WATERLINES

NEWSLETTER OF THE WESTERN REGIONAL AQUACULTURE CENTER WINTER 2009

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

Inside

WRAC on Track

Graham Young, WRAC Director

After some delay in publication of this edition of *Waterlines* because of personnel changes at the WRAC Administrative Office, we are definitely back with a bang, with the largest print run ever. In addition to 2,500 copies sent to our regular subscribers, as a proud sponsor of *Aquaculture America 2009*, a copy of this edition is included in the conference bag of all registered attendees. We welcome all delegates to our home city of Seattle.

On behalf of the WRAC community, I'd like to welcome our new program manager, Debbie Granger. (Debbie introduces herself on page 2.) Debbie has already had considerable positive impacts in the Administrative Office, and brings a proactive, efficient and enthusiastic approach to all she does.

It is with mixed emotions that I announce the retirement of Dr. Brian Allee as Director of the Alaska Sea Grant Program and from WRAC's Board of Directors. Brian served WRAC for 20 years, first as a member of the Industry Advisory Council and later on the Board. He was presented with a certificate in recognition of his dedication at the May 2008 Board meeting. Brian was Chair of the Board when I took over as Director, and was tremendously helpful, sharing time, knowledge, and wisdom as I got up to speed. He quickly became a valued colleague and friend. The idea that Brian has actually "retired" is one that anyone who knows him will treat with skepticism! On pages 6–7, Brian reflects on WRAC, and more. And, we welcome his successor on the Board, Ray RaLonde.

Graham Young

Penny Swanson

Dr. Fred Conte is leading the Strategic Planning Committee in a major revision of WRAC's *Manual of Operations* to reflect current practice and to emphasize the importance WRAC places both on sound, relevant science and transfer of that information to industry through a variety of outreach products. Many thanks to Fred for leading this effort.

In this edition of *Waterlines*, we continue our emphasis on communicating the rationale, aims, results, and impacts of our past and on-going research and extension projects. Dr. Jim Winton offers his perspective on the long history and impact of WRAC-sponsored research into infectious hematopoietic necrosis virus (IHNV). Three projects that started within the last year or two are also highlighted and these provide a good reflection of the range of research that WRAC has supported during the last 20 years.

In addition, Gary Fornshell summarizes some of the problems of gaining objective press coverage for aquaculture—in this case, the health benefits of farmed seafood.

Finally, we at the Administrative Office send you all our best wishes for 2009. ■

Funding provided by the US Department of Agriculture, Cooperative State Research, Education & Extension Service

First Impressions

Debbie Granger, WRAC Program Manager

As the recently hired Program Manager, I welcome this opportunity to share a few impressions of my first months with WRAC.

I am truly impressed with the dedication, enthusiasm, and sincerity that everyone involved with WRAC brings to implementing the mission. From the Board of Directors to all the members of the Industry Advisory Council and the Technical Committee to the numerous researchers, it has been a pleasure to meet you and observe your collegiality and professionalism as you work through the requirements involved in developing top-rate scientific and applied research projects.

I thank you for the patience you've shown me, as I've been learning about the workings of the WRAC program. The scientific terms involved in aquaculture are a long way from my work as a college and career counselor in a large high school! While my summers working as a crew person on our family reefnet fishing gear (a sustainable commercial fishery for sockeye salmon) in Puget Sound together with my husband's work in aquaculture and the seafood industry have acquainted me with the issues surrounding the world's seafood industry, there's still much to learn.

I am especially grateful to Graham Young for his thorough mentoring and patience with my endless questions as I transition into this position.

This issue of *Waterlines* provides an overview of the 20 years of the national Regional Aquaculture Center program as well as a few highlights of the Western Region (see pages 3–5). As we reflect on the rich history of WRAC, it's been very interesting and informative for me to learn of the many people who pioneered aquaculture endeavors in the West. I regret only being able to work with Brian Allee for one Board Meeting—his vision and hard work in establishing the WRAC program are impressive. (See interview, pages 6–7.)

I look forward to working with WRAC and helping continue the important task of sustaining and enhancing the aquaculture industry in the western states.

Best Fishes to all!

Gloria Hammel

Family commercial reefnet salmon operation with crewman, Bob Jewell

WRAC Celebrates 20 Years

Debbie Granger, WRAC Program Manager

As WRAC celebrates its 20th anniversary, it's important to reflect on the history and highlights of the national Regional Aquaculture Center (RAC) program as well as specific WRAC accomplishments.

Administered by the USDA's Cooperative State Research, Education, and Extension Service (CSREES), the federal RAC program offers a unique industry–academia partnership that serves as a cornerstone for federal funding of aquaculture-related research and development projects, extension programs, and demonstration activities of national or regional importance in the United States. Linked closely to numerous National Aquaculture Research Centers of the USDA Agricultural Research Service as well as to facilities and projects of the National Oceanic and Atmospheric Administration, the RACs have flourished for two decades.

Because RACs rely on industry representatives to identify problems facing aquaculture in each region, they are able to use the best scientific and educational expertise and facilities to fund projects that address these problems and thereby ensure accountability and coordination of resources.

Authorizing legislation and history

Amendments to the National Agricultural Research, Extension, and Teaching Policy Act of 1977 provided for up to five regional aquaculture research, development, and demonstration centers directed to perform aquaculture research, extension, and demonstration projects having a national or regional application. With an authorized appropriation of up to $7.5 million annually, the centers were strategically located geographically to represent regional aquaculture opportunities throughout the US.

The RACs began their first organizational activities in 1987 and the first research and extension projects were initiated in 1988.

Mission

The mission of the RAC program is to "support aquaculture research, development, demonstration, and education to enhance viable and profitable US aquaculture production for the benefit of consumers, producers, service industries, and the American economy."

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The Five RAC Regions

RAC Region	States	Host Institution/Director
Southern (SRAC) *http://www.msstate.edu/dept/srac*	Alabama, Arkansas, Florida, Georgia, Kentucky Louisiana, Mississippi, North Carolina, Oklahoma, Puerto Rico, South Carolina, Tennessee, Texas, US Virgin Islands, and Virginia	Mississippi State University *Dr. Craig Tucker*
North Central (NCRAC) *http://www.ncrac.org*	Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin	Michigan State University with co-administration by Iowa State University *Dr. Ted Batterson*
Western (WRAC)	Alaska, Arizona, California, Colorado, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington, and Wyoming	University of Washington *Dr. Graham Young*
Northeastern (NRAC) *www.nrac.umd.edu*	Washington D.C., Connecticut, Delaware, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont, and West Virginia	University of Maryland *Dr. Fred Wheaton*
Tropical and Subtropical (CTSA) *http://www.ctsa.org*	Hawaii, American Samoa, Commonwealth of the Northern Mariana Islands, Guam, the Republic of the Marshall Islands, the Federated States of Micronesia, and Palau	Oceanic Institute/University of Hawaii *Dr. Cheng-Sheng Lee*

—*continued from page 3*

WRAC's Organizational Structure

Group	Representation	Roles & Responsibilities
Board of Directors	Each Land Grant Institution from each state in the region plus one rep each from from the IAC & the two sub-committees of the TC	1. Meet semi-annually 2. Write and implement policies 3. Review recommendations from the IAC/TC and approves projects for funding and inclusion into the annual Work Plan
Industry Advisory Council (IAC)	Aquaculture-related producers, marketing & processing firms, associations & organizations	1. Meet annually 2. Identify significant problems facing aquaculture in the region 3. Submit project areas to the Board of Directors 4. Review research proposals and send recommendations for funding to the Board of Directors
Technical Committee (TC)	Research and extension professionals within the region	

Two foundation elements of the RAC program are: 1) industry involvement in all aspects of project development, ensuring funding of projects that directly impact commercial aquaculture efforts in each region, and 2) a regional team approach to foster integration of research, extension, and industry capabilities, avoiding duplication of effort and ensuring efficient use of resources.

Who are we and how do we work?

Each regional Center has a similar basic organizational and operational structure; the chart above is based on the procedures used at WRAC. In addition, the administrative office in each region coordinates proposal peer reviews, communications with committee members and researchers, coordination with researchers and their participating institutions, publicity, fiscal management, and facilitating the development of outreach publications and other outreach activities.

