University of Idaho
Aquaculture Research Institute
WINTER 2008/09

Matt Powell, Jacky Chan, Frank Liu, Mark Tsai, Chien-Hsien Kuo and Leo Ray of Fish Breeders of Idaho sampling tilapia at Leo Ray's farm

Collaborative research exchange with the National Chiayi University

Contributed by Dr. Matt Powell, Research Scientist, HFCES

Dr. Kuo releasing Redfish Lake Sockeye with Governor Butch Otter

For the past two summers the Hagerman Fish Culture Experiment Station has hosted researchers from the National Chiayi University in Taiwan for an intensive, 12-week exchange to study fish culture, genetics and physiology. This past summer, three Master of Science students from the Department of Aquatic Biosciences received travel awards along with their professor Dr. Chien-Hsien Kuo to study in Hagerman. Each student concentrated on developing research and laboratory skills to aid their graduate studies back in Taiwan. Several members of the Hagerman lab participated in the training including Joyce Faler and Jurij Wacyk with the U of I, Jeff Stephensen and Nate Campbell with the Columbia River Intertribal Fish Commission and Scott Snyder with the USDA. The Taiwanese students learned several molecular techniques including DNA and RNA isolation and microsatellite analysis, quantitative PCR, sequencing, probe/primer construction, as well as field and labatory sampling. They were also able to improve their English skills and got to know much more about aquaculture and the United States. The students participated in the release of Endandered Redfish Lake sockeye salmon along with Governor Butch Otter in September.

The collaborative research exchange with the National Chiayi University also allows for U of I students and faculty to travel to Taiwan and learn more about their growing aquaculture industry and the interests of the faculty at NCYU's Department of Aquatic Biosciences. In May, Dr. Matt Powell travelled along with 6 students from the U of I to Taiwan for a week of study and cultural exchange. Dr. Powell returned to Taiwan in October to discuss further possible research collaborations and to participate in a ceremony honoring Dr. Kuo and the National Chiayi University with a partnership award given by Governor Butch Otter.

From left to right: Jacky Chan, Matt Powell, Frank Liu, Chien-Hsien Kuo, Mark Tsai, Nate Campbell, Jeff Stephensen, Jurij Wacyk, and Joyce Faler

INSIDE

Aquaculture Research Institute

The ARI newsletter will be produced semi-annually and available online in Adobe Acrobat format through **www.webs.uidaho.edu/aquaculture/**. If you would like to be notified via email when the latest edition is available on our web page, please notify the editor at **aqua@uidaho.edu.**

We would be happy to in include appropriate contributions from those of you working in the field! Feedback and suggestions on how to improve this newsletter would also be appreciated.

This issue of the newsletter highlights various projects being conducted on the Moscow campus, the Hagerman Fish Experiment Station and includes various extension activities.

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In Search of a Vaccine Against Coldwater Disease

Contributed by Dr. Karen Plant, Postdoctoral Fellow, Fisheries and Wildlife Resources

Coldwater disease (CWD) is caused by *Flavobacterium psychrophilum* and was first described in 1946. It is one of the most important pathogens affecting salmonid fish worldwide and significantly impacts rainbow trout farms in Idaho, resulting in both mortality and reduced growth in surviving fish. The disease causes open lesions which can be observed on or near the peduncle (tail region) of affected fish. As the name suggests outbreaks of CWD mainly occur when water temperatures are colder, typically below 10°C, although the disease manifests in the Hagerman Valley in Southern Idaho, where water temperatures are a constant 15°C. In the rainbow trout farming industry in Idaho, most losses due to CWD occur in fish between 0.2 to 4 g. However, larger fish (10 to 20 g) can also suffer from CWD but a dual infection with infectious hematopoietic necrosis virus can be particularly acute. Currently treatment options are limited to good husbandry practices and the antibiotic, florfenicol. Consequently, our research is focused on identifying a suitable vaccine.

