WRAC

Western Regional Aquaculture Center

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

ANNUAL ACCOMPLISHMENT REPORT

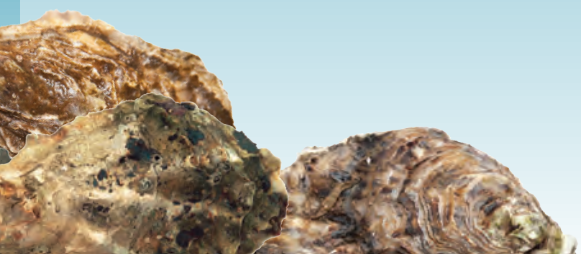
SEPTEMBER 1, 2010 TO AUGUST 31, 2011

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

MARCH 2012

USDA NIFA

United States Department of Agriculture
National Institute of Food and Agriculture



INTRODUCTION

This Annual Accomplishment Report for the Western Regional Aquaculture Center (WRAC) covers progress made from Sept. 1, 2010 through Aug. 31, 2011. WRAC is one of five regional aquaculture centers under the National Institute of Food and Agriculture, United States Department of Agriculture (NIFA/USDA). The Regional Aquaculture Centers are awarded funding from NIFA/USDA to support research, development, and demonstration projects in aquaculture. WRAC encompasses the 12 states in the Western Region of the United States—Alaska, Arizona, California, Colorado, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington, and Wyoming.

ACKNOWLEDGMENTS

WRAC acknowledges the contributions of the Principal Investigators and Participating Scientists involved in the projects reported in this 24th Annual Progress Report. Members of the WRAC Board of Directors, Industry Advisory Council (IAC), and Technical Committee (TC) have provided valuable input to the successful operation of WRAC during the past year. We particularly appreciate the assistance of the chairs of our Board, the IAC, and the TC, and those serving as Project Monitors.

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

Additionally, we thank the School of Aquatic and Fishery Sciences in the College of the Environment at the University of Washington for serving as the Host Institution for WRAC.

ORGANIZATIONAL STRUCTURE

Board of Directors

With representation from every land-grant institution from the 12 states in WRAC as well as one representative each from the IAC and the two subcommittees of the TC, the Board is the primary policy-making body for WRAC. The Board reviews and appoints members to the IAC and TC. The Board also reviews recommendations from the IAC/TC and approves projects for funding and inclusion into the annual Work Plan.

Industry Advisory Council

Composed of representatives of the industry and associated services, covering multiple sectors and geographic regions within the 12 WRAC states.

Technical Committee

Composed of two subcommittees:

- The ***Research subcommittee*** includes representatives from participating research institutions, state or territorial public agencies as appropriate, as well as nonprofit, private institutions.
- The ***Extension subcommittee*** includes representatives from state Extension Services—both Land Grant and Sea Grant.

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

PROGRESS REPORTS

Since the start of the regional aquaculture programs, WRAC has processed 24 Annual Work Plans (FY'87 through FY'11 funding) through NIFA/USDA. This current annual report covers the activities of the WRAC Administrative Center and progress made during the 24th year on all projects through August 31, 2011, listed below with funding levels for FY'11.

ANNUAL REPORTS

A. Cost-effective, Alternative Protein Diets for Rainbow Trout that Support Optimal Growth, Health, and Product Quality
   2nd project year: $118,645
B, Optimizing Dietary Protein and Energy Utilization to Improve Production Efficiency of Tilapia in the Western United States
   2nd project year: $66,947

TERMINATION REPORTS

A. Coldwater Disease Prevention and Control through Vaccine Development and Diagnostic Improvements
B. Determining Ripeness in White Sturgeon Females to Maximize Yield and Quality of Caviar

PROJECT REVIEW & DEVELOPMENT

Annual Budget: $58,000

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

PUBLICATIONS

Annual Budget: $24,000

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

ADMINISTRATIVE SUPPORT

FY'11 FUNDING LEVEL $191,619

The Administrative Center is located in the School of Aquatic and Fishery Sciences, College of the Environment at the University of Washington, which serves as the host institution. WRAC Administrative Center staff provide all necessary support services to the Board of Directors (Board), 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 24 Annual Work Plans (FY'87 through FY'11) to date for the various WRAC projects. Activities of the Administrative Center and funding for its operation rely upon the annual decisions of the Board prior to inclusion in the work plan.

The Administrative Center assists project Work Groups with the preparation of proposals, which, upon acceptance by WRAC, are included in the funding agreement between the National Institute of Food & Agriculture/United States Department of Agriculture (NIFA)/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 NIFA/USDA.

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

The Administrative Center also publishes *Waterlines*, an annual newsletter that has a mailing list of more than 2,400 recipients. *Waterlines* provides information on WRAC projects and general aquaculture news in order to educate the public on the importance of aquatic animal husbandry, as well as other WRAC activities, and to keep industry informed about current research projects and initiatives.

The Administrative Office has worked to significantly upgrade the WRAC website, which is available at *http://fish.washington.edu/wrac/*.

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

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

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

WRAC Board of Directors at NOAA Manchester Lab. Left to right: Kevin Fitzsimmons, Jerri Bartholomew, Steve Harbell, Chris Wilson, Walt Dickhoff, Ray RaLonde, Graham Young, Rossana Sallenave, Randy Robinette, Jeff Hetrick Debbie Granger

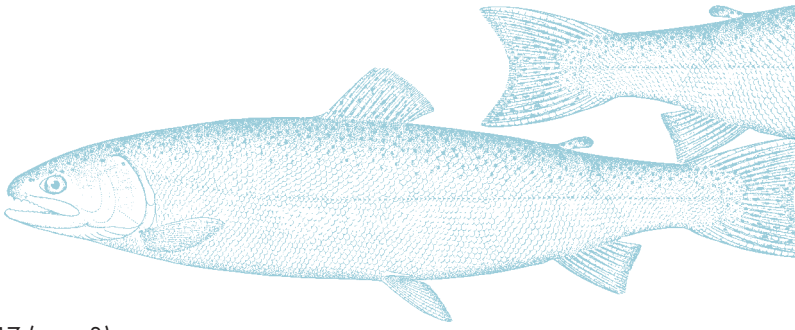
Cost-Effective, Alternative Protein Diets for Rainbow Trout that Support Optimal Growth, Health, and Product Quality

REPORTING PERIOD

September 1, 2010–August 31, 2011

AUTHOR

Wendy M. Sealey



FUNDING LEVEL

$119,864 (year 1); **$118,645 (year 2)**; $118,317 (year 3)

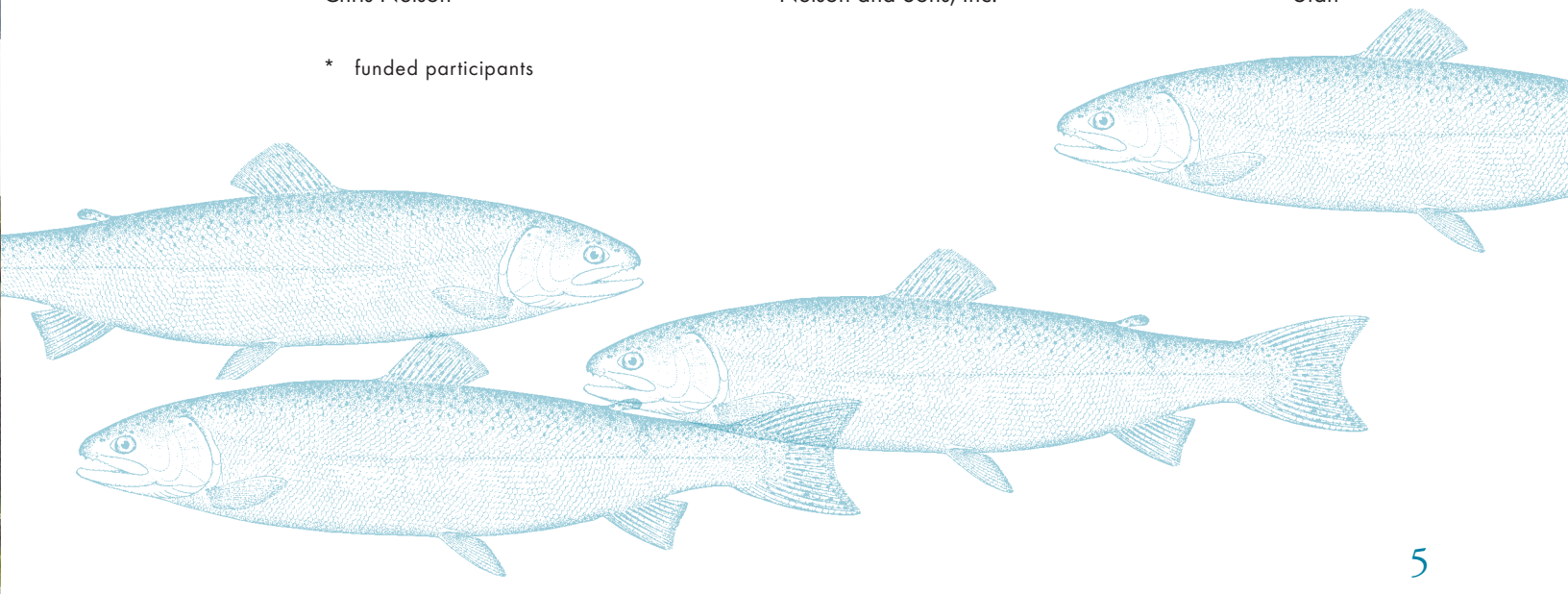
PARTICIPANTS

Wendy M. Sealey*	US Fish & Wildlife Service (USFWS), Bozeman Fish Technology Center	Montana
Carolyn Ross*	Washington State University	Washington
Christopher A. Myrick*	Colorado State University	Colorado
T. Gibson Gaylord*	USFWS, Bozeman Fish Technology Center	Montana
Frederic T. Barrows*	US Department of Agriculture, Agricultural Research Service Trout Grains Project	Idaho
Gary Fornshell* (*Outreach Coordinator*)	University of Idaho Extension	Idaho

