and appoints members to the IAC and TC. The Board of Directors also 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.

The 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 of Directors for new and continuing regional projects, project modifications, and project terminations.

## PROGRESS REPORTS

Since the start of the regional aquaculture programs, WRAC has processed twenty-two Annual Work Plans (for FY'87 through FY'09 funding) through USDA. This current annual report covers the activities of the WRAC Administrative Center and progress made during the twenty-second year on all projects through Aug. 31, 2009, listed below with funding levels for FY'09.

## **ANNUAL REPORTS**

- A. Determining Ripeness in White Sturgeon Females to Maximize Yield and Quality of Caviar  
2nd project year: \$100,001
- B. Coldwater Disease Prevention and Control Through Vaccine Development and Diagnostic Improvements  
2nd project year: \$80,043
- C. Economic Impacts of Private sector Aquaculture-Based Recreational Fishing in the Western USA  
2nd project year: \$99,624
- D. Physiological Changes Associated with Live Haul: Maintaining Healthy Fish  
4th project year: \$96,789
- E. Potential Threat of Great Lakes VHS Virus in the Western United States  
1st project year: \$35,063
- F. WRAC Publications  
\$24,000

## **TERMINATION REPORTS**

- A. Scale-dependent and Indirect Effects of Filter Feeders on Eelgrass: Understanding Complex Ecological Interactions to Improve Environmental Impacts of aquaculture
- B. Aquarius: Shellfish Sanitation Simulator, Rainfall and Water Quality Closure Rule Evaluator, Version 2.0

## **PROJECT REVIEW**

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.

## **PUBLICATIONS**

The WRAC Publications project (Item F 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 to produce the various publications.

# ADMINISTRATIVE SUPPORT

FY'09 FUNDING LEVEL

\$191,619

The Administrative Center is located in the School of Aquatic and Fishery Sciences at the University of Washington. The University of Washington 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 has become responsible for handling more detailed communications among investigators of various projects and for ensuring that the IAC and subcommittees of the TC are kept apprised of all ongoing activities.

The Administrative Center has processed twenty-two Annual Work Plans (FY'87 through FY'09) to date 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 and has a mailing list of over 2,700 recipients. *Waterlines* provides information on WRAC projects and general aquaculture news in order to educate the public on the importance of aquatic animal husbandry, as well as other WRAC activities.

Additionally, conference attendees at the Aquaculture America 2010 conference in San Diego, March 2010, received a two-sided WRAC FACTS sheet in their conference bags.

Other areas of support during this period, as in previous years, 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 and the TC in monitoring research activities and developing annual progress reports
- Coordination of the external review of proposals for technical and scientific merit
- Development of liaisons with appropriate institutions, agencies, and clientele

- Preparation of testimony, in coordination with the four other Regional Aquaculture Centers, for annual submission to the House Appropriations Subcommittee on Agriculture, Rural Development and Related Agencies in Washington, DC
- Participation in the National Coordinating Council (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

# ECONOMIC IMPACTS OF PRIVATE SECTOR AQUACULTURE-BASED RECREATIONAL FISHING IN THE WESTERN USA

<b>REPORTING PERIOD</b>	September 10, 2008–September 9, 2009		
<b>AUTHORS</b>	Craig Bond and Daniel Deisenroth		
<b>FUNDING LEVEL</b>	\$198,698 total budget over two years		
<b>PARTICIPANTS</b>	<i>Principal Investigator</i>		
	Craig A. Bond*	Colorado State University	Colorado
	<i>Co-Principal Investigators</i>		
	Steve Davies*	Colorado State University	Colorado
	John Loomis*	Colorado State University	Colorado
	Hossein Farzin	University of California, Davis	California
	Andrew Seidl*	Colorado State University	Colorado
	<i>(Outreach Coordinator)</i>		
	<i>Collaborators</i>		
	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
	Timothy Rakitan*	University of California, Davis	California
	Jay Griebing*	Colorado State University	Colorado
<b>INDUSTRY ADVISORS</b>	Kenneth Cline	Cline Trout Farms, Inc.	Colorado
	Rebecca Cooper	Cline Trout Farms, Inc.	Colorado

\* funded participants

## 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 estimate anglers' preferences for various attributes of fisheries, such as catch rates and pristine value. This will help to demonstrate the substitutability of angling across various fisheries, for example from private to public sites, and will aid policymakers in creating regulatory policies that are more impartial to the ASRF industry.

## **PROGRESS AND PRINCIPAL ACCOMPLISHMENTS**

Initial funding was received in late January 2008. Since that time, the project has generally progressed in accordance with the original timeline, though we decided to delay surveying ASRF direct customers (objective 1) until after the 2009 summer fishing season, thus slightly augmenting the original timeline. We anticipate a final report distribution on or around May 2010.

### ***Objective 1***

The accomplishments for objective 1 will be subdivided into three sections: ASRF Producers, ASRF Direct Customers, and Recreational Anglers.

#### ***ASRF Producers***

1. Most ASRF producers in the western United States received surveys by September 2008. However, a new set of previously unidentified ASRF permit holders in California was revealed to the CSU research team in late 2008.
2. The information gathered has been summarized in an economic development report that describes key aspects of the ASRF industry, such as demographic data, cost structure, sales outlets, location of purchases, and the geographic distribution of permit holders.
3. The information was also compiled into IMPLAN input-output software in order to create a preliminary estimate of the economic contribution of the ASRF industry. Using IMPLAN, we estimate that for an annual \$57 million industry such as the ASRF, the economic contribution is roughly \$110 million in the western United States, and that the ASRF industry supports approximately 1682 full-time jobs. This estimate does not take into account the "forward linkages" associated with ASRF production (economic activity generated from the final use of ASRF products, such as in private fisheries or by recreational anglers). During months 22–30, the CSU team will compile information from recreational anglers and ASRF direct customers in order to more completely assess the economic contribution of the ASRF industry in the western United States.

#### ***Recreational Anglers***

1. The CSU team, with the help of Rebecca Cooper and Ken Cline, created a survey instrument that was used to collect data from recreational anglers.
2. The CSU team conducted two focus groups on the survey instrument.
3. Cooper and Cline, with the help of Jeremy Liley, identified the 29 private fisheries (e.g., fishing clubs, ranches, homeowner's associations, other private property) that would be willing to have surveys distributed to their anglers.
4. Supplemental funding from a similar project was leveraged in order to facilitate a more robust angler sample.
5. Roughly 1400 surveys have been distributed in Colorado by the CSU research team, and roughly 700 in California by the UC Davis research team at public lakes, rivers, streams, and ponds. They have also been distributed at fishing clubs, dude ranches, homeowners' associations, and other private property. When the project is completed, we anticipate having distributed a total of 2200 surveys in both states.



6. We anticipate that the project will stay on schedule and that data gathering from ASRF direct customers will commence in mid-October (Month 22).

### ***ASRF Direct Customers***

While no data has been collected to date, a survey instrument has been created and will be taken to focus groups shortly. Furthermore, as outlined in the next section, a sampling frame has been created for this population and surveys will begin to be distributed during October, November, and December 2009 in order to align with the completion of the 2009 fishing season.

### ***Objective 2***

1. The CSU team has further refined its ASRF sampling frame by including an additional 100 aquaculture permit holders from California.
2. The CSU team, with the help of Cooper and Cline, created a sampling frame for recreational anglers in both California and Colorado. Nearly 60 policy relevant recreational angling sites, along with nearly 2000 recreational anglers in both California and Colorado, have been identified. All of the aforementioned individuals have been surveyed.
3. Finally, with the help of Rebecca Cooper and Ken Cline, a list of over 1000 direct customers to the ASRF industry have been identified in Colorado. These individuals will be receiving surveys starting mid-October, 2009.

### ***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.
2. A second identified area of research is the nature of the regulatory structure of the ASRF industry, especially as the regulators tend to be production competitors. From an economic standpoint, this is an extremely unusual structure, which likely leads to incentives that may not be in line with maximization of social welfare. The team intends to explore this relationship in a theoretical paper that will document the various incentives that result from this structure.

### ***Objective 4***

1. Outreach objectives, in terms of preparation and dissemination of research results, have been distributed through various media during 2009. Presentations have been made to the Colorado Aquaculture Association and to the Western Agricultural Economics Association by Daniel Deisenroth about the economic contribution of the ASRF industry.
2. Furthermore, in addition to two extension pieces authored in 2008, Craig Bond and Daniel Deisenroth have authored 4 extension articles in *The Fishline* (published by the CO Aquaculture Association) and one economic development report in order to broaden the audience which is exposed to the results of this study.
3. In order to inform anglers about the nature of the project and the reasons for collecting the data on recreational fisheries, we created a FAQ website about the project (<http://dare.agsci.colostate.edu/csuagecon/anglersurvey>).

## **USEFULNESS OF FINDINGS**

The team of researchers at CSU has compiled a list of active ASRF producers and used it to gather information regarding basic operations, expenditures, labor, and demographics. This data has been used to demonstrate the economic contribution of the ASRF industry to the western United States. We have evaluated the direct effect of the ASRF through sales, as well as the indirect and induced effects of these businesses on their local economy. The

indirect and induced effects are found by using statistical models to trace the economic impacts of backward linkages (up the supply chain) and forward linkages (down the supply chain), and estimating a “multiplier.” The multiplier gives the amount of dollars an industry will produce for an economy for every one dollar put into that industry. For example, an output multiplier of 1.89 indicates that for every dollar put into the industry, \$1.89 is returned to the local or regional economy. This multiplier is used to estimate that the \$57 million ASRF industry contributes roughly \$108 million of economic activity annually to the western United States. Similar logic is applied to estimate that this industry contributes roughly 1682 jobs to the region.

This information has directly benefited the 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. Furthermore, information regarding the geographic distribution of the ASRF industry should prove useful in terms of policy decisions.

These economic contribution estimates account for all dollars spent on ASRF inputs, such as fish feed, equipment, and labor, collectively known as “backward linkages.” Money spent on inputs to ASRF production trickles through a local or regional economy and ultimately leads to an output multiplier of 1.89. However, one shortcoming of this type of analysis is that it does not take into account economic activity generated by the final users of ASRF products.

The direct customers of the industry, along with recreational angling at many venues, depend critically on the presence of the ASRF industry. Failing to take into account the economic activity generated by these sectors would potentially grossly understate the true economic contribution of the industry.

Therefore, researchers have created a sampling frame for ASRF direct customers and recreational anglers, and have collected data from thousands of recreational anglers from around the West. This data, coupled with data collected from ASRF direct customers in late 2009, will be used to estimate the economic contribution of these sectors that is derived from the ASRF industry. By May 2010, these estimates will be combined with previous estimates to estimate the total economic contribution of the ASRF industry in the western United States.

## **WORK PLANNED FOR NEXT YEAR**

Work has generally progressed in accordance with the original timeline, with a few delays. A final report will be distributed in May 2010. Work planned for next year includes:

1. Complete data collection of recreational anglers in Colorado and California (months 22–24).
2. Create outreach material relating to the economic contribution of recreational fishing that comes as a result of the presence of the ASRF industry (months 22–27).
3. Distribute surveys to direct customers of ASRF producers via mail (months 22–24).
4. Create outreach material relating to the economic contribution of direct customers (months 22–27).
5. Integrate the results from all three groups (ASRF producers, direct customers, and recreational anglers) into input-output models, such as IMPLAN in order to estimate the total economic contribution (months 22–30).
6. Prepare the final economic report (months 22–30).
7. Continue investigation into the scale and scope of non-profit Northwest suppliers and impacts of the regulatory and competitive environment on the aquaculture industry, possibly including the relationships between private and public hatcheries, interstate trade regulations, and Native American reservation policies (months 22–30).
8. Prepare and disseminate the research findings in peer-review academic journals, extension publications, popular press publications, and conference and meeting presentations (months 22–30).

## **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, Deisenroth D. Colorado State University to lead effort to quantify economic contribution of recreational fish producers. *The Fishline* 2008 <http://www.colaqua.org/articles.htm>.

Bond CA, Deisenroth D. Estimating the economic impacts of the aquacultural suppliers of recreational fish. *Waterlines* 2008;15(1):16–17.

Deisenroth D, Bond CA. The aquacultural suppliers of recreational fish: A look at the freshwater recreational fish industry in the western United States.(Appendix 5). 2009

Deisenroth D, Bond CA. Phase One of Colorado State University study on The Economic Impacts of the Aquacultural Suppliers of Recreational Fish Nearing Completion. *The Fishline* 2009 <http://www.colaqua.org/articles.htm>.

Deisenroth D, Bond CA. Update: estimating the economic contribution of the aquacultural suppliers of recreational fish (ASRF) in the western United States. *The Fishline* 2009, <http://www.colaqua.org/articles.htm>.

Deisenroth D, Bond CA. Update: estimating the economic contribution of the aquacultural suppliers of recreational fish (ASRF) in the western United States” *The Fishline* 2009, <http://www.colaqua.org/articles.htm>.

Deisenroth D, Bond CA. Moving forward with the economic contribution of the aquacultural suppliers of recreational fish” *The Fishline* 2009, <http://www.colaqua.org/articles.htm>.

### ***Publications in Print***

The economic contribution of the aquacultural suppliers of recreational fish in the western United States”

- Presented at the WAEA annual meetings in Lihue, Hawaii, June 2009.

A preliminary look at the aquacultural suppliers of recreational fish in the western United States”

- Presented at the Colorado Aquaculture Association meetings at Mount Princeton, Colorado, January 2009.