Rainbow trout with CWD peduncle lesion

A CWD survivor with spinal compression

Using *F. psychrophilum* proteins known to be immunogenic to rainbow trout through previous work carried out in Professor Ken Cain's laboratory, our goal is to identify which of these are able to protect fish against CWD. Demonstration of protection from infection with *F. psychrophilum* could potentially result in a vaccine for this disease. In order to achieve this, the genes that encode the immunogenic proteins must be cloned into a suitable vector which will express the proteins in *Escherichia coli*. This enables large amounts of the protein to be produced and lysis of the bacteria allows the protein to be released so it can then be purified. Protein expression, purification and refolding are complex but once the unique conditions required for each protein are identified the correctly folded, purified proteins are injected into rainbow trout. The proteins are injected into the peritoneal cavity and after eight weeks the fish are challenged by sub-cutaneous injection with *F. psychrophilum*. During this period it is hoped the fish have built up an adequate immune response that will provide some degree of protection. Antibody responses are measured as an indicator of protection. There are many immunogenic proteins to choose from and the hunt for a vaccine is ongoing. Results from this project are in press and have been presented at national meetings.

This work, along with other work at the University of Idaho and Washington State University, is promising and has led us to a recently patented product that we hope to find a commercial partner for lincensing.

Culturing large amounts of E.coli expressing the proteins of interest

Using Sterile, Female Rainbow Trout to Produce Sockeye Salmon Eggs

By: Dr. Joseph Cloud, Department of Biological Sciences, University of Idaho

Redfish Lake sockeye salmon is a unique population that is on the federal endangered species list. A number of state and federal agencies are involved in maintaining the genetic diversity of this population. The resulting efforts of their activities have been successful and have resulted in increased returns of sockeye salmon to Idaho. Our research project is aimed at providing a backup system to insure the genetic makeup of the population; in short, our goal is to be able to re-establish the genetic diversity of the population in the event of a catastrophic loss.

Cryopreservation and long-term storage of animal germplasm started many years ago with the freezing of bull sperm, originally developed to support the dairy industry. Slight modifications of this original technology resulted in a methodology to freeze and store sperm from a wide variety of fish species including sockeye salmon. While a sperm bank can be used in conjunction with a back-crossing scheme (using eggs from a related stock) to re-establish the nuclear genome of a parent stock, the contribution of the original mitochondrial DNA would be lost. Since the mitochondrial genome may be a very important component of the athletic ability of fish populations and since Redfish Lake sockeye salmon have a long migration to their spawning ground (and are truly great athletes), the contribution of the mitochondrial DNA is probably a very important component in defining the population.

For many years, investigators throughout the world have attempted to freeze fish eggs. However, cryopreservation of fish eggs or embryos has not been successful because they are too large and too complex. Recent research results from the laboratory of Dr. Goro Yoshizaki at the Tokyo University of Marine Science and Technology suggested that fish testes contain germinal stem cells that could colonize embryonic ovaries and that they had the capability to develop into oocytes. Since it is know that sexually immature testes can be cryopreserved, these results provide a new direction – cryopreserve cells that have the potential to develop into eggs. (For reasons that are not clear, sexually immature, salmonid ovaries are not viable following cryopreservation).

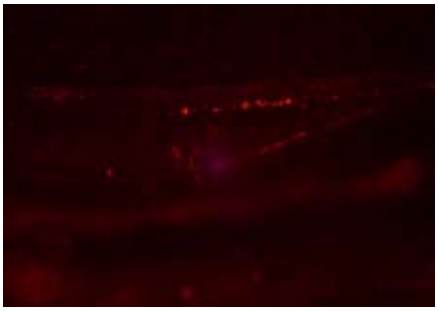
A project was developed to cryopreserve sexually immature testes from Redfish Lake sockeye salmon and to test whether cells from these testes would colonize the ovaries of sterile (3N) female rainbow trout and develop into Redfish Lake sockeye salmon eggs. This project amounts to applying the information derived by Dr. Yoshizaki to solve a practical problem. This resultant project is a collaboration of the Idaho Department of Fish and Game, the National Oceanographic and Atmospheric Administration (Dr. Penny Swanson), the University of Idaho and the Tokyo University of Marine Science and Technology.

Sexually immature testes harvested from male smolts have been cryopreserved and are being stored in liquid nitrogen. Cells derived from these frozen tissues have been injected into female rainbow trout embryos. Preliminary observations indicate that the injected cells can survive within the rainbow trout abdominal cavity for two months and can colonize the developing ovaries. Time will reveal whether the sterile surrogate females will produce fertile sockeye salmon oocytes.