PROJECT MONITOR

Chris Nelson	Nelson and Sons, Inc.	Utah

* funded participants

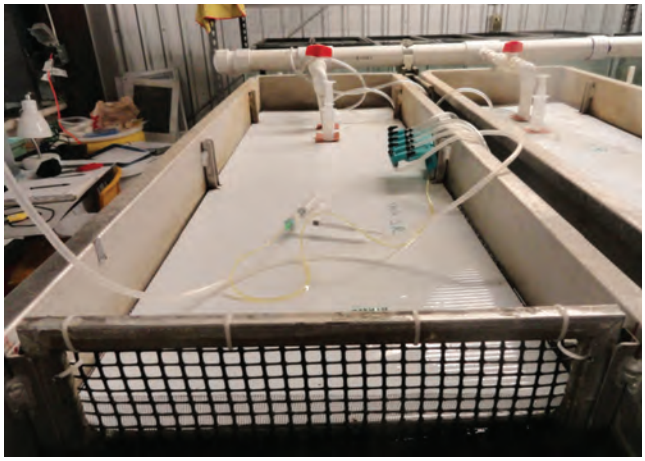


PROJECT OBJECTIVES

1. Identify commercially available alternate ingredient combinations that can meet the production needs of rainbow trout.
   a. Chemical analyses of alternative ingredients, USFWS-Gaylord (year 1–completed)
   b. Digestibility of alternative ingredients in extruded diets, USFWS-Gaylord and Sealey and USDA-Barrows (year 1–completed)
   c. Preliminary growth and respirometry trials with blended alternative feedstuffs, Colorado State University (Colorado State), USFWS, and USDA (year 1—ongoing).
2. Refine alternative feedstuff blends and examine the benefits of amino acid supplementation, Colorado State, USFWS-Gaylord, USDA (year 2).
3. Examine the effects of alternative feedstuffs on product quality and fish health.
4. Conduct on-farm trial of alternative feedstuff formulations at Magic Springs Farm, SeaPac of Idaho, (year 3).

5–8. Outreach, University of Idaho, (years 1–3):

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



Whole tank respirometer.

Courtesy of Alternative Protein Project Work Group

ANTICIPATED BENEFITS

Objective 1a and 1b. The aquaculture industry will be able to formulate trout diets using the alternative ingredients tested on an equal digestible nutrient basis. The data from this year's studies (Objective 1c) will further that knowledge, provide an increased understanding of the amino acid needs of trout, and identify appropriate ingredient combinations to meet those needs. Taken together, the outcome will increase the ability to produce effective feeds while maintaining fish growth, health, and product quality.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1a. Chemical analysis of alternative ingredients—*completed*

Objective 1b. Digestibility of alternative ingredients in extruded diets—*completed*

Analyses of the nutrient composition of commercially available and novel ingredients evaluated in digestibility trials in year 1 were completed. Proposed digestibility trails have been completed; evaluation of other potential ingredients, including algae and improved plant ingredients (low gossypol cottonseed meal) continue as funding allows, on a cost-sharing basis by USDA, ARS, and USFWS. Results from these completed WRAC-funded evaluations and co-supported analyses have been compiled into a database: "Nutrient digestibility of fish feed ingredients," available through *rick.barrows@ars.usda.gov*.

Objective 1c. Preliminary growth and respirometry trials with blended alternative feedstuffs—*ongoing*

Objective 2. Refine alternative feedstuff blends and examine the benefits of amino acid supplementation—*ongoing*

In order to assess ingredient/diet palatability and growth potential of fish fed the test ingredients identified and analyzed in Objectives 1a and 1b, a preliminary feeding trial was conducted using a plant-based feedstuff (high-protein dried distillers grains [HPDDG]); concurrently, two feeding trials at Bozeman Fish Technology Center (Bozeman Center) and Colorado State are currently underway using a blend of plant- and animal-based feed ingredients defined in year 1 to address Objectives 1c and 2 simultaneously.

DDGS Trial at Bozeman Center—*completed*

Experimental Design: A 2 x 2 factorial feeding trial that examined protein source (Menhaden fishmeal [MFM] or HPDDG)

and mycofix supplementation (yes or no) was conducted; a control diet (40% digestible protein, 20% crude lipid) was compared to a test diet, in which HPDDG completely replaced MFM (18% inclusion) on a digestible protein basis. Diets were balanced for available lysine, methionine, threonine, and total phosphorus. Biofix plus was supplemented (0.2%) to subsamples of each protein-base diet via vacuum-assisted top coating in the dietary oil portion. All four diets were then fed to four replicate tanks per treatment of juvenile rainbow trout, initial weight (39.2g + 1.0g) for nine weeks in a 15°C recirculating system. Bulk fish weight and feed intake were recorded every three weeks. At nine weeks, three fish per tank were sampled for proximate composition.

Results: Analyzed protein ADCs were 81%, 88%, and 83% for Wentworth, Valero, and HPDDG, respectively. However, rainbow trout growth performance results demonstrated significant negative effects of complete fishmeal replacement by HPDDG on growth (P=<0.0002) and FCR (P=<.0001). In contrast, no significant benefit of Biofix plus supplementation or significant interaction between protein source and Biofix plus supplementation was observed.

Conclusions: The protein ADCs and amino acid AACs of HP-DDG, coupled with its higher protein content relative to other DDG products, suggested an increased potential to replace fishmeal in rainbow trout diets. However, even when diets were balanced for digestible protein, lysine, methionine and threonine and supplemented with Mycofix, growth performance was still compromised, indicating that additional research is necessary to elucidate alternative explanations for rainbow trout's limited ability to utilize high dietary levels of DDGs.

Blends with supplemental AA Trials at Bozeman Center and Colorado State (Objectives 1c and 2—*ongoing*)
Experimental Design: Fish from a common lot were obtained as in-kind industry support from Trout Lodge in December, 2010. The fish were divided and sent to Bozeman Center and Colorado State. A defined starter diet was then formulated and produced by Bozeman Center staff to ensure common dietary history until the study could be initiated. Ten test diets were formulated and manufactured in adequate quantities to support the concurrent feeding trials at both locations. At conclusion of the feeding trials (mid-September), fillet samples will be obtained for assessment of quality (Objective 3a); blood and histology samples will also be obtained to assess dietary effects on fish health (Objective 3b).

Rainbow Trout (*Oncorhynchus mykiss*).

Eric Engbretson, Eric, US Fish and Wildlife Service

Diet Formulation and Manufacture: Diets were formulated on a digestible-energy and available-amino-acid basis as a 5 x 2 factorial experiment. Five ingredient combinations were used, consisting of: 1) (fishmeal diet, FMD) Menhaden fishmeal special select, soybean meal 48%CP, corn protein concentrate, poultry by-product meal, pet food grade, and blood meal; 2) (animal product diet, APD), poultry by-product meal—pet food grade, soybean meal 48%CP, corn protein concentrate, feather meal, blood meal; 3) (plant product diet, PPD) soy protein concentrate, corn protein concentrate, and soybean meal 48%CP; 4) (novel plant protein diet-NPD) soy protein concentrate-Hamlet protein, corn protein concentrate, and high protein distillers dried grains; 5) (plant products with future potential-PFP) ultralow oligosaccharide defatted soybeans, spirulina, corn protein concentrate, barley protein concentrate. Two nutrient concentrations were targeted: 1) To meet amino acid targets of Rainbow trout (Hardy 2002) using approximately 45% crude protein (40–42% digestible protein) and 2) To meet the ideal amino acid balance of rainbow trout muscle for Lys, Metm, and Thrm using approximately 40% crude protein (37–38% digestible protein).

Diets were formulated on an available-amino-acid-basis using a mixture of protein feedstuffs defined in objective 1b for which amino acid availabilities are known. All diets were manufactured at Bozeman Center laboratory, using a twin-screw cooking extruder and dried to a final moisture level of less than 7%.

Bozeman Center study: Each diet was randomly assigned to three tanks of fish. Fish were fed by hand to apparent satiation three times each day, 6 days per week for a total of 12 weeks.

All fish within a tank are being counted and weighed as a group every 3 weeks.

Colorado State study: Each diet was assigned randomly to three tanks containing 15 fish. Fish were individually marked using a visual implant elastomer tag with a unique 3-digit alpha-numeric code, implanted in the eyelid adipose tissue. Fish were fed by hand to apparent satiation twice each day, 7 days per week, for a total of 12 weeks. All fish within each tank were individually identified, measured, and weighed every three weeks. This feeding trial will conclude in Oct. 2011, with respirometry trials conducted at 6 weeks post-feeding and 12 weeks post-feeding.

Preliminary Results Bozeman Center: Significant effects of ingredient blends, nutrient concentration target, and interactions are being observed. At 9 wks post-feeding, neither adjusting the nutrient targets for the fishmeal-based diets nor the animal product diets appear to affect fish performance. Adjustment of nutrient targets does improve performance of trout fed the three diet combinations based on plant ingredients. The improvements in growth when amino acids are supplemented to an ideal protein basis make fish performance when fed the PPD and PFP diets equivalent to FMD. Fish fed the APD and NPD diets supplemented to ideal protein levels had reduced performance.

Preliminary Results Colorado State Study: Differing growth rates are being observed among the fish being fed the 10 different blends and nutrient target combinations. Comparing these results to the Bozeman work is not yet possible because the Colorado State researchers are blinded to the diet formulations. However, at 6 wks post-feeding, diets 8, 5, and 2 are exhibiting the highest growth rates, ranking 1, 2, and 3 respectively. Fish fed diet 1 appear to be experiencing reduced performance. Current differences among most treatments means are not statistically significant at the P=0.05 level. Data describing fish performance at 9 wks post-feeding will be collected on 17 Sept. 17, 2011.

