WESTERN REGIONAL AQUACULTURE CENTER

WRAC

ANNUAL ACCOMPLISHMENT REPORT

FOR THE PERIOD SEPTEMBER 1, 2006 TO AUGUST 31, 2007

APRIL 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, 2006 through Aug. 31, 2007. WRAC is one of five regional aquaculture centers under USDA, for which funding is 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 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 for new and continuing regional projects, project modifications, and project terminations.

Since the start of the regional aquaculture programs, WRAC has processed 20 Annual Work Plans (for FY'87 through FY'07 funding) through USDA. This annual report covers the activities of the WRAC Administrative Center and progress made during the 20th year on all active projects through Aug. 31, 2007, listed below with funding levels for FY'07.

A. 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

B. Physiological Changes Associated with Live-Haul Maintaining Healthy Fish
   2nd project year: $81,856

C. Development and Evaluation of Starter Diets and Culture Conditions for Three Subspecies of Cutthroat Trout and Gila Trout
   2nd project year: $95,677

D. Scale-Dependent and Indirect Effects of Filter Feeders on Eelgrass: Understanding complex Ecological Interactions to Improve Environmental Impacts on Aquaculture
   4th project year: $82,368

E. Immunological Mechanisms of Intensively-reared Warmwater and Coolwater Finfish
   4th project year: $100,000

F. National Aquaculture Extension Conference—April 30–May 4, 2007, Cincinnati, OH
   $18,200

G. WRAC Publications
   $24,000

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

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

Administrative Support

FY'O7 FUNDING LEVEL $191,619

The WRAC Administrative Center staff provides 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 20 Annual Work Plans (FY'87 through FY'07) to date for 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 about pertinent activities related to regional and national aquaculture in general and WRAC in particular.

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

Other areas of support during FY 2006–2007, 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 Administrative Centers and key administrators from USDA
- Coordination of special sessions for Regional Aquaculture Centers at aquaculture meetings
- Solicitation and coordination of recommended nominees to the IAC and TC
- Recruitment of Administrative Center staff, as authorized by the Board of Directors
- Close communication with other fisheries and aquaculture programs to track various aquaculture activities throughout the western region

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

Reporting Period	September 1, 2006–August 31, 2007 (Year 1)		
Authors	James D. Bowker		
Funding Level	First Year Request[1]	$29,864	
Participants	James D. Bowker*	US Fish & Wildlife Service	State??
	Vaughn Ostland*	Kent Sea Tech Corporation	California
	Steve Harbell (*Ext. Rep.*)	Washington State University	Washington
Technical Advisor	Jerri Bartholomew	Oregon State University	Oregon

* funded participants

[1] Although the initial notice of approval of funding was received April 10, 2006, no funds were available in the 2006 calendar year.

PROJECT OBJECTIVES

The purpose of this research project is to determine whether an alternate (i.e., higher concentration and/or longer duration) treatment regimen (other than 10 mg florfenicol/kg fish body weight administered on 10 consecutive days) is more efficacious in controlling mortality in hybrid striped bass (HSB) caused by *Streptococcus iniae*. The specific objectives for this study are listed below. Objectives that are relevant to Year 1 funding are italicized.

Objective 1.

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

Trial 2. *Refine methodologies identified in Trial 1 to consistently yield a mean cumulative mortality of 50% in HSB of a different age exposed to isolates of S. iniae. Evaluate reproducibility of IP injection dose or immersion dose/exposure duration to yield approximately 50% cumulative mortality.*

Objective 2.

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

Objective 3.

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

Objective 4.
Demonstrate and substantiate that the most efficacious treatment regimen identified in Objective 3 is also effective when florfenicol is administered to HSB naturally infected with *S. iniae* (i.e., field trial).

ANTICIPATED BENEFITS

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

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

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

On February 13, 2007, the principal investigators met at the Kent SeaTech (KST) Corp. HSB farm in Mecca, California, to start the first trial of Objective 1. The goal of this trial was to establish baseline information about *S. iniae* immersion and IP injection challenge models. We conducted a disease challenge study on large fingerling HSB and tested three different injection and three different immersion doses, and identified the challenge methodology (either injection or immersion) and the dose that resulted in about 50% mortality in test fish. Results from this study were used to help define the injection or immersion dose to be used in Trial 2 to achieve approximately 50% mortality with minimal variability in small fingerling HBS.

On September 10, 2007, the second trial of Objective 1 was conducted at the KST HSB farm. The goal was to refine the immersion challenge dose/methodology to achieve approximately 50% mortality with minimal variability in HBS mortality. We conducted an immersion disease challenge on smaller fingerling HSB and tested the mid-dose concentration of the challenge broth used in Trial 1 (the dose that resulted in approximately 50% mortality) at three different durations to test the reproducibility of findings from Trial 1 and to evaluate whether subtle changes (i.e., mortality) occur with increasing exposure duration.

Experimental Design/Methods—Objective 1 Trial 1.
To ensure inter-experiment reproducibility for this project, bacterial "working seeds" were produced and frozen for all subsequent challenges. From a single working seed, both the injection and immersion challenge inocula were prepared. Groups of fish were either injected with or immersed in a pre-determined dose of *S. iniae* to determine the challenge methodology (immersion or injection) and dose that resulted in approximately 50% mortality at the end of the study (i.e., nine days post-challenge).

The *S. iniae* bacterial working seed was prepared using standard procedures. Interperitoneal injection and immersion challenge inoculum were prepared and administered to naïve HSB (mean length,17.9 cm; mean weight, 52.1 g) that had not been previously exposed to *S. iniae*.

The trial consisted of two experiments (Experiment #1–injection; Experiment #2 – immersion) designed to test the following general null hypothesis: Ho: $\mu_{control} = \mu_{low\ dose} = \mu_{mid\ dose} = \mu_{high\ dose}$ (no difference in mean percent total mortality between treatment conditions - control, low dose, medium dose, and high dose in each experiment). In this trial, tanks of fish were considered the experimental unit. In each experiment, three replicates of each treatment condition were allocated among 12 tanks in a completely randomized design. Completely randomized procedures were also used to allocate fish to tanks.

Thirty fish were stocked into each test tank. Each experiment was monitored for nine days and began immediately after all fish in the study had been challenged with *S. iniae*. At the end of the experiment, mean mortality associated with each treatment condition was evaluated by statistically comparing mean percent total mortality between treatment groups.

At the end of Experiment #1, mean relative mortality of HSB injected with *S. iniae* was ≥ 98% in all treatment groups, except in the control group, in which mortality was 0%. Virtually all mortality observed in this experiment occurred on post-challenge days 2–3. The challenge dose estimated to have been delivered to test fish in a 0.1 mL injection volume in Experiment #1 ranged from 2.2E+04 to 2.73E+05 *S. iniae* CFUs.

In Experiment #2, mean relative mortality of HSB that were immersion exposed to a low dose of *S. iniae* was 44%, in the mid-dose tanks—53%, and in the high dose tanks—70%. Virtually all mortality observed in this experiment occurred on post-challenge days 3–5. The challenge dose of *S. iniae* (CFUs/mL) estimated to have been delivered to fish in their test tanks ranged from 1.48E+07 to 5.08E+07 for the low to high dose, respectively. From this trial, we chose the immersion model as the more appropriate challenge model, and the mid-dose concentration as the dose most likely to produce 50% mortality in the exposed population.

Experimental Design/Methods—Objective 1 Trial 2.
The goal of this study was to maximize the reproducibility of the immersion challenge model. To ensure comparable reproducibility with results from Trial 1, bacterial "working seeds" used in Trial 1 were also used in Trial 2. Groups of fish were immersed in a pre-determined dose (i.e., mid-dose) of *S. iniae*. The objective was to determine the mid-dose/exposure duration that resulted in approximately 50% mortality at the end of the study (i.e., nine days post-challenge) and resulted in a mortality rate profile similar to that seen in natural disease infections.

The *S. iniae* bacterial working seed was prepared using standard procedures. Immersion challenge inoculum were prepared and administered to naïve HSB that had not been previously exposed to *S. iniae*.