**SUPPORT**

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		UNIVERSITY	INDUSTRY	OTHER	FEDERAL	OTHER	
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

<b>REPORTING PERIOD</b>	September 1, 2008–August 31, 2009		
<b>AUTHOR</b>	Ken Cain and Doug Call		
<b>FUNDING LEVEL</b>	First Year funding	\$81,555 (received February 2008)	
	Second Year funding	\$80,043 (received March 2009)	
	Third Year funding	\$81,637 ( <i>approved 10/08</i> )	
	Fourth Year Request	\$81,639	
<b>PARTICIPANTS</b>	Kenneth Cain*	University of Idaho	Idaho
	( <i>Working Group Chair</i> )		
	Douglas Call*	Washington State University	Washington
	Scott LaPatra*	Clear Springs Foods, Inc.	Idaho
	Gary Fornshell*	University of Idaho	Idaho
	Greg Weins	US Department of Agriculture	West Virginia
	Amy Long	University of Idaho	Idaho
	Rashesh Kumar	Washington State University	Washington
<b>TECHNICAL ADVISOR</b>	Gael Kurath	US Geological Survey	Washington
<b>INDUSTRY ADVISOR</b>	Jim Parsons	Troutlodge, Inc.	Washington
<b>GRADUATE STUDENT</b>	Amy Long	University of Idaho	Idaho
<b>FACULTY PARTICIPANT</b>	Devendra Shah	Washington State University	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).
  - Candidate recombinant proteins will be tested in vaccine trials.
2. Validate quantitative diagnostic assays (ELISA and ovarian fluid filtration [FAT]).
  - Correlate assay results to risk of vertical transmission or disease susceptibility.
  - Establish threshold levels for culling broodstock and/or eggs.
3. Based on results from objective 2:
  - Develop other assays (e.g., real-time quantitative PCR) for quantification of infection in ovarian fluid.
4. Develop an integrated outreach program to meet stakeholder needs.
  - Based on results obtained 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 and control of CWD through vaccination or implementation of new disease management strategies at broodstock facilities.

### **ANTICIPATED BENEFITS**

Coldwater disease (CWD) has become one of the most significant disease problems in commercial trout aquaculture in recent years. It is a worldwide problem, and in the Pacific Northwest alone, losses from CWD can range from 18% to 30% with estimated economic impacts in Idaho alone reaching approximately \$10 million. 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. This approach has worked well for bacterial kidney disease. In addition to benefits associated with developing improved disease management strategies, identifying antigens that may be targeted for vaccine development will be important. If effective vaccine targets are identified, the long-term goal of developing a commercial CWD vaccine would provide a tool to prevent CWD at aquaculture facilities. Currently, such preventative measures do not exist and control relies on antibiotic use.

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

### **PROGRESS AND PRINCIPAL ACCOMPLISHMENTS**

Funding for this project became available in February 2008, and a PhD student (Amy Long) was recruited in May 2008 and a Postdoctoral Fellow (Rajesh Kumar), worked on this project from July 2008 to July 2009 at the University of Idaho (Cain) and Washington State University (Cain) and Washington State University (Call), respectively. A Work Group meeting (conference call) was held in July 2008 and another will be held in late September to discuss results to-date and upcoming needs for next year.

#### ***Objective 1: Identify potential vaccine candidates using in vivo-induced antigen technology.***

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 serum. The antibodies used in the screening process are absorbed against *in vitro* grown *E. psychrophilum* to isolate antibodies that are specific for *in vivo* expressed proteins. For this system to work, we require (1) successful expression of proteins from *E. psychrophilum*, (2) suitable convalescent antisera, (3) a suitable positive control, and (4) sufficient analytic sensitivity, which is a function of total protein expressed and reactivity/affinity of the convalescent antisera.

To date, we have demonstrated that we can detect our positive control protein (a putative *in vivo*-expressed protein, ACP21) using an experimental format that is compatible with library screening. This was an important development, but subsequent efforts yielded mixed results with significant obstacles due to poor analytic sensitivity. We might be able to increase analytic sensitivity by moving the library from an *E. coli* expression host to a *Vibrio parahaemolyticus* expression host based on findings in our lab, but preliminary work indicates that we will continue to encounter high variance between screening experiments and unreliable detection of our positive control protein even with a *V. parahaemolyticus* expression host. We surmise that after absorption against *in vitro*-grown *E. psychrophilum*, the remaining antibody titer is simply too low for reliable detection of expressed proteins in a 96-well format (this can be improved dramatically with larger volume culture, but this alternative is not practical for library screening).

Based on our efforts to date, we propose an alteration in our experimental plan. We recently developed a strain of *E. psychrophilum* (strain 259-93B.17) that has been completely attenuated as demonstrated by injection challenge trials in rainbow trout. Furthermore, we have shown that immunization with strain B.17 is sufficient to confer signifi-

cant protection against *E. psychrophilum* challenge. Preliminary characterization of B.17 relative to the parent strain CSF259-93 shows that there are a number of differentially expressed proteins that are of immediate interest with respect to development of vaccine targets. These results also suggest that attenuation is due to alteration of global transcriptional regulation and that further investigation into the mechanism of attenuation could yield valuable information about how to develop attenuated bacteria more efficiently and with a higher degree of stability. Finally, we have preliminary data showing that recombinant proteins expressed in *V. parahaemolyticus* can be delivered to fish as a crude lysate preparation, and this strategy appears to induce a protective immune response. This latter finding supports our earlier expectations and adds further credibility to our original strategy of using *Vibrio* as the expression host. The ability to use crude lysates to test vaccine candidates represents a large cost savings and our data also suggest that *Vibrio* could be used as a low-cost, alternative adjuvant for testing vaccine candidates.

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

We have continued to work on optimizing the ELISA and the membrane filtration FAT (MF-FAT) for detecting *E. psychrophilum* in kidney tissue and ovarian fluid. Optimization of the MF-FAT has been successful, and the detection limit of the assay has been calculated. By spiking sterile filtered ovarian fluid with known concentrations of *E. psychrophilum* CSF 259-93, we were able to set the detection limit at  $8.8 \times 10^3$  CFU ml<sup>-1</sup>. Once preliminary optimization of the MF-FAT was completed, ovarian fluid samples from rainbow trout and steelhead broodstock reared at two different hatchery facilities were analyzed. Of the 118 samples collected, 38 were determined positive for *E. psychrophilum* infection. We have also developed an indirect FAT (IFAT) using MAb FL43 conjugated to Alexa Fluor® 488 that can readily detect *E. psychrophilum* in tissue imprints as well as swabs from whole fish homogenates.

Optimization of the sandwich ELISA for detection of *E. psychrophilum* in kidney tissue has been more complicated. The assay was redesigned and the biotin-streptavidin system incorporated in an attempt to increase sensitivity; however, sensitivity has not increased as desired. With biotin labeled monoclonal antibody FL43, the assay is able to detect 106 CFU ml<sup>-1</sup> in kidney samples spiked with *E. psychrophilum*. Our original work (Lindstrom et al., 2009) reported what appeared to be greater sensitivity ( $\sim 2 \times 10^3$  CFU ml<sup>-1</sup>). Upon further evaluation, it is appears that this reported difference may be due to methods used in bacterial enumeration rather than a true loss in sensitivity. We are confirming this, but we know that strains of *E. psychrophilum* have a tendency to auto-agglutinate and therefore original plate counts may have underestimated the number of starting bacterial. Experiments to confirm this and find the best method for enumerating bacterial populations are underway.

To further evaluate assay sensitivity on infected fish, we have recently initiated a disease challenge experiment in which juvenile rainbow trout have been infected with two different doses of *E. psychrophilum*, high and low, and samples are being taken from the fish throughout the challenge to provide data on how ELISA measurements vary over the course of a disease challenge.

**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 of an article describing this project that was published in *Waterlines* [Vol 15(1)].

## USEFULNESS OF FINDINGS

Findings thus far, along with previous development of the monoclonal antibody (FL43) has led to a license agreement between the University of Idaho and an antibody company (Immunoprecise) in Vancouver, BC. This company has now produced FL43 and it is available commercially to diagnostic laboratories, research labs, and others wishing to test for CWD. Based on further results from this WRAC project, Immunoprecise will develop ELISA and FAT kits for the industry and research labs.

## WORK PLANNED FOR NEXT YEAR

### ***Objective 1: Identify potential vaccine candidates using in vivo-induced antigen technology.***

Transitioning the IVIAT library to a *Vibrio parahaemolyticus* expression host library presents a potential solution to the problems of insufficient analytic sensitivity, but preliminary work on this alternative indicates that we will still encounter a low signal to noise ratio because we will still need concentrated (1:50) antisera for antigen detection. We propose to redirect this portion of the project to focus instead on two aims: (1) Characterize the differentially expressed proteins between the virulent strain CSF259-93 and the attenuated daughter strain B.17. Proteins exclusive to strain CSF259-93 represent potential virulence factors so identification of these proteins will further our knowledge of *E. psychrophilum* pathogenicity while providing additional targets for subunit vaccine development. (2) Determine the mechanism responsible for attenuation of the B.17 strain. Our working hypothesis is that attenuation results from altered global transcriptional regulation and that this pattern of change is repeatable, predictive of attenuation, and can be engineered independently from rifampicin passage. To test this hypothesis and the alternative mechanisms (expression differences due solely to changes in RNA polymerase activity or to changes in environmental sensing pathways) we will complete our proteomic and sequence analysis to identify transcriptional binding sites that we will then use as “bait” to capture and identify transcriptional regulators involved in this process. We will use parallel rifampicin passage experiments to determine if the same pattern of transcriptional alterations is a repeatable phenomenon. Proteomic and sequence analysis will be used to confirm changes in the passaged strains and we will determine if strains with similar characteristics to 259-93B.17 are attenuated. Finally, if resources permit, we will generate deletion mutants to confirm their role in altered transcriptional regulation and attenuation. The rationale for this work is that we will know the precise mechanism involved in attenuation, which means that we will have the opportunity to design a more robust attenuated strain (less likely to revert to virulence), but more importantly, we will identify a predictable means for engineering attenuated strains with the clear implications that this procedure can be used to create attenuated strains of other important bacterial pathogens.

### ***Objective 2: Validate quantitative diagnostic assays (ELISA and ovarian fluid filtration FAT)***

In the upcoming year, we will use the ELISA and MF-FAT to screen broodstock reared at Troutlodge, Inc. for *E. psychrophilum*. Beginning this fall and continuing throughout the year, kidney tissue and ovarian fluid will be collected from up to 60 3-year-old broodfish. Samples will be transported to UI, analyzed using culture techniques and either ELISA (kidney tissue) or MF-FAT (ovarian fluid). While the samples are being tested at UI, fertilized eggs from the sampled broodstock will be kept in isolated incubators at Troutlodge. ELISA, MF-FAT, and culture results will be used to select progeny from up to 10 broodstock showing either high or low levels of *E. psychrophilum* infection. When fish reach the eyed stage, they will be shipped to UI for further experiments and grouped according to broodstock infection level. Fish will be reared to approximately 0.5 g at which point they will be separated and stocked in triplicate into 20 l tanks. Fish will then be subjected to a controlled stressor for a minimum of 28 days in an attempt to induce a *E. psychrophilum* outbreak. Random sampling of the fish will be done throughout the experiment to determine if *E. psychrophilum* is present. All mortalities will be examined for clinical symptoms of coldwater disease and attempts will be made to isolate *E. psychrophilum* from the kidney, liver, and spleen. Additionally, kidney and spleen imprints will be collected and screened for the presence of the bacterium using IFAT. These experiments will allow us to correlate the level of infection in the parent at time of spawning to the risk of a disease outbreak in the progeny. Fish will be examined for *E. psychrophilum* before the start of every experiment. Progeny from broodstock which are negative for *E. psychrophilum* will be included in all trials to serve as negative controls.



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

Development of a real-time quantitative PCR (qPCR) that can determine *E. psychrophilum* concentrations in ovarian fluid is essential as it will allow for non-lethal sampling of fish. Additionally, real-time qPCR is a quantitative and precise method for determining concentration of bacterial cells in both tissue and culture. In the upcoming year, we will begin to develop a real-time qPCR assay that can detect *E. psychrophilum* in both ovarian fluid and kidney tissue. We intend to develop a probe that is specific to the gene that encodes the antigen that is recognized by monoclonal antibody FL43. The sequence of this gene is currently unknown; however, Dr. Call's lab is close to elucidating the sequence. Once that sequence is known and assuming that it is specific to *E. psychrophilum* and does not share homology with any other genes, we will begin developing protocols for extracting bacterial DNA from samples and optimizing the assay. Ovarian fluid and kidney tissue sampled from broodstock at Troutlodge and used for Objective 2 will be preserved at -80°C for use in the real-time qPCR assay. Additionally, both ovarian fluid and kidney tissue samples have been collected from Lower Elwha hatchery and Dworshak National Fish Hatchery and can be tested by the real-time qPCR. These samples can be used to evaluate *E. psychrophilum* levels at different facilities.

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

Increase awareness of project and research progress through popular press articles in aquaculture newsletters and presentations at aquaculture meetings.

## IMPACTS

Currently, the primary impact is the recent commercial availability of monoclonal antibody FL43. The agreement with Immunoprecise and its involvement will be pivotal in implementing findings from the diagnostic component of this research.

## PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

### **Refereed publications**

- LaFrentz BR, LaPatra SE, Call DR, Wiens GD, Cain KD. Proteomic analysis of *Flavobacterium psychrophilum* cultured *in vivo* and in iron-limited media. *Diseases of Aquatic Organisms* (In Press)
- Plant KP, LaPatra SE, Cain KD. Vaccination of rainbow trout (*Oncorhynchus mykiss*) with recombinant and DNA vaccines produced to *Flavobacterium psychrophilum* heat shock proteins 60 and 70. *J of Fish Diseases* 2009;32(6):521–534.
- Lindstrom NM, Call DR, House ML, Moffitt CM, Cain KD. A quantitative enzyme-linked immunosorbent assay (ELISA) and filtration-based fluorescent antibody test (FAT) as potential tools to screen broodstock for *Flavobacterium psychrophilum* infection. *J of Aquatic Animal Health* 2009;21(1):43–56

### **General articles**

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

### **Presentations**

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

**SUPPORT**

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL	TOTAL SUPPORT
		UNIVERSITY	INDUSTRY	OTHER	FEDERAL	OTHER		
2008-09	81,555							81,555
TOTAL	81,555							81,555

# PHYSIOLOGICAL CHANGES ASSOCIATED WITH LIVE HAUL: MAINTAINING HEALTHY FISH

**REPORTING PERIOD** September 1, 2008–August 31, 2009

**AUTHOR** John Colt

<b>FUNDING LEVEL</b>	2005–2006	\$87,156
	2006–2007	\$81,856
	2007–2008	\$86,299
	2008–2009	\$96,789

**WORK GROUP CHAIR** John Colt

<b>PARTICIPANTS</b>	John Colt*	National Marine Fisheries Service	Washington
	Eric Kroeger	National Marine Fisheries Service	Washington
	Mike Rust	National Marine Fisheries Service	Washington
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	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 2008–2009 included:

### *Tilapia*

The overall goal of this study is to improve the survival of tilapia transported to the live fish market. Specific objectives for this reporting period include:

1. Assess bodily injury to fish loaded onto trucks by fish pumps.
2. Compare gill histology from different farms to assess pre-transport fish fitness and the ability to tolerate transport.
3. Determine if food withdrawal for greater than 24 hours is beneficial in reducing ammonia concentration during transport.
4. Determine the impacts of carbon dioxide retention on survival and production quality.
5. Construct a production-scale hauling system and use time-lapse video to study fish behavior during transport.
6. Determine the variation of oxygen flow through fine bubble diffusers.