National impacts

The RACs have had significant impact across the nation, helping to advance the aquaculture industry in all regions.. Products have included hundreds of peer-reviewed publications, workshops, fact sheets, culture manuals, extension publications, abstracts and presentations at professional meetings, popular articles, Agriculture Experiment Station publications, conference proceedings, DVDs and videos, and book chapters.

RAC funding has also had a significant impact on education and scientific research capacity, contributing to student training and research opportunities that have culminated in 35 PhD dissertations and more than 100 MS theses.

WRAC highlights

WRAC is proud of its active partnership among industry, academia, and extension specialists within the 12 states in the western region. The region's major aquaculture sectors are shellfish, hybrid striped bass, tilapia, rainbow trout, and sturgeon. Input from industry (identification of priority needs) has allowed WRAC to fund research projects that provide direct benefit to commercial aquaculture ventures.

Shellfish research projects include:

- Improving Pacific oyster broodstock
- Investigating the ecological role of molluscan shellfish culture and potential impacts of various harvesting methods

Disease research projects include:

- Control of infectious hematopoeietic necrosis virus and bacterial kidney disease in commercially reared salmonids

- Investigating immunological mechanisms of intensively reared warmwater (primarily hybrid-striped bass) and coolwater finfish
- Investigating disease interactions between wild and cultured fish

Nutrition research projects include:

- Developing economical, high-performance, low-polluting feeds and feeding strategies
- Developing and evaluating starter diets and culture conditions for three subspecies of cutthroat and Gila trout

Finfish research projects include:

- White sturgeon domestic broodstock management
- Optimizing quality and shelf-life of sturgeon caviar
- Reducing effluent in flow-through raceways
- Studies on critical problems in the recirculation aquaculture industry

Current projects

WRAC continues its commitment to enhancing and sustaining the aquaculture industry in the western region.

Current projects include:

- Physiological changes associated with live haul: Maintaining healthy fish
- Economic impacts of private sector aquaculture-based recreational fishing in the western US
- Determining ripeness in white sturgeon females to maximize yield and quality of caviar
- Coldwater disease prevention and control through vaccine development and diagnostic improvements
- Potential threat of Great Lakes Viral Hemorrhagic Septicemia Virus (VHSV) in the western United States

Inter-regional efforts

Three RACs (WRAC, NCRAC, NRAC) have formally worked together to coordinate and fund projects focused on VHSV, and several RACs have contributed to support new aquaculture drug approvals. In addition, regular meetings of the National Coordinating Committee (RAC Directors and USDA-CSREES personnel) ensure that all centers are familiar with activities at each RAC.

In summary

The national RAC program has a rich and robust history. The ability to catalyze and engage industry participation to identify high-priority regional needs and problems offers a regional model and a unique structure for federally funded programs. Although strained by funding limitations (the annual funding level has never exceeded 60% of the authorized appropriation), and confronted with the many problems facing the industry today, the Centers empower industry to direct federal investments in a timely manner to solve important problems in local and regional aquaculture.

The organizational structure of the Centers and the process used for project development make the program highly accountable and have generated significant industry "buy-in" in determining the future and fate of aquaculture industry in the United States.

WRAC continues to garner input from industry and extension specialists, and to mobilize regional academic firepower to ensure direct application and impact to the region's aquaculture endeavors. As is true of the US as a whole, complex problems in the further development of the aquaculture industry in the West remain. Nonetheless, the western industry has proven to be adaptable and innovative in the face of challenges, and WRAC is proud of its record of partnership with industry and academia in support of the continued development of safe, nutritious, affordable, and ecologically sustainable aquaculture products.

Brian Allee Resigns from WRAC

Debbie Granger interviews Brian Allee

Dr. Brian Allee retired in mid-2008 as Director of the Alaska Sea Grant College Program and, at the same time, resigned from the Board of Directors of WRAC. Brian has enjoyed a rich and diverse career in fisheries for more than 35 years—in positions in both the public and private sectors.

He holds a doctorate in fisheries from the University of Washington. Trained as a fisheries behavior scientist, Allee is an expert in salmonid restoration and has extensive experience in habitat restoration, bioengineering, adult and juvenile fish passage, fish screening biocriteria, fish physiology and supplementation, fish hatcheries, aquaculture, water supply and treatment, and biological design criteria. He has worked with salmonids, oysters, clams, and shrimp in freshwater, estuarine, and marine ecosystems in Washington, Alaska, Oregon, California, Florida, and Brazil. He has had broad management experience supervising large and small organizations in the public and private sectors.

Along with scientific expertise, Brian brings an enthusiastic willingness to work collaboratively with all stakeholders (fishermen, aquaculturists, community organizers, state and federal agencies) to solve complex problems within the local and regional marine environment.

Observing Brian in action at the May 2008 Board meeting, one was immediately impressed with his ability to take complex problems and synthesize the discussion into very simple, practical, and clear points. He listens intently, asks probing questions, provides information on all sides of the issue, and then formulates a plan for action. It's very obvious that he cares deeply about the future of aquaculture in the western states.

Brian recently spoke with Debbie Granger about his journey: working in fisheries, his 20+ year involvement with WRAC, and his retirement from Alaska Sea Grant.

Tell us about yourself, your background, and your family.

My grandparents emigrated from Norway and settled on a farm outside Bellingham, Washington on the Mount Baker Highway. After spending a number of years in Seattle, my mom and dad left Seattle and settled in Oakland, California. I was born in San Francisco, grew up in Oakland, and spent some of my summers in the Nooksack River Valley north of Bellingham on my Norwegian uncle's farm. While I was in Washington, I would also visit my aunt and uncle in Sekiu, Washington on the Olympic Peninsula and when I was 14 years old I worked a summer for my uncle, Al Olson, at his fishing resort. This experience cleaning salmon and recreational fishing boats stimulated my interest in fisheries.

After receiving my undergraduate degree in Zoology at UC Berkeley, I entered the College of Fisheries at the University of Washington. I was fortunate enough to be supported by the Cooperative Fisheries Unit and to do my PhD research at the Big Beef Creek Field Station on Hood Canal. During my tenure at Big Beef Creek, I was exposed to aquaculture projects funded by Washington Sea Grant and a private salmon venture. I have had the good fortune to work in the fisheries field in Oregon, Washington, and Alaska for the public, private non-profit, University, and private sectors for the last 37 years.

Brian Allee (left) accepts recognition certificate from Ron Hardy, Chair, WRAC Board of Directors

Photo: Debbie Granger

I am married to my junior high school sweetheart, Angela, and between us we have 8 children and 2 grandchildren. One of my sons, Scott, works as a law enforcement special agent for NOAA Fisheries in Juneau, Alaska.

How did you get your start in marine sciences?

After graduate school, I worked for the Quinault Indian Nation, located on the southwest corner of the Olympic Peninsula, on a number of fisheries projects, including the impacts of logging on fisheries in streams and rivers within the Quinault Indian Nation trust lands and on salmon restoration and enhancement. I was also asked to participate and testify in the US vs. Washington fishing rights case before Judge Boldt on behalf of the Quinault Indian Nation regarding the sockeye and steelhead fishery. As a young graduate, new to fisheries, it was thrilling to be part of this important work.

Can you share with us a bit about the early days of WRAC?

I was a participant in the first WRAC planning meeting of the IAC/TC at the Crown Plaza Hotel in Seattle in 1987. At that time, I was a member of the IAC, representing the private-nonprofit ocean ranching sector in Alaska.

In the early days, the industry, extension, and research groups had a very similar opinion on the research priorities and there was great enthusiasm and camaraderie among the three groups for research projects. Perhaps this was because WRAC was a new source of funding for aquaculture research and fewer states were involved at that time. I know I always felt grateful for any new funding that would engage university research expertise on industry problems. I was proud to work with other "founding fathers," developing the governance structure, the policies, and procedures to ensure WRAC's continued success. The early program also benefited from the strong leadership of Ken Chew as Director.

Of which projects and activities in your career are you the most proud? What do you want people to remember about your contributions to WRAC?

There have been a number of fine research projects, but the one that stands out to me is the sturgeon broodstock project. I believe WRAC funding was influential in the work group because it leveraged funds and helped research scientists work together, creating an opportunity for industry to develop in the Sacramento valley near the University of California, Davis. I was also very supportive of other long-term research projects such as oyster genetics and IHN research.

What are your thoughts about the future of aquaculture research, the aquaculture industry, and the future of WRAC?