Objective 5. Develop a project website on the WRAC homepage and update regularly—*ongoing*

Gary Fornshell has scheduled a trip to Spokane in early fall to develop a project website.

Objective 6. Present research results in cooperation with field day and meetings—*ongoing*

Wendy Sealey has presented preliminary research results at USTF (2010) and will again at USTF (2011). A graduate student supported by the project, Chris Hooley, will present results of the DDG feeding trial and Gibson Gaylord will present results of the Bozeman Center blend trial at the Fish Feeds Nutrition Workshop in Arkansas in September and at USAS in Las Vegas in February.

Objective 7. Develop at least one WRAC Extension publication—*completed*

Work group members drafted and edited an extension publication, *"Evaluating ingredients for aquafeeds,"* that Gary Fornshell submitted to the WRAC office in August 2011.

Objective 8. Visit feed manufacture plants in the Western Region to present project results—*ongoing*

One directed site visit to Silver Cup Feeds in Tooele, Utah, was conducted in association with the annual Work Group meeting. Another directed site visit to Rangen Feeds has been proposed in association with the USTF meeting in Twin Falls, Idaho, in September.

USEFULNESS OF FINDINGS

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

WORK PLANNED FOR NEXT YEAR

Refinement of feedstuff blends as described in Objective 2 is currently being assessed simultaneously with Objective 1c and will continue based on results from the ongoing trial. Specifically, two additional feeding trials will be performed at Colorado State and

Bozeman Center (estimated start November 2011) to determine an optimized blend of ingredients and amino acid supplementation to maximize nutrient utilization and growth. Effects of these blends on nutrient assimilation and metabolic demands will be measured as previously described.

Based on the results of the ongoing study and the two additional feeding trials proposed for early year three, the on-farm trial (Objective 4) of alternative feedstuff formulations at Magic Springs Farm, SeaPac of Idaho will be started.

During the third year of the project, work to address the extension and outreach objectives will also continue. Specifically, the project website will be completed and additional directed site visits to trout producers and fish feed manufacturers will be conducted.

IMPACTS

To aide in conducting studies, one MS student at Colorado State under the direction of Chris Myrick (Chris Craft), one PhD student at Montana State University under the direction of Wendy Sealey (Omolola Betiku), and one technician under the direction of Gibson Gaylord have been fully or partially funded by the project.

PUBLICATIONS, MANUSCRIPTS, AND PAPERS PRESENTED

Papers presented

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

Sealey WM, Gaylord TG, Barrows FT, Ross C, Myrick C, Fornshell G. Alternative protein research in rainbow trout. USTFA Meeting, Twin Falls, ID, September 2011.

Outreach publications

Fornshell G. Evaluating aquafeed ingredients, submitted WRAC Office.

Abstracts submitted

Hooley CG, Rosenstrater KA, Gaylord TG, Barrows FT, Sealey WM. Examination of the effect of a mycotoxin deactivation product to improve growth and nutrient utilization in juvenile rainbow trout *Oncorhynchus mykiss* fed high protein distiller's dried grains. Fish feed nutrition workshop, Pine Bluff, AR, September 2011.

Hooley CG, Rosenstrater KA, Gaylord TG, Barrows FT, Sealey WM. Examination of the effect of a mycotoxin deactivation product to improve growth and nutrient utilization in juvenile rainbow trout *Oncorhynchus mykiss* fed high protein distiller's dried grains", USAS, Las Vegas, NV, February 2012.

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		UNIVERSITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
2010	119,864						119,864
2011	118,645		Troutlodge fish for studies(1000) Ingredient donations (4000)	46,380			170,025
TOTAL	238,509			46,380			289,889

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

REPORTING PERIOD

September 2, 2010–September 16, 2011

AUTHOR

Wendy M. Sealey

FUNDING LEVEL

$66,704 (Year 1
$66,947 (Year 2
$67,728 (Year 3)

PARTICIPANTS

Principal Investigators

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

PROJECT MONITOR

Chhorn E. Lim	USDA ARS Aquatic Animal Health Laboratory	Alabama

INDUSTRY ADVISOR

Tark Rush	Desert Springs Tilapia	Arizona

* funded participants

PROJECT OBJECTIVES

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

ANTICIPATED BENEFITS

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

PRINCIPAL ACCOMPLISHMENTS

Objective 1a—completed

In collaboration with the industry, common stocks and appropriate lines of tilapia for use in studies both in Arizona and Montana were identified. Prior to initiation of the project, PI (Wendy Sealey) relocated from University of Idaho in Hagerman, Idaho to the USFWS, Bozeman Fish Technology Center (Bozeman Center) in Montana, in late August, 2009. Wendy Sealey's move to Montana necessitated pursuing permitting approval for tilapia from the State of Montana prior to their importation. Exotic Species Permit (for research use) approval was granted to Sealey and the Bozeman Center on April 7, 2010. Fish arrived in Bozeman in June 2010.

Objective 1b—completed

Experimental diets have been formulated as proposed. Diet manufacture for both Bozeman Objective 1c studies and the University of Arizona small fish trial Objective 1c is complete. Diets were manufactured at the Bozeman Center using cooking extrusion (DNDL-44, Buhler AG, Uzwil, Switzerland) and dried in a pulse bed drier (Buhler AG, Uzwil, Switzerland).

Objective 1c (Bozeman Center and University of Arizona, Year 1) As previously described, researchers at both Bozeman Center and University of Arizona will test the diets produced in objective 1b in late year 1 and early year 2 for their ability to promote growth in two different size classes of tilapia. Specifically, one size class will evaluate the ability of the diets to meet the needs of small tilapia (one gram to ten grams) when first transitioned from starter feed to a grow-out diet while the other size class will represent the last portion of the grow-out cycle (200 g to 600 g).

Objective 1c (Bozeman Center progress, small fish study, completed) A 3 X 3 factorial design was used with practical-type diets formulated to contain three levels of dietary protein (28%, 32%, and 36%) and three levels of dietary lipid (3%, 6%, and 9%). Juvenile tilapia (34.5 ± 0 .4 g initial weight) were fed one of the nine diets, three feedings/d to apparent satiation, six d/wk for 12 wks. Fish were weighed and counted every three weeks and feed consumed recorded weekly. At the conclusion of the feeding trial, three fish per tank were sampled for proximate composition analyses. One week post-conclusion of the feeding trial, tilapia remaining in each tank were subjected to a simulated live haul in which fish were transferred to a static water insulated container (2 lbs/gallon) with supplemental oxygen for 24 h, and then returned to their source tank and allowed to recover for an additional 48 h. Hematocrit, glucose, lactate, and cortisol measurements were collected at time 0, 24 h, and 72 h.

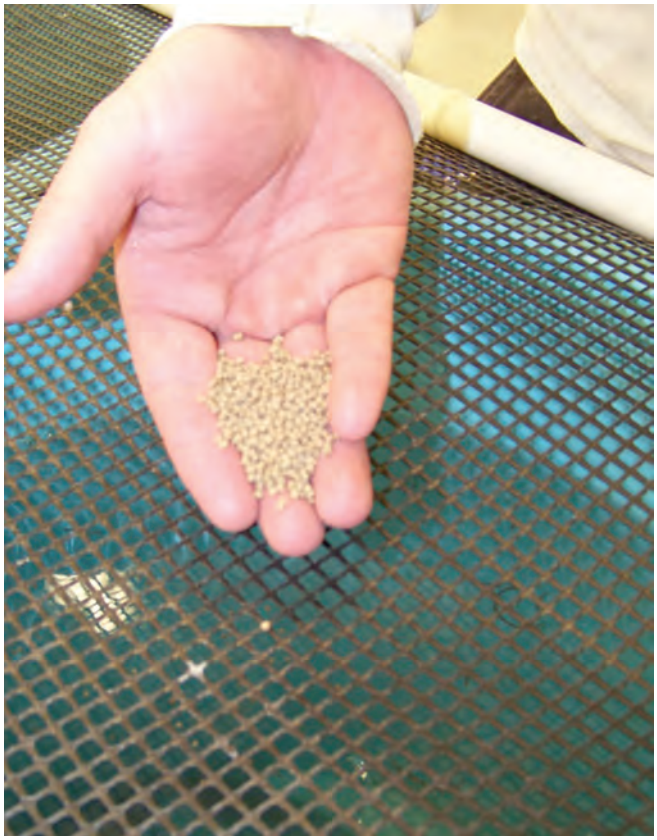
Increasing dietary protein significantly improved tilapia weight gain (P=0.01), feed conversion (FCR, P=0.03), feed intake (P=0.02), protein retention (P=0.01), and fillet yield (P=0.01). Increasing dietary lipid also significantly improved weight gain (P=0.05) and FCR (P=0.01), but at 9% decreased feed intake (P=0.02). Blood chemistry values were also altered by dietary protein and lipid levels. No significant interactions between dietary protein and lipid levels on growth performance or blood chemistry values were observed. Results of this study indicate a lack of dietary protein sparing by increasing

Extruded tilapia feeds produced at the Bozeman Fish Technology Center.

Optimizing Dietary Protein Project Work Group

dietary lipid at the levels investigated. Further, these data suggest that while increasing protein and lipid levels in tilapia diet formulations can improve tilapia production in high intensity systems, stress tolerance during live hauls may be reduced.

Objective 1c (Bozeman Center progress, large fish study, ongoing—end date October 10)
The same 3 X 3 factorial design described above was used; practical-type diets formulated to contain three levels of dietary protein (28%, 32%, and 36%) and three levels of dietary lipid (3%, 6%, and 9%). Mixed-sex tilapia (130± 4 g initial weight) were cultured as described above and fed one of the nine diets, two feedings/d to apparent satiation, six d/wk for 18 wk. Fish are weighed and counted every three weeks and feed consumed recorded weekly. At 18 wks post-feeding, fish will be sampled and exposed to the live-haul stress regime described above.