### *Trout*

The overall goal of this study is to improve the transport of trout. Specific objectives for this reporting period include:

7. Evaluate the use of video systems to determine fish distribution and behavior in hauling tanks.

8. Determine the impacts of hauling densities (1x, 2x, 3x) on survival in varying recovery conditions.
9. Conduct a preliminary assessment of the use of fluorescein dye techniques to assess bodily injury.

#### ***Outreach Activities***

10. 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: Assess bodily injury to fish loaded onto trucks by fish pumps—tilapia***

The fluorescein technique was used to assess the impact of a fish pump to load *Oreochromis mossambicus* (tilapia) at Pacific Aqua Farms (Niland, California). Very little injury was found in our entire sampling at any stage of the loading process at this farm. This is contrary to our findings at other farms in prior years where there was extensive injury demonstrated during the crowding, loading, and transport process. A small amount of injury was detected in individuals that had experienced the fish pump. This is most likely due to the sorter and/or broom used to “help” fish out of the pump. Overall, this injury seems insignificant as this grower has little issue with mortality. The fish pump is an alternative loading method that may reduce post-haul mortality.

#### ***Objective 2: Compare gill histology from different farms to assess pre-transport fish fitness and the ability to tolerate transport—tilapia***

In previous years, we observed high mortality of fish transported from one particular farm. Histological analysis of the gill tissue from these fish indicated some abnormalities (such as fusion of the gill lamellae and hyperplasia of the respiratory epithelium); which likely resulted in severely restricted intake of oxygen. To assess the potential impact of gill damage on hauling mortality, we collected gill samples from five different tilapia farms in Idaho and from Pacific Aqua Farms in California. We expect to begin the examination of histological sections of the gills in early September 2009.

#### ***Objective 3: Determine if food withdrawal for greater than 24 hours is beneficial in reducing ammonia concentration during transport—tilapia***

Groups of Nile tilapia averaging 182 grams per fish were fasted for periods of 0, 1, 3, 5, and 10 days, respectively. Afterwards, they were subjected to simulated transport for 18 hours and unionized ammonia was determined in the water in which they were transported. One day of fasting appears sufficient to prevent much ammonia buildup during the simulated transport. It appears that the standard industry practice of withholding food for a day or two prior to transportation is appropriate in terms of maintaining low ammonia levels, attributable to digestive processes, during transport.

#### ***Objective 4: Determine the impacts of carbon dioxide retention on survival and production quality***

The impact of alkalinity on pH and mortality of Nile tilapia (*Oreochromis niloticus*) was investigated with a simulated haul experiment. Market-sized fish (0.64–0.74 kg) were transported for 48 hours at a density of 180 g/L in insulated coolers. Pure oxygen aeration was provided with 30 cm x 3 cm Point Four ceramic diffusers and an oxygen flowrate of 3–4 lpm. The coolers were sealed during the entire simulated haul. The low alkalinity was 0.36 meq/L and the high alkalinity was 10.36 meq/L. DO, temperature, conductivity, and pH were monitored and logged at a 1-minute interval with YSI-556s. The initial pH in the low alkalinity was 8.2, then rapidly decreased to 6.7, gradually decreased to 6.4, and slowly increased to 6.5 by the end of haul. The initial pH in the high alkalinity was 8.5, rapidly decreased

to 7.7, gradually decreased to 7.3, and then remained relatively constant for the last 30 hours of the haul.

There was no mortality in the high or low alkalinity treatments at the end of the 48-hour haul. There was significant mortality in the other tanks that was not related to the treatments. The observed mortality appeared to be due to differences in oxygen flow rates that resulted in a wide variation in DOs and carbon dioxide concentrations. Because of these problems, it was necessary to modify the oxygen manifold used in this experimental work and take a careful look at the oxygen flow characteristics of fine bubble diffusers.

Based on the actual pH and TANs, the un-ionized ammonia concentrations were very low. Under the conditions of this simulated haul, carbon dioxide toxicity was much more important than ammonia toxicity. If aeration had been used rather than pure oxygen (resulting in higher pHs), it is likely ammonia toxicity would have caused significant mortality in all treatments. Additional experimental work is needed to examine ammonia toxicity when controlled levels of carbon dioxide stripping are provided.

***Objective 5: Construct a production-scale hauling system and use time-lapse video to study fish behavior during transport—tilapia***

A production-scale hauling tank was constructed from 1/4-inch plexiglass. The outside dimensions were 47"x 48"x 33" and is similar in size to the plastic totes used by some of the live haulers for tilapia. The camera is mounted 60" above the water surface. The digital cassette recorder can be used to record up to 60 minutes of video. The SecuritySpy software can be used to record digital time-lapse video of the fish. In contrast to the video work with trout, we elected to film in low-light conditions, not total darkness. Preliminary work has not demonstrated reduced dissolved oxygen in the corners of the hauling tank. Under production densities, only the fish in the upper layer can be seen. Work is on-going on this objective.

***Objective 6: Determine the variation of oxygen flow through fine bubble diffusers—tilapia***

Two of the most common types of fine-bubble diffusers used in commercial fish transport systems are ceramic plate (Point Four Systems, Inc.) and carbon stone. Ceramic plate diffusers typically produce smaller bubbles than carbon stone diffusers, and have better oxygen transfer characteristics. The oxygen flow characteristics of these two types of diffusers were studied using a mass flowmeter.

The gas flowrate through ceramic plate diffusers depends on pressure, length of use, and how the system is operated. For 30 psi and dry diffusers, the flowrate typically ranges from 7–8 lpm. If this diffuser is then placed into water (with the oxygen flowing), the resulting flowrate is reduced to 3–4 lpm due to surface tension and water depth. If the oxygen is turned off and the diffuser is allowed to soak in water for 5–10 minutes, the resulting oxygen flow may be as low as 0.50 lpm when the oxygen is turned back on. The oxygen flowrate slowly increases, but 20–40 hours may be needed before the oxygen flow returns to the 3–4 lpm rate. This reduction in flow appears to be due to water attached to the small pores of the ceramic diffuser and not from filling the hose and diffuser plenum with water. The flowrate variation for the carbon stone diffusers shows similar characteristics, but the magnitude of the impacts are smaller.

In multi-diffuser systems, small changes in individual diffuser head losses can result in uneven distribution of oxygen to the different diffusers. The installation of an individual flowmeter for each diffuser is needed to prevent uneven oxygen distribution. Adjustment of the metering valve on the flowmeter can assure that the flowrate to each diffuser is similar. Even for oxygen systems with individual flowmeters, the wide variation in observed flowrates could cause serious problems if it were necessary to increase the oxygen supply pressure above the diffuser burst pressure to push the required oxygen through a wet diffuser. To reduce the variation in oxygen flowrates, fine bubble diffusers should be run in the air for 30 to 60 minutes at the end of each trip and oxygen flow should be started prior to filling the hauling tanks.

***Objective 7: Evaluate the use of video systems to determine fish distribution and behavior in hauling tanks—trout***

Clumped or non-uniform distribution of fish within the hauling tank may result in localized low dissolved oxygen (DO) and high carbon dioxide concentrations. We attempted to use an underwater low-light video camera, but had no success obtaining reliable video. To overcome the difficulties with videoing fish in the dark, we used an underwater “point and shoot” camera that could take numerous time-delayed pictures at intervals spanning a transportation period. We assessed trout hauled from the Oregon Hatchery Research Center (OHRC) to a nearby pond, as well as from and Oregon Department of Fish and Wildlife’s Alsea Hatchery to Jefferson, Oregon (~2.5 hours). In neither haul, were we able to obtain decipherable images—all images were merely “close-ups” of fish. We were able to obtain distribution information on fingerling-sized spring Chinook during a transport from Marion Forks Hatchery (Detroit, Oregon) to OSU, which revealed that the fish were non-uniformly distributed during the haul. Our findings suggest that underwater time-lapse photography may be useful in assessing behavior of small salmonids during transport. However, to be useful for catchable-sized fish, transport densities would need to be lower than typical production loads.

***Objective 8: Determine the impacts of hauling densities (1x, 2x, 3x) on survival in varying recovery conditions—trout***

A density of 1.5-2 lb/gal is the industry standard for hauling trout. We tested the hauling of catchable trout of mixed diploid/triploid stock from the OHRC in Alsea to Corvallis at varying densities: 1x (1 lb/gal), 2x (2 lb/gal), and 3x (3 lb/gal). The haul was approximately 1 hour, and we then left the fish in the haul tanks for another 16.5 hours, resulting in a 17.5 hour transport experience. We then stocked fish from each treatment into “good” and “bad” water-quality holding tanks at OSU. “Good” tanks had water temperature of 13.5° C and DO levels were approximately 9.5 mg/L, and “bad” had water temperatures of 20° C and DO levels of approximately 6 mg/L. Mortality was monitored for 48 hours. Blood plasma was collected from pre-haul and post-haul-fish for cortisol analysis.

No mortality was observed in the 1x or 3x treatment immediately following the haul. All fish from the 3x treatment placed in the “Good” holding condition survived; however, mortality was observed in the 3x treatment placed in the “Bad” holding condition. This suggests that 3x (3 lb/gal) is an achievable hauling density, and survival is dependent on the receiving conditions. Further research is necessary to understand the role that the prior condition of the fish has on the effects of survival when hauled at higher densities. Additionally, we are interested in determining whether additives to the haul medium can further enhance the densities at which fish can be transported.

***Objective 9: Conduct preliminary assessment of the use of fluorescein dye techniques to assess bodily injury—trout***

We analyzed bodily injury using the fluorescein technique described in Objective 1. Overall, we detected very little bodily injury. Very minor spots were found but nothing too surprising for trout reared in a raceway. Similarly, after the haul, very little bodily injury was detected. The only noteworthy injury was that in three out of the five fish sampled from the 3x haul treatment there were eye injuries. No fish transported at 1x and only one from the 2x treatment had eye injury. This increased eye injury may be a consequence of the increased density in the 3x treatment, but this would have to be repeated to be certain.

***Objective 10: Outreach Activities***

Outreach activities are presented in the section “Work Planned for Next Year.”

**USEFULNESS OF FINDINGS**

The fish pump is an alternative loading method that may reduce post haul mortality. Changes in the normal 1-2 day feed withdrawal period are not needed. Alkalinity adjustment and better control of carbon dioxide stripping

may be an important improvement in hauling protocols. The use of video monitoring during hauling may be an effective way to monitor aeration and the overall health of the fish. Improvements in our understanding of oxygen flow through diffusers should result in improved efficiencies of oxygen use and fish survival. The use of higher hauling densities for trout may significantly reduce transportation costs and improve survival. The use of these higher densities remains to be tested and verified on a production scale.

## **WORK PLANNED FOR NEXT YEAR**

This section covers on-going work that has been funded by WRAC. A supplemental proposal has been prepared for future fifth-year work.

### ***Tilapia***

- Completion of the gill histology examination—OSU
- Completion of the carbon dioxide retention experiments for tilapia—NMFS
- Completion of the fish distribution/aerator studies for tilapia—NMFS
- Determination of the impact of pH shock on tilapia—NMFS

### ***Trout***

- Determine if additives to the haul medium affect survival of fish hauled at high density (3lb/gal) when placed in stressful recipient conditions—OSU

### ***Outreach***

1. Two workshops, one in California and the other in Idaho, where the live-haul industry is significant. These will probably occur after the project is completed, all the results are analyzed, and the Extension Publications are published. Participants will be trained in the use of the models that will be available from the WRAC website, and individual presentations will cover the recommended protocols. Some or all of the co-PIs may participate in the workshops.
2. Two WRAC Extension Publications. Work on the publications will probably begin toward the end of the project as research results and analyses become available. The first WRAC Extension publication will cover recommended pre-hauling handling, harvest, and hauling protocols. The second publication will include recommended protocols for final holding of fish at wholesale/retail outlets. The documents will be posted on the WRAC website as .pdf files.
3. User-friendly spreadsheet models (fish stress and mortality) will be available for downloading from the WRAC website. These will be completed at the end of the project by the other PIs.

## **IMPACTS**

The use of a fish pump to load tilapia appears to result in less injury than conventional methods. It appears that the standard industry practice of withholding food for a day or two prior to transportation is appropriate in terms of maintaining low ammonia levels during transport. The impact of alkalinity and pH on un-ionized ammonia and carbon dioxide is beginning to be understood on a practical basis. The use of video monitoring of fish during simulated hauling or actual hauling has the potential to improve our understanding of fish behavior and survival and product quality. The oxygen flowrate through the ceramic plate diffuser depends on pressure, length of use, and how the system is operated. It appears that trout can be transported at much higher densities than currently used by agency and commercial haulers.

**PUBLICATIONS, MANUSCRIPTS, AND PAPERS PRESENTED:**

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

Colt J, Kroeger E, Rust M. Impacts of alkalinity and carbon removal on the mortality and product quality of tilapia. In preparation.

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

Colt J, Schuur A, Cryer E, Miles T. In press. Modeling of multiple stocks and programs for master planning and feasibility studies. *Aquacultural Engineering*. doi:10.1016/j.aquaeng.2009.07.005.

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, Watten B, Pfeiffer T. Carbon dioxide transfer in aquaculture systems: standardization of analysis and reporting. In preparation.