I see a strong and challenging future for aquaculture research in the western region. I would like to see a continuing effort to fund applied industry-needed short-term research projects together with a balanced portfolio of long-term funded research directed at important industry problems. Of course, increasing the research funding base would be my greatest hope and I feel confident that our WRAC Director, Graham Young, is able to accomplish this goal. In addition, the future of WRAC is in good hands with a very competent and dedicated Board of Directors, together with industry, research and extension representatives. It is, of course, my opinion that WRAC is the best represented and most successful regional aquaculture center in the United States.

How would you compare aquaculture research when you were just starting out with current research?

Originally, the industry was smaller with fewer states involved so it seemed less complex. The industry is more diverse now and the research capacity in the 12 western states that comprise WRAC is substantial and very capable.

What plans do you have now?

After leaving Alaska this summer, I am living in Portland with Angela. In addition to spending time with our children and grandchildren, and a few fishing trips, I am working as a contractor half-time for NOAA Fisheries on a Columbia River Salmon Restoration project and enjoying the experience of being partially retired. I am also hoping to participate in some capacity in the Oregon Sea Grant program in the future.

However, I really do miss being a part of WRAC and the aquaculture research effort. I have always enjoyed the opportunity of working with so many talented and dedicated individuals within the WRAC program.

Dr. Ole Mathisen: A Real Fisheries Hero

Brian Allee

Dr. Ole Mathisen emigrated from Norway in 1946 with a Master of Science degree in Zoology. While working on his PhD in fisheries, he was a staff member of the highly respected Fisheries Research Institute at the University of Washington, chaired by W. F. Thompson. His interest in salmon research began with his first trip to Bristol Bay in 1948 to study the largest wild sockeye populations in the world and 59 years later he was still active in salmon research. Dr. Mathisen died March 12, 2007 in Friday Harbor, Washington.

Alaska was the major focus of Dr. Mathisen's energy and research during his tenure as a Professor in the College of Ocean and Fisheries Sciences at the University of Washington and later as a Professor in the School of Fisheries and Ocean Sciences at the University of Alaska Fairbanks. Dr. Mathisen was a Fulbright Scholar in Norway and Malaysia, a visiting scholar at the University of Moscow and the recipient of the Wally H. Noerenberg Award for Fisheries Excellence from the Alaska Chapter of the American Fisheries Society.

Ole Mathisen served on the WRAC Board of Directors with distinction from 1975–1998. He was able to do this by the force of his enthusiasm and his zest to make a contribution as a Board member rather than passively approving issues or funding recommendations from the Industry Advisory Council and Technical Committee. He had a way of motivating people to do more whether you were a student, professional or a Board member.

He was a paradox in Alaska. As a working professional he balanced his sustained interest and considerable research contribution in wild capture fisheries with his developing interest and enthusiasm in aquaculture. In this regard he was not a politician in Alaska. Although he helped to pioneer applied research supported by the fisheries industry they were diametrically opposed to aquaculture, with the exception of salmon ocean ranching and shellfish mariculture. Ole on the other hand, was always learning and integrating new knowledge based on his trips to Norway to observe their robust aquaculture research program. It was based on this exposure to aquaculture that he proposed and advocated for a marine farming research institute in Sitka, Alaska that drew severe statewide criticism. This never seemed to dampen Ole's enthusiasm and ideas for conducting research in area of new fisheries innovation and technologies. In spite of the negative environment, Ole would make annual trips to Norway and he would always come back with new ideas and concepts that he would debate with colleagues for application in Alaska. Clearly, Ole was committed to the concept that the United States should take advantage of its abundant natural resources for fisheries generally whether from wild-caught fisheries or aquaculture.

Courtesy of University of Alaska

Ole had a way of motivating people to do more whether you were a student, professional, or a member of the WRAC Board of Directors.

Dr. Mathisen was an inspiration to me personally and I believe one of the great contributors to fisheries research. If I had my way, I would recommend that his name be placed in the yet to be created Fisheries Hall of Fame. Come to think of it, let's create such a category in the American Fisheries Society.

It is in this spirit that I would like to challenge the collective community of fisheries industries, agencies, municipalities, stakeholder groups, universities, students, and citizens to contribute to the growth of fisheries science through the financial support of undergraduate students who aspire to excel in fisheries as Dr. Mathisen did. If you would like to so honor Dr. Ole Mathisen's dedication and commitment to Alaskan fisheries, you can make a financial contribution to the Ole Mathisen Scholarship fund at the University of Alaska Foundation as I did. ■

For more information about the Ole Mathisen Fisheries Scholarship, or to contribute, contact SFOS Development Officer Teresa Thompson at teresa@sfos.uaf.edu or by mail at PO Box 757220, Fairbanks, AK 99775-7220 or (907) 474-1867, or log on to the UAF Development Office website and give online at www.uaf.edu/giving/

Control of IHNV— 20 Years Later

Jim Winton, Chief, Fish Health Research Section, USGS Western Fisheries Research Center

Beginning in 1986 with the establishment of a series of Regional Aquaculture Consortia (RACs), the US Department of Agriculture (USDA) implemented a new approach to funding research in support of US aquaculture. Unlike the typical grant programs at the time, the strength of the RAC concept was the potential to fund long-term, multi-investigator research that could address the most difficult problems facing the industry. The industry-driven research, coupled with effective scientific peer review and a strong commitment to extension, ensured the work was relevant, of high quality, and was communicated effectively to industry.

In 1987, following the development of a set of problem statements that identified important needs of the aquaculture sector in the western states, interested scientists were invited to participate in a series of seven research work group meetings to develop proposals for long-term support. From these proposals, the Western Regional Aquaculture Consortium (later Center) selected four research projects for funding. One of these, "Development of Methods for Control of Infectious Hematopoietic Necrosis Virus in Commercially Reared Salmonid Fishes," ended in 1999. WRAC funding for this project totaled $1,635,500, although the leverage obtained from in-kind contributions of participating institutions made the project significantly larger. During the 12-year WRAC IHN project, a number of scientists and institutions participated, many for the duration (Table 1).

During the development of the initial IHN work plan, a large-scale attack on the problem was envisioned—with broad objectives similar to those that might be appropriate for research on the control of any virus disease of humans or animals. For the initial five-year project, the objectives were to develop methods of control of IHNV by 1) stimulation of specific immunity, 2) stimulation of non-specific resistance, and 3) application of drugs and chemicals; and to 4) determine the factors involved in the host-virus relationship.

The project began with an annual budget of $200,000 and this amount was again available in years 2 and 3. By year 4, flat funding for the RAC program and a desire by the WRAC Board of Directors to initiate additional projects resulted in a gradual reduction in funding. This reduction, coupled with increasing research costs, meant that the participating laboratories were receiving WRAC funding at a level below that needed to support even a single graduate student.

While the erosion of support nearly resulted in the dissolution of the IHN work group, the fact that many participating scientists and institutions had additional funding sources meant that the WRAC contribution was serving principally as "glue" to hold the group together. This proved critical because the WRAC work group meetings were an important avenue for maintaining communication and coordination among the researchers who were receiving substantial amounts of funding from other sources.

Based upon the success of the initial project, the IHN work group proposed an additional four-year project that was approved for funding. Designed as a continuation of the ongoing efforts, the objectives for years 6–9 were to:

- develop methods for inducing specific immunity to IHNV in rainbow trout
- determine the genetic basis of resistance to IHNV among different stocks of fish
- develop methods for inducing non-specific immunity to IHNV in rainbow trout
- develop novel methods for control of IHNV using antiviral drugs and chemicals
- understand the pathogenesis and virulence of IHNV in rainbow trout

—*continued on page 10*

Table 1. Principal investigators and institutions participating in the IHN project.

Robert Busch	Clear Springs Foods, Inc.; BioMed Research Laboratories
James Congleton	University of Idaho
Walt Dickhoff	University of Washington; NOAA Fisheries
William Eaton	University of Alaska; Washington Department of Fish and Wildlife
Gary Fornshell	University of Idaho
Ronald Hedrick	University of California, Davis
Marsha Landolt	University of Washington
Scott LaPatra	Clear Springs Foods, Inc.
Jo-Ann Leong	Oregon State University
Jim Parsons	Blue Lakes Trout Farm; Troutlodge, Inc.
Ray RaLonde	University of Alaska
Sandra Ristow	Washington State University
John Rohovec	Oregon State University
Jim Winton	Western Fisheries Research Center

—*continued from page 9*

Because USDA support for the RAC program remained under continued pressure, WRAC annual funding for the second IHN project was reduced even more. Again, the WRAC project was serving as glue for a productive research team that was working well together, but was largely funded by other sources.