Testicular cells are injected into the abdominal cavity of a recently hatched, sterile (3N), female rainbow trout. Because the hatchling has not developed a competent immune system at this age, the introduced cells (cells from a sockeye salmon) are not rejected. Rainbow trout are used as a surrogate because all-female embryos are available throughout the year. (See picture above)

Embryos 60 days post-transplantation still contain sockeye salmon cells; the orientation of injected cells (or cells derived from the injected cells) supports the conclusion that they have colonized the developing ovary.

(a) bright-field view;



(b) fluorescent view.

Doctoral Candidate Jurij Wacyk.

Major Professor, Dr. Ron Hardy

Jurij's main research interests are framed by the industry's need for fish meal replacements and desire to determine how dietary modifications can alter fish metabolism and consequently fish performance.

Jurij Wacyk was born and raised in Chile's capital, Santiago where he developed an early interest in science. This interest was sparked by Jurij's father, a veterinarian, who was studying gastrointestinal motility using small lizards as model animals for humans at the Medical School of the University of Chile.

Jurij received a degree in Agronomic Engineering with a specialization in Animal Production in 1997, and later completed his Masters program. His research involved trout nutrition, specifically comparing methodologies to determine digestibility values for alternative feed ingredients for the Chilean Aquaculture industry.

This path lead him to look for a PhD program in fish physiology/nutrition that considered not only the necessities of the industry, but would include combining classical nutritional approaches with modern molecular techniques. After a thorough search Jurij started a PhD program in 2004 at University of Idaho under the supervision of Dr. Ronald W. Hardy.

Jurij's main research interests are framed by the industry's need for fish meal replacements and desire to determine how dietary modifications can alter fish metabolism and consequently fish performance. This is relevant not only from a productive point of view, but from an environmental one as well. The more efficient fish utilize a particular diet the greater the reduction of aquaculture impacts in the ecosystem. Increasing diet efficiency is challenging due to the complexity of fish metabolism and its interactions with the diet.

There are two major dietary modifications that result from replacing fish meal with promising alternative--plant meals; (1) increased dietary carbohydrates and (2) modified amino acid profiles of the diet. To this we have to add the fact that carnivorous fish are recognized by their poor ability to utilize dietary carbohydrates (CHO).

The impact of this group of nutrients in fish metabolism is complicated. Depending on factors like the CHO form and level of inclusion, fish like rainbow trout (RBT) are able to use them and improve productive performance.

Several aspects of CHO metabolism have been explored in fish. Research on insulin receptor levels, glucose phosphorylation capacity and glucose metabolism controls have shed some light in how well fish can handle dietary sugars.

One of the advantages of using CHO as a source of metabolic energy is spare dietary protein. As in mammals, fish amino-transferases are key metabolic enzymes that remove amine of amino acids to free the carbon skeletons for supplying energy. So to gain further understanding on the protein sparing effect of dietary CHO, Jurij carried out a study to evaluate growth and plasma parameters along with changes in expression levels of alanine amino transferase (ALAT), aspartate amino transferase (ASAT) and glutamate dehydrogenase (GlDH) in RBT tissues.

Another less explored aspect of CHO metabolism in fish is related with the fact that certain levels of dietary CHO can become a burden for the fish. In mammals, including humans, it has been shown that chronically high dietary CHO can cause detrimental alterations in the REDOX environment in the liver among other things, leading to diseases like diabetes and obesity. Jurij is studying this aspect of CHO metabolism in trout using a suite of genes related to the generation of reductive power and REDOX environment modifications in the liver of these fish.

Mixing diets. Picture can be placed anywhere in article

Using a similar approach now with a fixed level of dietary CHO, he is also evaluating the effect of different amino acid levels in trout diets. For this, Leucine, isoleucine and valine, also known as branched chain amino acids were used to formulate experimental diets. Using a fish meal and a soybean protein background, increasing levels of the branched chain amino acid were used in order to evaluate fish performance as well as changes in gene expression.

This kind of amino acid is a known to be used as energy sources especially in fish muscle. So at this point the two studies connect. To evaluate this "connection" he is using the same set of genes along with some other aminotranferases to study the ability of the fish to use dietary protein, which is one of the most expensive parts of a fish diet.

Jurij's research should lead to optimized diet formulations for salmonids and increase aquaculture sustainability by providing reduced environmentally impact.