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

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

Kroeger E, Colt J, Rust M. Fine bubble diffusers: impacts of pressure, water, and handling on mass flowrates and transfer efficiency. In preparation.

Momoda TS, Chitwood R, Colt J, and Schreck CB. 2010. Effects of stress and injury associated with transporting live tilapia. Annual meeting, World Aquaculture Society, San Diego, California, March 1–5, 2010.

**SUPPORT**

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT				TOTAL SUPPORT
		UNIVERSITY	INDUSTRY	OTHER FEDERAL	OTHER	
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
2009	96,789	4,000		30,000		\$130,789
TOTAL	352,100	15,391		117,500		\$484,991



# DETERMINING RIPENESS IN WHITE STURGEON FEMALES TO MAXIMIZE YIELD AND QUALITY OF CAVIAR

<b>REPORTING PERIOD</b>	September 1, 2008–August 31, 2009		
<b>AUTHOR</b>	Molly Webb		
<b>FUNDING LEVEL</b>	\$100,001 (first year budget) + \$100,001 (second year budget)		
<b>PARTICIPANTS</b>	Molly Webb*	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*	Montana State University	Montana
	Gary Fornshell*	University of Idaho Extension	Idaho
	Linda Lemmon	Blind Canyon Aqua Ranch	Idaho
	Leo Ray	Fish Breeders of Idaho	Idaho
<b>TECHNICAL ADVISOR</b>	Fred Conte	University of California, Davis	California
<b>INDUSTRY ADVISOR</b>	Peter Struffenegger	Sterling Caviar, LLC	California

\* funded participants

## PROJECT OBJECTIVES

The long-term goal of this study is to develop a less invasive, faster, and better predictor of maturity than oocyte polarization index (PI) in sturgeon, and the overall objective is to correlate current predictors of maturity with instrumental and biochemical assays conducted at different stages of ovarian maturity in white sturgeon. The specific objectives 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, plasma protein levels, and crude chemical composition of eggs change with maturity (years 1–2)
3. Evaluate short wavelength near infrared spectroscopy (SW-NIR) and ultrasound as a non-invasive technique to predict fish maturity by taking spectra of gonads in fish (years 1–2)
4. Evaluate Fourier transform infrared spectroscopy (FT-IR) as a method to predict fish maturity from spectral measurements of blood and roe (years 1–2)
5. Using SW-NIR and plasma steroids, determine whether it is possible to detect the early signs of ovarian atresia to avoid sacrificing fish with inferior quality roe and use them during the next production cycle (year 1)
6. Conduct training and outreach programs at field sites in Idaho and California (years 3–4).

## ANTICIPATED BENEFITS

Developing an accurate and less invasive predictor of maturity will allow farms to select white sturgeon during the stages of late vitellogenesis and final maturation for their optimal caviar harvest time. Females harvested at the optimal time will have the greatest yield and highest quality caviar as assessed by firmness, flavor, and shelf life. An accurate predictor of maturity will also prevent the slaughter of fish that have started ovarian follicular atresia and allow these fish to be used for caviar production after the second ovarian cycle. Harvesting caviar at the optimal time of ovarian development will also result in an increase in yield for the 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 through 5. The first group of sturgeon was sampled to specifically address whether it is possible to detect early signs of ovarian atresia to avoid sacrificing fish with inferior quality roe (Objective 5, referred to as the Atresia Study). The second group of females was harvested for caviar to address whether stage of maturity can be assessed less invasively using the proposed tools (SW-NIR, FT-IR, plasma steroids measured by radioimmunoassay [RIA]) and to determine if there is a correlation between oocyte PI and caviar quality and yield (Objectives 1–4; herein referred to as the 2008 Caviar Study). In 2009, females in both California and Idaho were segregated by stage of maturity in the fall, and these segregated groups were harvested at different times in the spring, when each group would have fully grown eggs, to determine whether our alternative sampling techniques (FT-IR, SW-NIR, and plasma steroids) could accurately determine the oocyte PI. This study is herein referred to as the 2009 Caviar Study. As further analysis of the Atresia Study and the 2008 Caviar Study were conducted in Year 2, this information has been included in this report. This report is organized by study.

**Atresia study.** The sampling design for the Atresia Study is described in the Detailed Report. Plasma sex steroids, SW-NIR, and FT-IR can be used to detect early atresia. Discriminant function analysis (DFA) was used to determine that overall, 65% of females can be classified in the correct ovarian stage using plasma testosterone (T) and estradiol (E2), which exceeds the 33% probability of correctly classifying ovarian stage based on chance alone. The quadratic DFA was able to correctly classify 87% of normal (non-atretic) observations, 25% of early atretic observations, and 100% of mid-atretic observations using T and E2 as explanatory variables. The high misclassification rate of early atretic fish as mid-atretic fish did occur, but is not biologically relevant as the goal was to classify atresia and preferably early atresia. Therefore, 100% of the atretic fish were in fact correctly classified as atretic using plasma sex steroids.

Logistic regression modeling produced two separate significant models for predicting the probability of ovarian stage based on either T or E2 concentrations. Equations were generated that can be used to 1) predict the probability that a female white sturgeon has normal ovaries given a specific concentration of T or E2, and 2) avoid harvesting fish with atretic ovaries by setting a threshold value of T and/or E2 based on an acceptable probability of harvesting fish with normal ovaries.

In general, the model generated from the SW-NIR spectra predicted mature, vitellogenic, and early atresia status successfully for most eggs and fish. With the exception of two fish for which cross validation results yielded only 30% and 20% predictability, respectively, for early atresia, all other atretic fish were correctly classified anywhere between 50% and 100%. The current limitations on the ability of SW-NIR to predict onset of early atresia may be due to sampling and not necessarily to lack of a physical/chemical detectable signal.

**2008 Caviar study.** The sampling design for the 2008 Caviar Study is described in the Detailed Report. Morphometric analysis revealed larger eggs and lower oocyte PI in fish sampled in early May and June, indicating that they had reached full maturity and could provide optimal roe yield if harvested from late April through May. However, there was no significant difference in mean caviar yield among the harvest months, suggesting that either small sample size or other factors, rather than stage of maturity, affected caviar yield. Regression analysis of pooled samples did not reveal a correlation of caviar yield with oocyte PI or egg diameter, suggesting that fish maturity was not a main factor affecting roe yield in the population. Total weight of caviar from individual fish increased with body weight at a modest rate of 73 g kg<sup>-1</sup>. However, the caviar yield as a percent body of weight did not exhibit a significant correlation with fish weight, suggesting that there was no increase in production rate in faster growing fish. The lack of an increase in caviar yield in larger fish was concordant with a significant decrease of caviar yield, expressed as a percent weight of both ovaries. The caviar yield per ovarian weight ranged 25%–75% in the population and was the best predictor of caviar yield per body weight. The lack of a relationship between the oocyte PI and caviar

yield was likely due to the highly variable caviar yield in the population caused by ovarian adiposity, particularly in larger fish. The best producers of roe were the females in the low fat group: they had a lower mean body weight and condition factor, but produced the highest caviar yield per body weight and ovary weight. These relationships will be further examined in a different year class and farm (Idaho) in the 2009 Caviar Study.

Plasma T concentrations differed significantly only between February and May, while there were no significant difference in plasma E2 concentrations among dates. Plasma E2 was significantly correlated with caviar yield. However, even though this model was significant, the R-squared value was fairly low. Plasma E2 was also correlated with gonadosomatic index. As E2 increased, gonadosomatic index also increased, which means as the ovary weight to body weight increased so did plasma E2. Gonadosomatic index was fairly well correlated with yield. Therefore, plasma E2 may be correlated with yield because it is an artifact of the correlation between gonadosomatic index and yield.

Though modest correlations were achieved in cross-validation mode using the SW-NIR spectra, in most cases, the current SW-NIR approach to predict oocyte PI and caviar yield was not satisfactory. Further analysis of the data will involve including the 2009 California and Idaho datasets and exploring with the research team additional parameters that may yield improved correlation to spectra.

**2009 Caviar study.** In 2009, we examined a management practice of segregating the stock by stage of maturity in the fall and then harvesting segregated groups at different times in the spring when each group would have fully grown eggs. Oocyte PI and ED have been completed for both the California and Idaho samples. The steroid RIAs are almost complete for California but have not been initiated for Idaho. Proximate analysis on half of the subsamples for California caviar and all of the Idaho caviar have been completed. The FT-IR analysis for the samples collected in the fall of 2008 have been analyzed, and preliminary analysis indicates that the plasma steroid, lipid, and vitellogenin content of fish from both states, representing different watersheds, broodstock, and cultivation practices, are similar. A model was established to determine the actual PI values using FT-IR, with a significantly high correlation coefficient ( $R=0.9803$ ) and a low standard deviation ( $Stdev=0.01$ ) indicating that only a single model may be needed to predict oocyte PI values in cultivated white sturgeon. We were also able to modify the FT-IR method so that it was possible to use raw sera, removing the requirement to dry the serum sample on the glass slides or the membrane filter overnight as we did in the past. This means that it is possible to collect FT-IR in the field and to predict the actual PI value of a female within 15 minutes.

**Outreach.** The Work Group meeting was held in Bozeman, Montana, on August 11–12, 2009 to discuss the previous year's results, plan work for the following year, and discuss any technical challenges. When the new WRAC website is launched, the project web page will be posted soon after with the Work Group's presentations and popular press articles as well as peer reviewed publications.

## USEFULNESS OF FINDINGS

The radioimmunoassay of plasma steroids, spectral analysis of plasma by FT-IR, and abdominal scans by SW-NIR were suitable for detecting follicular atresia, and spectral analysis of plasma by FT-IR may be suitable for detecting maturity stages. These techniques may be used in lieu of a biopsy and the calculation of oocyte PI. However, the analysis of caviar harvest in 2008 in California found that the stage of maturity (PI) does not necessarily correlate with higher caviar yields. Adiposity of the sturgeon ovary appeared to be a major factor that negatively affected caviar yield, particularly in larger, faster growing fish. It should be noted that this analysis does not invalidate the importance of detecting maturity stage by non-invasive methods because it was based on sampling a single 7-year old, early-maturing age cohort reared on a high-energy, high-fat salmon diet (EWOS). Similar sampling methods were used in 2009 during the caviar harvest of 8-year old fish on the same farm, as well as during the caviar harvest in Idaho, where fish were reared on different diets. The samples are currently being processed and this new data will be compared with the results of the present analysis.

High amounts of ovarian fat (up to 15% body weight) were reported in the early maturing age cohorts of wild paddlefish and were also associated with lower fecundity compared to older, iteroparous fish (Scarnecchia et al. 2007). However, the reports on fatty ovaries in wild sturgeon during spawning migration upriver are rare and are largely limited to the hiemal spawning race of Russian sturgeon migrating in the rivers of the Caspian Sea during the summer and fall in early stages of maturity to spawn during the spring and summer of the following year (Dettlaff et al. 1993). Adipogenesis involves both hyperplasia and hypertrophy that may occur at different stages of ontogeny (Rosen & MacDougald 2006). Moreover, it is markedly influenced by the hormones and growth factors associated with ovarian cycle (insulin and IGF-1) and by the genotype (Quillet et al. 2005). It will be important to clarify whether the enhanced adipogenesis observed in our 2008 sampling in California is a result of a natural development or that of an imbalance between energy intake and energy expenditure in farmed sturgeon.

### **WORK PLANNED FOR NEXT YEAR**

In 2009–2010, we will randomly sample 120 females at Sterling Caviar and 60 females in Idaho in November. The fish will be sorted into two groups (low PI n=50 and high PI n=50 in California and low PI n=10 and high PI n=10 in Idaho), using the PI value predicted from the plasma sex steroids, SW-NIR, and FT-IR techniques, with FT-IR being the most promising predictor at this time.

As already pointed out, one of the challenges associated with the implementation of the SW-NIR method to predict atresia or other parameters such as oocyte PI may be due to inhomogeneity of the gonads. Therefore in the third year of the study, we will try to ascertain how homogeneous the gonads are and what the variability of spectra collected on a given abdominal area is. An additional study will look at the visual properties of skin and muscle layers and their contribution to the abdominal spectra.

### **IMPACTS**

The initial studies on the farms indicate that sex steroid levels, SW-NIR and FT-IR can detect the onset of early atresia and FT-IR may be able to determine stage of maturity (PI). This provides for useful tools that may act as an alternative method for pre-screening caviar females prior to harvest. The application of these new methods under field and commercial conditions will be further tested and refined. Some of these methods, for example SW-NIR, may be applied to monitor ovarian fat accumulation (Folkestad et al., 2008) for optimizing caviar yield and selective breeding for lean ovaries.

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## PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

### *Manuscripts In Prep*

- Lu X, Doroshov S, Webb M, and Rasco B. In prep. A review of white sturgeon (*Acipenser transmontanus*) female reproductive physiology and its importance for caviar production.
- Lu X, Webb M, Talbott M, Van Eenennaam J, Palumbo A, Doroshov S, Struggenegger P, Rasco B. In prep. Distinguishing maturity of white sturgeon (*Acipenser transmontanus*) by spectroscopic measurement (Fourier Transform Infrared Spectroscopy) of fish blood plasma. Submitted for review to Work Group.
- Lu S, Talbott M, Webb M, Van Eenennaam J, Linares-Casanave J, Doroshov S, Rasco B. In prep. A study of morphological and immunochemical parameters associated with ovarian atresia and quality of caviar in white sturgeon (*Acipenser transmontanus*) females by Fourier transform infrared spectroscopy (FT-IR) and multivariate analysis. Submitted for review to Work Group.
- Servid SA, Talbott MJ, Van Eenennaam JP, Doroshov SI, Struffenegger P, Webb MAH, and Cavinato AG. In prep. Rapid and non invasive characterization of ovarian follicular atresia in cultured white sturgeon by near infrared spectroscopy.
- Talbott MJ, Webb MAH, Van Eenennaam JP, Linares-Casenave J, Doroshov SI, Guy CS, and Struffenegger P. In Prep. Plasma sex steroid concentrations during onset of ovarian atresia in farmed white sturgeon, *Acipenser transmontanus*.

