WESTERN REGIONAL AQUACULTURE CENTER

WRAC

ANNUAL ACCOMPLISHMENT REPORT

FOR THE PERIOD SEPTEMBER 1, 2005 TO AUGUST 31, 2006

AUGUST 2008

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

Introduction

This Annual Accomplishment Report for the Western Regional Aquaculture Center (WRAC), covers progress made from Sept 1, 2005 through Aug. 31, 2006. 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.

The primary policy-making body for WRAC is the Board of Directors, which has established an Industry Advisory Council (IAC) and a Technical Committee (TC). The members of both groups are reviewed and appointed by the Board of Directors. The IAC is composed of representatives of the industry and associated services, covering multiple sectors and regions. The TC is composed of representatives from participating research institutions, state Extension Services, and other state or territorial public agencies as appropriate, as well as from nonprofit, private institutions. 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.

Since the start of the regional aquaculture programs, WRAC has processed nineteen Annual Work Plans (for FY'87 through FY'06 funding) through USDA. This current annual report covers the activities of the WRAC Administrative Center and progress made during the nineteenth year on all projects through Aug. 31, 2006, listed below with funding levels for FY'06.

Annual Reports

A. Physiological Changes Associated with Live-Haul Maintaining Healthy Fish
   1st project year: $89,456

B. Development and Evaluation of Starter Diets and Culture Conditions for 3 Subspecies of Cutthroat Trout and Gila Trout
   1st project year: $99,991

C. An Evaluation of the Effectiveness of Various Florfenicol Treatment Regimens to Control Mortality Caused by Streptococcus Iniae in Cultured Hybrid Striped Bass
   1st project year: $29,864

D. Immunological Mechanisms of Intensively-reared warmwater and coolwater finfish
   3rd project year: $100,000

E. Scale-Dependent and Indirect Effects of Filter Feeders on Eelgrass: Understanding complex Ecological Interactions to Improve Environmental Impacts on Aquaculture
   3rd project year: $82, 368

Termination Reports

A. Optimizing Quality and Shelf-Life of Sturgeon Caviar

B. Investigation into the Solution of Critical Problems of the Recirculation Aquaculture Industry in the Western Region

WRAC Publications

$24,000

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.

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

Administrative Support

FY'O6 FUNDING LEVEL $198,419

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 nineteen Annual Work Plans (FY'87 through FY'06) 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.

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 & 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 Adminresearchers, the aquaculture industry, and the public sector. Funds for this project cover actual printing costs and the necessary editorial and graphics expertise to produce the various publications.

Physiological Changes Associated with Live Haul: Maintaining Healthy Fish

Reporting Period September 1, 2005–August 31, 2006

Author John Colt

Funding Level $87,156 for 2005–2006

Participants

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

* funded participants
** salaried participants

PROJECT OBJECTIVES

The project objective during 2005-2006 included:

1. Document physical and chemical changes that occur in the fish transportation process.
2. Document physical and chemical charactisics of the retail holding systems.
3. Construct experimental transport systems to simulate long-haul conditions. Develop standardized hauling conditions and compare with observed data.
4. Develop methods to rapidly assess stress in cultured tilapia.
5. 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

Water Quality and Modeling Area

Physical and water quality data was collected from three tilapia farms, two haulers, and 18 retail stores in the Vancouver, BC area. There were significant differences in hauling protocols and resulting water quality values. The water quality in the retail stores was highly variable depending on type of systems and operations. The most serious water quality problems at the retail stores include low temperature, low pH, and high bacterial counts. Problems were also found with dissolved oxygen (DO), Oxidation-Reduction Potential, gas supersaturation, and un-ionized ammonia.

Physiological Impacts of Hauling

On November 3, 2005, tilapia were transported from Bruneau, Idaho, to Corvallis, Oregon, by truck. The size of the fish received ranged from 1 to 500 g. Many of the fish showed signs of parasite infestation within several days of arrival. Parasites were identified as *Trichodina spp., Gyrodactylus spp., Costia spp.* and an unknown type. Some fish also had an unknown fungus (species other than *Saprolegnia*). After fish began dying, we treated them with a salt dip. Fish appeared healthy after the treatment.

After a two-month acclimation at our Oregon State University (OSU) facility, we conducted a simulated hauling/holding experiment to determine if we could replicate the mortality seen during commercial hauling and holding in retail facilities. Four groups of fish (each duplicated) were treated as follows: positive control (no transport, good quality holding water after transport), negative control (no transport, poor quality holding water after transport), positive transport (transport, good quality holding water after transport) and negative transport (transport, poor quality holding water after transport). Transport was simulated based on the practices of Epicenter Enterprises Inc., Challis, Idaho. Our transport system was scaled down by a factor of 150. Poor quality water for holding was based on the worst conditions that we observed at five different retailers in Richmond, BC. Fish subjected to good quality holding water were simply placed back into the tanks that they were originally held in.

To simulate loading and hauling, fish in the transport groups were first subjected to a 2-hr crowding stress (lowered water), followed by a 1-min. net stress. Fish were then placed in 4 L plastic containers at a density of 300g/L containing 3g salt/L. The water was supplied with oxygen and quickly became supersaturated. The containers were then placed on orbital shakers (25 rpm) for 14 hr. To simulate arrival, delivery to retailers, and placement into retail holding water, orbital shakers were turned off for 5 hr and then turned on for an additional 3 hr. Fish were subjected to de-watering for 3 min. by placing them in perforated plastic buckets. Good quality holding water groups were placed back into their original holding tanks and poor quality holding water groups were placed in aquaria at a density of 50g/L.

Mortality was seen in only 2 out of the 8 replicated treatments. Mortality in 1 of the duplicates from the positive transport group was 4.5% (1 fish) and it was 39.2% (11 fish) in one of the negative transport duplicates. Further inspection of the fish revealed that most of the mortality occurred in smaller fish. To determine if there was a size effect because of the large range in the weights of the fish used, we repeated the experiment using more uniformly sized fish. Only the negative transport group conditions were repeated. Fish for this experiment (n=30) weighed 41.3 + 1.4 g and ranged between 24-61 g. No mortality was observed in either duplicate after transport simulation and holding in poor quality water.

In order to compare the effects of different treatments of transport and holding on tilapia, we need to have a method that is capable of detecting differences between stressed and unstressed fish. Research was conducted to determine if reflex tests are adequate measurements to compare differences between stress treatments, using juvenile tilapia. This test, the "RAMP" (Reflex Action Mortality Predictor) method, developed by M.W. Davis (*michael.w.davis@noaa.gov*) has been successfully used to predict by-catch mortality after trawl netting. We were unable to detect any differences using the reflex tests for measurement. Tilapia did not appear or behave stressed. Using market-sized fish at different stages of transport may provide different results.

Outreach Work

The work group held its annual meeting August 16, 2006, in Corvallis, Oregon. Based on the data collected thus far and published literature, the group prioritized critical locations, actions, and parameters that are most likely to negatively impact fish health, survivability, and product quality. On August 23, 2006, PI John Colt and Extension Coordinator Gary Fornshell met with three Idaho tilapia producers/live-haulers to further refine critical locations, actions, and parameters that are most likely to negatively impact fish health, survivability, and product quality.

USEFULNESS OF FINDINGS

Water Quality and Modeling Area

The physical and water quality data collected in 2005–2006 has defined the broad extremes of conditions for hauling

and retail holding. This information has been used to select physical and water quality parameters for the simulated hauling work. For some parameters such as gas supersaturation or pH, this work can be used directly to suggest changes in water management or treatment that are needed to improve fish survival in retail holding.

Physiological Impacts of Hauling

We have not been able to replicate the mortality seen in tilapia following commercial transport and holding in retail facilities. Based on our observations from our transport simulations, the fish were severely stressed yet none died during transport. Furthermore, placing fish in what we believe to be the poorest water quality that fish encounter at commercial retailers also did not result in any mortality. It appears that either some aspect of the commercial transportation process, holding conditions at retailers that are unknown to us, or a combination of both is causing the mortality seen during live haul and retail holding. We also believe that the condition of fish prior to transport is a very important factor for determining mortality. The fish used in our transport study may have been in much better condition (i.e., lowered parasite load) than fish from commercial operations.

Outreach Work

On August 23, 2006, John Colt and Gary Fornshell met with three Idaho tilapia producers/live-haulers to discuss the information collected by this project. In a very proprietary and competitive business, these three individuals allowed discussion of all the data collected by the project. This increased level of trust and cooperation will be critical for our future research and for the adoption of any transport improvements.