By the end of year 9, the project had developed several important products, including candidate vaccines and a chemical approach for control of IHN. To move this work into the field, a final two-year project was negotiated and funded to:

- conduct field tests of recombinant vaccines for induction of immunity to IHNV
- determine the synergistic effects between IHN and bacterial coldwater disease
- conduct field tests of low levels of elemental iodine for control of IHNV
- develop extension products and project synopsis

Funding from WRAC remained at about the previous levels for two years, and a one-year extension was funded at $9,000 to complete the outreach portions of the work in year 12. However, at this point there was agreement among the WRAC Board and the IHN work group members that the research had reached a logical end point for WRAC funding.

Leverage calculations & research impacts

Beginning in year 6, the IHN work group independently started to calculate the leverage from this project (this later was adopted as a requirement for all projects). It soon became obvious that the IHN program was operating on a total amount that was approximately six times the contribution of the WRAC budget. This leverage was comprised of direct sources such as grants from other agencies (e.g., Bonneville Power Administration, USDA competitive grants program) and in-kind contributions such as the IHN-specific portions of the operations costs of research facilities and the salaries and benefits for the principal investigators, technicians, or students at the universities, agencies, and companies involved. In total, these in-kind and matching funds totaled more than $3.7 million during the six years for which the IHN work group calculated (Table 2).

As is true for much scientific research, the most significant impacts from this project have only become apparent with time. Among the most important products and impacts are:

- *83 publications in peer-reviewed journals*
  The scientific information developed provided a major expansion of our knowledge of the disease and the causative virus. For example: the full genome sequence of IHNV was determined (first full genome of a fish pathogen); the genetic analysis of strains has shed insight into the biology of fish rhabdoviruses; the antigenic analysis of IHNV proteins has revealed important determinants for virulence and immunity; and assays for the immune response have increased our understanding of mechanisms needed to protect fish from virus diseases in general.
- *More than 100 presentations and technical articles*
  All were effective in communicating the findings of the project to the broader scientific and fish health community.
- *Two all-day workshops in Hagerman Valley, Idaho*
  These were well attended by commercial trout growers and extension specialists, and provided a substantial increase in the level of knowledge about IHN among those in the rainbow trout industry.
- *Improvements in the diagnosis of IHN or detection of the virus*
  These improvements have become standard methods used by national (e.g., American Fisheries Society; US Fish and Wildlife Service; Department of Fisheries and Oceans, Canada) and international (e.g., Office International des Epizooties, World Organization for Animal Health) agencies and organizations responsible for fish health. For example, the PCR assay for IHNV developed by the WRAC project was the first application of this technology in fish health and, possibly, in any area of fisheries science.
- *Development of tools and reagents*
  Many of the tools and reagents developed during the project are widely used in research and commercial applications. These include monoclonal antibodies, PCR primers, and DNA probes. In addition, some of the reagents (e.g. conformation-specific, neutralizing mono-

Table 2. Leverage of WRAC funds in years 6–11.

Year	WRAC	Other	Total
93–94	130,000	663,000	793,000
94–95	130,000	686,229	816,229
95–96	126,100	953,016	1,079,116
96–97	126,000	520,913	646,913
97–98	124,000	520,913	644,913
98–99	127,400	439,774	567,174
Total	763,500	3,783,845	4,547,345

clonal antibodies) have proven critical for commercial companies engaged in development or evaluation of killed IHN vaccines as they only bind to IHN antigens having the correct structure to stimulate a protective response.

- *Novel control methods for IHN*
  Several candidate vaccines and low-level iodine treatments have received commercial interest. Most significant was a highly efficacious DNA vaccine (Table 3) that recently has been marketed by Novartis Animal Health in Canada with US licensing planned. This product is among the most effective vaccines ever developed for finfish.

Although the WRAC project was not able to end IHN as a problem for the US aquaculture industry, it is important to realize that the project is still providing progress toward the eventual control of this disease. Compared with research efforts on virus diseases of humans or domestic livestock, the $1.6 million project was modest yet resulted in significant success, including critical research tools, a large body of scientific information, and several candidate vaccines and chemical methods that have resulted in commercially viable products. The recent licensing of a highly-effective DNA vaccine, initially developed by the WRAC project, promises to provide control of IHN where the vaccine can be delivered economically. Already in use in Canada among Atlantic salmon farms where hand injection of high-value smolts is commercially viable, new-generation delivery methods will be needed to realize the benefits of this vaccine in small rainbow trout. Research on mass delivery methods, some of which have been recently funded by WRAC, suggests this goal is not beyond reach. ■

Table 3. Results of challenge trials using rainbow trout immunized with various vaccine preparations developed during the WRAC IHNV project and their respective controls. Only the β-propiolactone-killed IHNV preparation, the DNA vaccine and the attenuated virus provided protection. Results are presented as percent mortality in replicate groups of trout challenged with waterborne IHNV at high and low doses.

Treatment	Description of Preparation	Average % Mortality at High Challenge Dose (105 PFU/mL)	Average %Mortality at Low Challenge Dose (103 PFU/mL)
PBS	Phosphate buffered saline control for vaccination	93%	54.5%
CMV-Luciferase	DNA vaccine control with the luciferase gene inserted into a plasmid	86%	44.5%
CMV-IHNV-G	DNA vaccine with the IHNV glycoprotein (G) gene inserted into a plasmid	2%	1%
β-propiolactone Inactivated	Whole IHNV grown in tissue culture and inactivated with β-propiolactone	1%	3%
Attenuated IHNV	IHNV passaged repeatedly in tissue culture to attenuate	45%	20%
Native IHNV glycoprotein	Native IHNV G removed from virus grown in cell culture	84%	48%
Empty ISCOM control	Immumostimulatory complexes (ISCOMS) with no inserts	87%	44%
ISCOM-native IHNV G	ISCOMS containing purified IHNV G arrayed on surface	91%	46.5%
IHNV G from	IHNV G expressed in baculovirus/insect Baculovirus cell culture	89%	40.5%
Baculovirus ISCOM	Baculovirus control in ISCOM formulation	83%	56%
pATH vector control	Subunit vaccine control with no insert in the TrpE fusion protein	82%	39.5%
pXL3-pLON3	Recombinant subunit vaccine with IHNV G expressed as a TrpE fusion protein	89%	49.5%

Maximizing Yield and Quality of Caviar

Molly Webb[1] and Serge Doroshov[2], USFWS, [1] Bozeman Fish Technology Center, and

Culture of Pacific white sturgeon started in California in the early 1980s, with the objective of producing meat for the food market. Production of caviar was an uncertain target because of late sexual maturity of sturgeon in the wild. However, accelerated maturation of farmed sturgeon and reduced caviar import from the Caspian Sea made caviar production on sturgeon farms economically feasible.

Currently, several farms in California produce more than 15 metric tons of caviar annually, and production has expanded into Idaho. Previous WRAC projects and support from federal agencies have made major research contributions on sturgeon reproduction, nutrition, genetics, health management and food science, providing a foundation for sturgeon husbandry and caviar production.

Fred Conte

Project goals

This WRAC project aims to optimize yield and quality of caviar in sturgeon farms in California and Idaho. Optimal yield and quality is obtained only when the eggs have reached their full grown size in the ovary. However, there is significant variation in the ovarian cycle among females, so that one fish could have fully grown eggs in February and another in June.

The only means to assess maturity and properly time caviar harvest is to conduct a surgical ovarian biopsy to determine egg polarization index (PI), which is an accurate indicator of ovarian maturity. This technique is stressful to fish, time consuming, and not effective for handling large numbers of fish. Application of this technique often results in decreasing harvest and quality due to ovarian follicular atresia (resorption of eggs). Even the early stage of atresia causes a reduction in the firmness, flavor, and shelf life of caviar. Harvesting early (when a female is still months away from maturation) to avoid atresia results in inferior caviar due to small egg size and a significant reduction in caviar yield. Thus, determining the proper time when fish are fully ripe *and* preatretic has significant economic benefits to sturgeon farmers.

