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WFRC Research News

(Lab news you can use to thrive and survive)

Editor: Gary A. Wedemeyer *Spring Issue: May, 2005*

In This Issue…

Lab News You Can Use

by Lyman Thorsteinson

In this issue, many of you will learn that Rip Shively (Klamath Falls Field Station) and Dennis Lynch (USGS Oregon Water Science Center) were recently awarded the Secretary's Four C's Award (Consultation, Cooperation, Communication, and Conservation). This is a very prestigious award, and with it, Rip and Dennis are nationally recognized and honored for their leadership in science and advocacy of science-based natural resource management in the Klamath Basin. In receiving the award, Rip stressed the team effort at KFFS, an attribute that always has been a hallmark of research at the WFRC.

Also honored in this issue is Jim Petersen (Columbia River Research Laboratory) for his Fulbright Award.

Recent memoranda from the Director's Office have highlighted future funding horizons in the upcoming years and, more immediately, restructuring in the USGS. In the first instance, the best-case scenario is for level funding and thus our current Center strategic planning is crucial to our efforts for sustainability and, optimistically, growth.

Bureau restructuring has much to do with successful implementation of the Bureau Planning Model, improvements to existing business practices, and integration of USGS science. Center impacts will likely be produced by internal changes in project funding decisions, funds flows, and communication chains within the Bureau.

My understanding is that each Region will be subdivided into three sub regions. Alaska will become a separate region. A Regional Executive will be assigned to each new sub region with oversight responsibilities for all cost-centers therein, reporting directly to a Regional Director. Most of the WFRC would fall within a Pacific Northwest sub region comprised of Washington, Oregon, and Idaho. High-level discussions have primarily addressed the roles and responsibilities of Headquarter (Director's Office, Associate Directors, and Program Coordinators) and Regional (Directors and REX's) and have not resolved issues regarding cross-boundary implications of the restructuring. It seems clear that the "Matrix" is evolving and that "multi-matrix management" is coming to the USGS soon.

Since our last newsletter, Jim Winton, Rip Shively, and I met with Headquarter and Departmental officials to describe a need for an Integrated Fish Health Program in the Klamath Basin. There are endemic fish disease issues in both upper and lower basins and infection pressures on endangered species are changing in altered habitats.

Our work continues in planning for a new CRRL facility and we have issued a contract to conduct a feasibility study for a new laboratory in the Columbia River Gorge. An open house is being planned for the CRRL on August 27 and we are hoping to host a media event and congressional visit. The Center will, in conjunction with the Office of Communications, play host to a congressional tour during the first week of July. I know that I have burdened you all with requests for fact sheets, brochures, and strategic plans, but our dissemination of this information will be critical this summer.

Finally, I have received numerous requests to have the WFRC Research News posted on the Center website (http://wfrc.usgs.gov) in pdf format. I support making this information available in this manner and assure you all that your fearless editor and I will be especially careful in our reviews to assure that personal information is properly screened and not publicly released.

The WFRC Immunology Research Group, Part 2: Eric Landis.

by Gary Wedemeyer

Eric was born in rural Lancaster, Pennsylvania (Amish country) and was fortunate to grow up in a house with a good-sized stream running through the property. As a child, Eric spent many happy hours fishing in his own back yard. He read his first biology book in second grade, and by 8th grade, Eric was pretty much hooked on fish. By age 10, he had decided he wanted to be a fisheries biologist when he grew up.

After graduating from high school in 1996, Eric moved to Florida to attend college. In 1998, he

transferred to San Diego where he earned a BS in Molecular Biology, together with a Phi Beta Kappa Key, from the University of California (S.D.) in 2001. His first biology job was as a research assistant at the Scripps Institute of Oceanography studying *Vibri cholerae* conjugation and ecology. He then moved to the Kent SeaTech Aquaculture Co. in San Diego where he conducted breeding, nutritional, and pathogenesis studies on stripped bass.

Although research on stripped bass aquaculture, and life living aboard a sailboat in San Diego Bay were both attractive, Eric nevertheless decided he wanted further training in fish immunology, and settled on the molecular and cellular biology program at the University of Maryland (U.M.) in Baltimore. In 2002, he became the newly appointed Dr. John Hansen's first graduate student sequencing and characterizing the genes of the rainbow trout (*O. mykiss*) major histocompatibility complex (MHC).

In 2004, Dr. Hansen joined the WFRC staff bringing his research grant, Eric, and post doc Dr. Kerry Laing with him to Seattle (see WFRC Res. News, Vol. 3(3), 2004). Eric had already completed his course work before he left, and just last month traveled back to U.M. to take his orals (needless to say, he passed).

Eric's research project here at WFRC is on the transcription regulation of the MHC class I and II genes of the rainbow trout. Rainbow trout occupy a unique global niche as an important food fish and as a valued sport fish for recreation. Unfortunately, hatchery trout populations have become increasingly jeopardized by disease outbreaks due to the intensive culture conditions required. Aside from the economic impacts of disease on commercial aquaculture and conservation hatchery operations, there is a degree of concern that hatchery disease problems may spread to wild populations of fish as well. Consequently, the immune system of rainbow trout (and of fish in general) has become an important topic of research in laboratories around the world.