WORK PLANNED FOR NEXT YEAR

The work planned for next year will be discussed in terms of (a) water quality and modeling area, (b) physiological impacts of hauling, and (c) outreach work:

Water Quality and Modeling Area

1. Continue and complete physical and water quality sampling at the retail level. This will result in a peer-reviewed manuscript on physical and water quality characteristics for retail holding facilities.
2. Conduct detailed water quality sampling during 24–30 hr hauling trips. Because of driver resistance, it has not been possible up to this point to (1) monitor water quality on a continuous basis in the tilapia transport systems or (2) obtain water samples for laboratory analysis at regular intervals. The ability to conduct detailed water quality monitoring on a long trip would greatly aid in understanding water quality changes that occur during the hauling process. This work may be conducted with Leo Ray using channel catfish.
3. Develop quality simulation models for alkalinity/pH/carbon dioxide/UIA for hauling and retail holding systems.
4. Make recommendations for an improved hauling mixture (possible inclusion of NaCl, KCl, CaHCO3 or CaCl2, and pH adjustment).
5. Obtain water samples from John Lambregts' farm and evaluate potential impacts on tilapia. John has had specific problems hauling from this site that may be related to the high alkalinity and pH of his water supply.
6. Support continuing work conducted at OSU.

Physiological Impacts of Hauling

7. Assess physical injury incurred by fish during transport.
8. Assess parasite load of fish before and after transport.
9. Determine if physical injury and/or parasite load affects survival.
10. Determine if a salt dip prior to transport to remove parasites results in reduced mortality.

Outreach Work

The outreach objectives are scheduled towards the end of the project; however, close collaboration with project PIs and industry cooperators will continue to ensure project progress and access to data.

BUDGET REQUESTS FOR 2006–2007

The following budget is requested for 2006–2007:

National Marine Fisheries Service (Colt)	$10,118
Oregon State Universtiy (Schreck)	73,238
University of Idaho (Fornshell)	800
Total	$84,156

This budget request includes $2,300 budgeted for Doug Ernst for 2005–2006. The OSU budget includes $7,782 for two months of Rob Chitwood's time. Rob runs the day-to-day operation of the tilapia facility and up to this point has not been funded on this project. He has assisted with water quality collection in Idaho and Vancouver and the research work at OSU. The salary for the coming year would include Doug Ernst's salary for 2005–2006 ($2,300 + $4,300) and $1,182 transferred from the NMFS budget.

PUBLICATIONS IN PRINT & MANUSCRIPTS

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

National Marine Fisheries Service, Oregon State University, University of Idaho. 2006. Working Paper No. 2: Water Quality in Farm, Hauling, and Retail Holding Systems.

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		University	Industry	Other Federal	Other	Total	
2006	87,156	3,891		27,500			$118,547
Total	87,156	3,891		27,500			$118,547

Development and Evaluation of Starter Diets and Culture Conditions for 3 Subspecies of Cutthroat Trout and Gila Trout

Reporting Period	October 1, 2005–September 1, 2006		
Author	Christopher Myrick		
Funding Level	**First Year Funding Received**	**$99,991**[1]	
	Second Year Request	**$95,677**	
	Third Year Request	$91,162	
	Fourth year Request	$94,304	
Participants	Christopher A. Myrick* (*Working Group Chair*)	Colorado State University	Colorado
	Gary Fornshell *(Extension Rep.)*	University of Idaho	Idaho
	Greg Kindschi*	USFWS Bozeman Fish Technology Center	Montana
	Ken Cline* (*Industry Collaborator & Advisor*)	Cline Trout Farms	Colorado
	John Seals*	USFWS Mora National Fish Hatchery & Technology Center	New Mexico
Industry Advisor	Chris Nelson	Nelson & Sons, Inc.	Utah
Technical Advisor	Rick Barrows	USDA/ARS	Montana

* voting, work group members

[1] First-year funds were not received by the participating institutions until October 1, 2005, or later. The late arrival of funds prevented the participants from conducting any research during the 2005 season because the cutthroat and Gila trout eggs were no longer available.

PROJECT OBJECTIVES

The purpose of this research project is to improve the growth, quality, and survival of cutthroat trout and Gila trout with the ultimate goal of providing fish culturists and feed manufacturers with information that can be used to improve the production of these species. The specific objectives of this study are listed below. Objectives that are relevant to Year 1 are italicized.

1. *Determine the effect of feed texture and formulation on survival, growth, and quality of cutthroat and Gila trout.*
2. Determine the effect and interaction of diet texture and formulation on trout growth, survival, and quality when reared at different water temperatures under laboratory conditions.
3. Determine the effect of rearing density on trout growth, survival, and quality.
4. Conduct production-scale evaluations of the best diet–temperature–density combinations identified in the first three objectives. This will also allow us to test our assumption that a diet developed for 2–3 strains of cutthroat trout will provide superior performance for other untested cutthroat trout strains (e.g., Rio Grande cutthroat trout, *O. clarkii virginalis*, and greenback cutthroat trout, *O. clarkii stomias*) than diets developed for rainbow trout.
5. *Develop outreach products to provide fish culturists and feed manufacturers with information on optimal growth temperatures, optimal rearing densities, and diet formulations for inland cutthroat trout subspecies and Gila trout.*

ANTICIPATED BENEFITS

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

Gila trout were reclassified as threatened (from endangered) this year. The final rule, Federal Register, Vol. 71. No. 137 page 40,657 was published on July 18, 2006 and took effect on August 17, 2006. Included is a special 4d rule that allows the affected states to establish regulations for the angling of Gila trout. This means that any Gila trout produced in excess of those required for recovery efforts may be used by the states of Arizona and New Mexico in catch and release or limited catch and keep waters. It is also possible that the down-listing of Gila trout will make them available to a limited number of private growers who are interested in providing a specialty product to their clientele. The new classification (threatened) allows states to use sport fish restoration money to propagate and stock these fish. In either case, increased survival and growth of this particular species under cultured conditions is now of greater concern because of the increased demand for progeny.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Year 1 (2006)

In 2006, the Bozeman Fish Technology Center (Bozeman Center) purchased five commercial feeds, and developed and manufactured two experimental feeds for the diet trials with Colorado River, Snake River, and Yellowstone cutthroat trout, and Gila trout. The commercial feeds were selected because pilot work completed in 2004–2005 demonstrated that some commercial feed formulations out-performed the initially proposed experimental formulations. All seven diets were packaged by feed size, labeled with numbers only so fish culturists were blinded, and shipped for testing at the three sites (Bozeman Center–Snake River and Yellowstone cutthroat trout; Mora Fish Technology Center (Mora Center)–Gila trout, and Colorado State University (Colorado State)–Colorado River cutthroat trout).

The initial feeding portion of the diet study for Snake River and Yellowstone cutthroat trout began on June 16, 2006, at the Bozeman Center in a 56-tank (7 diets x 4 tanks/diet x 2 strains) experimental system. There were 150 Snake River cutthroat trout and 135 Yellowstone cutthroat trout in each tank at the start of the study. Water temperature has been maintained at 10°C. This study will continue for 16 weeks or until October 16, 2006.

The Colorado River cutthroat trout diet study began at Colorado State on August 1, 2006, in an 8-tank (8 diets x 1 tank/diet) system. The eighth diet used in this trial is a live diet, *Artemia spp.*, which was fed to 1 treatment for 2 weeks before they were weaned onto diet #5. This represents a deviation from the initial design and is the result of a severe delay in the delivery of the 32 experimental tanks. The fish were then transferred to the 32 experimental tanks on September 1, 2006. Fish are being held at 10.5°C. Preliminary results suggest that diets are producing significant differences in growth rates and survival during the first 30 days of the study. The study will continue for a minimum of 16 weeks.

The Gila trout diet study began at the Mora Center on May 1, 2006, in a 24-tank (8 diets x 3 tanks/diet) system, at an initial density of 200 fish per tank. The treatments in this study were the same as those in the Colorado River cutthroat trout study. The fish were held at 14–16°C. The 120-day diet trial was completed during the last week of August 2006, when 15 fish from each treatment were scored using Goede's Fish Health/Condition Assessment (Goede and Barton 1990). Preliminary analysis of results shows that diet had a significant effect on growth rate, survival, and condition. The project staff at the Mora Center are now preparing to analyze the data.

Subsequent year studies concerning the other objectives dealing with optimal rearing water temperature, optimal rearing density and production-scale evaluations will be conducted using the optimal diet(s) determined from this first-year study. The optimal diets may be site-specific, but because one of the goals of this project was to determine

whether one diet formulation would suffice for the various species and strains of inland trout, for which we are prepared. No outreach products have been produced at this time because the work group is waiting for the completion of the first set of studies. As studies are completed and data are analyzed, this information will be presented at technical meetings and submitted for possible publication to meet outreach requirements.

USEFULNESS OF FINDINGS

Data will benefit the culture and quality of rearing these unique native fish species. Increasing our knowledge of the culture conditions and diet requirements for these species will enable commercial fish farmers to supply fish to their clientele, and will ultimately allow recreational fishermen to have greater fishing opportunities on both private and public waters. In turn, this will allow for commercial trout growers and feed manufacturers to expand their marketing capabilities. Certain commercial feed manufacturers and private and public hatchery personnel now know that this study is being conducted. Consequently, work group investigators have been receiving inquiries as to the status of this diet trial because there is much interest in knowing the outcome. Obviously, feed manufacturers have much to gain or lose pending the performance of their product. Additionally, fish culturists are interested identifying the best diets for the species in which they specialize. Additionally, fish culturists want the best available feed for their particular fish performance. However, no information has or will be disseminated until data analysis is complete.