### *Presentations*

- Cavinato AG, Servid SA. Determining ripeness in white sturgeon females: a nonlethal method to maximize yield and quality of caviar, Aquaculture America 2009, Seattle, WA, February 2009.
- Lu X, Rasco B. 2009. The use of Fourier transform infrared spectroscopy to predict reproductive readiness of white sturgeon (*Acipenser transmontanus*) and affects on caviar quality. IFT Annual Meeting, June 5–10, 2009. Anaheim, CA.
- Lu X and Rasco B. 2009. The use of Fourier transform infrared spectroscopy to predict maturity stages and caviar quality in white sturgeon (*Acipenser transmontanus*). Aquaculture America. Feb. 15–18, 2009.
- Lu X and Rasco B. 2009. A study of morphological and immunochemical parameters associated with ovarian atresia and quality of caviar in white sturgeon (*Acipenser transmontanus*) females by Fourier Transform Infrared Spectroscopy and multivariate analysis. Pacific Fisheries Technologists. Feb. 22–25, 2009. Portland, OR. Sponsor Oregon State University.
- Servid SA, Cavinato AG. Determining ripeness in white sturgeon females: A nonlethal method to maximize yield and quality of caviar, 237th American Chemical Society National Meeting, Salt Lake City, UT, March 2009.
- Talbott MJ, Van Eenennaam JP, Linares-Casenave J, Doroshov SI, Guy CS, Webb MAH. 2009. Investigating non-invasive methods to predict follicular atresia and spawning readiness in white sturgeon. Oral presentation at the AFS 139th Annual Meeting, August 30–September 3, 2009, Nashville, Tennessee.
- Talbot M, Van Eenennaam J, Linares-Casenave J, Doroshov S, Guy C, Webb M. 2009. Determining morphological and immunochemical parameters associated with early ovarian follicular atresia in White sturgeon (*Acipenser transmontanus*) females. Poster presentation at the 8th International Congress on the Biology of Fishes, July 28–August 1, Portland, Oregon.
- Talbot M, Van Eenennaam J, Linares-Casenave J, Doroshov S, Guy C, Webb M. 2009. Determining morphological and immunochemical parameters associated with early ovarian follicular atresia in White sturgeon (*Acipenser transmontanus*) females. Oral presentation at Aquaculture America 2009, February 15–18th, Seattle, Washington.
- Van Eenennaam J, Linares-Casenave J, Talbot M, Webb M, Struffenegger F, Conte F, Doroshov S. 2009. The relationship between white sturgeon caviar yield and stage of maturity. Abstract submitted to Aquaculture 2010, March 1–5, 2010, San Diego, California.

**SUPPORT**

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		UNIVERSITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
2007-08	100,001		18,500		79,986	98,486	\$198,487
2008-09	100,001	5,000	18,500		79,226	102,726	\$202,727
2009-10							
2010-11							
TOTAL	200,002	5,000	37,000		159,212	201,212	\$401,214

# POTENTIAL THREAT OF GREAT LAKES VHS VIRUS IN THE WESTERN UNITED STATES

<b>REPORTING PERIOD</b>	September 1, 2008–August 31, 2009		
<b>AUTHOR</b>	Gael Kurath		
<b>FUNDING LEVEL</b>	First Year Funding	\$ 72,097 (received July 2008)	
	<b>Second Year Funding</b>	\$ 35,063	
	Total Funding	\$107,160	
<b>PARTICIPANTS</b>	Gael Kurath <i>(Working Group Chair)</i>	USGS Wester Fisheries Research Center (WFRC), Seattle	Washington
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	Paul Herschberger	USGS–WFRC, Marrowstone Marine Station	Washington
	Carolyn Friedman*	University of Washington	Washington
	Jerri Bartholomew* <i>(Outreach)</i>	Oregon State University	Oregon
	Chang Hoon Moon <i>(Postdoctoral Fellow)</i>	University of Washington	Washington
	Evi Emmenegger	USGS-WRFC, Seattle	Washington
<b>INDUSTRY ADVISOR</b>	Scott E. LaPaftra	Clear Springs Foods, Inc.	Idaho.
<b>TECHNICAL ADVISOR</b>	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 project'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. Dr. Chang Hoon Moon has been working on the project since August 2008 as a postdoctoral fellow at the University of Washington, working in the Kurath laboratory at the USGS Western Fisheries Research Center (WFRC) in Seattle. Evi Emmenegger, a USGS GS12 level research microbiologist at the WFRC, has also contributed very significantly to the project. As the designated specialist in exotic and invasive pathogens requiring Biosafety Level 3 (BL3) containment, Ms. Emmenegger was the lead researcher for the virulence trials conducted in the WFRC BL3 wetlab facility. During this year, progress has been made on all objectives.

**Virus and host fish stocks:** Standardized virus stocks of VHSV isolates representing genotypes IVb (Great Lakes), IVb (Atlantic Coast, New Brunswick), IVa (Pacific Coast), and I (Europe) were prepared in year 1 and have been used throughout the research conducted in year 2. Stocks of pathogen-free yellow perch, rainbow trout, Chinook salmon, and Pacific herring have all been obtained, reared, and used successfully for *in vivo* experiments in year 2.

**Objective 1. Assemble and distribute biosecurity information currently available for dealing with VHSV.** Currently available biosecurity information for the European VHSV type I and the Great Lakes VHSV type IVb strains has been assembled and a PowerPoint presentation focusing on biosecurity has been prepared by the outreach coordinator, Jerri Bartholomew. This presentation has been given orally and it is now being made available on the WRAC website. Four general oral presentations on VHSV have been given by project investigators during year 2.

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

During this year, we have continued to use the general quantitative real-time polymerase chain reaction (qRT-PCR) assay that detects both VHSV types IVa and IVb, developed by Dr. Kyle Garver of the Pacific Biological Station in Nanaimo, British Columbia. The assay works well and has been used in objectives 3 and 4 below for general detection of VHSV. A genotype-specific qRT-PCR assays that specifically detects type IV a only, or type IVb only, has been designed using all known gene sequences from type IVa and IVb isolates of VHSV, but it has not been tested.

**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.**

The research in support of this objective has all been completed and is being written for publication in a peer-reviewed journal by the first author, Evi Emmenegger. Susceptibility of four fish species to disease and mortality due to Great Lakes VHSV type IVb (designated VHSV IVb-GL) has been determined in controlled wet laboratory challenge studies, where virus was delivered by intraperitoneal (IP) injection at two different viral doses. The challenge doses were 10<sup>3</sup> and 10<sup>6</sup> plaque forming units (PFU) per fish, and are referred to as “low”, and “high” doses, respectively. For comparisons of different VHSV genotypes, West Coast VHSV type IVa and European VHSV type I strains were tested simultaneously. In addition, an Atlantic Coast VHSV type IVb strain from New Brunswick (IVb-NB) was included in these challenge studies when possible. All infection studies were conducted on juvenile fish (2–5 g) at 12°C 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. In all cases mortality in mock-infected control groups was negligible.



***Virulence trials by IP injection in yellow perch***

Yellow perch are used in this project as a positive control host for the Great Lakes VHSV IVb, since epidemics have occurred recently in yellow perch in the Great Lakes. During year 1 of the project, we conducted two pilot studies that confirmed our ability to re-create the disease process seen in the field, and provided data on optimal challenge doses. During year 2, the full-scale virulence trial was conducted using triplicate groups of yellow perch challenged by IP injection with high and low doses of VHSV strains representing genotypes I, IVa, IVb-GL, and IVb-NB. In the high dose treatment groups, average final cumulative percent mortality (CPM) ranged from 84%–100%, and low dose treatments groups ranged 30%–93%. Relative virulence of the four VHSV strains, in order from highest to lowest, was IVa > IVb-GL > IVb-NB > I. In general, by the IP injection delivery route, yellow perch were highly susceptible to all strains, but the low dose treatment indicated it is least susceptible to VHSV genotype I.

***Virulence trials by IP injection in rainbow trout***

Rainbow trout are used due to their significance as a major western aquaculture species, and also as a positive control host for VHSV genotype I, which has caused severe epidemics in European Rainbow trout farms over the last 60 years. The Clear Springs stock of rainbow trout were tested in virulence trials conducted in the same manner as described above for yellow perch. In the high-dose injection groups CPM was 3-86%, and in low dose groups, CPM was 2%–98%. Relative virulence of the four VHSV strains, from highest to lowest, was I >> IVa = IVb-NB > IVb-GL. In general terms, by the IP injection delivery route, rainbow trout were highly susceptible to genotype I, and not very susceptible to genotypes IVa, IVb-GL, or IVb-NB.

***Virulence trials by IP injection in Chinook salmon***

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

***Modified virulence trials by IP immersion in herring***

Pacific herring were included in this project as a positive control host species for VHSV genotype IVa, which has caused large-scale marine epidemics of herring and pilchard off the West Coast. During our first year, pathogen-free herring brought to the Seattle WFRC lab from the WFRC Marrowstone marine station showed poor survival. During year 2, a successful trial was conducted in herring with modified conditions, including challenge by immersion rather than injection. In immersion challenges, the CPM ranged from 43%–80%. As expected, herring were very susceptible to genotype IVa, and they were only moderately susceptible to the other 3 VHSV genotypes.

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

This objective involves comparing infection levels of individual VHSV strains in different fish hosts regardless of the occurrence of disease signs or mortality. Quantification of virus in fish that died during the injection challenges described above showed that all VHSV strains replicated in all fish species. Virus titers in yellow perch, rainbow trout, and herring mortalities were 106–107 plaque-forming units per gram (PFU/g), and titers in Chinook salmon were slightly lower, at 104–105 PFU/g. Among fish that did not die, yellow perch, rainbow trout, and Chinook salmon from these challenges that were randomly sampled at 7 days post-challenge (dpc) also showed replication of all virus strains in all fish species, with virus levels 105–108 PFU/g. In herring sampled 14 dpc all virus strains persisted in most fish, but at 1–2 logs lower titers than in herring that died. Yellow perch and Chinook salmon survivors sampled 30 dpc showed all four virus strains persisted in at least some fish, but at reduced titers and lower prevalence, indicating viral clearance by some fish. In rainbow trout at 30 dpc only genotypes I and IVa persisted, in a small number of fish.

To compare *in vivo* growth of VHSV genotypes I, IVa, and IVb-GL, a time-course experiment measuring viral loads in rainbow trout challenged by immersion at 15°C has been completed. Virus replication peaked at day 3 for all 3 strains. The viral load of VHSV genotype I was 1–2.5 logs higher than genotype IVa at all time-points tested, from 0–10 days. VHSV IVb-GL was similar to genotype IVa at days 1–3, but thereafter it was cleared below detectable levels from day 5–10. This variation in viral growth among the 3 VHSV strains correlated very well with the levels of mortality in the virulence trials. Host immune gene response was also measured, and mirrored viral loads, being most notable in fish infected with genotype I. A similar time-course in yellow perch at 12°C has been conducted, and sample analysis is ongoing. Collectively, these data indicate that all four fish species tested can act as carriers and reservoirs of VHSV.

**Objective 5. Develop outreach materials to communicate project results.**

Five oral presentations and one poster on data from this project have been presented by project investigators at regional and national fish health meetings. In addition, a workshop for extension personnel is being held after the IAC/TC meeting this year.

## USEFULNESS OF FINDINGS

Informed response to emergence of VHSV in the Great Lakes.

## WORK PLANNED FOR NEXT YEAR

1. Assemble and distribute biosecurity information currently available 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 presented orally and on the website.
2. Develop diagnostic assays to differentiate Great Lakes VHSV IVb from endemic West Coast VHSV IVa. The genotype-specific qRT-PCR assays that have been designed will be tested for efficiency, sensitivity, and specificity, using tissues from fish that are singly infected or co-infected with two strains of VHSV.
3. Test susceptibility of yellow perch, rainbow trout, herring, and Chinook salmon to disease and mortality caused by Great Lakes VHSV IVb, West Coast VHSV IVa, and European VHSV I. This objective is complete and a publication is in preparation.
4. Test ability of relevant host species to act as carriers and/or reservoirs of different VHSV genotypes. Time courses comparing infection levels of different VHSV strains will be completed in both yellow perch and rainbow trout at 12°C. This will be published separately.
5. Develop outreach materials to communicate project results.

## IMPACTS

Outreach materials and presentation of project results at various venues have enhanced awareness and understanding of the emergence of VHSV in the Great Lakes among different interested parties, including academic fish health specialists and aquaculture groups.

## PUBLICATIONS, MANUSCRIPTS, AND PAPERS PRESENTED

### ***Publications in Print***

J. Bartholomew, Outreach product.

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

**Manuscript in preparation**

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

**Papers Presented: General outreach information on VHSV and biosecurity in aquaculture**

Kurath G. VHSV: The history and basics of the virus and the disease. 33rd Eastern Fish Health Workshop, VHSV Continuing Education Session, 4/4/08, Atlantic Beach, North Carolina.

Kurath G. How we got here: A review of VHS in the US. Invited talk for special “Producers session” on VHSV at Aquaculture America, 2/17/09, Seattle, Washington.

Kurath G. Fish RNA viruses: Epidemiology and evolution. Invited guest lecture in Fish Disease course at Oregon State University, 2/23/09, Corvallis, Oregon.

Kurath G. History and current affairs of VHS virus. Invited talk in special VHSV session at the International Conference on Aquatic Invasive Species, 4/23/09, Montreal, Canada.

Bartholomew J. Problems presented by emerging pathogens: Potential threat of Great Lakes VHS virus in the Western United States. Hagerman Fish Culture Experiment Station, 8/14/09, Hagerman, Idaho.

**Papers Presented: Research results from WRAC VHSV project (\* indicates presenter)**

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

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

Kurath G\*, Emmenegger EJ, Moon CH. Susceptibility of Pacific salmonids, yellow perch, and koi to viral hemorrhagic septicemia virus strains from the Great Lakes, Pacific and Atlantic coasts of Canada, and Europe. Aquaculture America, Invited talk for session on RAC research on VHSV. 2/17/09, Seattle, Washington.