In 2006, researchers and extension specialists from Idaho, Montana, Washington, Oregon, and California, teamed up with Sterling Caviar LLC, in California, Fish Breeders of Idaho, and Blind Canyon Aqua Ranch, Idaho, to develop a less invasive, faster, and better predictor of maturity and egg quality in sturgeon. To achieve this goal, investigators need a better understanding of the biochemical and physiological changes that occur during maturation and how these changes correlate with egg quality and yield.

MSU graduate student Mariah Talbott scans abdomen of sturgeon with SWNIR probe at Sterling Caviar LLC. Linda Lemmon (Blind Canyon Aqua Ranch, Idaho) observes procedure.

Montana State University, [2]University of California, Davis

Professors Barbara Rasco (School of Food Science, Washington State University) and Anna Cavinato (Eastern Oregon University) are examining non-invasive short-wave near infrared spectroscopy (SWNIR) and Fourier transform infrared spectroscopy (FTIR) as tools for assessing maturity based on biochemical changes in the ovary and blood during vitellogenesis (yolk formation), egg maturation, and follicular atresia. Sturgeon biologists Drs. Molly Webb (Bozeman Fish Technology Center and Montana State University) and Wendy Sealey (University of Idaho) will evaluate reproductive development in sturgeon and the ability of non-invasive techniques and immunochemical assays to replace the egg PI in the assessment of maturity.

The team anticipates that new non-invasive methods will improve stock management and caviar yield and quality in sturgeon aquaculture of the Western Region.

Preliminary results

This project is still in the early phase, but initial results indicate that stage of maturity (egg PI) and follicular atresia can be successfully detected by FTIR, SWNIR, and immunochemical assays, and the spectral data show a correlation with the egg PI, plasma steroids, calcium, and proteins. Whether and how these techniques can be applied to optimize caviar harvest will be determined over the following years of the project.

The ability of producers to produce a uniform product, standardize processing conditions (i.e. salt absorption), and maximize caviar yield by harvesting fish when the eggs have the appropriate firmness and size would be an important advance for the industry. Optimization of harvest time may extend the production season and reduce the need to hold caviar in storage for holiday sales. Conservation propagation programs for endangered sturgeon species will also benefit from the improved staging of fish for breeding. ■

Sturgeon in the News

Two recent articles in the consumer media have featured the recent WRAC-sponsored research of Rasco and colleagues aimed at optimizing quality and shelf-life of sturgeon caviar.

An article appearing in *WSU Today*, May 9, 2008, shares an overview of the importance of sturgeon caviar to the Northwest and the world. The article states that despite strict regulations, the vulnerable—and valuable—sturgeon has been greatly over fished and is on the endangered list in many parts of the world.

Rasco is among those working to help save the sturgeon in an international effort that could provide spin-off benefits for aquaculture in the Pacific Northwest. She believes one answer to the problem is to promote US sturgeon production—especially the fledgling white sturgeon industry taking shape in southern Idaho.

The researchers on this project hope to develop technical information—specifically techniques to determine optimum reproductive maturity for harvesting female eggs—that will not only help conserve global sturgeon populations but will also provide US sturgeon growers with new tools to produce and market caviar.

To learn more about *WSU Today*'s coverage of this research, visit *http://impact.wsu.edu/people/rasco.htm*.

Also, an article in the *Lewiston Tribune* (June 23, 2008) showcased the work of Rasco's collaborative research with Washington State University and University of Idaho and the work of Leo Ray, owner of an Idaho fish farm.

Ray began producing caviar from white sturgeon at his Hagerman, Idaho, fish farm several years ago. He credits the work from aquaculturists at the College of Southern Idaho and the University of California, Davis for figuring out how to spawn sturgeon in captivity. With the additional work of scientists at the Hagerman Fish Research Center regarding harvesting techniques for caviar production, Ray says sturgeon caviar production could provide a niche rural industry in Idaho.

Rasco believes that Northwest production of sturgeon will take some of the pressure off of the Caspian Sea fishery as people become familiar with caviar products that are just as good, if not better, than those from Europe and Asia, and from a sustainable resource.

Good News Gets Little Press

Gary Fornshell, Extension Educator, University of Idaho Extension

Have you heard the good news?

Seafood is good for you. It is so good for you that the American Heart Association recommends two servings (particularly fatty fish) per week. Also, the US Department of Health and Human Services 2005 Dietary Guidelines recommend increased consumption of fatty fish, especially salmon, trout, and herring.

Ever since scientists noticed surprisingly little heart disease among Eskimos who eat a lot of fatty fish, they have been intrigued by fish fats, specifically omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), found in cold water fish such as salmon, trout, herring, and sardines. Our bodies cannot produce these essential fatty acids, but they are necessary for our bodies to function well; they are key components of cell membrane formation and affect blood clotting and inflammation, and they contribute to optimal brain and vision development in unborn babies and infants.

Considerable scientific evidence suggests that eating seafood reduces the risk of cardiovascular disease and stroke, and helps protect against heart attacks and sudden death. Currently, there are no dietary omega-3 fatty acid requirements, but one study suggests an intake of a minimum of 250 mg/day of EPA and DHA appears sufficient for reducing risk of coronary disease.

Recent research also suggests that eating seafood may reduce the incidence of depression, preterm birth, Alzheimer's disease and cognitive decline, and inflammation caused by rheumatoid arthritis. Current research from the United Kingdom and Australia concludes that eating a diet high in oily fish actually protects against Type-2 diabetes. Recently, a team of researchers from the University of Adelaide reported that giving premature babies doses of an omega-3 fatty acid (DHS) can stop the development of mental retardation.

The FDA recently released a comprehensive, peer-reviewed draft report assessing the latest research on the effects of seafood on heart health and baby brain development (see *http://www.cfsan.fda.gov/~dms/mehgovi.html*).

Consumers lack confidence

While seafood is a low-fat, nutrient-rich, high-quality protein food that is widely available, only about 20% of Americans eat it twice a week or more. Why is that? Food choices are complex decisions involving psychological, social, cultural, and economic factors. Taste, price, availability, and convenience top the list of factors influencing purchasing decision.

Courtesy of G. Fornshell

Gary Fornshell

Surveys indicate that consumers lack confidence and knowledge about purchasing, handling, storing, and cooking seafood. For example, only 27% of one survey's respondents agreed that it is easy to judge seafood freshness. Consumers are also confused and concerned about the benefits and risks of eating seafood. In response to the statement, "pregnant women should eat seafood," 38% disagreed and 41% were unsure. Mercury was mentioned by 57% of consumers who indicated they had heard negative messages. Of consumers who reported hearing positive information, 47% mentioned omega-3 fatty acids, fish oils, or healthy fats.

Consumers receive information on seafood from three main sources: the media, family and friends, and the internet. There is evidence that media preferentially choose to focus on risks and events that are rare, novel, vivid, and dramatic. It is widely recognized that media impacts public perception of risk.

Coverage ignores benefits

In a recently published paper, investigators studied US newspaper coverage of farmed salmon after two studies reported on the contaminant levels of farmed and wild salmon. The first study was released in July 2003 by the Environmental Working Group, Washington, DC, which claimed that eating farmed salmon would significantly increase the risk of developing cancer or fetal birth defects. The second study, published in the journal *Science* in January 2004, also claimed an increased risk of cancer and defects in fetal and child development due to farmed-salmon consumption. However, both studies had significant experimental design flaws and

were met with skepticism from scientists. Yet, popular media essentially ignored the skepticism of the scientific community and agency seafood consumption guidelines.

An analysis of the text in the popular media concerning articles reporting about cancer risks showed an increase from 2% to 39% of all farmed salmon text after the first study and up to 50% after the second. The health benefits of farmed salmon were given little attention, with less than 10% of text—and this text was almost always portrayed in the context of potential risks. Media coverage emphasized the more severe risks— 70% of the stories about the first study and 90% about the second highlighted cancer risks.

The analysis of US newspaper coverage suggests that the public received a largely uniform negative message about farmed salmon and that was based primarily on the two controversial studies. During this period, multiple studies were published, including a report by the European Food Safety Authority, that concluded there was no difference between farmed and wild fish with respect to contaminants and consumer safety; yet the US media continued its narrow focus and the European report received very little coverage.