Besides using these studies as part of his PhD work, Jurij plans continue this research back in Chile as a part of an initiative between the Chilean Aquaculture industry and U. of Chile to improve Chile's productive sustainability.

Idaho tiptoes into caviar market with "best choice" rating

Reprinted from Summer 2008 *People & Places* magazine

by Marlene Fritz

At Fish Processors of Idaho in Hagerman, owner Leo Ray aspires to produce four to five pounds of caviar—one of the world's costliest delicacies—from each of about 100 female white sturgeon this year. By 2011 he estimates he'll be harvesting eggs from 700 mature females.

With the U.S. banning imports of Caspian Sea caviars from overfished beluga sturgeon, Fish Processors of Idaho and another Hagerman firm, Blind Canyon Aquaranch, are positioning themselves to help close the supply-demand gap with Idaho-grown

(continued on page 6)

Brad Beckman for Programs and People magazine.

Teaching

Although the Aquaculture Research Institute (ARI) is not an academic department, we provide educational and research opportunities to students at both the undergraduate and graduate level. Because of the diversity of aquaculture sciences, graduate degree programs with an aquaculture emphasis may be designed for post-baccalaureate students enrolled in fisheries, biology, animal sciences, agricultural economics and engineering programs.

ARI Director Ron Hardy is a Professor in the Department of Animal and Veterinary Sciences (College of Agricultural and Life Sciences) and an adjunct professor in the Department of Fish and Wildlife Resources (College of Natural Resources), Associate Director for campus programs Kenneth Cain is an Associate Professor in Fish and Wildlife Resources, and Associate Director for Hagerman Matt Powell is an Assistant Professor in the Department of Animal and Veterinary Sciences. Each serves as major professor for graduate students enrolled in their respective departments. They also teach undergraduate and graduate courses, offer special topic classes and independent study projects.

products. Linda Lemmon, executive secretary of the Idaho Aquaculture Association and a co-owner of Blind Canyon Aquaranch, notes that Monterey Bay Aquarium Seafood Watch—which helps consumers make environmentally sustainable purchases—rates both caviar and meat from farmed white sturgeon as "Best Choices." Awaiting its first major caviar harvest between 2009 and 2013, Blind Canyon Aquaranch is currently emphasizing sales of sturgeon meat. Without a doubt, producing caviar is a high-risk venture, Lemmon says. "You get into danger when you start counting your pennies before your fish are ready to make caviar."

Brad Beckman

WHY CAVIAR IS A HIGH-RISK EFFORT

It takes four to six years and time-consuming, labor-intensive, profit-trimming biopsies to distinguish female sturgeon from males. It takes 8 to 10 years before females start producing harvestable eggs.

Harvest timing is dicey: if harvest comes too soon, eggs will be shy of their flavor peak; if it comes too late, females will have reabsorbed their eggs—and along with them the profits producers hope to make.

At the University of Idaho's Hagerman Fish Culture Experiment Station, animal scientist Wendy Sealey says developing "practical, reliable, and non-invasive techniques for determining sex and maturation is critical to improving the production efficiency of sturgeon."

GENDER, EGG RIPENESS: NEW TESTS MAY HELP

Sealey is part of a multi-state trial that's examining the ability of non-invasive light-based techniques—near-infrared and Fourier transform infrared spectroscopy—and immunochemical assay to replace surgical methods in determining egg ripeness. Funded by the Western Regional Aquaculture Center, the project is led by researchers at the U.S. Fish and Wildlife Service and Montana State University and includes the University of California at Davis, Eastern Oregon University, and WSU as well as the UI. Sealey's role is to test Ray's and Lemmon's females several times a year during 2009 to 2011 to verify models developed for California production, while UI Extension aquaculture educator Gary Fornshell heads up outreach.

Brad Beckman

In a separate UI-WSU Aquaculture Initiative project, Sealey and Fornshell are also evaluating ultrasound as a means of quickly and inexpensively telling the much-wanted girl sturgeon from the meat-market-bound boys. Working with them are Hagerman's Matt Powell and College of Southern Idaho's Terry Patterson.

"If you're in the caviar business, you really don't need males," says Fornshell, "but sturgeon are an extremely difficult fish to sex. There are no external characteristics, no assay methods, no chromosome determinations."