WORK PLANNED FOR NEXT YEAR (Year 2)

The research scheduled for the 2007 season includes the second set of experiments investigating the interactions between water temperature (10–20°C) and diet on trout growth, survival, and quality. The Bozeman Center will use a Thermal Challenge System to conduct side-by-side growth trials with Snake River and Yellowstone River cutthroat trout at temperatures of 10–20°C in 2°C-increments to determine the optimal growth temperature at satiation rations. This temperature range was selected because a study by Bear (2005) on the closely related westslope cutthroat trout found that the upper incipient lethal temperature was 19.6°C. The Bozeman Center will also conduct a pilot density trial on Snake River and Yellowstone River cutthroat trout at 10°C using a separate 56-tank system to obtain data that can be used to better design the temperature x density trials planned as part of Objective 3.

A system similar to the Bozeman Center's Thermal Challenge System will be constructed at Colorado State during the winter and early spring of 2007, using computer-controlled mixing valves to regulate water temperature to the target levels. Once the system is assembled, Colorado State will conduct a similar growth trial with Colorado River cutthroat trout to identify the optimal growth temperature when fish are fed satiation rations.

The Mora Center doesn't have the infrastructure needed to manipulate temperatures to the same extent that Colorado State or the Bozeman Center can, so it will conduct a combination diet x density trial that begins to address the third objective of this project. A new 28-tank system will be installed so that the physical equipment used in the 2007 trials is more similar to that used at the Bozeman Center and Colorado State. The Mora Center is also securing additional belt feeders so that its feeding protocols more closely match those at Colorado State and the Bozeman Center.

The project website should be up by November 2006. Thereafter, annual progress reports and any other pertinent information will be posted. The website will be advertised on the WRAC website, aquaculture association websites, newsletters including *Waterlines,* and through appropriate state and federal agencies. The website will also serve as the distribution point for the outreach publication. We also expect that representatives from the three research sites will present results of their 2006 diet studies at local and regional meetings of industry associations and professional societies. All project results will be subject to an internal (work group) review and quality control and assurance process before they are disseminated.

IMPACTS

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

PUBLICATIONS, MANUSCRIPTS, AND PAPERS PRESENTED

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

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		UNIVERSITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
2006	99,991	5,000	5,000	81,500	5,500	97,000	$196,991
TOTAL	99,991	5,000	5,000	81,500	5,500	97,000	$196,991

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

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

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

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

Reporting Period	April 1, 2005–March 31, 2006		
Author	James D. Bowker		
Funding Level	J. Bowker	$14,000	
	V. Ostland	$13,564	
	S. Harbell	$ 2,300	
Participants	James D. Bowker*, MS	US Fish and Wildlife Service	Montana
	Vaughn Ostland*, PhD	Kent Sea Tech Corporation	California
	Steve Harbell*, PhD	Washington State University Cooperative Extension	Washington

* voting, work group members

PROJECT OBJECTIVES

1. Using isolates of *Streptococcus iniae*, determine which route of infection (immersion or IP injection) of hybrid striped bass (HSB) will consistently yield a mean cumulative mortality of 50% in the exposed group with the least statistical variation among replicates. Also, identify important dose-dependent variables, such as time to onset of first morbidity, time to first mortality, and total cumulative mortality.
2. Using the optimal dose and exposure route described above, determine the most effective treatment dose of florfenicol to control mortality in HSB experimentally infected with *S. iniae* fed either 0, 10, 15, or 20 mg florfenicol/kg fish/d for 10 days. This data will identify the lowest treatment dose that results in the least cumulative mortality during the 10-day trial.
3. Using the lowest treatment dose that resulted in the least cumulative mortality (identified in Objective 2), determine the most effective treatment duration of florfenicol to control mortality in HSB experimentally infected with *S. iniae* fed for either 0, 10, 15 or 20 days. This data will identify the shortest treatment duration that results in the least cumulative mortality during the 10-day trial.
4. Demonstrate and substantiate that the most efficacious dose determined in Trial B for the most efficacious duration determined in Trial C of the laboratory studies controls mortality when administered to HSB naturally infected with *S. iniae*.

ANTICIPATED BENEFITS

The primary direct benefit to the aquaculture industry is to determine whether the standard florfenicol dosage (i.e., 10 mg active drug/kg fish body weight administered daily for 10 consecutive days) is optimal for controlling mortality in HSB caused by *S. iniae*. If we conclude that a dosage other than the standard dosage is more effective, then we will encourage the sponsor (Schering Plough Animal Health, Corp., Union, NJ) to move forward with expanding the existing label for warmwater fish to include this dosage for this disease claim. The sponsor has recently requested from the US Food and Drug Administration's (FDA) Center for Veterinary Medicine's (CVM) Aquaculture Team

that residue depletion data and target animal safety data generated on tilapia be considered representative for all scaled warmwater fish. Approval of a more efficacious therapeutic dosage, if administered in a timely manner, will result in fewer mortalities of this commercially important fish species.

A major indirect benefit will be the development of a disease challenge model to initiate an outbreak of *S. iniae* in HSB that might be suitable in regulatory science. CVM's Aquaculture Team has indicated that conducting field studies to evaluate the effectiveness of fish therapeutants in which the disease of interest was experimentally induced in fish may satisfy some of the effectiveness data requirements. However, the criteria that must be met prior to acceptance of a disease challenge model method are extensive and include consistently obtaining a mortality rate profile mimicking that of a natural infection and demonstrating that the method is robust and works in the hands of various investigators.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

S. iniae working seeds were produced and frozen so that they will be available when we start infection studies in the next few weeks.

USEFULNESS OF FINDINGS

WRAC budget support for the project was delayed. No findings to report at present.

WORK PLANNED FOR NEXT YEAR

No changes in work direction or emphasis are planned. Responsibilities and assignments are as described in the original study proposal.

IMPACTS

WRAC budget support for the project was delayed until after the final reporting period. At present, no appreciable progress has been made and the project has yet to benefit the aquaculture industry.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

None to date

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		University	Industry	Other Federal	Other	Total	
4/05–3/06	29,864		12,000	24,000		36,000	$65,864
Total	29,864		12,000	24,000		36,000	$65,864

Immunological Mechanisms of Intensively Reared Warmwater and Coolwater Finfish

Reporting Period April 1, 2005–March 31, 2006

Author Vaughn Ostland

Funding Level $100,000 in Year 3

Participants

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

* voting, work group members

PROJECT OBJECTIVES

1. Develop a suite of reagents and assays that can be used to quantify the specific humoral and cellular immune responses of warmwater and coolwater finfish that could serve as indicators of healthy finfish immune systems and assess the efficacy of novel vaccines and methods of immunization.
2. Develop a better understanding of the role of the humoral, cellular, and mucosal arms of the fish immune system and the induction of both non-specific and specific immune mechanisms following exposure to bacterial, viral, and/or parasitic pathogens or their antigens.
3. Determine the role of environmental factors and physiological conditions on the immune response of warmwater and coolwater finfish. Evaluate finfish immune function in response to intensive culture and how various management practices affect immune function.
4. Assess the immune response of warmwater and coolwater finfish to selected candidate vaccines following delivery by novel methods for mass immunization.
5. Transfer the tools and research findings from this project to industry.

ANTICIPATED BENEFITS

Infectious disease continues to impede the growth and expansion of finfish aquaculture in the US. This research will develop key immune reagents and assays that will serve to quantify the immune response of hybrid striped bass and rainbow trout, 2 representative warmwater and coolwater finfish species presently cultured in the US. With these tools, we can then identify how the immune system responds during an infection or following immunization. This knowledge will assist aquaculture managers in understanding the effect of different rearing practices on finfish immune function, ultimately leading to improved fish health, disease prevention, and efficacious vaccination.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

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

Task 1. Develop ELISA to measure specific antibody responses by hybrid striped bass and rainbow trout to S. iniae or A. hydrophila.

Subtask 1a. Production of mouse monoclonal antibodies against HSB antibody.
Due to technical problems this subtask was dropped and replaced with subtask 1b.

Subtask 1b. Production of polyclonal antibodies to HSB IgM
Preliminary ELISA and immunoblots experiments indicate that we have successfully produced a polyclonal antibody in rabbits that recognizes deglycosylated heavy chain (HC) of hybrid striped bass (HSB) IgM in both serum and purified IgM. After further protein A affinity purification of the polyclonal antibody, this pilot batch of reagent will be sent to the Western Fisheries Research Laboratory for further assay development and optimization.

Subtask 2. Selection of reference bacterial strains.
This subtask has been completed.