Graduate student Chris Hoole feeding tilapia.

Optimizing Dietary Protein Project Work Group

Objective 1c (University of Arizona progress, small fish study, ongoing—end date November)
Small tilapia were stocked into 200-liter tanks and fed each of the experimental test diets in a common recirculating system where each receives the same water supply inside a temperature controlled greenhouse. Twenty-five (25) fish were group-weighed and randomly stocked into each of the replicate tanks (25 fish per replicate x 3 reps per diet). Initial feeding rate was 8% of biomass per day and was split into three feedings per day. Intermediate weights (25 fish per tank) are being determined every 14 days, and the feeding rate and amount are adjusted for each treatment as appropriate. Water quality characteristics (dissolved oxygen, temperature, pH, ammonia-N, and nitrate-N) will be determined and recorded on a regular basis. Each test diet produced will be fed to the triplicate groups of tilapia for a minimum of three months. At the conclusion of the growth trial, nutrient retention efficiencies for each of the various diets will be determined. Similar protocols will be followed and the feeding trial repeated with the larger size class of tilapia with stocking densities reduced accordingly to maintain suitable water quality.

USEFULNESS OF FINDINGS

Preliminary results of the small fish studies indicate a lack of dietary protein sparing by increasing dietary lipid at the levels investigated. Further, these data suggest that while increasing protein and lipid levels in tilapia diet formulations can improve tilapia production in high intensity systems, stress tolerance during live hauls may be reduced.

WORK PLANNED FOR NEXT YEAR

Objective 2: Evaluate the ability of vitamin supplementation to improve growth performance at different protein:lipid ratios

Objective 2a: (University of Idaho & USDA, ARS, Year 2–3). A vitamin premix that meets the minimum published requirements for tilapia will be formulated (NRC 2011). Those vitamins that have been reported to improve performance of tilapia in high intensity systems will then be added to a minimum of two of the diet formulations showing the most promise from year 1 results (small fish diet, 36:9 g; large fish diet, size to be determined). Vitamins to be examined include vitamin B6, niacin, vitamin C, and vitamin E. Each vitamin will be supplemented at deficient, defined requirement and super-requirement levels both individually and as a combined supplementation.

Objective 2b: (Bozeman Center and University of Arizona, Year 2–3) One growth trial will be conducted as previously described in Objective 1c for each institution. At the conclusion of the growth trial additional fish sampling will be conducted to define vitamin status. Specifically, fish in all treatments will be dissected and examined for signs of vitamin deficiency. Additionally, tissue levels of tested vitamins will be determined.

Objective 3: Evaluate formulations identified with potential by laboratory testing in pilot-scale on-farm trials (Year 3 post-conclusion of the vitamin trials)

Objective 3a: (Bozeman Center and USDA, ARS) Formulate practical-type diets that reflect the beneficial results of the year 1 and 2 experimental trials. Facilitate commercial production of the test formulations for Objective 3b.

Objective 3b: (University of Arizona and Desert Springs, Year 3) Conduct on-farm trials with at least two formulations showing promise in the laboratory trials and include appropriate commercial controls. Desert Springs Tilapia in Hyder, Arizona, will serve as the industry partner. In collaboration with this partner a replicated trial will be conducted with tilapia from fry to harvest.

Objective 4: Work to address the extension and outreach portion of the grant will begin. Specifically, a project webpage with preliminary results will be developed.

IMPACTS

The first year's results provide justification for further evaluation of higher protein and lipid levels than typically used in pond culture for intensively raised tilapia. To date, two MS students are currently being trained through the project: one at University of Arizona and one at Montana State University.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

Hooley CG, Barrows FT, Paterson JA, Sealey WM. 2011. (Poster presentation) Optimizing dietary protein and lipid levels of tilapia (*Oreochromis niloticus*) cultured in high intensity systems. Montana Nutrition Conference, Bozeman, MT. (Won 3rd place poster).

Hooley CG, Barrows FT, Paterson JA, Sealey WM. 2011. (Oral presentation) Examination of the effects of dietary protein and lipid levels on growth and stress tolerance of tilapia *Oreochromis niloticus*. Fish Feeds and Nutrition Workshop, Pine Bluff, AR (abstract submitted)

Hooley CG, Barrows FT, Paterson JA, Sealey WM. 2011. (Oral presentation) Examination of the effects of dietary protein and lipid levels on growth and stress tolerance of tilapia *Oreochromis niloticus*. USAS 2012, Las Vegas, NV. (abstract submitted)

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		UNIVERSITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
2010	66,704						66,704
2011	66,947			46,777			113,724
TOTAL	133,651			46,777			180,428

Coldwater Disease Prevention and Control through Vaccine Development and Diagnostic Improvements

TERMINATION REPORT

PROJECT WORK PERIOD

May 1, 2008–present; no-cost extension approved through September 30, 2012

AUTHOR

Ken Cain and Doug Call

FUNDING LEVEL

$81,555 (first-year budget)
$80,043 (second-year budget)
$81,637 (third-year budget)
$81,639 (fourth-year budget)

PARTICIPANTS

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

PROJECT MONITOR

Gael Kurath	US Geological Survey	Washington

INDUSTRY ADVISOR

Jim Parsons	Troutlodge, Inc.	Washington

GRADUATE STUDENT

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

FACULTY PARTICIPANT

Devendra Shah	Washington State University	Washington

REASON FOR TERMINATION

End of 4-year project, funds terminated.

* funded participants

Fish images: US Government Printing Office Pamphlet 1996-792-501: Lake Washington Ship Canal Fish Ladder; US NOAA

PROJECT OBJECTIVES

The goals of this project are to evaluate strategies that would aid in developing more effective ways of managing coldwater disease (CWD) at aquaculture facilities. This has included developing and validating improved diagnostic assays and exploring vaccine development by identifying possible bacterial gene targets and expanding work on an existing attenuated vaccine. Presently, disease management is difficult at many facilities and there is no commercial vaccine available for *Flavobacterium psychrophilum*, the causative agent for CWD.

The specific objectives for this project are to:

1. Identify potential vaccine candidates using comparative proteomic analysis of an attenuated strain of *F. psychrophilum* and determine if crude cell lysate can be used as a subunit vaccine delivery vehicle in the absence of adjuvant. This objective was modified to include studies of both subunit vaccines and the mechanism responsible for attenuation of strain CSF259.93.B17.
2. Validate quantitative diagnostic assays (ELISA and ovarian fluid filtration [FAT]) and assess utility for determining the risk of vertical transmission.
3. Develop alternative assays (e.g., real-time quantitative PCR) for quantification of infection in ovarian fluid.
4. Develop an integrated outreach program to meet stakeholder needs.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Funding for this project became available in February 2008; a PhD student (Amy Long) was recruited in May 2008, a postdoctoral fellow (Rajesh Kumar) worked on this project from July 2008 to July 2009; and a PhD student (Karol Gliniewicz) joined Dr. Call's lab in 2009. A program review of the project was conducted by Dr. Jerri Bartholomew in May 2010, and progress was reported to the WRAC Board. The primary Work Group members were involved in this review and it offered an opportunity to confirm upcoming plans and discuss results to date. This termination report provides a summary of results to date, but work is continuing through next year to effectively complete ongoing research and prepare outreach materials.

Objective 1: Identify potential vaccine candidates using comparative proteomic analysis of an attenuated strain of *Flavobacterium psychrophilum* and determine if crude cell lysate can be used as a subunit vaccine delivery vehicle in the absence of adjuvant. This objective was modified to include studies of both subunit vaccines and the mechanism responsible for attenuation of strain CSF259.93.B17.

An attenuated strain of *F. psychrophilum* (CSF259.93.B17) was originally produced by serial passage on agar plates that contain increasing concentrations of an antibiotic called rifampicin. Resistance to this drug is normally conferred by point mutations in the *rpoB* gene, which produces a subunit of the RNA polymerase holoenzyme. We verified that CSF259.93.B17 has an expected mutation in this gene. We have also completed the comparative proteomic analysis for CSF259.93 (wild-type) and attenuated strain, for which we identified eight and six proteins that were uniquely up-regulated in the wild-type and attenuated strains, respectively. Using a western blot procedure with our 2-dimensional gels and mass spectrophotometry, we also identified the antigen that is targeted by our diagnostic antibody, FL-43. We completed a subunit vaccine trial using recombinant FP1493 followed by challenge using CSF259.93, but detected no evidence for a protective immune response despite elevated serum titer against FP1493. A paper describing this work has been submitted for peer-review.

Our working hypothesis is that CSF259.93.B17 is attenuated because the mutation in the *rpoB* gene interferes with the ability of the RNA polymerase holoenzyme to bind to promoter sequences or it interferes with the polymerase interaction with sigma factors. To test this hypothesis we are generating a "knock-in" mutant. If proteomic and attenuation changes are recapitulated by the knock-in experiment, this

Rainbow trout fingerlings.

Gino Santa Maria/photos.com

will support the hypothesis. In the past year, we conducted a 454-sequencing experiment of the wild-type and attenuated strains. This experiment identified 24 nonsynonymous mutations in the genome of the attenuated strain (there were only 8 for the wild-type strain). This high degree of mutation raises the possibility that attenuation results from mutations in genes other than *rpoB*, although we cannot ascertain if the mutation process was due to long-term selection on agar plates or to the influence of rifampicin selection pressure. To examine this alternative further, we have generated new passaged strains with and without rifampicin for a second genome analysis (in progress).