This trial, which started on September 10, 2006, consisted of an immersion experiment and was designed to test the following general null hypothesis: Ho: $\mu_{control} = \mu_{10\ min\ exposure} = \mu_{15\ min\ exposure} = \mu_{30\ min\ exposure}$ (no difference in mean percent total mortality between treatment). Three replicates of each treatment condition were allocated among 12 tanks in a completely randomized design. Thirty fish were stocked into each test tank. Tanks of test fish were exposed to a *S. iniae* challenge broth at a concentration similar to the mid-dose used in Trial 1 Experiment #2 for 0, 10, 15 or 30 minutes. The experiment was monitored for nine days and began immediately after all fish had been challenged with *S. iniae*.

At the time the report was drafted, trial specifics (e.g., mean fish size, approximate challenge concentration, etc.) and preliminary results (e.g., mortality, measured broth concentration, feeding behavior, etc.) were not available.

USEFULNESS OF FINDINGS

1. Findings from the first trial showed that the immersion disease challenge methodology was more appropriate than the injection methodology for infecting fish in a manner to evaluate the effectiveness of fish therapeutants (lower mortality and greater interval between challenge and the on-set of mortality). In addition, results from this trial demonstrated that the mid-dose was adequate for producing the desired level of mortality.
2. We anticipate that findings from the second immersion experiment will verify that the *S. iniae* broth dose/exposure duration identified in Trial 1 was adequate for infecting HSB to a level that resulted in approximately 50% cumulative mortality. We anticipate that findings will also show that challenge duration will have subtle effects on mortality, indicating that adjusting exposure duration can be used

to further refine the methodology to achieve subtle adjustments in the desired level of mortality.

3. We anticipate that results from the second immersion experiment will provide us with the tool needed to successfully challenge test fish in a manner to adequately evaluate the effectiveness of different dose and duration treatment regimens of florfenicol to control mortality in HSB caused by *S. iniae*.

WORK PLANNED FOR NEXT YEAR

Two trials are planned next year (September 2007–August 2008 [Year 2]) to evaluate the effectiveness of different dose and treatment duration regimens of florfenicol to control mortality in HSB caused by *S. iniae*.

Trial 1. We will evaluate the effectiveness of 10, 15, and 20 mg florfenicol/kg fish body weight to control mortality in HSB caused by *S. iniae* (experimentally induced in test fish).

Trial 2. We will evaluate the effectiveness of the treatment dose identified in the previous trial as the most efficacious for 10, 15, or 20 days to control mortality in HSB caused by *S. iniae* (experimentally induced in test fish).

Results from these two trials should provide us with the "most" efficacious treatment regimen to control mortality in HSB caused by *S. iniae* that had been experimentally induced. Such results will be substantiated in field trials using production HSB naturally infected with *S. iniae*.

IMPACTS

Disease challenge model developed that may be used by others to evaluate therapeutants, vaccines, and other disease management strategies. Such a model will allow for more expedient evaluation of drugs and biologics needed by the industry to maintain and propagate healthy populations of fish. Caution must be taken by others to ensure the virulence of the bacterial working seed used in other trials is comparable to that used in the above-described trials.

PUBLICATIONS IN PRINT & MANUSCRIPTS

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

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		University	Industry	Other Federal	Other	Total	
1/07–8/07	29,864		12,000	24,000		36,000	$65,846
Total	29,864		12,000	24,000		36,000	$65,846

Physiological Changes Associated with Live Haul Maintaining Healthy Fish

Reporting Period	September 1, 2006–August 31, 2007		
Author	John Colt		
Funding Level	$87,156 for 2005–2006 $81,856 for 2006–2007		
Workgroup Chair	John Colt		
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 objectives during 2006–2007 were to:

1. Document physical and chemical changes that occur in the fish transportation process.
2. Document physical and chemical characteristics of the retail holding systems.
3. Construct experimental transport systems to simulate long-haul conditions.
4. Develop a simulation model for the hauling and holding conditions
5. Develop methods to rapidly assess stress, physical injury, and parasite loads in cultured tilapia.
6. Evaluate changes in hauling protocols and systems on survival and product quality.
7. 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

Document physical and water quality at the retail level.

Monitoring of physical and water quality in retail holding systems has shown a wide variation in param-

eters, some of which may have a significant impact on survival and product quality. A manuscript is in preparation to document our findings.

Develop experimental hauling system and monitor water quality during hauling trips.
Continuous monitoring of pH, temperature, conductivity, dissolved oxygen, and oxidation-reduction potential during experimental hauls has shown that we do not fully understand the impacts of tilapia on water quality. It has been assumed that the pH would decrease during hauling due to excretion of metabolic carbon dioxide. Experimentally, it was found that the pH initially dropped, but then actually increased for the majority of the trip. In addition, both the alkalinity and conductivity were higher at the end of the trip. The increase in pH may be due to excretion of ammonia and the possible excretion of bicarbonate and will require additional experimental work to clearly determine what is the primary mechanism responsible for the increase in pH.

Develop quality simulation models for alkalinity/pH/carbon dioxide/UIA for hauling and retail holding systems.
A simulation program was developed to improve our understanding of the water quality changes that occur during hauling. This program estimates oxygen consumption, carbon dioxide excretion, and ammonia excretion as a function of temperature and activity. Carbon dioxide stripping by pure oxygen is estimated. The pH of the solution is computed by trial and error from the basic alkalinity–carbonate equation. The temperature model computes the conductive heat transfer based on a one-dimensional steady-state solution, estimates the metabolic heat production using 13.68 J/mg of oxygen, and accounts for both the direct cooling of the pure oxygen flow and the evaporative cooling component of this flow. The program predicts the rapid decrease in pH at the start of hauling, followed by an increase in pH over the haul that was observed in the experimental hauling.

The accuracy of this model strongly depends on values of metabolic production factors used and the specific geometry of the hauling tanks and liquid oxygen (LOX) distribution system. More information is needed on the following parameters: (a) change in alkalinity over the hauling trip, (b) thermal characteristics of the hauling tanks and LOX distribution system, (c) impact of metabolic activity on oxygen transfer and carbon dioxide stripping, (d) variation of oxygen consumption and ammonia production during the hauling trip.

Develop improved hauling mixture.
Tilapia are typically hauled in a simple salt (sodium chloride) solution of approximately 2–5 g/kg, depending on individual hauler. Based on Prosser (1973), an "isotonic" hauling solution was developed. A spreadsheet was developed to allow haulers to compute the amount of different salts needed. After discussion with two haulers and demonstration of the spreadsheet, at least one of the haulers has tested a potassium supplemented hauling mixture. Information on post-hauling survival for this media is not available at this time.

Use fluorescein to assess physical injury incurred by tilapia during transport.
Following published protocols, tilapia were submerged in water containing 0.2 mg/ml fluorescein. Skin was examined under UV light to allow visualization of damage and injury. This technique was tested with marked fish and with netted fish. Quality images were obtained from intentionally marked tilapia. The net stress experiment was successful and allowed visualization of bodily damage to the fish. The fluorescein dye indicated that puncture wounds from the spines of other fish and abrasions from the net were the primary cause of injury.

Compare different hauling media and recovery conditions on survival during and after transport.
Simulated hauling was conducted using two hauling media. The first was well water with 3 g/L NaCl added. This hauling medium is typically used by commercial growers. The second "saltmix" media was well water with specified ion concentrations (in mg/L): sodium–3,473, chloride–4,893, potassium–189, calcium–133, magnesium–56, and bicarbonate–200. Mortalities were observed in the NaCl group im-

mediately following transport but not in the saltmix group. Blood gas values were not different between hauling or recovery treatments nor was the water quality. We did observe expected differences in blood gas data between hauling and recovery treatments due to the increased stress during the haul. The use of a "saltmix" can reduce the immediate mortality following hauling.

Determine effects of a pre-transport salt-dip on survival after transport.
Fish that were salt-dipped were placed in water containing 3% Sea Salt (Instant Ocean) for one minute before loading into transport tanks. The control fish were not dipped. All the fish were hauled in a conventional hauling mix of 3 g/L. The fish were hauled from Idaho to Oregon State University for post-hauling evaluation. Tilapia were analyzed for bodily injury via fluorescein dye. Skin samples were taken from two moribund fish three days post-transport for histological analysis of both hemotoxylin and Eosin and Gram stained sections. Tilapia that were not pre-treated with a salt dip showed accelerated mortality following transportation compared to those that received a salt-dip. Control fish began to die within 24 hours of transport. Mortality for tilapia pre-treated with the salt-dip was not evident until nearly 72 hours post-transport. Overall mortality was also lower for salt-dipped tilapia, which had a total of 13 mortalities, compared to 18 for the control group. The use of a pre-haul salt dip reduced the mortality of tilapia for the first 72 hours following hauling.