Subtask 3. Preparation of *S. iniae* and *A. hydrophila* antigens.
This subtask has been completed.

Subtask 4. Production of hyperimmune serum to *S. iniae* and *A. hydrophila*.
This subtask has been completed.

Subtask 5. Optimization of enzyme-linked immunosorbent assays for trout and hybrid striped bass antibody.
This subtask has been completed for the rainbow trout ELISA component. Affinity purified polyclonal antibodies have been forwarded to the Western Fisheries Research Laboratory for further ELISA development and optimization of the HSB ELISA (see subtask 1).

Task 2. Modify immune assays developed for salmonids for use with warmwater fish.

Subtask 1 and 2. Blood and tissue collection and humoral measures of immune function.
This sub-task is complete for rainbow trout using a monoclonal antibody and the method published. An ELISA for quantification of HSB immunoglobulin levels will be developed using polyclonal antibodies in Year 4.

Subtask 3. Cellular measures of immune function.
A standard phagocytosis assay was developed for both rainbow trout and HSB that uses flow cytometry of stained cells. The method will be suitable for publication and will be made available to the research and aquaculture communities.

Task 3. Develop quantitative PCR-based assays for fish cytokine gene expression.

An initial quantitative PCR assay (qPCR) was performed during this reporting period with the hepcidin primers and probe. There was a greater than 1000-fold increase in hepcidin gene expression in HSB infected with *S. iniae* at 24 and 72 hours post-injection compared to controls Other qPCR assays for HSB genes involved in infection and immunity are in development at this time. These assays will constitute an important set of tools for assessment of the health and immune response in this species.

Objective 2: Determine the immune response kinetics of HSB and rainbow trout to S. iniae and A. hydrophila.

Task 1. Produce a standard challenge model for infection of hybrid striped bass and rainbow trout with S. iniae and A. hydrophila.

A standard challenge model is nearly complete for *S. iniae* and *A. hydrophila* infections in HSB. Minor refinements are needed to achieve an LD50 for *S. iniae* to fulfill the goal of subtask 3. This should be completed in the next few weeks. Preliminary dose range finding experiments for *A. hydrophila* in HSB have indicated that a much higher dose will be required to achieve an acceptable level of mortality. This is not a significant technical adjustment and it is anticipated that this will be completed in the next month. A standard challenge model for infection of rainbow trout with *S. iniae* is in progress. The standard challenge model for *A. hydrophila* in rainbow trout has been completed.

Subtask 1. Growth of *S. iniae* and *A. hydrophila* cultures and preparation of bacterins.
Subtask completed.

Subtask 2. Determination of *in vivo* dose of *S. iniae* and *A. hydrophila* bacterins needed to produce antibody response in HSB and rainbow trout.
This research will begin early in Year 4.

Subtask 3. Determine the LD50 of *S. iniae* and *A. hydrophila* to HSB and rainbow trout
With a few technical refinements, the LD50 for *S. iniae* in HSB is nearly complete. This should be completed in the next month. With minor technical adjustments, the LD50 for *A. hydrophila* in HSB should be completed in the next month. The LD50 of *S. iniae* for rainbow trout will begin early in Year 4. This should be completed in approximately 1 month.The LD50 for *A. hydrophila* in rainbow trout has been completed. The mean LD50 value has been estimated at 3.37 x 107 CFU.

Task 2. Compare immune response and cytokine gene expression of HSB and rainbow trout injected with live or bacterin versions of S. iniae and A. hydrophila.

Research for both the HSB and rainbow trout component will begin early in Year 4. We are in the process of finalizing our protocols to begin experimentation.

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

Task 1. Determine the effects of loading density on immune function and cytokine gene expression of HSB and rainbow trout.

With the pending completion of relevant immune function assays, research for both the HSB and rainbow trout component will begin early in Year 4. We are in the process of finalizing our protocols to begin experimentation.

Task 2. Determine the effects of rearing temperature on immune functions of HSB and rainbow trout.

Research for the rainbow trout component can begin immediately. Research for both the HSB and rainbow trout component will begin early in Year 4. We are in the process of finalizing our protocols to begin experimentation.

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

In year 3, we began testing of a proprietary method for delivery of DNA vaccines using automated technologies funded by a Washington Technology Center grant. Preliminary results using a DNA vaccine against infectious hematopoietic necrosis were encouraging; however, more work needs to be done.

Success in this collaborative effort is expected to result in the availability and acceptance of a novel vaccine delivery technology that will significantly improve the health and disease resistance of fish reared at, or released from, federal, state, tribal and private sector facilities. This will provide a significant new benefit to fish stocks in aquaculture, resource enhancement, and threatened/endangered captive broodstock programs.

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

We have prepared a revised draft of the first outreach product for this project (a comprehensive overview of the non-specific and specific aspects of the fish immune system, and key cellular components of the immune response). This product will introduce specific and relevant phagocyte assays and their utility for assessing fish health. While revisions are still needed, it is anticipated that this product should be ready for publication and dissemination within the next few months.

We have also begun to consider a draft of a second outreach product based on the immunologically relevant cellular assays that could be further refined and employed to assess HSB cellular immune function. While recognizing the fact that this research is both technically complex and requires specialized equipment and reagents, it may be possible to disseminate this information to the scientific community. Nevertheless, we recognize that there is still a significant amount of effort needed on this manuscript before it is ready for publication. In con-

sultation with our Extension specialist, the suitability of disseminating this type of information over the next few months will be considered.

During the second of 2 of the Immunology Working Group teleconferences that were held during Year 3, our TA proposed a potential third outreach product that would overview the most common and economically significant infectious diseases of warmwater fish reared in the Western region. The intended target audience would be aquaculturists, fishery biologists, resource managers, students as well as the general public. Further plans to assess the suitability of disseminating this work will also be discussed with our Extension specialist during the first quarter of Year 4.

USEFULNESS OF FINDINGS

From a functional fish immunology perspective, this research has begun to identify many similarities and differences among the rainbow trout and HSB immune systems. We are finally beginning to realize that an assay developed for rainbow trout may not necessarily be developed as easily for hybrid striped bass. The true usefulness of this project will be realized when we begin to study the effect of common rearing practices on immune function of warmwater and coolwater finfish reared in the western region of the US. In the interim, the development of many novel tools for assessing both humoral and cellular immunity in rainbow trout and hybrid striped bass are being made available to the research community and the aquaculture sector.

WORK PLANNED FOR NEXT YEAR

Research effort over the next two months will be focused on complete Objectives 1 and 2. When completed we can then begin to finalize the protocols, and initiate and complete the experiments proposed for Objective 3.

IMPACTS

Not appropriate at this time.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

None to report at this time.

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		University	Industry	Other Federal	Other	Total	
1	100,000	7,000[a]	25,530[b]	25,000[c]		57,530	$157,530
2	100,000	7,000[a]	25,530[b]	25,000[c]	1,000[d]	58,530	$158,530
3	100,000	7,000[a]	25,530[b]	25,000[c]		57,530	$157,530
Total	300,000	21,000	76,590	75,000	1,000	57,530	$473,590

[a] in kind salary for Friedman (0.1 FTE)
[b] in-kind support from Kent SeaTech, Clear Springs Foods (see proposal)
[c] in-kind salary, ancillary laboratory equipment, facilities, reagents for Winton
[d] in kind support from NMT

Scale-Dependent and Indirect Effects of Filter Feeders on Eelgrass: Understanding Complex Ecological Interactions to Improve Environmental Impacts of Aquaculture

Reporting Period	April 1, 2005–March 31, 2006 (does not include 2006 field season)		
Authors	Jennifer Ruesink, Sally Hacker		
Funding Level	Year 1: $79,607 Year 2: $83,415 Year 3: $82,368 Total: $245, 390		
Participants	Brett Dumbauld	USDA-ARS	?????
	Sally Hacker*	Oregon State University	Oregon
	Steve Harbell	Washington State University	Washington
	Jennifer Ruesink*	University of Washington	Washington
Graduate Students	Kirsten Rowell	University of Washington (2004)	Washington
	Heather Tallis	University of Washington (2003–04)	Washington
	Lorena Wisehart	Oregon State University (2003–05)	Oregon
	Elizabeth Wheat	University of Washington (2005–2006)	Washington
	EricWagner	University of Washington (2005–06)	Washington
	Margo Hessing-Lewis	Oregon State University (2005–06)	Oregon
	Sara Frame	University of Washington (2006)	Washington
Undergraduate Students	Michelle Briya	Oregon State University (2004–05)	Oregon
	Ashley Lyons	Oregon State University (2005–06)	Oregon
	Thatcher Jones	Oregon State University (2005-present)	Oregon

* voting, work group members

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.