Gaining Consumer Trust

Because consumer purchasing habits may be dictated by fear, it is important to gain consumers' trust through better informational efforts and transparent policies. To be successful and to develop appropriate educational messages, it is necessary to understand the basis of consumer decisions. Frequent seafood consumers (defined as those who eat seafood once or more per week) look at quality and health as positive factors, while price is a negative factor influencing their seafood consumption. In fact, "diehard" seafood lovers continue to eat seafood and even food safety issues do not deter them. However, consumers who eat seafood less frequently say environmental issues are a concern, and getting these consumers to increase their consumption beyond the occasional restaurant meal remains a challenge.

With all of the good news about the health benefits of eating seafood, science must do a better job of disseminating the good news in a directed effort to counter the negative and often sensational reporting of the popular media. Extension outreach efforts should target moderate and infrequent seafood consumers to build their confidence in buying and preparing seafood. Providing consistent, correct information is the best way to build consumer confidence and knowledge. ■

For More Information

http://www.aboutseafood.com/health-nutrition/pregnancy
Lists the benefits of eating seafood for pregnant women and their babies and links to summaries of the latest studies and articles.

http://www.seafood.net.au/health/benefits.php
Links provide detailed information on the benefits of eating seafood for specific medical conditions.

http://www.bmj.com/cgi/content/full/bmj.39561.501007.BEv1
Research article: Adherence to Mediterranean diet and risk of developing diabetes: Prospective cohort study. *BMJ* 2008;336:1348-1351.

http://www.nmfs.noaa.gov/fishwatch/seafood_and_health.htm
Comprehensive site offering practical information on seafood health benefits, cooking and storage tips, and more.

Suggested reading

Amberg SM and TE Hall. 2008. Communicating risks and benefits of aquaculture: A content analysis of US Newsprint Representations of Farmed Salmon. *Journal of the World Aquaculture Society* 39(2): 143–157.

Hicks D, L Pivarnik, and R McDermott. 2008. Consumer perceptions about seafood—an internet survey. *Journal of Foodservice,* 19: 213–226 (early online).

Mozaffarian D and EB Rimm. 2006. Fish intake, contaminants, and human health: Evaluating the risks and the benefits. *JAMA* 296(15): 1885–1899.

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Estimating Economic Impacts

Craig Bond, Department of Agricultural and Resource Economics, Colorado State University

Economists typically estimate such impacts and contributions by tracing the backward flows—or backward linkages—of goods and services between sectors of the economy

Recently, WRAC funded a project to determine the economic impacts and contributions of aquacultural suppliers of recreational fish (ASRF) in the western United States. This project will be administered by a team of researchers at Colorado State University led by Dr. Craig Bond.

Economists typically estimate such impacts and contributions by tracing the backward flows—or backward linkages—of goods and services between sectors of the economy (such as industries, households, etc). Backward linkages are simply connections between producers and their suppliers.

As an example, assume a trout producer in Colorado spends $10,000 on food and equipment per year, 50% of which is sourced within the state, and another $10,000 annually on labor and benefits, where all employees live in the state. These are termed "direct expenditures" and are the first round of spending from the producer to suppliers of inputs.

Thus, of the $20,000 per year for direct expenditures, approximately $15,000 accrues to "local" firms and households in the state. These firms and households, in turn, purchase supplies (inputs) for their own businesses and households; again a proportion of these "second round" expenditures stays in the state. For example, the local firms that supplied food and equipment buy labor and other inputs for their businesses, some proportion of which is sourced from firms and households in Colorado. Similarly, some proportion of the labor income originally paid to the Colorado workers by the trout producer is spent locally on items such as groceries, pet food, and entertainment. This money continues to spread around the regional economy in third and subsequent rounds of spending, with the resultant impacts termed "indirect" and "induced" effects of the original spending.

Using a representation of the regional economy called an "input-output" model, in which many industrial and household sectors and their associated production functions are represented, the end result of each dollar of spending can be estimated. So, if the original $20,000 spent by the Colorado trout producer translates into $35,000 worth of regional spending overall, then the indirect and induced effects totaled $15,000, and the "multiplier effect" on output is 1.75. Similar multipliers can be calculated for other economic variables of interest, such as value-added or employment.

Of course, backward linkages do not tell the full story. The connections between producers and their customers are called "forward linkages." Dude ranches, private ponds, and government organizations all purchase fish from the ASRF and are included in this category. Finally, the end users of the product—namely, the anglers—are at the conclusion of the supply chain, and are impacted by the aquaculture industry as well.

From an economic standpoint, anglers are the most difficult point in the chain to evaluate, mainly because it is difficult (though not impossible) to quantify the dollar amount of "fun," or to use economic terms, "surplus," that anglers enjoy. Of course, many anglers also spend money to go fishing (more than $700 per year on average), but this doesn't take into account the additional net benefits they enjoy from the activity. While not all recreational fishing trips can be directly attributed to private aquaculture activity, certainly there would be an impact if the industry was not engaged in recreational fish production.

Our project will use input-output analysis and a variety of other statistical methods to estimate the backward and forward linkages, and thus the economic impacts and contributions of the aquacultural suppliers of recreational fish. The key to our efforts will be the collection of data regarding production aspects of ASRF producers, a sample of their direct customers, and a sample of the anglers that fish Western waters.

Each set of firms/households will be asked to complete a survey that provides relevant information to the research team for analysis. For example, the producer survey will ask general questions regarding operational practices, and specific questions regarding costs of operation. While this data is relatively sensitive in nature, it is extremely important to accurately represent the direct expenditures of the industry in order to properly estimate the related indirect and induced effects.

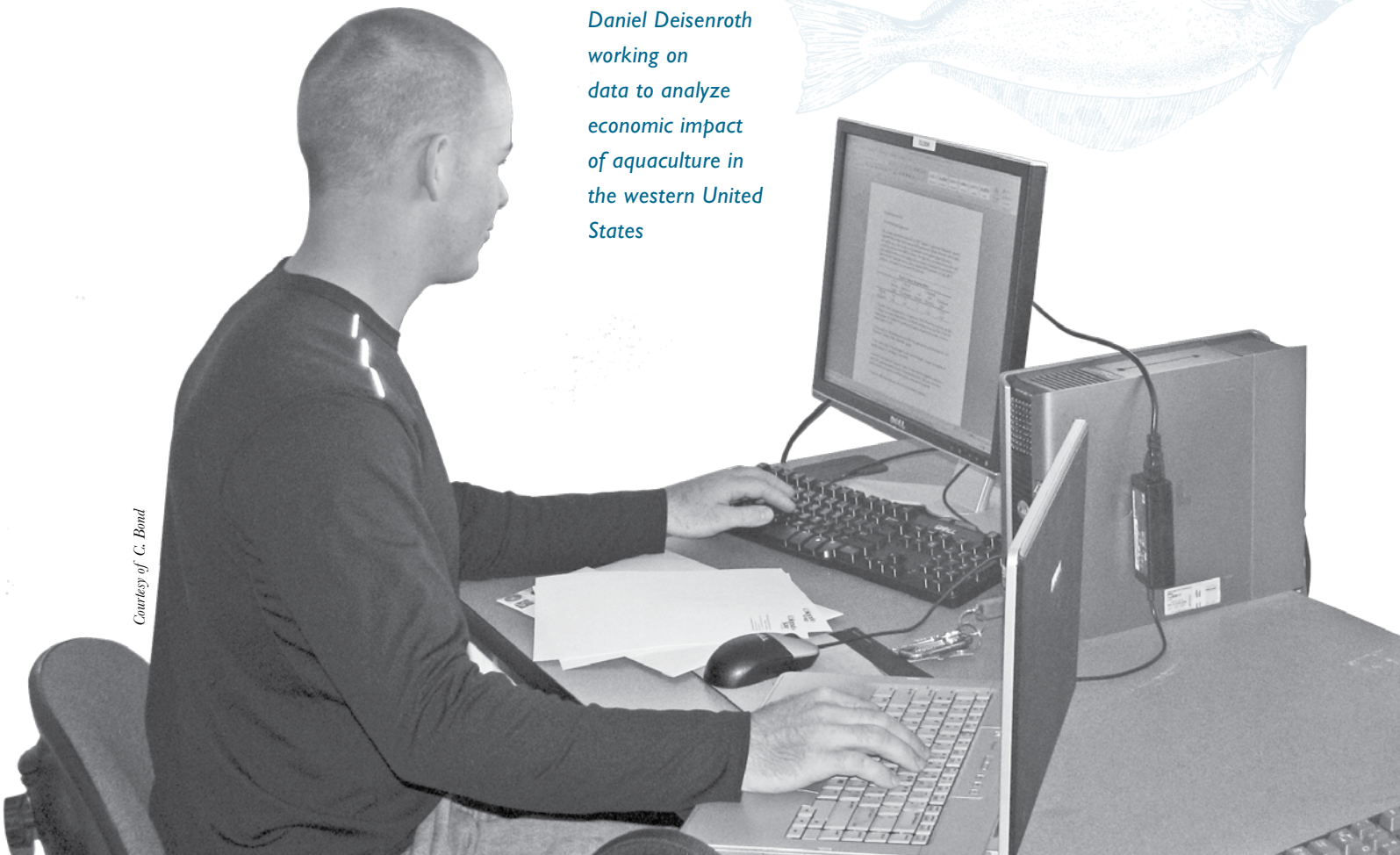
All information will be kept confidential in accordance with the appropriate laws and regulations and results will be pooled to maintain the confidentiality of the producers. We will use state-level input-output models to estimate the overall contributions to the Western economy, aggregating where necessary in order to conceal the identity of specific producers.