Assess physical injury and pathology of tilapia during loading, hauling, and retail holding.
Tilapia from Idaho were sampled before crowding, after crowding, after loading into the truck, upon arrival in Richmond, BC, and after 24 hours and 48 hours of holding in two different retailers. Fluorescein analysis was used to assess bodily injury. Agar plates were streaked with mucus from skin and tissue from the posterior kidney to determine bacterial load. Wet mounts were also taken from the skin mucus, gills, and kidney for bacterial and parasite analysis. Histological samples were taken from skin and kidney for gram staining to identify bacteria.

Fluorescein analysis revealed puncture wounds and abrasions in all tilapia sampled directly from the raceway, before crowding. After arrival at Richmond, BC, tilapia sampled directly off of the truck had similar bodily injury as pre-loading with more noted abrasions to the caudal and pectoral fins. Tilapia 24 hours and 48 hours post-holding in the retailers did not look much different after fluorescein exposure than the fish sampled at unloading; however, at this point, visual signs of sickness (lesions and hemorrhaging on skin) were apparent to the naked eye. Samples are currently being processed for histological analysis.

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

WORK PLANNED FOR NEXT YEAR

1. Monitor water quality in hauling and retail stores in the San Francisco area.
2. Determine the impacts of tilapia on the carbonate-pH system and ionic composition of hauling water under simulated hauling conditions.
3. Determine experimentally the thermal characteristics of the hauling tanks and distribution system on production hauling systems.
4. Determine the oxygen consumption of tilapia using a mass-balance approach on the gas phase in simulated hauling experiments.
5. Determine ammonia excretion rate of tilapia by sequentially measuring the TAN concentration.
6. Determine the impact of hauling on the volumetric mass transfer coefficient (K_La) for oxygen and carbon dioxide.

7. Evaluate both pre-hauling salt treatment and improved hauling mix.
8. Repeat the salt dip experiment with fish from an additional farm.
9. Document further the fluorescein dye technique to assess loading and transport damage to tilapia.
10. Document further parasite load and pathology on the survival of fish at the retail stores.
11. Assess aeration and hauling protocols to improve the survival of transported fish.
12. Initiate work on trout transport issues. Initially, identify and assess key problems via contacts of industry cooperators and the US Trout Growers with the assistance of Gary Fornshell.
13. Develop outreach products and industry training activities.

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		University	Industry	Other Federal	Other	Total	
2006	87,156	3,891		37,500			$128,547
2007	81,856	3,500		30,000			$115,356
Total	169,012	7,391		67,500			$243,903

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

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

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

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

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

Colt, J., Watten, B., Michael Rust, M. 2007. Modeling carbon dioxide, pH, and un-ionized ammonia relationships in serial reuse systems. Submitted to *Aquacultural Engineering*.

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

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

Reporting Period	October 1, 2006–September 1, 2007		
Author	Christopher Myrick		
Funding Level	**First Year Funding Received**	**$99,991**	
	Second Year Funding Received	**$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

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 and Year 2 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 of fish. Recreational fishermen and outfitters will also benefit by having more fishing opportunities available for catching native fish. Indirectly, all of this benefits local and surrounding communities, providing services in the areas where these native fish are found or propagated. Another indirect benefit is improving the recovery and restoration of these species, hopefully leading to the delisting of Gila trout from the endangered species list and preventing other strains of cutthroat trout from being listed.

Gila trout were reclassified as threatened (from endangered) 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 which 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. All the diets were packaged by feed size and labeled with numbers so that fish culturists were blinded. Feeds were distributed for testing at Colorado State University (CSU), the Mora National Fish Hatchery and Technology Center (Mora Center), and the Bozeman Center.

The Snake River and Yellowstone cutthroat trout initial feeding trials were conducted over 18 weeks at a water temperature of 10°C with initial fish densities of 150 Snake River and 135 Yellowstone cutthroat trout per tank. The diet types significantly affected fish weight, total length, survival, and feed conversion. Diet #5 (one of the commercial diets) provided the greatest growth for both Snake River and Yellowstone cutthroat trout, while fish fed Diet #2 had the poorest performance. Survival of fish fed Diet #5 exceeded 97% for both Snake River and Yellowstone cutthroat trout. Based on these results, Diet #5 is recommended for improved initial feeding performance.

The Colorado River cutthroat trout initial feeding trials were conducted over 120 days at a water temperature of 10.5°C, with initial fish densities of 150 fish per tank. Colorado State University used the same five diets as the Bozeman Center, but also included an eighth diet, referred to as Diet 5-8, where one group of fish fed Diet #5 was fed live Artemia for two weeks before being weaned onto Diet #5. Fish fed Diet #5-8 experienced the greatest growth, roughly 2.8 times that of fish fed the poorest performing diet, Diet #2. If the Artemia-supplemented diet is excluded, fish fed Diets #1 and #5 had the greatest

growth. Survival was highest for fish fed Diet #5-8 (88%), followed by the unsupplemented Diet #1 and Diet #5. Based on these results, both Diets #1 and #5 are recommended to improve initial feeding performance of Colorado River cutthroat trout.

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). The results with the Gila trout were similar to those seen for the cutthroat trout subspecies; the highest growth was observed in fish fed Diets #3, #4, and #5 (Diet #3 was the highest, but it was not significantly higher than Diets #4 or #5), while Diet #2 produced the lowest growth. Overall survival was poor, but was also affected by diet, with Diets #1 and #5 having higher survival rates (42.5% and 41.0%, respectively) than the other diets. Although Diets #3, #4, and #5 showed the highest growth rates, these results may be confounded by the relatively low survival, which is perhaps an artifact of the experimental system used in 2006. The system was extensively modified for the 2007 experiments to address this issue.

The outreach component of the project, coordinated by the University of Idaho, got underway in late 2006 with the initial development of the project website. Website development continued into the first quarter of 2007. A description of the project, first year results, and IAC/TC PowerPoint presentation were loaded onto the website. Project prinicipal investigators provided input on the website. The website will be posted on the WRAC website.

Year 2 (2007)

In 2007, the Bozeman Center again served as the point of distribution for the diets used in the feeding trials. Based on the results from the 2006 diet trials, the Bozeman Center used Diet #5 for the temperature and density studies conducted on Snake River and Yellowstone cutthroat trout. Colorado State University requested Diets #1 and #5 because the performance of the two diets was nearly identical. The Mora Center requested Diets #3, 4, and 5, but the unfortunate loss of eyed eggs during incubation prevented Gila trout trials from being conducted during 2007.

The Bozeman Center is currently conducting comprehensive density and temperature studies with Snake River and Yellowstone cutthroat trout. The density study is comparing the performance of both strains of cutthroat trout when reared at densities of 50, 100, 150, 200, 250, 300, and 350 fish per tank at a fixed water temperature of 10°C. Fish of both strains are also being reared at 10, 12, 14, 16, 18, and 20°C to determine the optimal rearing temperature for fish growth and survival. Each of these density and temperature studies will continue for 16 weeks. Preliminary results (week 8) show no differences in Snake River and Yellowstone cutthroat trout performance due to density. Rearing temperature does appear to affect fish size, with 16°C producing larger fish of both subspecies at this time.

Colorado State University is currently conducting a temperature–diet study with two diets (#1 and #5, both supplemented with live Artemia for the first two weeks) and temperatures of 10, 12.5, 15, 17.5, and 20°C. Preliminary results (Day 60) suggest that Diet #5 is producing higher growth rates than Diet #1. The temperature x diet study will continue for 120 days. Additionally, a temperature effect is apparent, with the highest growth rates occurring between 15 and 17.5°C.

Several suggestions were made at the 2006 work group and IAC/TC meetings about Mora Center's rearing set-up and design. In place of aquaria used in 2006, 30 opaque fiberglass troughs were purchased as rearing units and plumbed. In addition, 20 belt feeders were obtained to provide a more even feed rate over hand feeding. The system was successfully installed, but because of an unanticipated problem with the Gila trout eggs, it was not put into use in 2007.