ANTICIPATED BENEFITS

This project focuses on the ecological impacts of two filter-feeding bivalves: Pacific oysters (*Crassostrea gigas*) in Willapa Bay, and geoducks (*Panopea abrupta*) in Puget Sound. Both species are cultured in areas that also support native

eelgrass (Zostera marina). Because eelgrass is an ecologically important and protected species, it is essential to know how bivalve aquaculture affects it. Our conceptual model includes both the "press perturbation" of enhanced bivalve densities and the "pulse perturbation" of disturbance associated with planting and harvest. Two key issues are the resistance of eelgrass to press perturbations—response variables are density and growth rate—and the resilience to pulse perturbations—recovery through seed germination and asexual branching. The anticipated benefits from the project include documentation of positive and negative effects that allow growers to develop best management practices for coexistence of aquaculture and eelgrass.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

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

It is a simple truism that filter feeders eat particulate organic matter. Nevertheless, the magnitude and spatial scale of their effects are context-specific. We examine depletion at the scale of aquaculture beds in a variety of contexts (location, phytoplankton concentration, water flow, water depth, oyster density). The method involves small, shallow drifters that remain associated with a parcel of water and carry multiple sensors: a GPS to track location, a salinity sensor to confirm that the drifter stays with the same water, and a fluorometer to track phytoplankton. We also collect water samples throughout each drift and measure chlorophyll-a directly on a benchtop fluorometer. In 2005, we carried out 20 drifts on flood tides and detected significant depletion of particles across oyster beds in shallow water. This opens up the possibility for oysters to improve light conditions for eelgrass, but in Willapa Bay, we see little evidence of phytoplankton "blooms" that would substantially reduce light—the highest summer chlorophyll concentrations are generally <5 μg/L. Under the supervision of Jennifer Ruesink, UW graduate student Beth Wheat will complete this work as part of her PhD thesis.

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.

We have taken two approaches to understanding how shellfish affect sediment properties: 1) comparative studies of areas subjected to different aquaculture practices, and 2) experimental studies at smaller scales in which we manipulate shellfish, nutrients, and eelgrass independently.

Comparative studies—In Willapa Bay, eelgrass experiences both press and pulse perturbations. Higher oyster densities are associated with lower eelgrass densities; in addition, disturbance from aquaculture practices reduces density—particularly by mechanical dredge. However, sustainable (but lower) densities of eelgrass are present in all aquaculture types. UW gradute student Heather Tallis' manuscript is in revision for Estuaries and Coasts.

In Puget Sound, beds of geoducks generally have lower organic content and higher porewater nutrients than beds of eelgrass.

Experimental studies, Puget Sound—In June 2004, we established a three-factor design (+/- eelgrass, +/- geoducks, +/- fertilizer) in 1 m^2 plots, and we have measured sediment characteristics, and eelgrass density and growth rate at seasonal intervals. In response to the pulse perturbation of eelgrass removal, recovery occurred exclusively by branching and rhizome extension (not seed germination),began in spring 2005 after an 8-month lag, and was complete by spring 2006. In response to the press perturbation of geoducks, eelgrass density was lower in summer, but because eelgrass shoot densities naturally decline in winter, there was no effect of geoducks that season. We measured growth rate throughout 2004–2006 and did not observe any differences across treatments. Two factors may account for this lack of "fertilization" effect—first, porewater nutrients are naturally high (60-90 μM NH4+); second, eelgrass growth is apparently limited by factors other than nutrients, specifically light in winter and desiccation in summer. Two graduate students are responsible for different parts of this project, under the supervision of Jennifer Ruesink: Kirsten Rowell (2004–2005) and Sara Frame (2006).

Experimental studies, Willapa Bay—In June 2004, we established six treatments in 2x2 m plots with or without eelgrass at two locations to explore the important mechanisms that may be driving the patterns found in the comparative studies above. The treatments are: bare, shell addition, fertilizer addition, shell and fertilizer addition, medium-density oysters (30–50% cover), and high-density oysters (50–80% cover). The experimental plots were censused for adult and seedling eelgrass abundance and growth. In response to the pulse perturbation of eelgrass removal, recovery occurred via both asexual growth and seed germination but showed an 8-month lag; it was complete by spring 2006. In response to press perturbations, eelgrass declined to a level inversely proportional to the cover of either oyster or shell. Over the 2005–06 winter, oysters at one location became cemented together to form hummocks, which have completely excluded eelgrass; but at the other location, the oysters were scattered by winter storms and eelgrass is now increasing in density. These results prompt the idea that aquaculture activities—because they maintain oyster cover <30%—actually prevent the formation of hummocks that would locally exclude eelgrass. As in Puget Sound, we have observed little variation in eelgrass growth rates across treatments, probably because porewater nutrients are not limiting. Intriguingly, however, NH4+ concentrations are actually lowest where oysters were added at high density, a result that could occur if biodeposits facilitate denitrification. This work is the responsibility of UW graduate student Eric Wagner (adult eelgrass and sediments) under the supervision of Jennifer Ruesink.

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

We studied seed production, germination, and seedling growth and survival of eelgrass under different oyster aquaculture practices: dredging and off-bottom longline culture. To study germination we added seeds to two different aquaculture types, as well as eelgrass reference areas, in paired control and eelgrass removal plots. Germination of experimentally added seeds was highest in dredged areas, where adult shoot densities were lowest. Seedlings survived better and were bigger in plots where adult plants had been removed. We also found high natural seedling recruitment in dredged beds compared to longline beds and reference areas. We estimated mean seed production per $0.25m^2$ area and found this to be highest in dredged beds and lowest in longlines. We propose that the greater recruitment in dredged beds is due to both enhanced seed densities, because many shoots become reproductive, and removal of neighboring adult plants. Low success in longlines may be due to a combination of physical factors, including increases in sediment accretion and significantly lower redox values. Dredging can enhance or at least maintain seed density and seed germination, but longline aquaculture appears to significantly reduce eelgrass recruitment. These results have been submitted as a manuscript to Marine Ecology Progress Series. Under the supervision of Sally Hacker, OSU graduate student Lorena Wisehart has collected and compiled data on eelgrass phenology, and how seedling germination and survival vary across Willapa Bay and under different aquaculture types.

USEFULNESS OF FINDINGS

WRAC demonstrated tremendous foresight in funding this eelgrass research (and previous molluscan shellfish research) because it constitutes some of the only scientific data relevant to two emerging environmental decisions: regulation of geoduck aquaculture in Puget Sound (primarily occurring at the state level), and development of a Regional General Permit for shellfish aquaculture (primarily occurring through Army Corps of Engineers). In light of these developments, a critical result from our work is that eelgrass is not the same everywhere. Thus, it will be important—but difficult—to craft regulations that are context-specific, rather than blanket protections. In Willapa Bay, shellfish aquaculture is a stable, >100-year-old industry in a bay that fosters truly amazing eelgrass production (100x oyster production), and eelgrass populations are resilient to perturbations because they have effective seed production and germination. In south Puget Sound, geoduck aquaculture is an expanding industry in an area with little eelgrass. The eelgrass that is present is not resilient, because almost all population dynamics occur through vegetative growth, not through seeds. However, populations are so small that avoiding them would not remove much land from production—depending on buffer widths utilized.

We have communicated our findings at public events (Shelton oyster festival, Nahcotta seafood festival), industry meetings (Sea Grant conferences for shellfish growers, Pacific Coast Shellfish Growers Association), and eelgrass meetings (including both the National and the Pacific Estuarine Research Societies), and we have answered questions from groups such as People for Puget Sound and state and county natural resource departments.

WORK PLANNED FOR NEXT YEAR

Objective 1

1) Test for changes in water properties as water moves across the tideflat: We will continue to drift over oyster beds with different oyster densities, water flows, water depths, and initial phytoplankton concentrations.

2) Test for bed-scale variation in oyster growth, related to food gradients across tideflats. We will outplant oysters to multiple (~10) sites along a tideflat and expect that sites more distant from the channel will have slower-growing, low-condition oysters (holding tidal elevation constant). We will measure several factors at each outplant site: chlorophyll, water flow, and sediment.

3) Test for changes in water properties at subtidal (longer than tidal) time scales. Recent oceanographic modeling by Neil Banas (Oceanography, UW) suggests a long "residence time" for water on Willapa's tideflats—essentially this water cycles on and off the tideflat and undergoes little replenishment with new water. If so, then oysters may remove material from the water over many tidal cycles, and this would be evident in food-depleted water on tideflats relative to channels.

We will also quantify, using both field and laboratory experiments, the relationship between eelgrass growth and light within Willapa Bay. In particular, we are interested in whether eelgrass is ever light-limited and under what circumstances. These experiments will involve detailed light measurements at different sites using Pendant light meters and Li-cor quantum sensors. Coincident with these measurements, we will quantify eelgrass growth and biomass as well as sediment conditions (redox potential, salinity, silt:sand ratio, porewater nutrients). In the laboratory (at Hatfield Marine Science Center), we will conduct growth experiments in which eelgrass is exposed to different phytoplankton concentrations (and thus different light levels). The goal will be to understand under what phytoplankton concentrations is eelgrass light-limited and whether these concentrations are ever achieved within Willapa Bay. Ultimately, we hope to relate these data with the oyster feeding experiments outlined above to determine whether oysters could facilitate eelgrass growth indirectly through increases in light.