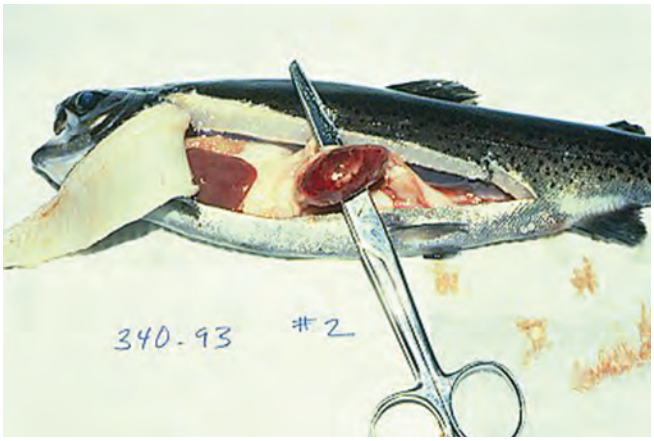
With the identity of FP1493 known and the fact that we know that its expression is influenced by iron availability, we determined if growing the B17 strain in iron limited media would improve the competency for inducing a protective immune response. Coho salmon were therefore vaccinated with either B17 grown in TYES or in TYES with an iron chelator (2,2-bipyridyl; DPD). Injection and immersion vaccination strategies were tested in this trial. The live attenuated vaccine protected Coho salmon against a virulent strain of *F. psychrophilum* in both the immersion and injection trials. Antibody titers were significantly higher in immunized fish versus non-immunized fish at 4, 6, and 12 weeks post-vaccination. Overall, statistically significant protection for immersion immunized fish was only observed in groups that were vaccinated with B17 grown under iron-limited conditions consistent with improved competency of the vaccine strain.

Objective 2: Validate quantitative diagnostic assays (ELISA and ovarian fluid filtration [FAT]) and assess utility for determining the risk of vertical transmission.

Both the ELISA and MF-FAT have been validated as diagnostic assays (Long et al., in review). In addition, bacteriological culture and a commonly used nested PCR protocol (Taylor 2004) were also validated using a wide range of field samples. The ELISA has the highest diagnostic sensitivity (0.97) and specificity (0.98). The sensitivity and specificity of the ovarian fluid MF-FAT were both low.

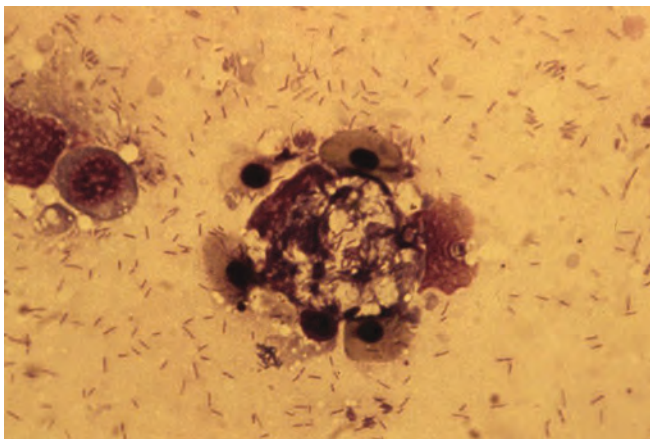
Two separate trials were used to examine the link between broodstock infection levels and risk of bacterial CWD outbreaks in progeny; one trial with rainbow trout and the other with Coho salmon. Using our diagnostic assays, we selected five families for each trial that had varying levels of infection. Eyed eggs from each family were shipped to the University of Idaho (UI) and progeny were sampled for *F. psychrophilum* upon arrival and then on a regular basis for the next two months in both trials. *F. psychrophilum* was detected within eggs upon arrival at UI and after disinfection, indicating that vertical transmission of the bacterium had occurred. Once fish reached an appropriate mass, stress experiments were initiated in an attempt to induce a BCWD outbreak in progeny and relate this to broodstock infection level. We also conducted a susceptibility trial using the Coho and found some evidence for differences between families..

While the link between broodstock infection levels and risk of progeny outbreaks is still unknown, the ELISA can be



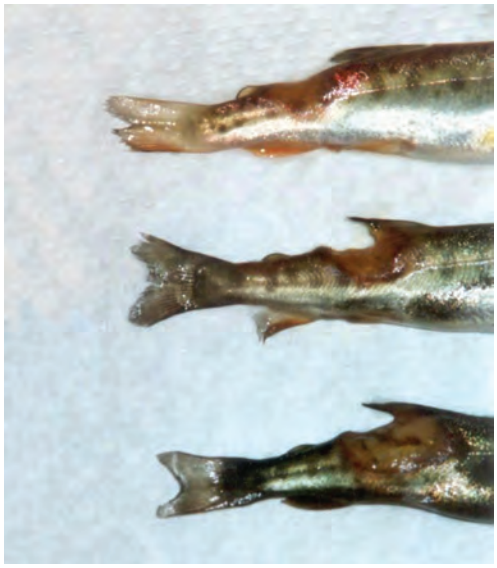
Swollen spleen from a fish infected with coldwater disease.

Courtesy of Clear Springs Foods, Inc.



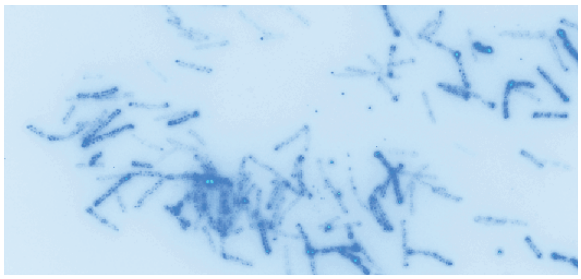
Long thin gram negative rods of *F. psychrophilum* in an imprint of the spleen of an infected fish.

Courtesy of Clear Springs Foods, Inc.



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

Benjamin LaFrentz



F. psychrophilum.

Kenneth Cain

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

Courtesy of Clear Springs Foods, Inc.

used to assess antigen levels in broodstock and progeny. The ELISA and other diagnostic assays, including nested PCR and quantitative PCR, can be used as part of a health management plan to decrease the overall frequency of *F. psychrophilum* infected fish at a facility. Additional work is underway to evaluate broodstock samples from six hatcheries as part of an effort to determine prevalence of *F. psychrophilum* in spawning populations. Early analysis indicates that prevalence is generally higher in broodstock from hatcheries where fish are returning to spawn than those from commercial rearing facilities.

Objective 3: Develop alternative assays for quantification of infection in ovarian fluid.

Development of a quantitative PCR assay for ovarian fluid is underway. The target gene for the assay putatively encodes the outer membrane protein (FP1493) that is the target of MAb FL43. While we have been able to develop the assay for pure bacterial cultures and detect the gene, extraction of bacterial DNA from ovarian fluid has proven difficult. We are currently optimizing the extraction technique and anticipate completing this in the next two to three months. Attempts to optimize the ELISA for ovarian fluid were unsuccessful because of a consistently poor signal to noise ratio.

An extension of this work is continuing in partnership with the company "Infoscitex," a USDA SBIR Phase I grant subaward. The work includes the development of a qPCR assay that uses aptamers designed to the outer membrane of *F. psychrophilum*. Four targets have been identified and isolated, which was the goal of Phase I. If Phase II is funded, we will begin work on detecting the targets in ovarian fluid as well as tissue samples.

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

Outreach activities have resulted in two articles in *Waterlines* and additional information highlighted in the fall issue of *Trout Talk*. There have been media releases and reports on our vaccine work along with numerous presentations at professional and other meetings. In summer 2011, we participated in the University of Idaho's Center for Research on Invasive Species and Small Populations Research Experience for Undergraduates program (NSF funded). An undergraduate from Eckerd College was selected to work on the CSF 259-93.B17 vaccine efficacy in the Coho salmon project. In addition to gaining valuable lab experience, the undergraduate student also presented the results of her project to her peers at the conclusion

of the program. In July 2011, we led a workshop at the Salmon Disease Course at Oregon State University. During the day-long workshop, participants carried out an ELISA with MAb FL43 using kidney from fish injected with *F. psychrophilum*. Participants in the course represented state, provincial, tribal, and federal agencies as well as industry, including Schering-Plough and Marine Harvest. Additional WRAC publications will be developed following completion of current studies.

IMPACTS

There is a strong need for public and private aquaculture facilities to have additional control and management options for CWD. One deliverable from this project is the commercialization of monoclonal antibody FL43 through ImmunoPrecise Antibodies, Inc. This is now being sold to research labs and aquaculture companies in the un-conjugated form or conjugated to FITC or HRP. Diagnostic assays to cull infected broodstock are established and protocols for the capture ELISA and FAT have been distributed to fish health labs in the region. Furthermore, we have provided these protocols to ImmunoPrecise to be distributed to customers when they purchase FL43 and they are available as downloadable pdfs directly from its website. The other deliverable will hopefully be a commercialized vaccine for CWD. The B17 vaccine was patented by the University of Idaho in June 2010 and is currently being field tested for efficacy. The UI press release about the vaccine was sent to a broad array of stakeholders, including Idaho trout growers. Recent experiments showing enhanced protection of the B17 vaccine are viewed as potential enabling technology and a provisional patent application was filed in August, 2011 to protect improved methodology.

RESULTS & IMPACTS

- Attenuated vaccine effective in rainbow trout and Coho salmon.
- Growth of B17 under iron limited media appeared to enhance vaccine efficacy.
- The commercialization of monoclonal antibody FL43 through ImmunoPrecise Antibodies, Inc. This is being sold to research lab and aquaculture companies.
- A potential commercial vaccine for CWD. The B17 vaccine was patented by the University of Idaho in June 2010 and is currently being field tested for efficacy.

RECOMMENDED FOLLOW-UP ACTIVITIES

We will complete the experiments outlined above to determine the mechanism that is responsible for attenuation of CSF259.93.B17. Ongoing work will continue through next year to complete development of qPCR assays and validate for use on ovarian fluid. We will complete evaluation of the mechanisms associated with attenuation and wrap up work aimed at relating broodstock infection levels to risk of disease in progeny.