When the results from the producer survey are in, the research team will begin distributing a survey to the dude ranches, private ponds, ranches, and other direct customers of the ASRF. This second survey will ask similar questions and use similar techniques to estimate the economic contributions of these industries. Finally, a third survey will go out to the anglers to collect information that will allow us to estimate the total benefits associated with the portion of fishing that can be attributed to the aquaculture industry.

The documentation of the contributions of the ASRF industry on the Western economy will serve an educational purpose for regulatory agencies, the general public, and other researchers. These groups will learn about the magnitude, scale, and scope of the direct, indirect, and induced benefits of the industry, including the associated economic multipliers associated with the production activity and welfare contributions to anglers from private aquaculture-supported fisheries

This information could then be used to publicize the positive economic impact of the industry on the Western US economy and its impact on the welfare of anglers across the region to relevant government agencies and the general public. In addition, these same groups will learn about the regulatory and policy issues facing the aquaculture industry, and the potential impact of various regulatory and competitive changes on the welfare, growth, and incentives of the industry as a whole.

The research team is hopeful that this project will prove useful to the ASRF, as well as to related industries and government institutions. If you have any questions regarding this study, please visit the project website at *http://dare.colostate.edu/wracimpact.html,* or contact Dr. Craig Bond at 970-491-6951 or *craig.bond@colostate.edu*. ■



Graduate student Daniel Deisenroth working on data to analyze economic impact of aquaculture in the western United States

Courtesy of C. Bond

Control and Prevention of Coldwater Disease

Ken Cain, Department of Fisheries & Wildlife Resources, University of Idaho

Presently, coldwater disease management is difficult and there is no commercial vaccine available

In recent years, coldwater disease (CWD) has become one of the most significant problems worldwide in commercial trout aquaculture. In the Pacific Northwest, mortality from CWD can range from 18% to 30%. Federal, state, and tribal hatcheries rearing a variety of salmonids (steelhead and Coho salmon in particular) also suffer dramatic losses.

Presently, CWD management is difficult and there is no commercial vaccine available. This disease is caused by the gram-negative bacterium *Flavobacterium psychrophilum*, and while antibiotics and chemotherapeutics have been used for control and can provide some benefit, there is concern over deformities in survivors and the development of antibiotic resistant strains of *F. psychrophilum*.

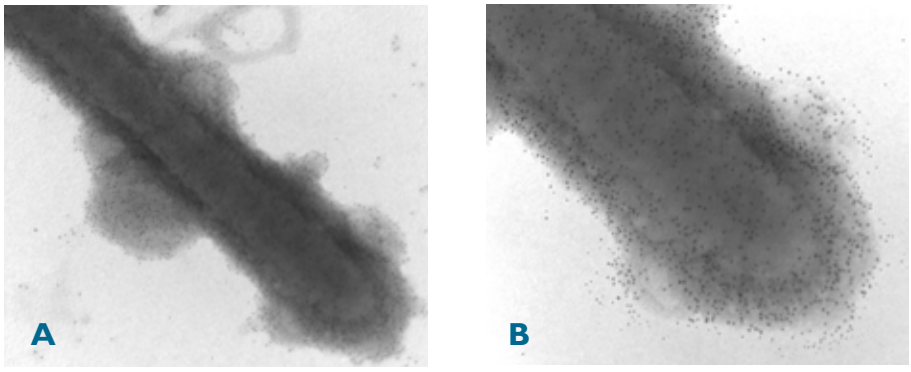
WRAC-funded project

WRAC funding is supporting a four-year project to develop more effective CWD management strategies and identify possible bacterial genes that may be targeted for vaccine development and testing. The project involves a number of investigators and collaborators, and links researchers and facilities in academia and industry. The CWD work group consists of Ken Cain (University of Idaho), Doug Call (Washington State University), Scott LaPatra (Clear Springs Foods, Inc.), Jim Parsons (Troutlodge, Inc.), and Gary Fornshell (University of Idaho). PhD student Amy Long joined the Cain lab in May 2008, and Rajesh Kumar, a postdoctoral associate, was recruited by Dr. Call.

Investigators will take a two-pronged approach. First, they will build on efforts to target vaccine candidate antigens through identification of important proteins at the gene level. The work proposed complements other research in our laboratories, thereby the likelihood of identifying protective antigens and developing an efficacious vaccine will be maximized.

Second, because colonization of eggs and vertical transmission of *F. psychrophilum* from broodstock to progeny has been demonstrated and is implicated as a source of outbreaks in early life stages, researchers will validate quantitative diagnostic assays (recently developed in these laboratories) as potential tools for selecting and culling infected broodstock or eggs. Culling broodstock and eggs with high infection loads may reduce subsequent disease outbreaks. It is also possible that at facilities where vertical transmission does not appear to contribute to outbreaks, broodstock infection levels may have a genetic link and correlate to increased susceptibility of progeny to CWD.

Researchers hypothesize that identification of heavy infection loads in broodstock, and the culling of these carriers or their eggs, will result in an overall reduction of CWD outbreaks over time. Culling eggs from infected adults is a routine strategy for controlling bacterial kidney disease (BKD) caused by *Renibacterium salmoninarum*, and has resulted in significant reductions in BKD over the past decade at hatcheries rearing Chinook salmon. Such an ap-



Courtesy of Ken Cain, University of Idaho

Electron micrograph of F. psychrophilum cells immunogold labeled to show outer glyocalyx or "slime" layer

Subcutaneous injection of F. psychrophilum to induce coldwater disease in rainbow trout as means of evaluating vaccine efficacy

proach for the control of CWD could reduce occurrence at early life stages, and the diagnostic assays that will be validated through this WRAC project will become essential tools for this type of control strategy and early detection and treatment of juvenile fish.

This unique multi-investigator approach will substantially increase the likelihood of transferring practical tools (deliverables) to the industry. Results and products developed from this project will have potential regional and worldwide applications that would greatly benefit the commercial and the public sectors of salmonid aquaculture.

Based on their previous studies, investigators hypothesize that antibodies in the serum of fish challenged or naturally exposed to *F. psychrophilum* can guide selection of recombinant protein vaccine candidates produced through a large-scale expression library from *F. psychrophilum*. Many potential antigens will need to be selected and tested for this process to be successful. In addition, investigators surmise that the risk of CWD in aquaculture facilities can be minimized by culling eggs or broodstock that harbor a high burden of *F. psychrophilum*.

Objectives

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

The procedure we will use to identify potential vaccine candidates, "*in vivo*-induced antigen technology" (IVIAT), involves two primary tasks. On the genome side, genomic DNA from *F. psychrophilum* is extracted from cells grown in broth culture. The DNA is then fragmented (using restriction enzymes) and the fragments are cloned into three different expression vectors where each represents one of three possible translational frames. The vectors are then transformed into an expression competent strain of *E. coli*. These clones are arrayed onto agar plates that include a compound (IPTG) that induces expression of the inserted DNA fragment. Cells are lysed *in situ* and the recombinant peptide is probed with antisera from fish. Clones that react positively with the fish antisera are retrieved, sequenced, and potentially tested as vaccine candidates. *F. psychrophilum* genes will appear "foreign" to *E. coli*, but the method we are using capitalizes on an *E. coli* gene promoter so we do not have to depend on *F. psychrophilum* promoters and ribosomal binding sites to initiate transcription and translation.