Objective 2

Continue recording sediment properties and eelgrass density and growth in experiments in Puget Sound and Willapa Bay. We will record experimental results over 2 years (2004–2006). In July 2006 (after this reporting period), Taylor Shellfish helped "harvest" geoducks from experimental plots, and we will track initial impacts and eelgrass recovery. We will also carry out an eelgrass removal in December to test if recovery begins in spring vs. 8 months later. There are many remaining questions about eelgrass growth, which shows strong seasonal patterns but has not responded to experimental manipulation: Is eelgrass in Puget Sound affected by the pulse perturbation of desiccation during hot midday tides? Does eelgrass transplanted into aquaculture beds grow faster or slower? How is growth rate affected by thinning of the surrounding eelgrass bed or by trimming of a plant? Does thinning in fact also enhance flowering?

A major effort to address objective 2 is a Willapa Bay-wide sampling of multiple habitat types for eelgrass growth, porewater nutrients, sediment grain size, redox potential, and organic content, to scale up experimental results to a level relevant to aquaculture.

Objective 3

Much of what we initially planned for this objective has been achieved. Publications are underway. In 2006, we will

continue to explore the relationship between oyster dredging and eelgrass recovery by sampling beds of different dredge dates to understand the time course of eelgrass recovery. Multiple sites will be used (Nemah, Stony Point, Stackpole, Long Island) to understand whether there is a context dependent and spatial scale component. We will measure eelgrass seed densities, seedling and adult densities, and a number of physical measurements including light, redox potential, porewater sediment, silt, and salinity.

IMPACTS

1) Aquaculture in areas with eelgrass is currently more controversial in California than farther north. However, results from our research have been used to inform decision-making about aquaculture in Humboldt Bay, California. Jennifer Ruesink now serves on the Humboldt Bay Ecosystem-Based Management pilot project.
2) The Army Corps of Engineers has drafted a proposed Regional General Permit for shellfish aquaculture in California, Oregon, and Washington, in which they state "Impacts to native eelgrass (*Zostera marina*) within the project area that reduce the density or impair the health of native eelgrass are not authorized." Our results from Willapa Bay indicate that this restriction could have major consequences for the industry and may not be advisable given the health and rapid recovery of this plant in that estuary as opposed to other areas such as South Puget Sound.
3) Washington Department of Natural Resources—in addition to local agencies for Pierce and Kitsap Counties—is developing guidelines for intertidal geoduck aquaculture in Puget Sound. Jennifer Ruesink has provided research results and interpretation.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

Publications in Print, Manuscripts, and Theses

Wisehart, L.M., B.R. Dumbauld, J.L. Ruesink, S.D. Hacker. Spatial variation in eelgrass recruitment from seed: influence of seed production, physical factors, and adult neighbors. To be submitted in September 2006, Aquatic Botany.

Richardson NF, Ruesink JL, Naeem S, Dumbauld BR, Hacker S, Tallis H, Wisehart L. Abundance and functional diversity of sediment microbes across natural and oyster aquaculture habitats in a northeastern Pacific estuary. Submitted, Hydrobiologia

Wisehart, L.M., B.R. Dumbauld, J.L. Ruesink, S.D. Hacker. Importance of eelgrass life history stages in response to oyster aquaculture disturbance. Submitted, Marine Ecology Progress Series.

Tallis, H.M., J.L. Ruesink, B.R. Dumbauld, S.D. Hacker, L.M. Wisehart. Oysters and aquaculture practices affect eelgrass density and productivity in a Pacific Northwest estuary. In revision, Estuaries and Coasts.

Wisehart, L. M. 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.

Ruesink JL, Feist BE, Harvey CJ, Hong JS, Trimble AC, Wisehart LM. 2006. Changes in productivity associated with four introduced species: Ecosystem transformation of a "pristine" estuary. Marine Ecology Progress Series 311:203-215.

Papers presented

Wisehart, 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.

Wisehart, L. June 2006. Aquaculture and eelgrass interactions in the Pacific Northwest. Presentation to Oregon State University Marine Biology Course, Newport, OR.

Wisehart, L., S. Hacker, J. Ruesink and B. Dumbauld. March 2006. Oyster aquaculture may positively affect eelgrass (*Zostera marina* L.) through enhanced seed production and germination. National Shellfisheries Association Annual Meeting, Monterey, CA.

Wisehart, L., S. Hacker, J. Ruesink and B. Dumbauld. February 2006. Assessing the importance of early life history stages of eelgrass (*Zostera marina* L.) in response to aquaculture disturbance. Pacific Estuarine Research Society Annual Meeting, Friday Harbor, WA.

Wisehart, L., S. Hacker, J. Ruesink and B. Dumbauld. October 2005. Effects of oyster aquaculture on eelgrass recruitment. Estuarine Research Federation Meeting, Norfolk, VA.

Wisehart, L., S. Hacker, J. Ruesink and B. Dumbauld. September 2005. Interactions between pacific oyster aquaculture and eelgrass: the importance of early life history stages in response to disturbance. Pacific Coast Shellfish Growers

Association Meeting, Hood River, OR.

Craig, C., K. Rowell, J. Ruesink. Pacific Estuarine Research Society, February 2006. How does geoduck aquaculture affect eelgrass in south Puget Sound? (poster)

Ruesink, J. 14th annual meeting for shellfish growers, March 2006. How does geoduck aquaculture affect eelgrass in south Puget Sound? (poster)

Dumbauld, B. M. Wecker and J. Ruesink, Oyster Aquaculture in a Pacific Northwest (USA) Estuary: A Plea for Taking a Landscape-Ecosystem Perspective, National Shellfisheries Association meeting, March 2006.

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		University	Industry	Other Federal	Other	Total	
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	$102,531
2005	82,368	12,000	20,000	8,000	20,000	60,000	$142,368
Total	245,390	24,000	60,000	14,000	23,000	121,000	$366,390

Optimizing Quality and Shelf-Life of Sturgeon Caviar

Termination Report

Reporting Period	September 1, 2002–September 1, 2006 with no cost extensions through 2008		
Author	Barbara Rasco		
Participants	Barbara Rasco*, PhD, JD	Washington State University	Washington
	Thomas Wahl, PhD	Washington State University	Washington
	Fredick T. Barrows*, PhD	UW Fish & Wildlife Service	Montana
	Jean Xavier Guinard , PhD	University of California, Davis	California
	Daivd Reid, PhD	University of California, Davis	California
	Yi-Cheng Su, PhD	Oregon State University	Oregon
Technical Advisors	Peter Struffenegger, PhD, JD	Marine Harvest/Stolt Sea Farm	California
	Robert McGorrin, PhD	Oregon State University, Corvallis	Oregon

* voting, work group members

REASON FOR TERMINATION

Objectives completed.

PROJECT OBJECTIVES

1. Characterize the sensory attributes of caviar and determine what factors influence product flavor, texture, color and other sensory attributes.
2. Determine how to optimize quality factors through diet modification, rearing conditions, and timing of harvest
3. Evaluate thermal and non-thermal processing methods to improve the shelf-life of caviar; evaluate microwave and RF processes for pasteurization.
4. Evaluate freezing methods to improve the shelf-life of caviar, and evaluate enzyme pretreatment, cryogenic and partial freezing processes for preservation.

PRINCIPAL ACCOMPLISHMENTS

This project solved technological issues facing the development of caviar from cultured white sturgeon (*Acispenser transmontanus*), an important species in California and the Inland Northwest. We assessed quality and sensory attributes of white sturgeon caviar; a number of different processing technologies including thermal processing, freezing; and the use of processing aids and additives. We also studied dietary changes on product quality and factors affecting product safety. This study should provide useful data to develop, produce, and promote American caviar. The longer term impact of the study is to assist the development of domestic and international markets for U.S. sturgeon products and provide opportunities for product diversification.

Objective 1. Characterize the sensory attributes of caviar and determine what factors influence product flavor, texture, color, and other sensory attributes.

Since there is no standardized way to grade caviar, this study looked at the sensory attributes correlated with high- and low-quality caviar. The descriptive analysis panel developed a lexicon with 33 attributes and found 16 significantly

discriminated between the 22 commercial caviars tested. This reliable sensory lexicon can be used to evaluate caviar products from a variety of sources, feed regimes, and processing conditions, and has already been used to evaluate numerous samples provided by companies throughout the Western region. From evaluation of a large number of samples, it appears that the attributes selected for the lexicon provide a wide enough range to cover differences observed among caviars sturgeon samples (imported Beluga, Sevruga, Osetra, *Astara asetra*; American Sevruga; cultivated Osetra; white sturgeon, spoonbill sturgeon, hackleback, and paddlefish).