Outreach activities will be a primary focus over the next year and beyond. Currently, the patented vaccine is under field evaluation through a partnership with Aquatic Life Sciences who has signed an option agreement with UI to license the patent. If results are promising, it is expected that the vaccine could be commercialized and sold under a USDA conditional license approval as early as January 2012. If commercialization of this vaccine occurs, it may then be possible to determine long-term impact due to adoption and implementation of a vaccine to control CWD and subsequent reduction of mortalities due to CWD. A survey of the target audience or aquaculture vaccine manufacturers may provide the information needed for long-term impact evaluation. The results of the initial vaccine field trial will be presented at the Pacific Northwest Fish Health Protection Committee annual meeting and the joint US Trout Farmers Association and Idaho Aquaculture Association Fall Conference in September 2011. If the vaccine is effective, a new downloadable WRAC outreach publication that will briefly cover CWD: what it is, how to diagnosis it, its impact, and then, in great detail, how to use the vaccine and expected results based on the immunization/challenge and field results. In addition, a section will be added to the WRAC outreach publication describing the methodology of the diagnostic tools, how to apply the tools for rapid CWD diagnosis and/or implementation of a broodstock and/or egg culling program. The results would provide effective early detection of disease and treatment of juvenile

fish and possible long term reduction of disease if culling programs are implemented. The group will follow-up with ImmunoPrecise and fish health labs to quantify impacts through the sale and use of the monoclonal antibody FL43. One to two years after project completion a survey of the target audience will attempt to determine the extent of diagnostic tool use and if broodstock and/or egg culling programs are used to minimize CWD outbreaks. If the vaccine is commercialized, workshops for private and public salmonid hatchery personnel will be held to explain and demonstrate how to use the vaccine and incorporate the diagnostic tool for early detection of the disease. An impact statement will be written after an evaluation of the deliverables to industry and other stakeholders.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

Refereed publications

Plant KP, LaPatra SE, Call DR, Cain KD. Immunization of rainbow trout (*Oncorhynchus mykiss*) with *Flavobacterium psychrophilum* gliding motility protein N. Journal of Fish Diseases (In review).

Long A, Polinski MP, Call DR, Cain KD. Validation of diagnostic assays to screen broodstock for *Flavobacterium psychrophilum* infections. Journal of Fish Diseases (In review).

Gliniewicz K, Plant KP, LaPatra SE, LaFrentz BR, Cain K, Snekvik KR, Call DR. Comparative proteomic analysis of virulent and rifampicin attenuated *Flavobacterium psychrophilum*. Journal of Fish Diseases (In review).

LaFrentz BR, LaPatra SE, Call DR, Wiens GD, Cain KD. 2011. Identification of immunogenic proteins within distinct molecular mass fractions of *Flavobacterium psychrophilum*. Journal of Fish Diseases (In Press).

Plant KP, LaPatra SE, Call DR, Cain KD. 2011. Immunization of rainbow trout (*Oncorhynchus mykiss*) with *Flavobacterium psychrophilum* proteins elongation factor-Tu, SufB Fe-S assembly protein and ATP synthase. Journal of Fish Diseases 34, 247–250.

LaFrentz BR, LaPatra SE, Call DR, Wiens GD, Cain KD. 2009. Proteomic analysis of *Flavobacterium psychrophilum* cultured *in vivo* and in iron-limited media. Diseases of Aquatic Organisms 87:171-182. PMID: 20099411.

Lindstrom NM, Call DR, House ML, Moffitt CM, Cain KD. 2009. A quantitative enzyme-linked immunosorbent assay (ELISA) and filtration-based fluorescent antibody test as potential tools for screening *Flavobacterium psychrophilum* in broodstock. Journal of Aquatic Animal Health 21:43-56. PMID: 19485125.

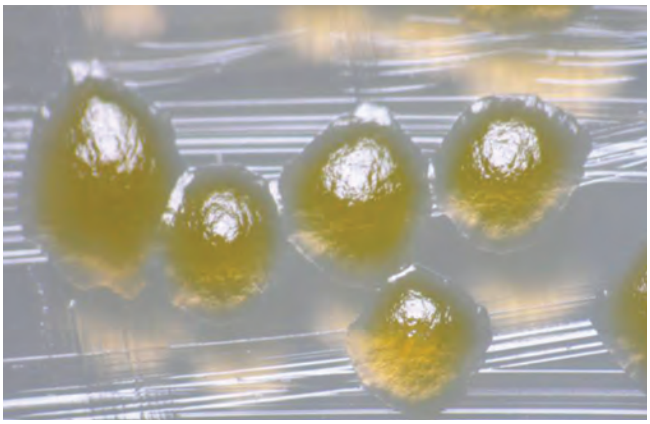
Plant KP, LaPatra SE, Cain KD. 2009. Vaccination of rainbow trout (*Oncorhynchus mykiss*) with recombinant and DNA vaccines produced to *Flavobacterium psychrophilum* heat shock proteins 60 and 70. Journal of Fish Diseases 32(6):521–34.

General articles

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

Cain KD, Call DR. 2010. Coldwater disease. Waterlines Newsletter, Spring 2010, p10.

Cain K, Call DR. Coldwater disease research. Trout Talk, Fall, 2011.



Raised yellow pigmented colonies of *Flavobacterium psychrophilum*

Ben LaFrentz

Fish suffering from coldwater disease

Ben LaFrentz

Presentations

Cain KD. Research overview and update. University of Tasmania. Launceston, Tas, Australia, Feb. 10, 2011.

Cain et al. A potential vaccine to control bacterial coldwater disease. US Trout Farmers Association and Idaho Aquaculture Association Fall Conference. Twin Falls, ID. Sept. 29–Oct. 1, 2011.

Cain and Zinn. BCWD vaccine development. 56th Pacific Northwest Fish Health Protection Committee Annual Meeting, Portland, OR. Sept. 21–22, 2011.

Gliniewicz KS, Cain KD, Snekvik KR, Call DR. The role of rpoB in the attenuation of *Flavobacterium psychrophilum* after passage with rifampicin. Poster presented, 10th Annual College of Veterinary Medicine Research Symposium, Pullman, WA, Oct. 14, 2009.

Gliniewicz K, Plant KP, LaPatra SE, Cain KD, Snekvik KR, LaFrentz BR, Call DR. Comparative proteomic analysis of virulent and rifampicin attenuated strains of *Flavobacterium psychrophilum*. American Fisheries Society Annual Meeting, Seattle, WA, Sept. 5–7, 2011.

Gliniewicz K, Snekvik K, Cain K, LaPatra S, Call D. Assessing the immune-protective potential of FP1493 against coldwater disease in rainbow trout. Poster presented, WSU Showcase, Pullman, WA, March 2010.

Gliniewicz K, Snekvik K, Cain K, LaPatra S, Call D. Assessing the immune-protective potential of FP1493 against coldwater disease in rainbow trout. Poster presented, American Society for Microbiology General Meeting, San Diego, CA, May 2010.

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

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

Long A, Call DR, Cain KD. Use of diagnostic assays to screen rainbow trout (*Oncorhynchus mykiss*) broodstock for *Flavobacterium psychrophilum*. Sixth International Symposium for Aquatic Animal Health and AFS Fish Health Section Annual Meeting. Tampa, FL, Sept. 5–9, 2010.

Long A, Call DR, Cain KD. Use of diagnostic assays to screen rainbow trout (*Oncorhynchus mykiss*) broodstock for *Flavobacterium psychrophilum*. Third Annual Western Division American Fisheries Society Student Colloquium, Moscow, ID, Oct. 14–16, 2010.

Long A, Polinski MP, Call DR, Cain KD. Validation of diagnostic assays to screen broodstock for *Flavobacterium psychrophilum* infection. Idaho Chapter, American Fisheries Society Annual Meeting. Boise, ID, March 2–4, 2011.

Swain MA, Long A, Fehringer TR, LaFrentz BR, Call DR, Cain KD. Vaccine efficiency in Coho salmon against *Flavobacterium psychrophilum*. Center for Research on Invasive Species and Small Populations. Moscow, ID, Aug. 4, 2011.

SUPPORT

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

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

TERMINATION REPORT

PROJECT WORK PERIOD

September 1, 2007–August 31, 2011

AUTHOR

Molly A. H. Webb

FUNDING LEVEL

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

PARTICIPANTS

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

PROJECT MONITOR

Fred Conte	University of California, Davis	California

INDUSTRY ADVISOR

Peter Struffenegger	Sterling Caviar, LLC	California

REASON FOR TERMINATION

Objectives completed and funding ended.

* funded participants

PROJECT OBJECTIVES

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

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

PRINCIPAL ACCOMPLISHMENTS

Objective 1. Determine how currently utilized morphological characteristics (oocyte PI, ovarian follicle size, gonadosomatic index, age, and live weight) correlate with caviar quality and yield.

The analysis of morphological characteristics and caviar harvested during 2008, 2009, 2010, and 2011 revealed relationships of caviar yield with body weight (BW), stage of maturity (oocyte PI), and egg diameter (ED) for age 7 fish in California ($P<0.05$); however, the coefficients of determination (R^2) were less than 0.10. There was a significant ($P<0.0001$) relationship between gonadosomatic index (GSI) and caviar yield for both age 7 and 8 cohorts in California, with R^2 values of 0.46 and 0.52, respectively. Unexpectedly, the stage of maturity (oocyte PI) was not a main factor affecting roe yield due to apparent confounding and interactive effects of other variables, particularly the highly variable caviar yield caused by extensive ovarian adiposity, which was pronounced in larger fish within age cohort. Three categories of ovarian adiposity (high fat, medium fat, and low fat corresponded well to the caviar yield per body weight (CY%BW) and caviar production per fish (CW). Generally, processed caviar yield was less than 50% of the ovarian weight for the high fat category, within 50- 65% for the medium fat category, and more than 65% for the low fat category.