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

The outreach component of the project continues to be coordinated by the University of Idaho. Presentations of the Colorado State University portion of the study were delivered to the 2007 meetings of the Colorado Aquaculture Association and the Colorado-Wyoming Chapter of the American Fisheries Society. An "Inland Native Trout Propagation" symposium was submitted by the Bozeman Center and approved for the 137th annual meeting of the American Fisheries Society; 10 papers were presented. The project website is ready for posting on the WRAC website, and the intent is to post annual progress reports and any other pertinent information as it becomes available. The URL will be advertised on aquaculture association websites; in newsletters, including *Waterlines*; and through appropriate state and federal agencies. The website will also serve as the distribution point for the Extension publication. The work group will have its second annual meeting September 13, 2007 in Ft. Collins, Colorado, to discuss second-year trials and coordinate the full-scale production trials.

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 will enable commercial fish farmers to supply fish to their clientele, and ultimately will allow recreational fishermen to have greater fishing opportunities on 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. Work group investigators have been receiving inquiries about the status of the diet trials because there is much interest in knowing the outcome. Obviously, feed manufacturers have much to gain or lose pending the performance of their product. Fish culturists want to know the best available feed, temperature, and rearing density to increase the performance of their fish. These recommendations will be passed on for 2008 and 2009 production-scale studies.

Although the workgroup has not yet disclosed the identity of the diets, the information on the positive benefit of even two weeks of diet supplementation with Artemia on the survival and growth of first feeding cutthroat trout has been mentioned during presentations, and has generated some interest from industry and hatchery personnel. This result also confirms the recently published findings of Arndt and Wagner (2007), who also saw improved growth of first feeding Colorado River cutthroat trout fed *Artemia nauplii*.

WORK PLANNED FOR NEXT YEAR (Year 3)

1. Colorado State University will conduct a density study on the Colorado River cutthroat trout using the optimal growth temperature and best diet identified in the 2007 diet x temperature growth trial.
2. The Mora Center will conduct the density trial that was not completed in Year 2 due to complications with the supply of Gila trout.
3. Data from the diet, temperature, and density studies will be analyzed and manuscripts developed. Technical assistance will be provided for the initiation and completion of production-scale studies during Years 3 and 4.

4. Results from the first two years of the study will be presented in a variety of industry and professional association forums, including the 2008 Colorado Aquaculture Association Meeting, the Colorado–Wyoming AFS meeting, the Aquaculture America 2008 meeting, and the US Trout Farmers Association Meeting.
5. Annual progress reports and any other pertinent information will be posted on the project website. The URL of the website will be advertised on aquaculture association websites; newsletters, including *Waterlines*; and through appropriate state and federal agencies.

IMPACTS

This project has already benefited the western aquaculture industry because it has brought a diverse group of researchers and industry representatives together. This group is working 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.

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		University	Industry	Other Federal	Other	Total	
2006	99,991	5,000	5,000	81,500	5,500	97,000	$196,991
2007	95,677	25,829	5,000	90,000	5,500	126,329	$222,006
Total	195,668	30,829	10,000	171,500	11,000	223,329	$418,997

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

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

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

PUBLICATIONS, MANUSCRIPTS, AND PAPERS PRESENTED

Brandt, M. M. and C. A. Myrick. 2007. Getting ahead in a cutthroat world—performance of Colorado River cutthroat trout fed eight starter diets. Symposium on Inland Native Trout Propagation at the 137th Annual Meeting of the American Fisheries Society, San Francisco, CA, September 2–6.

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

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

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

Myrick, C. A. 2004. Development and evaluation of starter diets and culture conditions for three 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.

Symposium Arranged

Inland Native Trout Propagation at the 137th Annual Meeting of the American Fisheries Society, San Francisco, CA, September 2–6. 10 presentations, moderator Steve Sharon, Wyoming Game and Fish Department Hatchery Supervisor.

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

Reporting Period	April 1, 2006– March 31, 2007 (*does not include 2007 field season*)		
Authors	Jennifer Ruesink, Sally Hacker		
Funding Level	Year 1	$79,607	
	Year 2	$83,415	
	Year 3	$82,368	
	Year 4	$82,368	
	Total	$327,758	
Participants	Brett Dumbauld	USDA-ARS	Oregon
	Sally Hacker*	Oregon State University	Oregon
	Steve Harbell	Washington State Univ. Extension	Washington
	Jennifer Ruesink*	University of Washington	Washington
	Graduate Students		
	Kristen Rowell	Univ. of Washington (2004)	Washington
	Heather Tallis	Univ. of Washington (2003-04)	Washington
	Lorena Wisehart	Oregon State University (2003-05)	Oregon
	Elizabeth Wheat	Univ. of Washington (2005-07)	Washington
	Eric Wagner	Univ. of Washington (2005-06)	Washington
	Margot Hessing-Lewis	Oregon State University (2005-07)	Oregon
	Sara Frame	Univ. of Washington (2006)	Washington
	Micah Morwith	University of Washington (2007)	Washington
	Sylvia Yang	University of Washington (2007)	Washington
	Undergraduate Students		
	Michelle Briya	Oregon State University (2004-05)	Oregon
	Ashley Lyons	Oregon State University (2005-06)	Oregon
	Thatcher Jones	Oregon State University (2005-07)	Oregon
	Jackie White	University of Washington (2005)	Washington
	Carrie Craig	University of Washington (2006)	Washington
	Marla Koberstein	University of Washington (2006)	Washington
	David Holden	University of Washington (2006)	Washington
	Chao-chung Tsai	University of Washington (2006-07)	Washington
	Jeremy Henderson	Oregon State Univ. (2007-present)	Oregon

PROJECT OBJECTIVES

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.

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.

Objective 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 eelgrass. 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 2005–06, we carried out 30 drifts on flood tides and detected significant depletion of particles across oyster beds in shallow water. In addition to this "mobile" perspective on water properties, we also collected "stationary" information at stations in line with tidal flow across a tideflat. These results show that water at the end of the ebb and beginning of the flood contains very little chlorophyll, presumably because this water passes directly over benthic suspension-feeders on the tideflat multiple times—in short, this water is "retained" on the tideflat and therefore has substantial opportunity for clearance by oysters. Taken together, we would expect the effects of oysters on water clarity (and indirectly on eelgrass growth) to manifest at very large scales, much larger than the scale of beds because of oysters' downstream legacy. This work provides an empirical underpinning for two recent predictions about the filtration capacity of oysters in Willapa Bay: Ruesink et al. (2006) suggest that cultured oysters could filter just 1–2% of the bay's volume daily, but Banas et al. (2007) predict complete filtration of the daily net tidal import of oceanic phytoplankton and infer that oysters are measurably reducing phytoplankton at the estuary-wide scale. 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.

Willapa Bay sediments—We collected sediment from six habitat types and along the estuarine gradient in Willapa Bay. Microbial cell density varied most strongly along the estuarine gradient, being positively associated with organic content. Aerobic microbial activity varied less strongly along the estuarine gradient and additionally showed habitat-specificity, being slightly lower in on-bottom oyster aquaculture.

Although sediment properties such as grain size and organic content did not vary by habitat type in this study, other experimental work suggests that biodeposits from oyster hummocks reduce grain size and increase organic content. This research is published in *Hydrobiologia*.

Willapa Bay eelgrass—We sampled eelgrass density, biomass, and growth across four habitat types: eelgrass beds, longlines, hand-picked, and dredged-ground aquaculture. Higher oyster densities are associated with lower eelgrass densities, and 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. This manuscript remains in preparation.

To examine the important mechanisms that may be driving the patterns found in our comparative studies, we established six treatments in 2x2 meter plots with or without eelgrass at two locations in June 2004. 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. 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.