Emmenegger EJ\*, Moon CH, and Kurath G. Susceptibility of western aquaculture species to different strains of VHS virus, including Great Lakes VHSV. Idaho Aquaculture Association, 6/09, Twin Falls, Idaho.

Kurath G\*. Epidemiology of fish rhabdoviruses part 2: VHSV. Invited guest lecture in Oregon State University Salmon Disease Workshop, 7/21/09, Corvallis, Oregon.

Moon CH\*, Emmenegger EJ, and Kurath G. Susceptibility of Pacific salmonids, yellow perch, and koi to four strains of VHS virus, including Great Lakes VHSV. Poster presentation at AFS Fish Health Section Annual meeting, 6/10/09, Park City, Utah.

**SUPPORT**

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		UNIVERSITY	INDUSTRY	OTHER	FEDERAL	OTHER	
2008	72,097						\$72,097
2008	27,903						\$27,903
TOTAL	100,000						\$100,000

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).

# SCALE-DEPENDENT AND INDIRECT EFFECTS OF FILTER FEEDERS ON EELGRASS: UNDERSTANDING COMPLEX ECOLOGICAL INTERACTIONS TO IMPROVE ENVIRONMENTAL IMPACTS OF AQUACULTURE

## TERMINATION REPORT

**PROJECT WORK PERIOD** July 1, 2003–June 30, 2010

**AUTHORS** Jennifer Ruesink and Sally Hacker

<b>PARTICIPANTS</b>	Brett Dumbauld	USDA-Agricultural Research Service	Oregon
	Sally Hacker*	Oregon State University	Oregon
	Steve Harbell	Washington State University	Washington
	Jennifer Ruesink*	University of Washington	Washington

<b>TECHNICAL ADVISOR</b>	Bill Dewey	Taylor Shellfish	Washington
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<b>GRADUATE STUDENTS</b>	Kristen Rowell	University of Washington (2004)	Washington
	Heather Tallis	University of Washington (2003–04)	Washington
	Lorena Wischart	Oregon State University (2005–present)	Oregon
	Sara Frame	University of Washington (2006)	Washington
	Micah Horwith	University of Washington (2007)	Washington
	Sylvia Yang	University of Washington (2007–09)	Washington
	S. Alexandra Hart	University of Washington (2007)	Washington
Orissa Moulton	Oregon State University (2008–present)	Oregon	

<b>UNDERGRADUATE STUDENTS</b>	Michelle Briya	Oregon State University (2004–05)	Oregon
	Ashley Lyons	Oregon State University (2005–06)	Oregon
	Thatcher Jones	Oregon State University (2005–07)	Oregon
	Jackie White	University of Washington (2005)	Washington
	Carrie Craig	University of Washington (2006)	Washington
	Marla Koberstein	University of Washington (2006)	Washington
	David Holden	University of Washington (2006)	Washington
	Chao-chung Tsai	University of Washington (2006-07)	Washington
	Jeremy Henderson	Oregon State University (2007-present)	Oregon
	Arwen Norman	University of Washington (2008)	Washington
	Kris Lawrence	University of Washington (2008)	Washington
	Hollis Baley	Oregon State University (2008)	Oregon
	Brady Ryan	University of Washington (2009)	Washington
	Johnny Fitzpatrick	University of Washington (2009)	Washington
	Emma Fuller	University of Chicago (2009)	Illinois
	Wyatt Rice-Narusch	Oregon State University (2009)	Oregon
Sea-oh McConville	Oregon State University (2009)	Oregon	

**REASON FOR TERMINATION** Objectives completed

## PROJECT OBJECTIVES

1. Test the ability of benthic filter feeders to remove particulates from marine waters, and the response of eelgrass in distribution or growth rate.
2. Test the ability of benthic marine filter feeders to increase the nutrient and organic content of sediments through production of feces and pseudofeces, and the response of eelgrass in distribution, growth rate, and tissue quality.
3. Test the response of eelgrass to filter feeders in terms of eelgrass seed recruitment, germination, and seedling success.

## PRINCIPAL ACCOMPLISHMENTS

### ***Objective 1. Test the ability of benthic filter feeders to remove particulates from marine waters, and the response of eelgrass in distribution and growth.***

Oysters “clean up” the water by removing phytoplankton through filter feeding. This is very easy to see in a small tank, but contentious debate surrounds the bio-filtering capacity of oysters in the field. Why? Primarily because much of the pelagic realm may be inaccessible to benthic organisms; their effects may be swamped out by resuspension and phytoplankton growth—particularly if metabolic waste products stimulate phytoplankton growth; and additionally, low temperatures and low food quality or quantity can cause bivalves to shut down pumping. Thus, we were challenged to show that there is any detectable effect of cultured bivalves on particulates in the water. This phenomenon is called top-down control in ecological parlance, reflecting that a consumer (“top” trophic level) strongly reduces the abundance of its resource (one trophic level “down”). We collected field data demonstrating top-down control of phytoplankton by cultured oysters, specifically reducing the amount of phytoplankton about 5% for every 100 m of water flow across the tideflat. This adds up for Willapa Bay’s tideflats, some of which reach 1 km in breadth. The effects of these bio-filters compound further because water passes back and forth across oyster beds for multiple tidal cycles, resulting in the early flood tide water having less than 10% of background phytoplankton levels in the bay (Wheat et al., unpublished).

Does eelgrass benefit from reduced phytoplankton (and likely improved water clarity)? We found little evidence that bivalves facilitate eelgrass via this mechanism. Because of the large scale of phytoplankton removal, a logical comparison to understand the response of eelgrass was between oyster and non-oyster beds. If improved light were a major determinant of eelgrass performance, we would have expected faster growth and higher biomass with oysters. Instead, shoots were both smaller and sparser on oyster beds (longlines, hand-picked, and especially dredged), although in some cases, they grew faster for their size (Tallis et al. 2009) and had higher seed germination and growth (Wishart et al. 2007). Overall, light limitation is more likely to be a factor at the lower limit of eelgrass, rather than in the low intertidal and shallow subtidal zones that overlap with aquaculture. Indeed, one of our major unexpected findings about intertidal eelgrass was its control by disturbance and recovery processes, rather than light or nutrient limitation.

### ***Objective 2. Test the ability of benthic marine filter feeders to increase the nutrient and organic content of sediments through production of feces and pseudofeces, and the response of eelgrass in distribution, growth rate, and tissue quality.***

The material that bivalve suspension-feeders remove from the water has three general fates: it is incorporated into the organism’s tissues for growth and reproduction; it is recycled to the water column as metabolic solutes; and feces and pseudofeces are deposited on the sediments. This last fate generates a prediction that bivalves could fertilize nearby eelgrass. Because the spatial scale of sediment change is much smaller than water column change, we were able to test the effects of bivalves on sediment and eelgrass performance in several experiments. We examined three pairs of species: *Crassostrea gigas* and *Zostera marina* in Willapa Bay (Wagner et al., unpublished); *Panopea abrupta* and *Z. marina* in south Puget Sound (Ruesink and Rowell, submitted); and *Ruditapes philippinarum* and *Z. japonica* in Willapa

Bay (Tsai et al. submitted). *C. gigas* at high density (70% cover) created sediments of fine grain size and high organic content, although, interestingly, this was the role of *Z. marina* in south Puget Sound. Also, across beds in Willapa Bay, there was no association of sediment type with aquaculture habitat, probably swamped out by factors other than bivalves influencing sedimentary processes (Richardson et al. 2008). *P. abrupta* occasionally increased porewater ammonium, but *R. philippinarum* at aquaculture densities did not.

Overall, bivalves certainly did change sediment structure or biogeochemistry in small-scale experiments, but these changes had no strong effect on eelgrass performance. Rather, the growth rates of eelgrass suggested strong seasonal effects (reduced shoot size and relative growth rate in winter; Ruesink and Rowell, submitted; Ruesink et al. in revision), effects of tidal elevation (reduced shoot size and relative growth rate at upper distribution limits), and effects of disturbance (reduced growth and size following tissue loss). Among the three studies, the only positive relationship of eelgrass growth to bivalves was with *P. abrupta*, and it is possible that larger shoot sizes (and therefore faster raw shoot growth) stemmed from reduced eelgrass density, rather than geoducks per se (Ruesink and Rowell, submitted). Chemical analyses of leaf tissue provided no evidence that plants were nutrient-limited. Nitrogen concentrations (3.3%) were well above those of *Z. marina* collected from nutrient-loaded estuaries on the East Coast of the United States. (2.0–2.8%), and Carbon:Nitrogen ratios (11.1) were correspondingly lower than in these East Coast estuaries (16.4–22.6), as well as in *Thalassia testudinum* that had (13.4) or had not (16.3) been fertilized with mussel biodeposits. In our study, %N and C:N of eelgrass did not differ significantly among bivalve treatments. Porewater ammonium concentrations in Willapa Bay (15–500  $\mu\text{M}$ ), south Puget Sound (15–155  $\mu\text{M}$ ), and throughout Puget Sound (5–150  $\mu\text{M}$ ) include values that should limit *Z. marina* growth (nutrient limitation below 100  $\mu\text{M}$ ), but water column nutrients are naturally high, and porewater values variable in time and space, both of which may damp out any small nutrient boost from bivalves.

A secondary set of information derived from these studies includes the magnitude of space competition between eelgrass and bivalves. We found space competition between *P. abrupta* (10  $\text{m}^2$ ) and *Z. marina* only in summer, when eelgrass shoot densities were naturally highest (this was a population with a high-density, small-size morphotype). We found space competition between *C. gigas* and *Z. marina* only at oyster densities above 10–20% cover, although in at least one case we also found underyielding, that is, less eelgrass than would be expected simply from space occupied by oysters. This space competition documented experimentally is consistent with observational studies showing negative relationships between oyster cover and eelgrass density or reproduction (Wisehart et al. 2007; Tallis et al. 2009; Hacker, Hessing-Lewis, Dumbauld, unpublished) and points out the importance of future work to describe the carrying capacity of eelgrass in aquaculture habitats.

**Objective 3. Test the response of eelgrass to filter feeders in terms of eelgrass seed recruitment, germination, and seedling success.**

Eelgrass often occurs at lower density where shellfish aquaculture is carried out. We documented this pattern for Willapa Bay (Tallis et al. 2009), and it has been reported widely for West Coast estuaries where aquaculture and eelgrass co-occur. These lower densities likely derive from a combination of space competition and removal due to aquaculture activities. We carried out observational and experimental studies at multiple spatial and temporal scales to determine the factors controlling eelgrass recovery. We consider asexual reproduction (rhizome branching) to influence shoot density over rather short distances, whereas effective sexual reproduction (seedling germination and survival) would be required for recovery of large, denuded areas. At the largest scale, we recorded sexual and asexual reproduction across 19 sites in Washington state. These data were somewhat sobering, given the small fraction of sites where eelgrass demonstrated successful sexual reproduction (6 of 19 had seedlings) in May 2007. Willapa Bay, Samish Bay, and parts of Hood Canal harbored seedlings. At all sites, eelgrass had high rates of rhizome branching (46% of shoots were new branches), indicating the importance of remnant shoots to support recovery. Within Willapa Bay, we carried out a similar study along an estuarine gradient from fully marine water to as far up-river as the distribution of eelgrass extended. Branching ranged from 4–63% across sites, and seedlings contributed

0–15% of shoots. This variability in sexual reproduction across sites in a single study helps explain some apparent inconsistencies in seedling densities that we documented across studies in Willapa Bay (0–4 per m<sup>2</sup>; Wischart et al. 2007, Ruesink et al. unpublished).

At scales of ha relevant to aquaculture, we compared life-histories and population dynamics for eelgrass in meadows vs. aquaculture beds. We found, not surprisingly, that native eelgrass was more abundant in non-aquaculture beds and oysters were more abundant within aquaculture beds. From 2007-2009, eelgrass abundance was virtually unchanged in the non-aquaculture beds (~80–95% cover) but increased with time since disturbance in the aquaculture beds until it reached roughly 30–40% cover at all sites. Within the aquaculture beds, oyster cover varied little over time or site, ranging from 20–35% cover (Hacker, Hessing-Lewis, Dumbauld, unpublished). Across habitat types, high seed production and seed densities occurred on dredged beds, but within this habitat, there was a negative effect of oyster cover (Wischart et al. 2007, Hacker, Hessing-Lewis, Dumbauld, unpublished). At the smallest (experimental) scale, we examined the mechanisms and pace of recovery after different types of disturbance (cutting, thinning, complete removal) in small 1–4 m<sup>2</sup> plots. Seedling germination and success (growth) improved at low eelgrass densities (Wischart et al. 2007). Cutting (removal of leaves) did not kill shoots but reduced their capacity for asexual reproduction (rhizome branching). Thinning (removal of shoots) increased both asexual (branching) and sexual reproduction (seedling size), which would accelerate recovery over that expected based on dynamics in eelgrass beds (Ruesink et al. unpublished).

Observations at our study sites reinforce that *Z. marina* is naturally dynamic: Over the course of this grant, it disappeared from a zone at Rocky Pt (Ruesink and Rowell, submitted), and increased overall in Willapa Bay (especially at higher tidal elevations, perhaps facilitated by *Z. japonica* holding water, Ruesink et al. in revision). It is not surprising, then, that eelgrass sometimes makes a novel appearance in aquaculture beds (where it gets noticed, in contrast to dynamics elsewhere). If appearance of eelgrass in aquaculture happens more often than by chance (a landscape-level, low-probability question), then this issue should be pursued through further mechanistic work to determine major controls on the distribution of seeds. We documented no facilitative effects of bivalves per se on eelgrass during the recovery process (where eelgrass was previously present), except perhaps in terms of higher seed density on dredged beds than in eelgrass. Overall, shellfish aquaculture appears not to erode the resilience of eelgrass, which can recover rapidly (for eelgrass) as shoot densities are augmented by branching and—in selected locations—seedlings. Caveats must be added that it would certainly be possible for the frequency and intensity of removal to exceed the intrinsic recovery capacity of eelgrass, and the presence of bivalves may further influence the carrying capacity for eelgrass.