Expert judges were asked to rate the caviar on a quality scale from zero to 100 taking into consideration all the sensory qualities such as taste, aroma, appearance, and texture. The experts included chefs, members of the caviar industry, and faculty of the Culinary Institute of America. Some of the judges had more experience with caviar than other judges, creating some disagreement about what constituted high- or low-quality. The judges rated the imported Osetra as the highest-quality and an imported Sevruga and domestic Spoonbill as the lowest quality. However, judges had different opinions on what traits constitute high-quality caviar. The results from the study indicate that caviar producers should strive for an egg that has a thicker skin and a buttery flavor and mouth-feel. The eggs should not have any bitterness, sourness, metallic flavors, grassy leaves, or high salt.

Expert ratings are good indicators of consumer likes and dislikes, but further work should be done with consumer testing. If manufacturers are looking to develop new products and are interested in how consumers will react, expert assessors will not necessarily describe the attributes that are relevant for product development. The use of new ingredients, processing techniques, and storage methods can cause conflict with experts' specifications regardless of how consumers may perceive the product.

Rheological tests can provide an instrumental assessment of product texture with correlations found between egg size and skin thickness; dissolvability, breakability, and firmness. Both the physical properties and sensory characteristics of caviar can vary by how the product is packed, and the location of the sample within a jar.

Objective 2. Determine how to optimize quality factors through diet modification, rearing conditions, and timing of harvest.

Large-scale feeding trials that evaluated antioxidants were conducted. Eggs from fish consuming a high antioxidant diet can differ in certain flavor, aroma, and texture characteristics by both instrumental and sensory tests, but these differences may not affect overall product quality or consumer acceptability or preference. For example, in one study, the breakability (tougher skins) and skin thickness of caviar from fish on the control diet were significantly greater than for eggs from fish fed an experimental diet containing antioxidants.

The fish fed the experimental diet produced eggs that were wetter in appearance and more bitter and that had a stronger aftertaste; however, there was an overlap in sensory attributes for samples between the two treatment groups. Eggs from fish raised on the experimental diet also had more viscous internal contents. The experimental eggs had a more consistent or uniform appearance in the jar compared to the control. Lastly, the experimental eggs separated in the mouth more easily and dissolved faster than the control diet eggs. These results are reproduceable between two different panels at different years for fish on similar diets. Antioxidants may have prevented the occurrence of off flavors and poor textures. Previous research suggests that the addition of antioxidants benefits the shelf life of the product and preserves the fresh flavor.

Objective 3. Evaluate thermal and non-thermal processing methods to improve the shelf-life of caviar; evaluate microwave and RF processes for pasteurization.

Dielectric and conventional thermal processing methods were developed for packed caviar in jars and plastic containers. Shelf life of caviar products could be extended by using different additives, with antimicrobial agents, particularly nisin, improving the effectiveness of thermal processing treatments by reducing both the treatment temperature needed to inactivate *Listeria monocytogenes*, the vegetative pathogen of greatest heat resistance in aquatic food products, and treatment time.

Objective 4. Evaluate freezing methods to improve the shelf-life of caviar, evaluate enzyme pretreatment, cryogenic, and partial freezing processes for preservation.

Freezing (including cryogenic technologies and super-cooling) were successfully developed. In addition, various pre-freezing treatments were evaluated as means to enhance storage stability and maintain product quality. The lack

of endogeneous transglutaminase in sturgeon roe means that treatment with various additives (e.g., polyphosphate, sulfate salts, calcium chloride) that is often effective for improving the texture of some fish roe products (e.g., ikura) through a cross-linking reaction of membrane proteins, will not be effective for sturgeon caviar. Protein composition of salmon and sturgeon roe products differ significantly.

IMPACTS

The WRAC-sponsored research has had positive impacts on the industry by providing processing and storage technologies to extend shelf life and maintain product quality and safety. Investigators have worked with individual aquaculture facilities on strategies to produce safe, high-quality caviar with an extended shelf life and have provided technical information needed to show that caviar from cultivated fish is a quality product, and as good as caviar from wild harvested fish. During the course of this study, the quality of caviar from U.S. producers has become more reliable, with consistently attainable product standards that are necessary for further market development.

Implementation of technologies developed in this project has resulted in major cost savings, potentially in the range of millions of dollars per year. For example, if caviar goes "sour" during storage, the value drops from roughly $1,000 per pound to $10 per pound.

Product quality is a critical feature for any aquatic food, and probably more important for higher value items. The demand for sturgeon caviar continues to exceed supply, and sturgeon remain subject to extreme over-exploitation throughout their natural range of distribution (Bledsoe and Rasco, 2006; Wirth et al. 2002). The quality of imported product has dropped over the past two years, despite a significant increase in price (Bledsoe and Rasco, 2006; Irwin and Marsh, 2005). Restrictions on the sale of beluga caviar will continue to provide an excellent opportunity for U.S. producers to meet market demand for caviar as foreign supplies tighten. The United States and European Union remain the largest import markets of beluga caviar selling in 2005 at retail prices of $750–1,500/100 g in high-end European retail markets and $450/100g in the U.S., substantially more than three years earlier. Prices are dramatically higher in 2006 at £229/50 g for beluga caviar, £123/50 g for sevruga and £159/50 g at retail stores in London in August. Because of trade restrictions and current high demand, there is every reason to believe that there will be a good market for sturgeon caviar (*Acipenser transmontanus*) produced in the United States.

It will be important to be able to predict how products from aquacultured sturgeon differ from wild fish and how to exploit these differences to one's advantage in the marketplace by promoting caviar with distinct attributes to specific markets. The flavor of caviar changes as it ages, with the conventional market for an aged product that has undergone a certain degree of auto-oxidation. However, there are new markets for fresher products that realistically can only be made from cultivated fish. For example, Prunier recently introduced a "48-hr" old Paris brand malosol caviar, selling in London at £141/50 g indicating that there are markets for a wide variety of caviar products.

RECOMMENDED FOLLOW-UP ACTIVITIES

Additional work should be conducted to assist sturgeon growers and processors to develop products for particular markets based upon sensory characteristics that can result from differences in fish diet, harvest technology, processing method, and storage conditions and time. Research is needed to assist growers to determine when fish are mature and when the optimal time is to harvest eggs for greatest yield and quality. The nutritional needs of these fish are still not well understood; also not well understood is how the diet could be modified to improve the flavor, texture and mouthfeel of the caviar, control fat content and color, and to shorten depuration periods needed to control for off-flavor development. Studies on husbandry or processing practices that could improve texture and flavor should be continued. Texture changes are often more important than flavor in assessing caviar quality, and how texture is affected by product source (water, temperature, age), fish diet, and processing and storage conditions should receive more attention. Experiments focused on reducing the membrane fragility of sturgeon eggs should examine agents other than enzymes. Studies on the safety and lowest effective levels of treatments necessary for controlling texture and microbial spoilage should be evaluated, as well as continued work on technologies and practices that could maintain product quality and safety.

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SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		UNIVERSITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
2002	57,000	17,400	56,000	19,200		92,600	$149,600
2003	57,000	17,400	51,500	25,000		93,900	$150,900
2004	57,000	9,000	56,500	19,250		84,750	$141,750
2005	57,000	5,000	56,500	12,500		74,000	$131,000
TOTAL	228,000	48,800	220,500	75,950		345,250	$573,250

Investigation into the Solution of Critical Probflems of the Recirculation Aquaculture Industry in the Western Region

Termination Report

Reporting Period	April 15, 2001–August 30, 2006		
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	Raul Piedrahita	University of California, Davis	California
Technical Advisor	Dallas Weaver	Scientific Hatcheries	California
Industry Advisor	Gary Grace	Red Top Aquafarm	California

* voting, work group members

REASON FOR TERMINATION

End of funding.