South Puget Sound—In June 2004, we established a three-factor design (+/- eelgrass, +/- geoducks, +/- fertilizer) in 1 m^2 plots within an existing ~2,000 m^2 eelgrass bed in south Puget Sound. These manipulations altered three sediment characteristics. The removal of eelgrass reduced organic content and silt:sand ratio, and the addition of geoducks tended to increase porewater ammonia towards optimal levels for eelgrass growth, consistent with measurements taken across habitat types. Constructed gaps in the eelgrass bed recovered over two years, exclusively due to vegetative regrowth from shoots at the edges. Geoducks also competed for space with eelgrass, reducing shoot density by about 40% in summer (no effect in winter). Overall eelgrass growth rates were not affected by geoduck or fertilizer treatments. After two years, the geoducks were harvested by commercial methods from the addition plots, which reduced eelgrass density by more than 70%. Recovery from this pulse perturbation required at least one year, but was difficult to gauge because the entire eelgrass bed declined in size and density, probably due to natural stressors (desiccation, waves). Two graduate students have been responsible for different parts of this project, under the supervision of Jennifer Ruesink: Kirsten Rowell (2004–2005) and Sara Frame (2006). A manuscript will be submitted in Fall 2007.

North Puget Sound—Experimental work on factors influencing eelgrass in Samish Bay is just beginning, carried out by Micah Horwith, 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 0.25 m^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 published in Marine Ecology Progress Series. Under the supervision of Sally Hacker, Lorena Wisehart, OSU graduate student, has collected and compiled data on eelgrass phenology and determined 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, more than 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. (But populations are so small that avoiding them would not remove much land from production—depending on buffer widths.)

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), as well as answered questions from groups such as People for Puget Sound and state and county natural resource departments.

WORK PLANNED FOR NEXT YEAR

Objective 1: Much of what we planned for this objective has been achieved, although additional drifts are planned for 2007 over oyster beds with different oyster densities, water flows, water depths, and initial phytoplankton concentrations.

Objective 2: Continue recording sediment properties, eelgrass density, and growth in experiments in Puget Sound and Willapa Bay. In July 2006, Taylor Shellfish helped "harvest" geoducks from experimental plots, and we will track initial impacts and eelgrass recovery.

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? Experiments will be conducted in Willapa Bay and Samish Bay in which eelgrass is manipulated by thinning and/or trimming. We will measure responses in terms of water flow, sediment properties, sexual and asexual reproduction, and shoot growth and morphology.

Objective 3: Much of what we initially planned for this objective has been achieved. Publications are underway. In 2007, we will continue to explore the relationship between oyster dredging and eelgrass recovery by sampling recently dredged beds and following them each year to understand the time course of eelgrass recovery. Multiple sites will be used (Nemah, Stony Point, Stackpole) 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.

In response to industry interest, we will also collect morphological and demographic information about eelgrass at multiple sites in Washington state. This wide-scale sampling should reveal the capacity of eelgrass for resilience (recovery after perturbation) to large-scale disturbance via recruitment from seed.

Outreach: We plan to contract with Lorena Wisehart, who completed her Master's degree working on this project, to develop a ~30-page handbook (and shorter executive summary) on project results, suitable for decision-makers. The intention is not simply to duplicate scientific papers, but to provide guidance on best management practices.

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.
2. The Army Corps of Engineers produced a nationwide permit for shellfish aquaculture in March 2007. This permit requires pre-construction notification prior to any aquaculture activities in eelgrass. Our results suggest that several factors should be taken into consideration: the degree of disturbance, the recovery capacity of the eelgrass, and the return time of disturbance (crop cycle).
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, *Aquatic Botany*.

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 preparation.

Ruesink, J.L., J.S. Hong, B.R. Dumbauld, S.D. Hacker, A.C. Trimble, L.M. Wisehart. Congener comparison of native (*Zostera marina*) and introduced eelgrass (*Z. japonica*) in Willapa Bay. In preparation.

Ruesink, J.L., K. Rowell. Geoduck clam (*Panopea abrupta*) aquaculture as press and pulse perturbations to eelgrass (*Zostera marina*). In preparation.

Richardson, N.F., J.L. Ruesink, S. Naeem, B.R. Dumbauld, S. Hacker, H. Tallis, L. Wisehart. 2007. Abundance and functional diversity of sediment microbes across natural and oyster aquaculture habitats in a northeastern Pacific estuary. *Hydrobiologia*, in press.

Wisehart, L.M., B.R. Dumbauld, J.L. Ruesink, S.D. Hacker. 2007. Importance of eelgrass early life history stages in response to oyster aquaculture disturbance. Marine Ecology Progress Series 344:71-80.

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, J.L., B.E. Feist, C.J. Harvey, J.S. Hong, A.C. Trimble, L.M. Wisehart. 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.

Frame, S. October 2006. Eelgrass and geoduck interactions. Pacific Coast Shellfish Growers Association Meeting, Vancouver, WA.

Dumbauld, B.R. October 2006. Pacific Coast Shellfish Growers Association Meeting, Vancouver, WA.

Ruesink, J.L. October 2006. Ecological interactions between oysters and eelgrass in Willapa Bay. Pacific Coast Shellfish Growers Association Meeting, Vancouver, WA.

Ruesink, J.L. October 2006. Ecological interactions of geoducks and eelgrass in south Puget Sound. Sound Science Series (invited), Shelton, WA.

Ruesink, J.L. November 2006. Conservation science in a coastal estuary—ecological impacts of shellfish aquaculture. Western Society of Naturalists (invited), Redmond, WA.

Ruesink, J.L. April 2007. Ecological consequences of Japanese oysters in western North America. International Oyster Reef Meeting (invited), Chiba, Japan.

Ruesink, J.L. July 2007. West coast oyster reefs as sentinel habitats. Coastal Zone 2007 (invited), Portland, OR.

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

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

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
2006	82,368	12,000	20,000	8,000	10,000	50,000	$132,368
Total	327,758	36,000	80,000	22,000	33,000	171,000	$498,758

J. Ruesink, supported by UW, worked on the Puget Sound portion of the project. Tides and data processing required (Apr/May 06 –40 hrs). At Willapa Bay, supported by the Mellon Foundation, she devoted 0.5 month to the drifting project. Advising, writing, and editing have required >100 hrs.

S. Hacker, supported by OSU, worked on eelgrass seedling and adult growth under natural and experimental conditions in Willapa Bay. Field dates of full day work with graduate student in Willapa Bay included: 80 hours. In addition, she spent 10-15% administrative time on the project (writing, editing, advising, etc.).

B. Dumbauld and technicians, supported by USDA-ARS, worked on eelgrass seedling and adult growth surveys and began eelgrass mapping efforts in Willapa Bay. (110 hrs). B. Dumbauld helped editing and writing (24 hrs)

A. Trimble, supported by Mellon Foundation, worked on the drifting project and provided field support during most low tide series. In 2006, he estimates 1 month.

Immunological Mechanisms of Intensively Reared Warmwater and Coolwater Finfish

Reporting Period	April 1, 2006—July 1, 2007		
Author	Vaughn Ostland		
Funding Level	$100,000 in Year 4		
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

PROJECT OBJECTIVES

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

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, two 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 response to vaccination.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

The majority of the research effort for Year 4 was focused on continuing to develop the immune reagents and their respective immune assays. The following summarizes the progress towards the successful accomplishment of each objective.

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.
As described in the Year 3 Annual report, we began to characterize the suitability of this new polyclonal antibody raised against HSB IgM heavy chain for development of an ELISA to measure pathogen specific antibodies produced by HSB following challenge or immunization. Both non-purified and affinity purified polyclonal rabbit antibodies against a deglycosylated HSB IgM heavy chain were forwarded to the Western Fisheries Research Center (WFRC) for further characterization to determine their suitability for use in an ELISA. Once again, after several in-depth experiments that were designed to tease out the nuances of the apparent cross-reaction of the polyclonal antibody by adsorbing the antiserum with *S. iniae* cells, we were unsuccessful in ascertaining why this "second generation" polyclonal antibody raised against a deglycosylated HSB IgM heavy chain, failed to discern hyperimmunized HSB sera that physically agglutinated killed *S. iniae* cells, but could not specifically recognize antibodies bound to homologous *S. iniae* antigen coated to the solid phase of a 96 well ELISA plate. To this day, we remain frustrated by our unsuccessful efforts to raise an antibody to HSB IgM that would be suitable for further ELISA development.