## IMPACTS

We recently summarized the ecological state-of-the-science with respect to effects of bivalve shellfish aquaculture on the West Coast of North America (Dumbauld et al. 2009). This document has informed debates about practices and regulations, as it collates and interprets two WRAC-funded research efforts along with other relevant work from the West Coast and worldwide. Two recent eruptions of scrutiny of bivalve shellfish aquaculture include continued oyster culture in Drakes Estero (Dumbauld serves on the National Research Council committee) and the expansion of intertidal geoduck aquaculture in Washington (WRAC and Industry funded prescient examination of impacts).

During this project, the aquaculture industry encountered a new federal regulation (NWP 48 in March 2007) through the Army Corps of Engineers, which has jurisdiction over dredging and filling in US waters. ACOE is required to consider and protect submerged aquatic vegetation. In the NWP, “The permittee must submit a pre-construction notification to the district engineer if... dredge harvesting, tilling, or harrowing is conducted in areas inhabited by submerged aquatic vegetation.” Two other federal agencies (USFWS, NOAA Fisheries) have provided biological opinions about how aquaculture practices may influence listed species and essential fish habitat. The economic risk to the industry is from spatial or temporal closures to aquaculture activities in eelgrass, which would reduce

the productivity of shellfish beds. Our results have demonstrated that a one-size-fits-all approach to aquaculture regulation ignores important spatial variation in how eelgrass responds to disturbance. For instance, in Willapa Bay, seedlings are relatively common and populations robust and expanding, whereas south Puget Sound harbors small patches of eelgrass that are susceptible to a variety of disturbances and show little capacity for recovery via sexual reproduction. To date, and in line with our results, it appears that shellfish aquaculture in eelgrass may only be restricted in south Puget Sound. Importantly, *Z. japonica* as an introduced eelgrass is not protected under NWP 48.

We made particular efforts to speak annually at the PCSGA conference and SG Conference for Shellfish Growers, which provided opportunities for feedback on results and interpretation from many growers. In contrast, we have had very little direct contact with agency regulators responsible for implementing and detailing NWP 48; it seems that most of the communication has occurred through industry.

## RECOMMENDED FOLLOW-UP ACTIVITIES

**Functional consequences:** In the current research effort, we have documented local changes in the distribution and abundance of eelgrass as a function of bivalve shellfish aquaculture. However, the functional consequences of these changes for other species and ecosystem functioning have not been determined. Presumably, attention is paid to eelgrass not because any human cares much about its long, green blades, but because it contributes to societal benefits (e.g., shoreline protection, fisheries). Does eelgrass at 30% of ambient density still provide adequate ecosystem services? Or, at a larger scale, how do economically important fishes perform in an eelgrass meadow vs. less dense eelgrass or an edge? Such questions may be addressed through specific tests of foraging efficiency, growth, and survival of nekton in different configurations of habitat.

**Landscape-level approach:** Ultimately, a landscape-level approach is critical to understanding what fraction of a particular habitat (say, intertidal eelgrass) has been modified by aquaculture activities.

**Polyculture:** Shellfish growers produce bivalves, and many incidentally also produce eelgrass. (This is particularly obvious where a bed is sprayed against burrowing shrimp, and more eelgrass accompanies better oyster growth and survival.) Society places a value on this eelgrass, but the economic benefit of growing eelgrass does not materialize in terms of a payment to the grower. Consequently, it may be more economically viable for a grower to remove eelgrass, for instance, to allow better drainage of a bed or easier harvest of shellfish. The whole principle of polyculture is to produce multiple crops simultaneously on a piece of ground, where the benefit in terms of production, profit, or sustainability is enhanced over a monoculture. Research is required to determine how much joint eelgrass and oyster production is possible (carrying capacity). These could involve cultivation of eelgrass for restoration efforts, or mitigation for eelgrass removed elsewhere.

**Eelgrass resilience:** Given the overwhelming attention to light and nutrient effects on eelgrass in previous studies, we were not fully expecting that disturbance and recovery dynamics would figure so prominently in understanding distribution and abundance of Washington's intertidal eelgrass. In light of this new information, it would be helpful to shift regulatory scrutiny away from the amount of eelgrass to the resilience of eelgrass. If eelgrass continues to recover after disturbances associated with aquaculture activities, then its resilience has not been eroded. But, essentially nothing is known about what governs eelgrass resilience. Some factors may be at the landscape level (how much total eelgrass is present, e.g., producing seeds), others may be at the level of the individual (are local environmental characteristics suitable for sexual and asexual reproduction). Methods to address the question of resilience would include transplanting shoots at different densities into aquaculture beds, and excavating shoots to determine their history of germination and branching.

### *Specific suggestions*

- Study light availability for eelgrass in and near low (often subtidal) dredged beds.
- Compare the importance of various factors influencing seed distribution, including the proximity of source



plants, sloughs and sinks that may be involved in seed transport, identity and distribution of seed predators, and duration of seed bank.

- Continue survey of long-term plots established in eelgrass and dredged beds in Willapa Bay (section 3B), to examine carrying capacity of eelgrass in aquaculture and non-aquaculture areas.
- Expand research to additional locations and types of aquaculture, particularly as needed to support decision-making. This suggestion responds to feedback we received throughout the project that our results for Washington state could not be extrapolated to other places where ecological data were necessary to inform decision-making. Our response is that such extrapolation is problematic even within Washington, as clarified by our work in different parts of Willapa Bay and south Puget Sound, as well as the survey of 19 sites throughout the state. We may have seen as much or more variation within Washington as we would have documented by (logistically, financially infeasible) studies all along the coast. Some generalities do emerge for West Coast shellfish aquaculture (summarized in Dumbauld et al. 2009), but a commitment to funding similar ecological research in new locations may be necessary for aquaculture development.

## **PUBLICATIONS, MANUSCRIPTS, AND PAPERS PRESENTED**

### ***Publications in print and Theses***

- Dumbauld BR, Ruesink JL, Rumrill SS. The ecological role of bivalve shellfish aquaculture in the estuarine environment: A review with application to oyster and clam culture in West Coast (USA) estuaries. *Aquaculture* 290:196–223.
- Richardson NE, Ruesink JL, Naeem S, Dumbauld BR, Hacker S, Tallis H, Wischart L. 2008. Abundance and functional diversity of sediment microbes across natural and oyster aquaculture habitats in a northeastern Pacific estuary. *Hydrobiologia* 596:269–278.
- Ruesink JL, Hong JS, Dumbauld BR, Hacker SD, Trimble AC, Wischart LM. Congener comparison of native (*Zostera marina*) and introduced eelgrass (*Z. japonica*) in Willapa Bay. In revision, *Biological Invasions*.
- Ruesink JL, Feist BE, Harvey CJ, Hong JS, Trimble AC, Wischart LM. 2006. Changes in productivity associated with four introduced species: Ecosystem transformation of a “pristine” estuary. *Marine Ecology Progress Series* 311:203–215.
- Tallis HM, Ruesink JL, Dumbauld BR, Hacker SD, Wischart LM. 2009. Oysters and aquaculture practices affect eelgrass density and productivity in a Pacific Northwest estuary. *Journal of Shellfish Research* 28:251–261.
- Wischart LM, Dumbauld BR, Ruesink JL, Hacker SD. 2007. Importance of eelgrass early life history stages in response to oyster aquaculture disturbance. *Marine Ecology Progress Series* 344:71–80.
- Wischart LM. 2006. Impacts of oysters on eelgrass (*Zostera marina* L.): Importance of early life history stages in response to aquaculture disturbance. MS Thesis, Oregon State University, Corvallis, OR.

### ***Manuscripts***

- Hacker SD, Hessing-Lewis M, Dumbauld BR. Effects of oyster aquaculture on eelgrass community structure across multiple spatial and temporal scales. In preparation.
- Hessing-Lewis M, Hacker SD. Macroalgal associations with eelgrass and oyster aquaculture in Willapa Bay, Washington. In preparation.
- Ruesink JL, Fitzpatrick J, Dumbauld BR, Hacker S, Trimble AC, Wagner EL, Wischart L. Response of intertidal eelgrass (*Zostera marina*) to different types and magnitudes of pulse disturbance. In preparation.
- Ruesink JL, K Rowell. Geoduck clam (*Panopea abrupta*) aquaculture as press and pulse perturbations to eelgrass (*Zostera marina*). Submitted. Ecological Applications
- Tsai C, Trimble AC, Ruesink JL. Interactions between two introduced species: *Zostera japonica* (dwarf eelgrass) facilitates itself and negatively affects *Ruditapes philippinarum* (Manila clam) on intertidal flats. Submitted. *Marine Biology*
- Wagner EL, Ruesink JL, Dumbauld BR, Hacker S, Wischart L. Density-dependent effects of oysters (*Crassostrea*

- gigas*) on intertidal eelgrass (*Zostera marina*) in small-scale experiments. In preparation.
- Wheat E, Trimble AC, Ruesink JL. Oysters (*Crassostrea gigas*) exert top-down control on pelagic resources across tideflats in Willapa Bay, Washington, USA. In preparation. Marine Ecology Progress Series
- Wischart, LM, Dumbauld BR, Hacker SD. Spatial variation in eelgrass recruitment from seed: influence of seed production, physical factors, and adult neighbors.
- Yang S, Wheat E, Horwith M, Ruesink JL. Implications of intertidal eelgrass (*Zostera marina* L.) life history variation for resilience to disturbance. In preparation. Aquatic Botany

### **Papers presented**

- McCoy L, Ruesink J. What are you eating? Willapa Bay Seafood Festival, May 2004 (poster)
- Wischart LM, Tallis HM, Hacker SD, Ruesink JL, and Dumbauld BR. The effects of oyster aquaculture on eelgrass (*Zostera marina* L.) biomass, density, and growth rates in Willapa Bay, WA. Pacific Estuarine Research Society Meeting, Port Townsend, WA, May 2004 (talk).
- Tallis H. Ecological Society of America annual meeting, August 2004 (poster)
- Ruesink JL. Research projects in south Puget Sound. Meeting with Squaxin tribal biologists, September 2004 (talk)
- Rowell K, White J, Ruesink J. October 2004. Interactions between geoducks and eelgrass. NSA/PCSGA
- Wischart L, Hacker SD, Ruesink J, and Dumbauld B. Eelgrass (*Zostera marina* L.) seed dispersal and recruitment inside and outside oyster aquaculture areas in Willapa Bay, WA. PCSGA/NSA annual meeting, October 2004 (talk).
- Wischart L, Hacker SD, Ruesink J, and Dumbauld B. Eelgrass (*Zostera marina* L.) seed dispersal and recruitment inside and outside oyster aquaculture areas in Willapa Bay, WA. International Seagrass Conference, Australia, October 2004 (talk).
- Ruesink JL. Direct and indirect effects of oysters and shellfish aquaculture on eelgrass (*Zostera marina*) in Willapa Bay, Washington. Humboldt Bay Marine Management Commission, December 2004 (talk)
- Dumbauld BR. December 2004. The ecological role and potential impacts of shellfish culture in the estuarine environment. Humboldt Bay Marine Mariculture Commission meeting, Dec. 14
- Ruesink JL, Rowell K. Geoduck aquaculture and eelgrass in south Puget Sound—a preliminary report. 13th Annual Meeting for Shellfish Growers, February 2005 (talk)
- Rowell K, Ruesink JL, White JM. Influences of geoduck aquaculture on eelgrass. Puget Sound Georgia Basin Research Conference, March 2005 (poster)
- Wischart L, Hacker SD, Ruesink J, and Dumbauld B. Does oyster aquaculture influence eelgrass recruitment? Pacific Estuarine Research Society, Coos Bay, OR, March 2005 (talk).
- Wischart L. June 2006. Impacts of oysters on eelgrass (*Zostera marina* L.): Importance of early life history stages in response to aquaculture disturbance. Thesis defense, Oregon State University, Corvallis, OR.
- Wischart L. June 2006. Aquaculture and eelgrass interactions in the Pacific Northwest. Presentation to Oregon State University Marine Biology Course, Newport, OR.
- Frame S. October 2006. Eelgrass and geoduck interactions. Pacific Coast Shellfish Growers Association Meeting, Vancouver, WA.
- Dumbauld BR. October 2006. Pacific Coast Shellfish Growers Association Meeting, Vancouver, WA.
- Ruesink JL. October 2006. Ecological interactions between oysters and eelgrass in Willapa Bay. Pacific Coast Shellfish Growers Association Meeting, Vancouver, WA.
- Ruesink JL. October 2006. Ecological interactions of geoducks and eelgrass in south Puget Sound. Sound Science Series (invited), Shelton, WA.
- Ruesink JL. November 2006. Conservation science in a coastal estuary—ecological impacts of shellfish aquaculture. Western Society of Naturalists (invited), Redmond, WA.
- Ruesink JL. April 2007. Ecological consequences of Japanese oysters in western North America. International Oyster Reef Meeting (invited), Chiba, Japan.
- Ruesink JL. July 2007. West Coast oyster reefs as sentinel habitats. Coastal Zone 2007 (invited), Portland, OR.

Ruesink JL. September 2007. Geoduck clam (*Panopea abrupta*) aquaculture as press and pulse perturbations to eelgrass (*Zostera marina*). Northwest workshop on bivalve aquaculture and the environment (invited), Seattle, WA.

Dumbauld BR. September 2007. Environmental effects of culture structures. Northwest workshop on bivalve aquaculture and the environment (invited), Seattle, WA.

Wheat E. November 2008. Drivers of local variation in oyster growth: food limitation or tidal circulation? Western Society of Naturalists.

Yang S. November 2008. Western Society of Naturalists.

Ruesink JL. August 2009. Evidence for sustainability of seagrasses responding to Washington's shellfish agroecosystems. Ecological Society of America, Albuquerque, NM.

Wheat E. August 2009. Drivers of local variation in Pacific oyster growth (*Crassostrea gigas*): food limitation or tidal circulation? Ecological Society of America, Albuquerque, NM.

Yang S. August 2009. Storm and stress: Balancing intertidal eelgrass recovery with repeated hydrodynamic disturbance. Ecological Society of America, Albuquerque, NM.

Ruesink JL., Tsai CC, Trimble AC. August 2009. Introduced habitat engineer facilitates itself and negatively affects a co-occurring non-native clam. Marine Bioinvasions Conference, Portland, OR.