MALES NEED NOT APPLY; ULTRASOUND GENDER TESTING

Ray calls sexing his 10,000 white sturgeon a "massive amount of labor." It demands two months and a 4- to 6-person crew every year. To test the ultrasound alternative, Sealey—hip deep in Magic Valley fish runs—will take numerous images of anesthetized fish. If ultrasound proves accurate, she'll train producers in the technique.

Lemmon predicts Sealey's research "will make a huge difference. If we can sex a sturgeon a lot quicker, we can maximize space for females and minimize space for males. That would allow farmers to expand production and become more efficient and competitive."

Growth in Idaho's caviar industry is likely to be cautious, says Lemmon. "It's going to depend on economics." Magic Valley fish farmers can reliably raise market-sized rainbow trout in 12 months, and that's what most of them choose to do. However, because sturgeon tolerate 70°F temperatures, she calls them an "attractive alternative" in geothermal waters or marginal aquaculture sites that are too warm for trout.

Masters Candidate Mark Polinski.

Major Professor, Dr. Ken Cain

Mark Polinski is originally from upstate New York, where he received a Associates degree in Ecology and Environmental Technology at Paul Smiths College. He then enrolled at the University of Idaho (UI), graduating with a Bachelors Degree in Fisheries. After completing his undergraduate education, Mark worked for three years as a commercial SCUBA diver on projects involving mapping and removing the invasive Eurasian water milfoil from various lakes across the United States. In 2007, he returned to the U of I to pursue a M.S. degree in Fish Health under the direction of Dr. Ken Cain. Mark's project is focused on determining disease susceptibility risks and developing diagnostic tools to aid in the development of aquaculture methods for burbot, (*Lota lota maculosa*).

Burbot, a freshwater cod, are in severe decline in the Kootenai River drainage, the only river system in Idaho to which this species is native. Historically this population was utilized by the Kootenai Tribe of Idaho (KTOI) for food and became an important cultural component for the tribe. To aid in the recovery of this population, the KTOI and the University of Idaho are working together to develop hatchery protocols and research information for a conservation aquaculture program for this species. Mark's research, funded by the KTOI and Bonneville Power Administration, will provide information to hatchery managers working with this species. It will also address disease issues associated with captive rearing of this species as well as transportation and stocking.

To improve viral diagnosis and to test potential disease susceptibility, Mark is working on developing and characterizing a laboratory cell line derived from burbot. Cell lines are critical for identifying and isolating viral diseases. The development of a burbot cell line allows for the potential diagnosis of diseases specific to this species that may not be observed on other standard fish cell lines. Additionally, to address concerns for spreading disease, he is conducting challenges of juvenile burbot with multiple fish pathogens to determine their degree of susceptibility and potential carrier status. Lastly, Mark has been investigating optimal methods for controlling fungal outbreaks during burbot embryo and larvae rearing in hopes to increase production capabilities. He has presented some of his current findings at the 49th Western Fish Disease Workshop and was awarded the best student paper award at the 2008 American Fisheries Society/Fish Health Section meeting in Newfoundland, CA.

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SCHEDULE OF EVENTS

February 1-4, 2009, Seafood Summit 2009, San Diego, California. For details: *www.Seafoodchoices.com.*

February 2-5, 2009, AFS State of the Salmon Conference., Vancouver, BC, Canada. Bringing the Future into Focus. The Fairmont Waterfront Hotel. For details: *www.stateofthesalmon.org/conference2009*

March 4-6, 2009, Idaho AFS annual meeting. Double Tree Hotel Boise. Boise, Idaho. February 15-19, 2009, Aquaculture America Meeting, Seattle, Washington. For details: *www.was.org*

March 15-17, Seafood processing America, Boston, Massachusetts. Biggest seafood show in US with many aquaculture products. For details: *www.bostonseafood.com*

May 3-7, 2009, AFS Western Division Annual Meeting, Albuquerque, New Mexico, Hyatt Regency. Evolution of the Western Landscape. Balancing Habitat, Land, and Water Management for Fish . For details : *www.wdafs.org*

May 25-29, 2009, World Aquaculture Meeting, Veracurz, Mexico, A Blue Revoulution to feed the world, For more information: *www.was.org.*