Characterization of Rabbit anti-HSB IgM HC Polyclonal Antiserum. In spite of the lack of the suitability of this polyclonal antibody for development of an ELISA, we have found that this antibody does have value for other enzyme immunoassays such as western immunoblots. To examine the intraspecies and interspecies utility of this polyclonal antibody for detecting the heavy chain region of IgM we probed hybrid striped bass (*Morone saxatilis* x *Morone chrysops*), striped bass (*Morone saxatilis*), white bass (*Morone chrysops*), largemouth bass (*Micropterus salmoides*) and rainbow trout (*Oncorhynchus mykiss*) serum with this antibody. Briefly, dilutions of serum were subjected to SDS-PAGE under reducing conditions before being transferred to a PVDF membrane using a standard immunoblotting protocol as recommended by the manufacturer (Invitrogen). The membrane was blocked with skim milk for 30 minutes and probed with a 1/1,000 dilution of deglycosylated rabbit anti-HSB IgM heavy chain (HC) serum for one hour. After washing, the alkaline phosphatase labeled secondary detection reagent was added, incubated for 30 min, and washed extensively to remove unbound reagent. The membrane was flooded with chromogen and developed for four minutes before washing with water. This antibody strongly recognized the HC region of the IgM (approx. 70 kDa) of all three species of the genus Morone examined but, not surprisingly, failed to recognize the IgM of largemouth bass or rainbow trout.

Application of this Polyclonal Antibody for Immunohistochemistry. We also tested the ability of this antibody to detect the presence of immunoglobulin secreted by splenic lymphocytes of HSB previously injection vaccinated with *S. iniae* or control HSB that had received an identical injection with phosphate buffered saline (PBS). Immunohistochemical staining was performed using a commercially available polyclonal antibody staining kit (SuperPicTureTM Polymer Detection Kit, Zymed Laboratories, San Francisco, California). Regardless of the dilution of primary antibody tested, we were unable to detect the presence of specific antibody secreting cells in the spleen of the HSB that were vaccinated with formalin-inactivated *S. iniae* cells. Moreover, at seven days post vaccination, there was evidence of low numbers of cocci still within the visceral body stores of the vaccinated HSB. Similar observations were not found in the control fish. There was evidence of weak staining (background?) within the lumen of blood vessels of all the organs examined in both the vaccinated and sham vaccinated fish. This preliminary work suggests that this polyclonal antibody may not be suitable for immunohistochemical studies.

Discovery of a Commercial Monoclonal Antibody That Recognizes HSB IgM. While attending the 5th International Symposium of Aquatic Animal Health in San Francisco during September 2006, Vaughn Ostland met with Dr. Kim Thompson, the Technical Director of Aquatic Diagnostics Limited (ADL), an inter-

national biotechnology company based in Stirling, Scotland. ADL develops and markets monoclonal antibodies (mAb) and mAb-based *in vitro* diagnostic kits to monitor fish and shrimp health. After our discussions of the major fish pathogens affecting HSB in the western US and our lack of success in developing antibodies against HSB IgM, Dr. Thompson offered to screen HSB serum with ADL's large panel of mAbs produced against many species of fish IgM. In November 2006, approximately 10 ml of HSB serum was sent to ADL via courier to begin this in-kind project. Approximately five months later, Ostland was contacted by Dr. Karen Snedden, lead scientist for this project, and she informed him that their Asian Sea Bass (*Lates calcarifer*) IgM mAb strongly recognized HSB IgM in an ELISA format. ADL was kind enough to supply us with two different mAb products free of charge (approximate total retail value of $600 USD), an unlabeled anti-Asian Sea bass IgM mAb (product number F02) and anti-Asian Sea bass IgM labeled with horse radish peroxidase (product number C2-HRP). Dr. Snedden felt that either of these products would be suitable for development of an ELISA that specifically recognized HSB IgM.

In April 2007, the Immunology Work Group chair traveled to the Western Fisheries Research Center in Seattle, Washington, to assist with further assay optimization to measure the cellular immune responses of adult hybrid striped bass following injection challenge with PBS or *S. iniae* (see below). During this time, we undertook a proof-of-principle experiment to test the ability of these newly acquired mAbs in an ELISA to recognize *S. iniae*-specific antibodies in the serum of HSB. This experiment demonstrated that both anti-Asian Sea Bass monoclonal antibodies strongly recognized the HSB anti-*S. iniae* antibodies present in the hyperimmune serum, compared to the control serum. This experiment also indicated that the strongest signal to noise (S/N) ratio was achieved with the 1:50 dilution of the HSB sera that was detected with a 1:50 dilution of HRP-labelled anti-Asian Sea Bass IgM monoclonal antibody. As we have reported numerous times before, the pool of 3E8 and 4D2 ascities fluid developed during this project, failed to demonstrate the presence of *S. iniae*-specific antibodies. In addition, a Western immunoblot of HSB serum was prepared as described for the rabbit polyclonal antibody against HSB IgM above, probed with a 1:50 dilution of the HRP-labelled anti-Asian Sea Bass IgM monoclonal antibody, and it was found to specifically but weakly recognize the HC region of HSB serum IgM.

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.

Subtask 5a. Optimization of ELISA for Hybrid Striped Bass Antibody
With the success of the proof-of-principle experiment using the commercially available anti-Asian Sea Bass IgM monoclonal antibody, we undertook additional experiments to optimize the ELISA for detection of pathogen-specific HSB antibodies.

Preliminary Efforts to Optimize Detection of S. iniae-Specific Antibodies
Since there was evidence of significant background issues when we conducted the proof-of-principle experiment using the unlabelled mouse anti-Asian Sea Bass IgM antibody, it was decided to concentrate our efforts on optimization of the mouse anti-Asian Sea Bass IgM.hrp labeled antibody. Furthermore, a

secondary anti-species conjugate would not be required because the horse radish peroxidase was directly labeled to the primary detection antibody (mouse anti-Asian Sea Bass IgM.hrp labeled antibody), thus this would reduce the number of steps and improve the speed of the assay.

Our preliminary experiments to optimize the ELISA with this monoclonal antibody indicated that a 1:50 dilution of HSB serum could detect *S. iniae*-specific antibodies with a 1:50 dilution of the mouse anti-Asian Sea Bass IgM.hrp labeled antibody (anti-ASB.hrp). Additional optimization experiments found at a fixed dilution of the detection antibody (1:50, mouse anti-ASB.hrp), the greatest OD was obtained with a 1:20 dilution of the HSB serum; however, the best signal to noise ratio occurred when the HSB serum was diluted 1:40.

Based on these results, we continued to optimize the dilution of the mouse anti-Asian Sea Bass IgM.hrp labeled antibody (mouse anti-ASB.hrp serum) against the dilution of HSB serum. It was found that a 1:10 dilution of HSB serum provided the greatest optical density with a 1:25 dilution of the mouse anti-Asian Sea Bass IgM.hrp labeled antibody. The optimal signal to noise ratio under the conditions tested in this experiment, was obtained with a 1:10 dilution of HSB serum and a 1:50 dilution of mouse anti-Asian Sea Bass IgM.hrp labeled antibody. Since the latter is a relatively expensive reagent, it is felt that the 1:50 dilution will be sufficient for further ELISA development but the dilution of the HSB serum may have to be varied to between 1:10 and 1:20 dilution. This of course, will be dependent on the size of the fish being studied because this will directly affect the volume of HSB serum available for any particular assay.

Preliminary Efforts to Optimize Detection of A. hydrophila-Specific Antibodies

Using the same methodologies described above, the mouse anti-Asian Sea Bass IgM.hrp labeled antibody was also optimized in an ELISA to detect *A. hydrophila*-specific antibodies in HSB serum. To detect *A. hydrophila*-specific antibodies under the conditions tested, we concluded that a soluble *A. hydrophila* antigen coating dilution of 1:100 followed with a 1:100 dilution of hyperimmune HSB serum yielded an optimal signal to noise ratio when detected using a 1:50 dilution of the mouse anti-Asian Sea Bass IgM.hrp labeled antibody.

While it is obvious that additional optimization will be required before this ELISA can be used to monitor and/or quantify IgM levels in HSB serum, it is felt that the general approach on how to measure pathogen specific antibodies has been established. It is likely that additional optimization of the initial dilution of the HSB serum will be necessary to identify low levels of pathogen-specific antibody in fish species belonging to the genus Morone.

This subtask in considered complete.