We examined the relationship between oocyte PI and caviar yield (% BW) within each category of ovarian adiposity and found there was a relationship in the medium fat group ($P=0.01$); however, the R^2 was only 0.0387. In California females, the three fattiness groups did not differ by GSI ($P>0.05$, one-way ANOVA and Tukey test), but the means for caviar weight and caviar yield per both BW and ovary weight (OW) were significantly different. The high fat group had the highest mean BW, smallest eggs (ED), and the lowest caviar yields per fish BW and OW (CY-BW and CY-OW). The best producers of roe were the females in the low fat group; while they had significantly lower mean BW and condition factor (K), they produced the highest yield of caviar (CY% BW and CY% OW).

The caviar harvest data from Idaho included fewer fish, but the comparisons with California revealed differences between the stocks of the two states. The Idaho fish were similar in BW but much older, had a lower K (0.73 versus 1.03), smaller ovaries, and a lower GSI compared to California fish. Despite the lower GSI in the Idaho stock, the mean caviar weight and the caviar yield per body weight were similar in both states because the Idaho fish ovaries were much leaner. The mean yield of caviar as a percent of ovarian weight was 71% in Idaho compared to 58% in California stock. The Idaho fish were much older (15-21 years) and produced larger eggs (3.4 mm diameter compared to 3.1 mm in California fish). Other factors to consider are generally cooler water in Idaho and the fact that fish were kept in the dirt canals for several years prior to this study, having access to a single demand feeder and various species of fish living in the canal. This restricted feeding and more naturalistic environment could also be a factor in the leaner ovaries and lower condition factor. It should be noted that the Idaho producers have observed more fatty ovaries in the younger year-class fish reared in the concrete raceways and fed prepared diets more regularly.

Though the main effect of the oocyte PI was not obvious in this study, this analysis does not invalidate the importance of detecting maturational stage by non-invasive methods.

Using the oocyte PI even though weakly correlated to caviar yield, to determine the optimal time to harvest fish did result in a 2% increase in yield in both California and Idaho, a significant gain.

The quality of caviar was also a critical component of this project. In the past, caviar processors have noted that using the same percent of salt throughout the processing season resulted in egg batches becoming too salty or not salty enough. It has long been assumed that the variation in saltiness following a standardized salt treatment could be associated to variation in egg maturity (oocyte PI) between females. If females could be harvested at more homogenous oocyte PI's then the standardized percent of salt used would result in less variation in quality of the caviar, in terms of saltiness.

Objective 2. Determine how plasma sex steroid, total calcium, plasma protein levels, and crude chemical composition of eggs change with maturity.

Blood plasma calcium and total protein were measured repeatedly in fish in 2007–2008. Plasma calcium and total protein did not differ among stages of maturity in these fish. These parameters were not measured in the following years of the study. Blood plasma T and E2 and crude chemical composition of eggs did not change with increasing maturity (measured by decreasing oocyte PI) at the time of caviar harvest. Blood plasma T and E2 were significantly lower in fish with atretic ovaries compared to fish with normal ovaries.

Objective 3. Evaluate short wavelength near infrared spectroscopy (SW-NIR) and ultrasound as a non-invasive technique to predict maturity by taking spectra of gonads in fish.

SW-NIR spectra were collected in California in 2008 and 2009 and in Idaho in 2009 to assess the ability of SW-NIR to predict stage of maturity (oocyte PI). For California, a model was developed using the intensity spectra collected on the abdomen, resulting in 84% of the fish with high PI values and 62% of the fish with low PI values correctly classified. In Idaho, a similar analysis was done using the intensity spectra collected on the abdomen—66% of the fish with high PI values and 82% of the fish with low PI values were correctly classified. SIMCA models were also built from one year (2008 or 2009) in an attempt to predict the PI value of the other year. However, results from these models are poor and lack the

Short wavelength near infrared spectroscopy probe is used to assess stage of maturity through the abdominal wall in white sturgeon.

Fred Conte

Mariah Talbott analyzing blood chemistry parameters in white sturgeon plasma to assess the use of specific analytes as indicators of stage of maturity.

Molly Webb

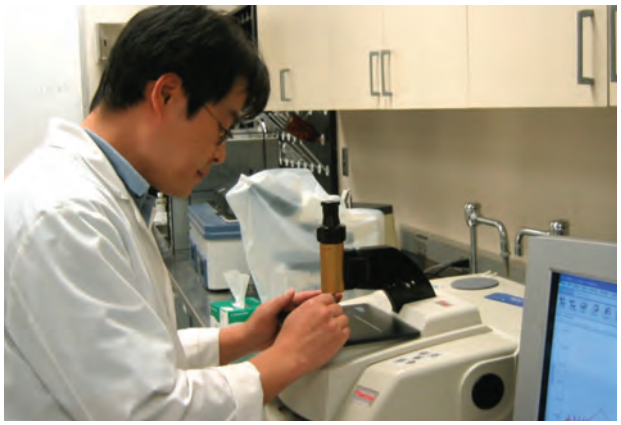
ability to predict from one year to the next. It is unclear at this time whether the transferability of the model is precluded by differences in the eggs from one year to the next or by instrumental irreproducibility. Other factors, such as limited year classes (individual fish variation) and limited sample size when compared to typical SW-NIR models, may also contribute to the lower predictability of the models developed in this study. Though sturgeon gonadal development is synchronous, we tested whether there were differences in the stage of maturity as seen by oocyte PI and SW-NIR spectra across and between gonadal lobes. There is no difference between anterior, middle, or posterior scans on a given fish, and the differences in oocyte PI were not biologically significant.

Adiposity seen in white sturgeon gonads.

Fred Conte

Objective 4. Evaluate Fourier transform infrared spectroscopy (FT-IR) as a method to predict fish maturity from spectral measurements of blood and roe.

We applied a rapid and less invasive vibrational spectroscopy method to collect spectral features of sturgeon female plasma and quantify and predict the actual PI values with the aid reference values and partial least squares regression chemometric models. Two types of partial least squares regression (PLSR) models were established and the advantages and disadvantages of both models were compared. The Plan A PLSR model was constructed from spectra from fish plasma from the years of 2007, 2008, and 2009 randomly selected with a leave-one-out cross validation to challenge the rigorousness of the calibrated model. The Plan B PLSR model was constructed similarly to the Plan A model except that 5 fish samples from the 2010 and 2011 data sets were randomly selected and used for model establishment and leave-one-out cross validation. The PI values of all fish in 2010 and 2011 were predicted based upon the Plan A model, while the remaining fish not used in model development from 2010 and 2011 were predicted based upon the Plan B model. Strictly speaking, only the Plan A PLSR model is "truly" predicting the PI values of the samples in the year of 2010 and 2011, and the Plan B PLSR model is only cross validating additional samples from 2010 and 2011. This is because Plan B incorporates spectral features of the fish sampled in 2010 and 2011 (n=5 each) into the calibration model, whereas Plan A)is based upon fish from prior years only. FT-IR can successfully predict oocyte PI with approximately 90% accuracy. Model maintenance and a total of 10 fish per year are necessary.



Fourier transform infrared spectroscopy is used as a method to predict stage of maturity from spectral measurements of white sturgeon blood plasma

Barbara Rasco

Objective 5. Using SW-NIR and plasma steroids, determine whether it is possible to detect the early signs of ovarian atresia.

Plasma sex steroid analysis, SW-NIR of abdomen and ovarian follicles, and FT-IR of plasma appear to be reliable predictors of atresia with variable success rates depending on the tool chosen. The use of plasma sex steroids analyzed by radioimmunoassay (RIA) resulted in 95% of the females with normal ovarian follicles (nonatretic) and 93% of the atretic females correctly classified (Talbott et al. 2010). Logistic regression equations were developed to predict the probability that a female white sturgeon has normal ovaries given a specific

concentration of T or E2. In California, females with a circulating concentration of T less than 35.1 ng/ml should not be harvested as they have a 95% probability of having initiated ovarian atresia. The SW-NIR spectroscopy resulted in a model that correctly predicted 115 body scans out of 120 body scans from atretic fish for a correct classification of 96%. The validation of this model, using a leave-one-out technique, showed that 55% of the body scans were correctly classified as atretic (Servid et al. 2010). Similarly, the classification rates of atretic scans on ovarian follicles were high for the model development (96%), and the validation of the model showed that 58% of the atretic scans were correctly classified. The FT-IR was capable of identifying atretic females with 70% accuracy (Lu et al. 2010).

Objective 6. Conduct training and outreach programs at field sites in Idaho and California.

The studies were conducted at aquaculture facilities in both Idaho and California with extensive support and participation of our industry partners. Industry partners were involved in field collection of all biological samples for laboratory analysis and on-site SW-NIR spectroscopy. The results of the project revealed that FT-IR can be used to successfully predict oocyte PI, and analysis of plasma sex steroids was the most reliable predictor of follicular atresia. These laboratory techniques are available for the aquaculture industry; however, given that oocyte PI did not correlate well with caviar yield, none of the current techniques were pursued for instrument-to-fish analysis. An outreach publication that describes the different methods of determining follicular atresia in female sturgeon is currently being written. To improve quality and yield of caviar in farmed white sturgeon, it is essential to correctly assess the stage of ovarian maturity and avoid harvesting females with atretic ovarian follicles.

IMPACTS

Relevance: Currently, the only means to assess ripeness of white sturgeon females and properly time caviar harvest is measurement of oocyte PI that requires surgical biopsy. This technique is accurate but invasive, stressful to fish, time consuming, and not an effective tool for handling a large number of female fish. The large caviar farms that harvest thousands of females cannot determine oocyte PI for every fish, resulting in decreased caviar yield and quality due to follicular atresia (phagocytosis of ovarian eggs). Even the early stage of atresia causes a reduction in the firmness, flavor, and shelf life of caviar, and sometimes the complete loss of the product.