PROJECT OBJECTIVES

1. To provide recirculation system design information specific to the needs of cool and cold water species cultured in the Western Region, Washington State University (WSU).
   - to develop nitrification design criteria for cool or cold temperature applications
   - to evaluate the fine solids removal performance of different biofilters
   - to optimize the design and operation of biofilters
2. To evaluate RAS design criteria at commercial production scales (WSU)
   - to evaluate the design criteria through a new commercial trout system
   - to evaluate the design criteria through a new commercial sturgeon system
3. To develop methods to control off-flavor compounds in RAS, University of California, Davis (UCD)
   - to develop a treatment method for controlling off-flavor compounds that is compatible with commercial RAS and economically viable
4. To provide guidelines (Best Management Practices) for meeting the new regulations written by the EPA. (***This is the primary outreach objective.***) (University of Arizona (UA), WSU).
   - to interview Western aquaculture producers and use existing literature to determine which effluent components will be most problematic with new EPA regulations.
   - to develop Best Management Practices that operators of RAS can utilize to improve their RAS and to meet effluent guidelines
   - to develop additional outreach materials that will inform users of RAS of improvements in equipment and techniques developed by the work group

PRINCIPAL ACCOMPLISHMENTS

Objective 1: To provide recirculation system design information specific to the needs of cool and cold water species cultured in the Western Region (WSU)

Nitrification rate as a function of total ammonia nitrogen (TAN) concentration, with and without the interaction of organic matter, was investigated for three types of biofilters of laboratory scale: floating bead filter (FBF), fluidized sand filter (FSF), and submerged bio-cube filter (SBF). The performance of each type of biofilter was evaluated using a 5-reactor series with synthetic solutions containing different carbon/nitrogen ratios (C/N=0, 0.5, and 2.0). The tests were run at representative cold water aquaculture system temperatures of 10, 15 and 20°C respectively. In year 2004, data from tests at 15°C and 20°C, C/N=0, 0.5, and 2 were analyzed to determine the effect of substrate concentration, biofilter type, and temperature on the nitrification rate. Data from the 10°C tests were analyzed in 2005 and combined with the results at 15°C and 20°C to provide nitrification design information for cold and cool water systems. The highlights of these results are as follows:

1) The Nitrification rate of all three types of biofilters could be modeled linearly (R2>0.9) as a function of TAN concentration without the interaction of organic carbon at low TAN concentrations ([TAN] <6 mgl-1).
2) Organic carbon had a significant ($p<0.0001$) inhibition on nitrification performance for all three types of biofilters. Key effects to nitrification performance were: as the C/N changed from 0 to 0.5, nitrification rates decreased dramatically and as C/N changed from 0.5 to 2 the nitrification rate of the floating bead filter (FBF) continued to decrease while the nitrification decrease was insignificant for the fluidized sand filter (FSF) and submerged biocube filter (SBF). With C/N=2, all three types of biofilters showed zero-order nitrification kinetics over a substrate concentration of 3~11mgTANl-1.
3) Without the interaction of organic carbon, the FBF had a nitrification rate of 12% and 50% higher than that of the FSF and SBF respectively; however the difference among them became less significant and even insignificant with an increase of organic carbon.
4) Temperatures of 15°C and 20°C had no significant impact ($p=0.283$, $\alpha=0.05$, $n=75$) on the nitrification rate of the three types of biofilters tested.

The results of this study provide useful information for the design of nitrification biofilters for cold water RAS applications.

Objective 2: To evaluate RAS design criteria at commercial production scales (WSU)

The cool/cold water RAS, served as a demonstration system and as the baseline system operated from January–July 2005 in the Aquaculture Education and Research Lab at WSU. The system was monitored for water quality, water consumption, energy consumption, and system performance.

Modeling of biofilm and Biofilter nitrification design recommendations for cold water RAS

A simplified analytical model was developed to solve the mass flux of ammonia and COD in multi-species biofilms. Based on the concept of equilibrium mass flux at the liquid-biofilm interface from the external and internal mass balance, this simplified model can be solved easily by an integrated interaction process within an Excel spreadsheet. A comparison of the performance of the simplified model against results from complex numerical solutions resulted in deviations of less than 10%. Comparison between bench scale data and the WSU cool/cold water RAS results indicated that the addition of organic matter can cause an 0~80% reduction in the nitrification rate of biofilters with the deficiencies associated with system scale-up causing another 10~80% reduction; therefore, the ammonia nitrification rate in a commercial scale production system can be determined as,

R_A (actual) = $\alpha*\beta*R_L$ (α = 0.2~1.0, β = 0.2~0.9)

Where, RL = the TAN removal rate from a pure culture bench scale biofilter, mg m -2 d-1; α = reduction coefficient due to the effect of organic matter; β = reduction coefficient due to scale-up deficiency.

This model provides useful information for the design of cool and cold water RAS. A complete discussion on biofilm model parameters selection is provided for the end users of this model.

Objective 3: To develop methods to control off-flavor compounds in RAS (UCD)

Geosmin and 2-methylisoborneol (MIB) have been reported as the major compounds causing "earthy" and "musty" odor in aquaculture waters and related "off-flavor" in fish. A preliminary extended aeration study was successful for the removal of geosmin and MIB concentrations of 100 ng/L in less than 24 hours. Further degassing tests, with clean tap water in the laboratory and using effluent water from an aquaculture farm, were performed to measure geosmin and MIB removal in a 1.83 m high, 0.14 m diameter packed aeration column. The tests were carried out in water spiked with a geosmin and MIB stock solution prepared in methanol. Removal rates did not vary significantly for the gas to liquid flow rate ratios tested (approximately 1 to 10 on a volume basis), but there were differences between the results obtained in the laboratory with clean tap water and those obtained using effluent water from an aquaculture farm. Tests were carried out at a hydraulic loading rate of 78 $m^3 m^{-2} h^{-1}$. The K values obtained for geosmin and MIB were 0.33 ± 0.06 m^{-1} and 0.66 ± 0.14 m^{-1} for the laboratory and farm trials, respectively. These K values would result in approximate removals of 60 and 80% of influent concentrations for a 2.0 m tall column using the laboratory and farm results, respectively.

Objective 4: To provide guidelines (BMP) for meeting the new regulations written by the EPA (US, WSU)

We have provided guidelines, blank forms, and example BMPs for meeting the new regulations at *http://ag.arizona.edu/azaqua/extension/BMPs/bmp.htm*. Development of outreach publications for individual states is continuing. We have developed and updated a comprehensive website that describes the release of the effluent guidelines that were being proposed by EPA: *http://ag.arizona.edu/azaqua/extension/BMPs/Final_EPA.html.* We also added documents from the National Sea Grant Law Center and the EPA regarding management practices to comply with the new regulations; links to compliance meetings held across the country are included in the website. Also, links to the recirculating aquaculture conferences and short courses and WRAC research reports were embedded as they were considered valuable sources of pertinent information. Guidelines (BMPs) for effluents in Arizona were developed and submitted to state agencies. Idaho has also developed a BMP document for intensive systems but does not include recirculating systems.

An additional aspect of the project is to develop an enterprise budget for a model recirculating system in the western US. The enterprise budget will be modified with two alternative scenarios incorporating the expenses and projected benefits of the off-flavor treatments developed at UC-Davis and the cool water nitrogen and biofilter technologies developed in the WSU aspect of the project. We anticipate having these models available by the time of the IAC-TC. Jim Durfey is planning to coordinate with the Idaho Research, Aquaculture Extension faculty and fish producers in Southern Idaho in early September to discuss findings of this project.

IMPACTS

Many industry members have been informed of the exact situation of the new effluent guidelines and requirements. There had been a large amount of mis-information and preliminary recommendations that were not implemented causing tremendous confusion. By providing accurate information and subsequently providing the tools for industry members to succinctly meet the requirements, we have helped smooth the transition.

Growers are aware of the need to continually improve management strategies of their water resources. We no longer have the inexhaustible supply of water for single pass style production systems. As the need for fish products increases, recirculation technologies will provide producers the tools with which they can increase production with the same water resources. The opportunity to development recirculation technologies through joint operation of private producers and public land grant institutions creates a unifying pathway to help growers transition in these technologies. As growers transition into recirculation systems, their attention to detail of inputs and outputs of their system will be more critical. As growers adopt these management strategies as outlined in the BMP's (*http://ag.arizona.edu/azaqua/extension/BMPs/bmp.htm*) they will become more familiar with a standardization occurring throughout industry.

RECOMMENDED FOLLOW-UP ACTIVITIES

The use of recirculation systems continues to grow and new problems will need the attention of the research com-

munity to ensure the economic viability of recirculation systems. Some of these include the development of systems that are more energy efficient, the treatment of effluents, and improvements in water treatment effectiveness to allow for reductions in water use. Additional presentations at local, regional, and national meetings will further inform the producers of how best to meet the regulations and implement new technologies that should improve their operations.

As a part of the extension and outreach, during the Fall of 2006 there will be visits with the growers to discuss these systems with Idaho research, extension and producers. A follow-up contact with these growers will be done to see what progress is being made to adopt these recirculation technologies.

PLAN OF WORK FOR REMAINING FUNDS

Remaining funds will be used to participate in conferences and meetings (travel funds) and to develop outreach products based on research results (i.e. student support, supplies, and computer equipment). This will include posting of additional information at the website and an outreach report on an enterprise budget for a typical recirculating system in the Western US.

In addition to the posting of information on the website, contact with the growers in a year following the termination will take place to see what adoption of these technologies has taken place.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		University	Industry	Other Federal	Other	Total	
2001–2002	59,645						$59,645
2002–2003	68,473	5,980[1]				5,980	$74,453
2003–2004	69,013		17,800[2]	15,000[3]		32,800	$101,813
2004–2005	63,804		7,000[2]	15,000[3]		22,000	$78,804
TOTAL	260,935	5,980	24,800	30,000		60,780	$321,715

1) Washington State University

2) AquaKing—Funds for intern to conduct research at recirculation tilapia farm in California.

3) USDA-ARS—Funds for Dr. Kevin Schrader's participation in the Off-flavor work.