Task 2. Modify immune assays developed for salmonids for use with warmwater fish.
Subtasks 1 and 2 (blood and tissue collection, humoral measures of immune function)

At this time, the methods to collect blood and to separate the putative HSB leucocyte populations by DiOC6(3) staining is complete, but we do not know what cell types correspond to the separated cell populations.

These subtasks are complete.

Subtask 3. Cellular measures of immune function.
Bactericidal Killing Assay

As reported last year, the bacterial killing assay will need further development before it can be used to monitor macrophage activity. Further research is planned during the next several months using existing funds.

Isolation of anterior kidney leucocytes

This subtask is complete for both juvenile rainbow trout and juvenile HSB.

Cytometric phagocytosis assay

As reported last year, cytometric phagocytosis assays originally developed for Chinook salmon (*Oncorhynchus tshawytscha*) leucocytes (Alcorn et al. 2002) were adapted for use on RBT and HSB. The RBT anterior kidney leucocytes used in the comparison of the cytometric versus chambered slide phagocytosis assays did not have as great a percentage of cells which ingested the FITC-*S. aureus* as expected. Similar cytometric studies with Chinook salmon produced phagocytic anterior kidney leucocyte percentages of about 40 to 50%. By cytometric assay, large granulocytes of HSB showed a progressive increase in the number of fluorescent particles. During Year 4, the phagocytosis assay was optimized further.

Ten hybrid striped bass (HSB) were reared at 18°C to approximately 500 gram each. These fish were used to compare the cellular immune response of PBS and *S. iniae*-injected fish during the first 72 hours after injection. Portions of the liver, spleen, and anterior kidney were also snap frozen for later gene expression studies. The gene expression in one or more of these organs will be used to correlate this study and the previous gene expression study using HSB done in Year 3.

Four fish were injected IP with 1.0 mL of PBS and six fish injected IP with an equal volume of *S. iniae* in PBS. After injection the fish were transferred to two separate 4-foot diameter treatment tanks. Two and 3 PBS and *S. iniae*-injected fish, respectively, were sampled 24 and 72 hours after injection. For each fish, samples of the liver, spleen and anterior kidney were taken for gene expression analysis. Leucocytes from the anterior kidney of each fish were purified and tested for phagocytic index, percent phagocytic cells, and chemiluminescent response.

There was no apparent difference among the results of the samples prepared from the HSB 24 hours after injection (Fig. 2, HSB #2–5). These results may indicate that the phagocytic response had not yet adjusted to the presence of *S. iniae* in HSB #3–5. This is supported by the similar response of HSB #11, which was not injected with anything.

Seventy two hours after injection, the percentage of phagocytic large granulocytes from the fish exposed to *S. iniae* (Fig. 2, HSB #8-10) appeared to be greater than the percentage of cells from the PBS injected fish (Fig. 2, HSB #6 and 7). This pattern was especially evident among the large granulocytes which had taken up 5 or more beads. However, this pattern does not seem to be due to an increase in phagocytosis among the *S. iniae* exposed fish, but rather a decrease in the percentage of phagocytic cells in the PBS-injected fish.

Chemiluminescence

The anterior kidney leucocytes from both the PBS and *S. iniae*-injected HSB produced a strong chemiluminescent (CL) response to PMA at both time points. When the fish were sampled 24 hours after injection, the PMA induced CL response of the leucocytes from PBS injected fish (HSB #1 and 2) were slightly greater than the CL response of the *S. iniae* injected fish leucocytes (HSB #3-5). By 72 hours after injection, however, the PMA induced CL response of the *S. iniae* injected fish leucocytes (HSB #8-10) was greater than the response from the PBS injected fish leucocytes (HSB #6 and 7). The increased response appears to have been due to a secondary peak in the PMA response of the *S. iniae* injected fish leucocytes.

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

Rainbow trout gene expression

Quantitative PCR assays to assess expression of a panel of rainbow trout cytokines have been developed at the Western Fisheries Research Center and the methods published for others to use (Purcell et al. 2004).

Hybrid striped bass gene expression

Research to identify genes of immunological relevance in HSB began in Year 3 and continued in Year 4. Because S. iniae is the primary pathogen of interest for the HSB research in this project, genes for cytokines involved in the inflammatory response were sought for development quantitative PCR-based

assays. To determine which HSB inflammatory response genes are modulated in their expression after exposure to *S. iniae*, tissues were collected from control and infected fish. Eight hybrid striped bass (HSB) were anesthetized in a MS222 bath and injected with PBS and another 8 fish were each injected with *S. iniae*. The PBS and *S. iniae* injected fish were placed in separate 8 foot diameter tanks for recovery and holding. The fish were not fed for the remainder of the experiment. At 24 and 72 hours post injection, 4 fish from the PBS and S. iniae injection groups were overdosed with MS222 and each fish was bled via the caudal vein and the blood sample was centrifuged over a 46% isotonic Percoll bed. The leukocytes at the blood/Percoll interface were collected and re-suspended in lysis buffer and vigorously triturated to break up the cells. The cell suspension was then stored at -80°C. To collect peritoneal macrophages, the peritoneal cavity of each fish was aseptically opened using a scalpel and scissors, the internal organs flushed with 10 ml of PBS and vigorously massaged with the tip of the syringe. The resulting suspension was processed, centrifuged, and the cell pellet re-suspended in buffer RLT to lyse the cells. The cell suspension was stored at -80°C. Several gill filaments and portions of the liver, spleen, posterior kidney, anterior kidney and brain were placed in separate cryovials, snap-frozen in liquid nitrogen and stored at -80°C.

RNA extraction from the liver tissue and subsequent cDNA synthesis were performed as described by Purcell et al. (2004). The final 20 ml cDNA synthesis reactions were diluted to a final volume of 100 ml. The results of a quantitative PCR assay (qPCR) for HSB hepcidin were presented during the last reporting period. The HSB hepcidin gene was chosen initially since it has been previously sequenced (Shike et al. 2002). Using the hepcidin primers and probe and the method described by Purcell et al. (2004), an increase in hepcidin gene expression of greater than 1000-fold was seen in the infected fish. Other qPCR assays for HSB genes involved in infection and immunity are in development at this time. Other genes of interest include those that code for inducible nitric oxide synthase (iNOS), interleukin 1 and interleukin 8, gamma interferon, tissue necrosis factor alpha and the 70 Kda heat shock protein (hsp70). In HSB these gene sequences have not been reported in SwissProt or GenBank, but the sequences of related fish species are available. For each gene, consensus sequences from related fish will be used to create degenerate PCR primers for subsequent cloning and sequencing of the orthologous HSB gene. Once the hybrid striped bass gene sequence has been determined, quantitative PCR primers and probes will be developed in the coming months.

Objective 2: Determine the immune response kinetics of hybrid striped bass 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 injection challenge model has been completed for both *S. iniae* and *A. hydrophila* infections in hybrid striped bass. A standard challenge model for infection of rainbow trout with *S. iniae* was not completed. 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.

No further research was conducted in Year 4.

Supplemental Research Generated From Data Obtained During Year 3

During Year 3, our research efforts to develop a standardized A. hydrophila challenge model in both HSB and rainbow trout, led us to conclude that a much greater dose (than was expected) of this pathogen was required to produce mortality in both species. Based on this finding, additional research has

been conducted in rainbow trout to determine whether fish that survived experimental exposure to A. hydrophila had developed sufficient immunological memory to provide protection following a subsequent reexposure to a virulent isolate.

The results from this research indicate that survivors of a previous A. hydrophila infection are almost completely resistant to reinfection by the same pathogen and the relative protection did not appear to be dependent on the dose of bacteria used in the primary challenge. Furthermore, we have found that passive immunization of rainbow trout with hyper-immune rainbow trout serum can provide significant levels of protection against challenges with lethal concentrations of A. hydrophila. This information will be useful in the development of an efficacious vaccine. In the next series of studies a killed A. hydrophila vaccine will be produced and fish will be immunized intraperitonealy or via the dorsal sinus route and evaluated for protection using the A. hydrophila standardized challenge procedure.