Specifically, the sturgeon industry was interested in new methods to predict sturgeon maturity (oocyte PI) as an alternative to the surgical biopsy and to identify females in the early stages of atresia.

Response: To achieve our goal, we needed a better understanding of the biochemical and physiological changes that occur during gonadal maturation and early atresia and how these changes correlate with roe quality. The methods should be non-invasive, minimally stressful to fish, and quick. Ideally, they would allow female fish to be sorted into groups based upon the degree of ripeness in the fall during late vitellogenesis followed by a quick examination of groups for early atresia just prior to selection for caviar harvest. In this project, we examined the ability of non-invasive SW-NIR, FT-IR, and immunochemical assays to identify early atresia in females and replace the surgical biopsy procedure currently used to determine ripeness in sturgeon.

Results: The radioimmunoassay of plasma steroids (Talbott et al. 2011), spectral analysis of plasma by FT-IR (Lu et al. 2011), and abdominal and follicular scans by SW-NIR (Servid et al. 2011) were suitable for detecting early atresia with 55–93% accuracy depending on the tool, and the FT-IR was determined to be the most accurate in determining maturational stage (oocyte PI) and may be used in lieu of the surgical biopsy technique (90% accuracy). The analysis of caviar harvest found that the stage of maturity manifested by

Collection of ovarian follicles from white sturgeon by minor biopsy to assess stage of maturity.

Fred Conte

the oocyte PI does not necessarily correlate with higher caviar yields. However, using oocyte PI even though weakly correlated to caviar yield to determine the time to harvest fish did result in a 2% increase in yield in both California and Idaho, a significant gain. Adiposity of the sturgeon ovary appeared to be a major factor that negatively affected caviar yield, particularly in larger, faster-growing fish. It should be noted that this analysis does not invalidate the importance of detecting maturity stage by noninvasive methods. Using the same percent of salt throughout the processing season has resulted in some egg batches becoming too salty or not salty enough. The variable saltiness is hypothesized to be associated with variation in egg maturity (PI) among females. If females could be harvested at more homogenous PIs, the standardized percent of salt used would result in less variation in quality of the caviar in terms of saltiness.

RESULTS AT A GLANCE...

- The FT-IR was determined to be the most accurate in determining maturational state (oocyte PI) and may be used in lieu of the surgical biopsy technique.
- The analysis of caviar harvest found that the stage of maturity manifested by the oocyte PI does not necessarily correlate with higher caviar yields.
- However, using oocyte PI even though it is weakly correlated to caviar yield to determine the time to harvest fish did result in a 2% increase in yield in both California and Idaho, a significant gain.
- Adiposity of the sturgeon ovary appeared to be a major factor that negatively affected caviar yield, particularly in larger, faster growing fish.

RECOMMENDED FOLLOW-UP ACTIVITIES

Sturgeon farmers in California and Idaho observe highly variable roe yield in mature sturgeon, associated with accumulation of fat in the ovaries. Environmental, genetic, and developmental factors can all affect caviar yield and quality, but the role of these factors is not well understood. Understanding these effects is essential for sustained production of high-quality sturgeon caviar in the Western Region. We proposed a collaborative study, with participation of four states of the region and four sturgeon farms, aimed at investigating these effects on ovarian adiposity, roe yield, and caviar quality in farmed sturgeon. This recent WRAC proposal was funded for the first year. An outreach publication to describe the different methods to determine follicular atresia in female sturgeon is currently in preparation. To improve quality and yield of caviar in farmed white sturgeon, it is essential to correctly assess the stage of ovarian maturity and avoid harvesting females with atretic ovarian follicles.

More robust PLS-DA analyses compared to SIMCA of the eggs and spectra collected on the abdomen is underway and will be completed this fall. A blind study was conducted to test whether a model built on spectra collected during the atresia study in California could be used to predict atresia in Idaho fish. Analyses are currently underway.

PUBLICATIONS, MANUSCRIPTS, AND PAPERS PRESENTED

Publications

Lu X, Webb M, Talbott M, Van Eenennaam J, Doroshov S, Rasco B. 2011. A study of morphological and immunochemical parameters associated with ovarian atresia and quality of caviar in white sturgeon (*Acipenser transmontanus*) utilizing fourier transform infrared spectroscopy (FT-IR). Aquaculture 315:298–305.

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

Lu X. 2009. Determining sexual maturity in white sturgeon (*Acipenser transmontanus*) to maximize yield and quality of caviar. Masters Thesis, Washington State University.

Servid SA, Cavinato AG. Non-invasive characterization of maturity status to optimize caviar yield and quality in white sturgeon. 2010-2011. Eastern Oregon Science Journal, Vol. XXI, in print.

Servid SA, Talbott MJ, Van Eenennaam JP, Doroshov SI, Struffenegger P, Webb MAH, and Cavinato AG. 2011. Rapid noninvasive characterization of ovarian follicular atresia in cultured white sturgeon (*Acipenser transmontanus*) by near infrared spectroscopy. Aquaculture 315:290–297.

Talbott M, Van Eenennaam JP, Linares-Casenave J, Doroshov, SI, Guy CS, Struffenegger P, Webb MAH. 2011. Plasma sex steroid concentrations during onset of ovarian atresia in farmed white sturgeon, *Acipenser transmontanus*. Aquaculture 315:283–289.

Talbott MJ. 2010. Determining morphological and biochemical parameters associated with ovarian follicular atresia and caviar quality and yield in cultured white sturgeon (*Acipensertransmontanus*). Master's Thesis, Montana State University.

Vixie B. 2011. Stress response and implications for white sturgeon (*Acipenser transmontanus*) cultivation: Qualitative detection of cortisol in caviar and roe by FT-IR and differentiating between soft and hard roe by SEM. Master's Thesis, Washington State University.

Presentations

Cavinato AG, Servid S, Van Eenennaam J, Doroshov S, Lu X, Rasco B, Talbott M, Sealy W, Webb M. Non-invasive means to determine maturity in white sturgeon females. 2011. 242nd American Chemical Society National Meeting, Denver, CO, August 2011. (Invited presentation).

Cavinato AG. Adapting research to meet regional needs. 2009. 237th American Chemical Society National Meeting, Salt Lake City, UT, March 2009. (Invited presentation).

Lu X, Webb M, Doroshov S, Rasco B. Distinguishing vitellogenin, sex steroids, and lipids in blood plasma and atresia of farmed white sturgeon (*Acipenser transmontanus*) by Fourier transform infrared spectroscopy: A potential tool for caviar production. Institute of Food Technologists 2010 Annual Meeting, poster presentation. Chicago, IL. July 17–21, 2010.

Lu X, Callahan G, Rasco B. Infrared spectroscopy, a novel and less invasive method to determine maturity in cultured white sturgeon (*Acipenser transmontanus*) females by predicting polarization index and follicle diameter. Institute of Food Technologists 2010 Annual Meeting, poster presentation. Chicago, IL. July 17–21, 2010.

Lu X, Wang J, Rasco B. Rapid determination of maturity in cultured white sturgeon (*Acipenser transmontanus*) females using plasma steroid concentrations by Fourier transform infrared spectroscopy and partial least squares model. Institute of Food Technologists 2010 Annual Meeting, poster presentation. Chicago, IL. July 17–21, 2010.

Lu X, Webb M, Doroshov S, Rasco B. Distinguishing vitellogenin, sex steroids and lipids in blood plasma and atresia of farmed white sturgeon (*Acipenser transmontanus*) by Fourier transform infrared spectroscopy: A potential tool for caviar production. Pacific Fisheries Technologists 61th Annual Meeting, poster presentation. Seattle, WA. Feb. 21–24, 2010.

Lu X, Callahan G, Rasco B. Infrared spectroscopy, a novel and less invasive method to determine maturity in cultured white sturgeon (*Acipenser transmontanus*) females by predicting polarization index and follicle diameter. Pacific Fisheries Technologists 61th Annual Meeting, poster presentation. Seattle, WA. Feb. 21–24, 2010.

Lu, X, Rasco, B. The use of Fourier transform infrared spectroscopy to predict maturity stages and caviar quality in white sturgeon females. Institute of Food Technologists 2009 Annual Meeting, oral presentation. Anaheim, CA. Jun 6–9, 2009.

Lu X, Rasco B. The use of Fourier transform infrared spectroscopy to predict ovarian atresia and caviar quality in white sturgeon (*Acipenser transmontanus*). Aquaculture America 2009 Meeting, oral presentation. Seattle, WA. Feb 15–18, 2009.

Lu X, Rasco B. A study of morphological and immunochemical parameters associated with ovarian atresia and quality of caviar in white sturgeon (*Acipenser transmontanus*) females by Fourier transform infrared spectroscopy and multivariate analysis. Pacific Fisheries Technologists 60th Annual Meeting, oral presentation. Portland, OR. Feb 22–25, 2009.

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

Servid SA, Cavinato AG. Novel non-invasive method to determine maturity in cultured white sturgeon (*Acipenser transmontanus*) females. 241th American Chemical Society National Meeting, Anaheim, CA, March 2011.

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

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

Van Eenennaam J, Linares-Casenave J, Talbot M, Webb M, Struffenegger P, Conte F, Doroshov S. The relationship between white sturgeon caviar yield and stage of maturity. Oral presentation at Aquaculture 2010, San Diego, CA, March 1–5, 2010.

SUPPORT

YEAR	WRAC-USDA FUNDS	OTHER SUPPORT					TOTAL SUPPORT
		UNIVERSITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
2007-08	100,001		18,500	79,986		98,486	198,487
2008-09	100,001	5,000	18,500	79,226		102,726	202,727
2009-10	100,001		19,000	71,023		90,023	190,024
2010-11	100,000		19,000	70,156		89,156	189,156
TOTAL	400,003	5,000	75,000	300,391		380,391	780,394

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

United States Department of Agriculture
National Institute of Food and Agriculture