The antisera used in the screening process will come from fish that have survived an artificial or natural CWD epizootic. We anticipate that the fish immune system will have been exposed to a very wide array of antigenic proteins, only a small proportion of which are applicable to protective immunity. This happens because some *F. psychrophilum* are undoubtedly lysed *in vivo*, providing a wide repertoire of immunogenic epitopes for antibody production, but not necessarily suitable for invoking a protective immune response.

Consequently, the antisera that we use must be absorbed against whole cells and whole cell lysate from *F. psychrophilum* that is grown *in vitro*. This absorption process removes antibodies that would normally react to a large number of epitopes that have little relevance to protection *in vivo*. Positive clones identified using absorbed sera would be indicative of proteins that are expressed during an actual infection. Recently, this approach has been used to identify 233 proteins from *E. coli* O157:H7 that are expressed during human infection. Based on relative genome size, we anticipate that up to 123 *F. psychrophilum* genes will be detected with this experimental design.

—*continued on page 20*

—*continued from page 19*

2. *Validate quantitative diagnostic assays (ELISA and ovarian fluid filtration FAT)*

To establish a biologically relevant assay for CWD control, it is essential to correlate risk of vertical transmission or disease susceptibility in progeny with assay results from broodstock and establish some threshold level for culling purposes. Therefore, we need to identify broodstock with various levels of infection and follow their progeny for signs of disease or susceptibility differences. We have partnered with Troutlodge (Sumner, Washington) who is the largest supplier of rainbow trout eggs in the world and will use our recently developed enzyme linked immunosorbent assay (ELISA) and ovarian filtration fluorescent antibody test (FAT) to screen broodstock and quantify levels of infection.

To assess the relative risk of vertical transmission it will be essential to relate infection levels in broodstock at the time of spawning to risk of disease occurrence in progeny. To do this, we will focus on female broodstock only, as it is unlikely that sperm would carry bacteria into the egg due to the size of the micropyle. Briefly, samples of kidney and ovarian fluid from up to 60 female broodstock will be collected at various times during the year. Fertilized eggs from these fish will be kept in isolated incubators. During the incubation period, results from ELISA (kidney tissues), FAT (ovarian tissues), and standard bacterial culture (kidney and ovarian fluid) will be obtained as a means of selecting appropriate groups.

Eggs from up to 10 individuals showing graded levels of *F. psychrophilum* infection (from low to high) will be transferred to UI when they reach the eyed stage. Each egg group will be identified based on the parent and infection level as measured by ELISA optical density (OD) and/or FAT cell counts/ml.

Progeny from each group will be reared separately to approximately 0.5 g in size. At that time, fish will be subdivided and stocked in triplicate into 20 l tanks (50 fish/tank). Fish will be subjected to various controlled stressors (low water [density], handling, oxygen, temperature, etc.) and monitored for 28 days in an attempt to induce a disease outbreak.

All mortalities will be examined to determine cause of death. Clinical symptoms consistent with CWD and isolation of *F. psychrophilum* in the absence of other known pathogens will provide strong evidence from vertical transmission. All progeny will be assayed for *F. psychrophilum* prior to stress trials and progeny from broodstock testing negative for *F. psychrophilum* will be included in all trials and serve as a negative control.

3. *Develop other assays (e.g., real-time quantitative PCR) for quantification of infection in ovarian fluid.*

The need to non-lethally sample broodstock to quantify infection levels has also been identified. Therefore, we plan to investigate the development of a molecular based real-time quantitative PCR assay that may provide quantification of bacterial loads in ovarian fluid.

Another possibility is that a biological significance related to disease outbreaks in progeny will only be found at very high levels of infection and a simple "on-site" assay may provide a rapid means of separating or culling egg groups. If this is the case, then we plan to pursue the development of a rapid antibody-based dipstick method for detection of CWD infection. A lateral flow immunoassay (LFIA) would allow easy detection and the minimum detection capabilities could be determined based on comparison to ELISA and FAT values. To develop such an assay, we will follow established methods used for *E. coli*, but will be able to use monoclonal antibodies that were recently produced by our laboratories.

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

Based on results obtained from this project and the deliverables made available to researchers and the aquaculture community, a number of outreach/extension products will be developed. These will be disseminated through a WRAC Extension publication, popular press outlets such as the *Ag Weekly* (Idaho), WRAC *Waterlines* (western region), and *Trout Talk* and *Fish Line* (national) and presentations at industry association meetings and the Pacific Northwest Fish Culture conference (attended by public sector hatchery personnel) to continually inform the target audiences about the project and its progress.

If diagnostic tools prove useful in tailoring disease management at broodstock facilities and/or an efficacious vaccine is developed from this work, then workshops will be conducted to transfer the technology to the industry.

This project will result in a number of deliverables that will benefit the aquaculture industry. The diagnostic assays developed and validated and the monoclonal antibodies produced will be made available to fish health laboratories, aquaculture companies, and researchers. In addition, if a recombinant vaccine shows efficacy, it will be pursued for use in commercial and public aquaculture sectors. Potential deliverables include 1) monoclonal antibody FL-43; 2) ELISA protocol; 3) FAT protocol; 4) QPCR probes and protocol; 5) rapid LFIA assay; and 6) a recombinant CWD vaccine. ■

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WRAC at Meetings—2008

WRAC Board members gathered together in May (Spokane and November (Salt Lake City) at their semiannual meetings. These photos were taken during field trips at each meeting. The "modified" Honda is a testament to the driving skills of WRAC's director.

Mark Your Calendar—2009

March

2–3 **17th Conference for Shellfish Growers—Washington Sea Grant**
Alderbrook Resort, Union, WA
www.wsg.washington.edu

15–17 **International Boston Seafood Show**
Boston, Massachusetts
www.bostonseafood.com/09/public/enter.aspx

22–26 **National Shellfisheries Association (NSA) 101st Annual Meeting**
Savannah, Georgia, USA
www.shellfish.org/101stAnnualMeeting

April

7–9 ***Annual Pac-Rim Shellfish Sanitation Conference***
Olympia, WA
Bob.woolrich@doh.wa.gov

28-30 **European Seafood Exposition 2009 Brussels, Belgium**
http://www.euroseafood.com/09/public/enter.aspx

May

10-13 **Aquaculture Canada 2009 Aquaculture: Meeting the Challenges**
Nanaimo, BC, Canada
http://www.aquacultureassociation.ca/ac09/

19–25 **World Aquaculture 2009**
Veracruz, Mexico
https://www.was.org/Main/Default.asp

June

9-11 **US Fish & Wildlife Service—15th Annual Aquaculture Drug Approval Coordination Workshop**
Little Rock, Arkansas
http://www.fws.gov/fisheries/aadap/inadworkshop09.htm

13 **Idaho Aquaculture Association Conference**
Twin Falls, ID
Contact: Linda L. Lemmon, Executive Secretary
PO Box 767, Hagerman, ID 83332
phone: 208-837-4808
email: *iaa@northrim.net*

14–19 **7th International Conference on Molluscan Shellfish Safety**
Nantes, France
http://www.icmss09.com/

July

5–7 **Genomics in Aquaculture Symposium**
Bodø, Norway
http://www.gia2009.com/

August

14–17 **Aquaculture Europe 2009 European Aquaculture Society**
Trondheim, Norway
http://www.easonline.org/index.php?option=com_content&task=view&id=82&Itemid=1

October

Tbd **Pacific Coast Shellfish Growers Association Annual Meeting**
http://www.pcsga.org/pub/news_events/PCSGA_NSA_AC/AC_home.shtm

Backwash

WRAC wants to hear from you. Tell us who you are and what you would like to see in *Waterlines*. If you are a new reader and want to be added to our mailing list, complete the form below and mail to:

Waterlines
Western Regional Aquaculture Center
University of Washington
School of Aquatic and Fishery Sciences
Box 355020
Seattle, WA 98195-5020

Or forward via email: *wrac@u.washington.edu*
or fax: 206-685-4674.

If you are receiving duplicate copies, please let us know.
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Country

Comments

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Waterlines is a annual publication intended to inform the general public and various aquaculture groups regarding WRAC activities and regional news. These include highlights of USDA/CSREES-funded research and extension projects; a calendar of scheduled meetings and events; and articles regarding aquaculture and related topics appropriate to the Western region. Readers are encouraged to submit material for inclusion in the newsletter. Publication of material in *Waterlines* does not imply endorsement by WRAC.

Submit material to:
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