Subtask 3. Determine the LD50 of S. iniae and A. hydrophila to HSB and rainbow trout

The LD50 of *S. iniae* for HSB was estimated to range from 2.95 x 105 to 3.93 x 105 (mean 3.44 x 105) CFU in a 0.1 ml injection volume. The LD50 of *A. hydrophila* for HSB was estimated to range from 5.53 x 106 to 4.15 x 106 (mean 4.84 x 106) CFU in a 0.1 ml injection volume. The LD50 of S. iniae was not determined. The LD50 for A. hydrophila in rainbow trout has been estimated at 3.37 x 107 CFU.

Task 2. Compare immune response and cytokine gene expression of hybrid striped bass 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.

The polyclonal antibodies raised against the HC of HSB IgM were unsuitable for an ELISA. Our preliminary efforts to optimize the ELISA for HSB antibodies using the commercially available monoclonal antibody left insufficient time to study the effects of hatchery practices on immune functions of rainbow trout and hybrid striped bass therefore this Objective was not addressed during Year 4.

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.

As indicated in the Annual Report for Years 2-3, we tested two proprietary methods for mass vaccination of finfish developed by Northwest Marine Technologies, the world leader in the design and manufacture of equipment for the automated mass coded wire tagging of salmonids. This collaborative research provided the first proof of principle for the safety and efficacy of these methods under laboratory conditions. No research on this Objective was planned for Year 4; however, with permission of Northwest Marine Technologies, we are preparing an outreach product to highlight this work.

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

Based upon the progress in Objectives 1 through 4, we have prepared outlines or revised drafts for three outreach products for this project. These are:

1. Measurement of the innate cellular immune responses of hybrid striped bass and rainbow trout (Appendix 1).
2. Enzyme-linked Immunosorbent Assay (ELISA) for assessing the humoral immune response of rainbow trout and hybrid striped bass (Appendix 2).
3. Testing of novel mass delivery methods for fish vaccines (Appendix 3).

A fourth outreach product is planned once the macrophage killing assay is developed. A tentative title will be: Development of a macrophage killing assay for assessing the cellular immune status of rainbow trout and hybrid striped bass.

During an Immunology Working Group teleconference held in Year 3, our TA proposed a potential fifth outreach product that would overview the most common and economically significant diseases of warmwater fish reared in the Western region. The intended target audience for this type of product could be tailored towards is aquaculturists, fishery biologists, resource managers, and students as well as the general public. Further plans to assess the suitability of disseminating this work will be discussed in the next 4 months.

USEFULNESS OF FINDINGS

From a functional fish immunology perspective, this research has begun to identify many similarities and differences among the rainbow trout and hybrid striped bass immune system. What we realized all too well is that a tried-and-true assay developed for rainbow trout may not necessarily be developed as easily for HSB. 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 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 HSB are in the process of being made available to the research community and the aquaculture sector.

WORK PLANNED FOR NEXT YEAR

Not applicable, project funding exhausted.

IMPACTS

It is anticipated that the outreach components of this project will enable interested individuals to conduct relevant immune response measurements in hybrid striped bass and rainbow trout utilizing some of the approaches and tools developed during this project.

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	
2003	100,000	7,000 a	25,530 b	25,000 c		$157,530
2004	100,000	7,000 a	25,530 b	25,000 c	1,000 d	$158,530
2005	100,000	7,000 a	25,530 b	25,000 c		$157,530
2006	100,000	7,000 a	25,530 b	25,000 c	2,000 d	$159,539
Total	100,000	28,000	102,120	100,000	3,000	$633120

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 Northwest Marine Technologies
e. in-kind contribution (reagents, salary) from Aquatic Diagnostics Limited, Stirling Scotland

4th National Aquaculture Extension Workshop and Conference

Project Termination Report for the Period September 1, 2006–August 31, 2007

Funding Level $18,200 (January 1, 2006–December 31, 2007)

Participants	Kevin Fitzsimmons	University of Arizona	Arizona
	Raymond LaLonde	University of Alaska	Alaska
	Steve Harbell	Washington State University	Washington
	Gary Fornshell	University of Idaho	Idaho
	Jim Bergeron	Oregon State University	Oregon
	James Bennage	Sheridan College	Wyoming
	Fred Conte	University of California, Davis	California
	Jon Boren	New Mexico State University	New Mexico
	Chris Myrick	Colorado State University	Colorado
	Terry Messmer	Utah State University	Utah
National Steering Committee			
	Steve Harbell	Washington State University	Washington
	Charles Pistis	Michigan State University	Michigan
	Michael Rice	University of Rhode Island	Rhode Island
	Michael Schwarz	Virginia Tech University	Virginia
	George Selden	Univ. of Arkansas–Pine Bluff	Arkansas
	Clyde Tamaru	University of Hawaii	Hawaii
Conference Ex-Officio Advisory Committee			
	Kevin Fitzsimmons	University of Arizona	Arizona
	Gary L. Jensen	USDA Coop. State Research Education, and Ext. Service	Washington, DC
	Andy Lazur	NOAA National Sea Grant College Program	Washington, DC
	Maxwell H. Mayeaux	USDA Coop. State Research, Education, and Ext. Service	Washington, DC
	Joseph E. Morris	Iowa State University	Iowa
	James Murray	NOAA National Sea Grant College Program	Washington, DC
	Graham Young	University of Washington	Washington

1 WRAC has provided funding along with the four other Regional Aquaculture Centers for four national aquaculture extension meetings; the first was called a National Aquaculture Extension Workshop whereas the second through fourth were called National Aquaculture Extension Conferences. This termination report is for the fourth meeting which was held April 30-May 4, 2007 in Cincinnati, Ohio.

REASON FOR TERMINATION
The project objectives were completed.

PROJECT OBJECTIVES
1. Learn successful approaches to problem-solving through case studies that can be replicated in other states (i.e., lessons learned).
2. Demonstrate and conduct hands-on experience with state-of-the-art computer applications for improving delivery of extension programs.
3. Identify national extension priorities and critical issues with development of corresponding action plans for implementation.
4. Identify potential interregional extension projects, such as curriculum development or national decision-support databases.
5. Share educational materials and programs in addition to expertise.
6. Strengthen regional and national communication networks to improve services to our clientele.
7. Examine successful extension components and outcomes to research projects and develop approaches to improve integration nationwide.
8. Develop a collective strategy to define extension's role in measuring impacts of RAC projects and collaboration with others in academia and the private sector.
9. Improve business management skills related to aquaculture and enhance knowledge concerning marketing of aquatic products.

PRINCIPAL ACCOMPLISHMENTS
The 4th National Aquaculture Extension Conference was organized by a national Steering Committee which was comprised of representatives from each RAC, the National Association of County Agriculture Agents, US Department of Agriculture's Cooperative Research, Education, and Extension Service, and the National Office of Sea Grant.

The conference was held in Cincinnati, Ohio, April 30–May 4, 2007. 83 people from 38 states and territories attended. WRAC participants included Graham Young, Fred Conte, Steve Harbell, Ray LaLonde, Gary Fornshell, and Kevin Fitzsimmons.

Multiple presentations made during the conference addressed many of the objectives. Two workshops, dealing with the media and working with *www.grants.gov*, helped attendees gain new skills. The key note speaker, Dr. Steve Otwell from the University of Florida, spoke on seafood marketing and consumption issues, identified as a critical issue by the Steering Committee.

A field trip to Jungle Jim's, a large, unique international market northwest of Cincinnati, Ohio that employs re-circulating technology to hold live fish, improved attendees' knowledge concerning marketing of aquatic products. Socials and local tours gave attendees time to share programming ideas and expertise.

A website (*http://southcenters.osu.edu/aqua/extension%20conference/extension%20conference.htm*) with the conference agenda linked to abstracts, attendee list, and photos was created and made accessible to all.

RECOMMENDED FOLLOW-UP ACTIVITIES
Attendees will be sent a post-conference evaluation survey to determine how the conference has impacted their actions or activities. Plans for the next conference are being coordinated by the Steering Committee.

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		University	Industry	Other Federal	Other	Total	
2006–07	**18,200**			**20,000**			**$38,200**
Total	**18,200**			**20,000**			**$38,200**

* Each of the four other Regional Aquaculture Centers contributed $5,000 to the conference and some travel grants were supported by NOAA's National Sea Grant College Program. WRAC provided travel grants to WRAC participants as well as providing $5,000 towards conference costs.