Ruesink JL. September 2009. Multiple hypotheses about effects of shellfish aquaculture on eelgrass. PCSGA/National Shellfisheries Association, Portland, OR.

Wheat E. September 2009. PCSGA/National Shellfisheries Association, Portland, OR.

## SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		UNIVERSITY	INDUSTRY	OTHER	FEDERAL	OTHER	
2003	79,607	>6,000	20,000	>3,000		29,000	\$108,607
2004	83,415	>6,000	20,000	>3,000	>3,000	32,000	\$115,415
2005	82,368	12,000	20,000	>3,000	20,000	55,000	\$137,368
2006	82,368	12,000	20,000	>3,000	10,000	45,000	\$127,368
2007		30,500		>3,000		33,600	\$33,500
2008		20,600			12,000	32,600	\$32,600
2009		38,800			3,000	41,800	\$41,800
TOTAL	327,758	125,900	80,000	30,000	33,000		\$596,658

University sources include salary for S. Hacker and J. Ruesink.

Federal sources include salary for B. Dumbauld.

Other sources include salary for A. Trimble from Andrew W. Mellon Foundation.

# AQUARIUS: SHELLFISH SANITATION SIMULATOR, RAINFALL AND WATER QUALITY CLOSURE RULE EVALUATOR VERSION 2.0\*

## TERMINATION REPORT

**PROJECT WORK PERIOD** September 2006–February 2009

Because WRAC has no protocols developed for Rapid Response Grants in the WRAC Manual of Operations, this Termination Report covers the work performed from September 2006 through February 2009, the period for which the WRAC funds were made available and used.

**AUTHOR** Fred S. Conte

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\*\* (Funded)

## PROJECT OBJECTIVES

The long-term goal of this project was to develop a version 2.0 of the Aquarius simulation and statistical analytical software designed for public health agencies and the shellfish industry to evaluate water quality closure regulations for the shellfish industry, based on the National Shellfish Sanitation Program. This project has regional, national, and international application. With the success of Aquarius version 1.0\*\*\*, a series of meetings were held with industry and state and federal agency representatives of the regional Pacific Rim Shellfish Sanitation Conference, in which they were asked what improvements they would like to see in a new version of the software program. State and federal shellfish regulators and industry in the western states expressed enthusiasm for an expanded program, and identified 12 additional components that they considered essential to a fully mature program that could be used as a decision-making tool in their processes for initiating changes in rainfall closure regulations.

\*\*\* Conte, FS and A Ahmadi. 2005. Aquarius: Shellfish sanitation simulator; rainfall and water quality closure rule evaluator. Department of Animal Science. Copyright 2004–2006. Regents of University of California. Software program and program manual. 40p.

Aquarius Version 2.0 has a similar basic format as exhibited by Version 1.0; however, the program has been enhanced to accommodate increased parameters in the simulation mode and incorporate additional statistical analyses. Version 2.0 incorporates 12 primary changes as follows:

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\* A second software program, “Weather Processing Computer Programs” not included in the grant proposal was also produced as a companion program for Aquarius v.2.0, and may also be used to convert data associated with any other rainfall program datasets.

**Primary Rules:** In Version 1.0, the user can operate the program using only one primary rule. Version 2.0 will allow the user to run up to three primary rules. Growing areas vary in the number of primary rules assigned by the health department. Most have one primary rule, but some may have two to three primary rules.

**Sample Type:** In Version 1.0, the user can operate the program using only one sample type. The health service agencies often separate their own archived data sets from other agency water samples, or even water samples data that are taken by commercial growers participating in cooperative sampling programs. Version 2.0 will allow the user to isolate individual sample types, or combine up to four samples in a single analysis.

**Cumulative Rainfall:** In Version 1.0, rainfall is fixed at 10-day accumulative. In Version 2.0, cumulative rainfall will not be fixed and the user will be able to change the cumulative rainfall to any number of days. Health departments are now considering changing the traditional 10-day accumulative rainfall to 7-day accumulative rainfall. This feature allows the health service to apply a greater variability in accumulative rainfall.

**Rainfall Resolution:** In Version 1.0, the rainfall data are imported from daily values. In Version 2.0, the rainfall data will be improved from daily, hourly, or 6-hourly values. Most rainfall monitoring stations have archived data on a daily basis, but recently, agencies are considering 6-hour and 1-hour intervals. In California, there are six production bays, and in only one of the six bays is data accumulated on 6-hour intervals. Rainfall from the remaining five bays is now reported on an hourly basis. These criteria are also being considered by other state and international health service agencies.

**No Rain:** Version 1.0 has an option to treat trace rainfall below 0.01 inch as zero rainfall. Version 2.0 will allow the user to adjust the 0.01 inch to any amount.

**Adverse Condition Data:** Version 1.0 does not allow the user to isolate the adverse condition data. Version 2.0 will allow the user to isolate and run only data taken during adverse conditions. This conforms to traditional health department protocols for conservative analysis placing public health as the first priority.

**Sample I.D.:** Version 1.0 does not allow tracking of sample IDs. Version 2.0 allows tracking of sample IDs. Health departments assign a unique sample ID to each official sample, which can be traced for authentication and validation.

**Rainfall Data Format:** In Version 1.0, the rainfall data format is saved in Excel. In Version 2.0, the rainfall data format will be saved in General Comma Separated Values (CSV), which allows data to be freely exchanged between different systems. Almost all archived rainfall data can be downloaded in this format.

**Fecal Coliform Format:** In Version 1.0, the fecal coliform data format is saved in Excel. In Version 2.0, the fecal coliform data format will be saved in General Comma Separated Values (CSV). Almost all archived fecal coliform data can be obtained in this format.

**Confidence Level:** In Version 1.0, the statistical package only allows analysis at the 95th percentile. In Version 2.0, the statistical package will have options to run at the 95th, 98th, and 99th percentile, thus allowing greater sensitivity and greater choice for health-related decisions.

**Non-Parametric Test:** Sometimes samples do not follow the normal distribution pattern, and hence the T-test alone may not be applicable. In these cases, the program will provide equivalent non-parametric tests which are not sensitive to normality of the sample distribution, but at the same time, can detect statistically the difference between two scenarios.

**Type II Error:** Version 1.0 only applies Type I error, but Version 2.0 handles both Type I and Type II errors. Type II errors occur when the new rule is actually worse than the current rule in terms of health protection, but the program would report the rules as equivalent. This type of error is primarily due to an insufficient number of

samples. The side effect of such error is significant in terms of public health, because the agency would be relaxing the rule without sufficient samples to justify the decision. The Type II error is usually around 1.0 to 2.0 percent, and a higher value should force the agency to resample the growing site to obtain sufficient sample numbers. Version 2.0 will calculate the number of samples required for 1.0 to 2.0 percent Type II error.

**Grace Period:** The accumulation of fecal coliform in a bay usually results from fecal coliform washing into the bay due to runoff from the watershed. Because watersheds differ in their conformations, two bays with the same rainfall closure rules may not accumulate fecal coliform at the same rate. If conditions permit, a slower accumulating bay may be granted a grace period that allows continued harvest for a time period after a given rainfall accumulation trigger occurs. Version 2.0 will allow establishment of a grace period in analysis.

All objectives were completed, including two additional statistical programs determining adequacy of sample sizes, and application of the upper limit of the 95% confidence interval for compliance sampling. In addition, a second software program, Weather, was produced as a companion program for preparation of weather data sets for application in Aquarius v.2 and any other weather data program.

## **TECHNICAL SUMMARY AND ANALYSIS**

In September 2006, after receiving WRAC funds, a core development team including University of California Davis (UCS) and California Department of Public Health (CDPH) personnel was formed; the UCD responsibility was to design and build the program, and the agency responsibility was to Beta-test the program using real data and cross-check the accuracy of the program against its own statistical analysis and procedures.

The software was developed and constructed during 2007 through 2008. All 12 proposed improvements to the program were completed, including the addition of two statistical programs that are used to determine if the sample size in the data sets are of sufficient size to provide a correct analysis. The program is written in the Visual Foxpro database programming language and uses the Visual Basic for Application (VBA) codes for communicating with Microsoft Excel. The major upgrades in the new version not only include the two additional sample size statistical programs, but an upper limit of the 95% confidence interval that is used by the agency to determine, with greater sensitivity, adequacy of compliance analysis. Aquarius v.2 expansions include improved data filtration options, and the inclusion of additional parametric and non-parametric statistical analyses that increase the reliability of the decision-making process necessary for public health objectives. Rainfall data can show cumulative daily, 6-hourly, hourly, and Tip-data format, and there are options for Three-Tube Test, Five-Tube Test, 12-Tube Test, Membrane Filter (MF), and Restricted 3-, 3-, and 12-Tube Test. An option was incorporated to use data from Wet Antecedent Conditions only, and options to define these conditions. Options for statistical parameters include choices of Alpha and Beta level for Statistics. There are also options to apply either the standard NSSP method to meet the NSSP criteria, in which the Geometric Mean must be less than 14 and the Estimated 90th percentile must be less than 49 for 3-Tube Test, or a NSSP-CI method in which CI stands for Confidence Interval. The second choice is a more sensitive option described earlier in which the NSSP method is modified by using the upper limit of the 95% confidence interval. This is only used for compliance sampling, but not used to consider a rule change. After extensive beta-testing of the program by the public health evaluation team, Aquarius v2.0 was finalized and adopted by the CDPH in January 2009.

A second software program was developed as a direct spin-off of Aquarius v2.0, the Rainfall Processing Program v.1.0 (RPP). This was not proposed in the grant proposal, but was added at no cost to WRAC. The RPP program was also developed in a cooperative project with personnel of the Shellfish Sanitation Branch of the CDPH. The CDPH and all other federal and state shellfish sanitation agencies spend numerous hours converting databases by hand to formats that could be used in analytical spreadsheets and/or software. The primary problems involved are human error caused by duplicate data, the need to reverse data entries without error, and conversion of tip rainfall

data to cumulative rainfall data without error. The RPP program is composed of four modules designed to prepare databases for use in any analytical rainfall program, and has specific application to Aquarius v2.0. The Dup Program is designed to remove duplicated data in any dataset. The Sum Program is designed to calculate cumulative tip rainfall data from hourly or semi-hourly rainfall data and at the same time removes the duplicate rows from any weather file. The Rev module is designed to reverse the sorting order of rainfall data. The Tip Program is designed to convert rainfall data collected from tipping-bucket and converts the data into hourly rainfall, and even by seconds, into datasets. The RPP program was beta tested and adopted by the CDPH in February 2009.

Based on the successful adoption of Aquarius v2.0 and the RPP v1.0 by the CDPH, funds were provided by WRAC to conduct three regional workshops in the spring of 2009 to extend the technology to shellfish producers in California (Bodega Bay) and Washington (Union City), and to state and federal shellfish regulators at the Pacific Rim Shellfish Sanitation Conference (Olympia, Washington). Representatives of the CDPH were co-presenters during the workshops at the Pacific Rim Shellfish Sanitation Conference and the Bodega Bay shellfish producer's conference. The software programs are currently being used by the Washington shellfish industry to analyze rainfall harvest regulations in Puget Sound in Washington State. Personnel of the Federal Food & Drug Administration are also evaluating the program.

## **PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED**

### ***Publications***

- Conte FS and Ahmadi A. 2009. Aquarius: Shellfish sanitation simulator rainfall and water quality closure rule evaluator, version 2.0. Copyright 2009-2010, Regents of University of California. Software and Manual. p. 114.
- Conte FS and Ahmadi A. 2009. Weather Processing Computer Programs. Copyright 2009-2010, Regents of University of California. Software and Manual. p. 17.
- Conte FS and Ahmadi A. 2009. AQUARIUS: a simulation program for evaluating water quality closure rules for the shellfish industry. *In: Bregt A, Wolfert S, Wien JE, Lokhorst C (Eds.), EFITA Conference 09. Proceedings of the 7th EFITA Conference, Wageningen, The Netherlands, 6–8 July 2009, Wageningen Academic Publishers, Wageningen, pp. 311–318.*

### ***Presentations***

- Conte FS and Ahmadi A. 2006. AQUARIUS Version 2.0: Expansion of Simulation and Statistical Analysis to Improve Evaluations of Shellfish Harvest Closure Rules. 4th World Congress on Computers in Agriculture. Orlando FL. July 24 to 26, 2006.
- Conte FS and Ahmadi A. 2007. AQUARIUS Version Release 2.0: Expanded Analytical Tool for Industry and Agencies to Evaluate Shellfish Harvest Closure Rules. Proceedings of the National Aquaculture Extension Conference. Cincinnati, OH. May 1-5, 2007.
- Conte FS and Ahmadi A. 2009. AQUARIUS: A Simulation Program for Evaluating Water Quality Closure Rules for Shellfish Industry. Proceedings of the 7th World Congress of Computers in Agriculture and Natural Resources (WCCA2009), June 22-24, 2009, Reno, Nevada, USA. pp 5.
- Conte FS and Ahmadi A. 2009. RPP-Rainfall Processing Program. Proceedings of the 7th World Congress of Computers in Agriculture and Natural Resources (WCCA2009). June 22-24, 2009, Reno, Nevada, USA. pp 4.

### ***Published Abstracts***

- Conte FS and Ahmadi A. 2006 AQUARIUS Version 2.0: Expansion of simulation and statistical analysis to improve evaluations of shellfish harvest closure rules. 4th Annual Conference on Computers in Agriculture. Orlando, FL. p. 5 (Published Abstract)
- Conte FS and Ahmadi A. 2007. AQUARIUS Version release 2.0: Expanded analytical tool for industry and agencies to evaluate shellfish harvest closure rules. Aquaculture 07, San Antonio, TX. (Published Abstract)

- Conte FS and Ahmadi A. 2009. AQUARIUS: A Simulation Program for Evaluating Water Quality Closure Rules for Shellfish Industry. Proceedings of the American Society of Agriculture and Biological Engineers. Reno, NV. (Published Abstract)
- Conte FS and Ahmadi A. 2009. Weather Processing Computer Programs. Proceedings of the American Society of Agriculture and Biological Engineers. Reno, NV. (Published Abstract)