The MHC genes in rainbow trout have now been identified and the WFRC immunology research group has recently sequenced a number of bacterial artificial chromosomes (BACs) containing rainbow trout MHC class Ia and Ib regions. However, their transcriptional regulation and direct involvement in immunity is unknown.

To address these issues, Eric's research will establish levels and tissue distribution of transcription for each of the MHC genes in naïve and infectious hematapoietic necrosis virus (IHNV) infected fish.

At present, rainbow trout are being infected with IHNV under standard conditions and RNA extracted from tissue samples at three time points post-infection. A quantitative reverse transcriptase polymerase chain reaction (qPCR) assay is being used to determine the number of transcripts of each gene per μg of tissue. Based on previous work, the MHC class I pathway genes are expected to be upregulated and MHC class II genes to be downregulated in a tissue-specific manner in response to viral infection.

In mammals, the function and expression of class Ib genes are variable but are thought to play an important role in cellular immunity. The tissue specific levels of class Ib gene expression in

rainbow trout has yet to be addressed and will provide insight into the functions of these genes.

Eric is also biochemically and functionally defining the promoter features of the MHC genes that are essential for basal and virally induced transcription. Once defined, the protein binding abilities of individual basal and inducible promoter elements will be biochemically assessed using electromobility shift assays (EMSAs).

Eric expects to finish his Ph.D. dissertation research in about 2 years and then would like to find a research position in aquaculture or academia. He lives with his wife Christine and 2-month old son Ezra, and in his "spare time" likes to fly fish and indulge in his hobby/avocation, Art.

(Ed. note: although still a graduate student, Eric has already presented fish immunology papers/posters at three international conferences and has co-authored several publications with John Hansen -- including a recent one in the prestigious Proceedings of the National Academy of Science.)

Petersen Receives Fulbright Award

by Gary Wedemeyer

Dr. Jim Petersen, Director of the WFRC-Columbia River Research Laboratory, will be participating in the Fulbright Senior Specialist Program, visiting the Discovery Bay Marine Laboratory (DBML) in Jamaica in November, 2005.

The Discovery Bay Marine Laboratory is a branch of the University of the West Indies, offering training and research facilities for scientists working in Jamaica and in the western Caribbean Sea.

The Fulbright Senior Specialist Program, conducted through the U.S. State Department and the Council for International Exchange of Scholars, provides support for scientists from the United States to conduct training or research at foreign institutions. Fulbright awards are intended to foster both scientific and cultural exchange among scientists.

During his visit to DBML, Jim will be teaching a workshop on fish energetics modeling, his area of expertise, to local and visiting scientists at the Jamaica lab. He will also be giving lectures and seminars to local NGOs, school groups, and visiting researchers. Jim will also be assisting with portions of the fisheries class being taught at DBML by faculty from the University of the West Indies.

Shively Receives DOI 4-C's Award

by Gary Wedemeyer

In recognition of outstanding contributions in establishing strong and effective relations with a broad array of stakeholders in the scientific understanding and management of natural resources in the Upper Klamath Basin, WFRC's Mr. Rip Shively, and Mr. Dennis Lynch (USGS Oregon District WRD), have received the prestigious Secretary of the Interior's Four C's Award (Consultation, Cooperation, Communication, and Conservation).

Rip is Station Chief and lead scientist at the WFRC Klamath Falls Field Station (KFFS). Dennis Lynch is the District Chief of the Oregon District (WRD) and was delegated the authority of USGS Lead Scientist for Klamath Basin issues in 2000 by the Western Region Director.

The Klamath Basin of Oregon and California has long been a focal point of conflict between traditional water uses and needs related to endangered fishes. Upper Klamath Lake has been supplying water for agriculture through the U.S. Bureau of Reclamation Klamath Project since the early 1900's. However, Upper Klamath Lake also provides habitat for two endangered fish populations: the Lost River and shortnose suckers.

Rip and his team are conducting extensive research in Upper Klamath Lake as related to the ecology, behavior, and population dynamics of endangered suckers. Field work is conducted February through November and is based on the pioneering efforts of Gary Scoppettone and his crews at Klamath Lake from 1994-1998. Following the advice Rip received from "Scopp" when he first moved to the the Klasmath Basin, the KFFS staff try to approach each task with extreme vigor!

Working with Mr. Dennis Lynch of the USGS Water Resources Discipline (WRD) has provided nationally recognized leadership in the study of endangered species behavior and habitat, and the understanding of the hydrologic system and water quality in the Upper Klamath Basin.

Both Lynch and Shively are nationally recognized experts in Upper Klamath Basin issues, and water-resources and fisheries science. Through their positions as scientists-in-charge of their respective offices and lead scientists for USGS in the Basin they have made extraordinary contributions to DOI and the States of Oregon and California in resource management and protection goals.

Through their personal research, and research by their staffs, controversy and legitimate differences in expert opinions regarding the scientific issues facing Klamath Basin resource management are being recognized and addressed, consensus on key issues is being achieved, and complex technical information is being effectively translated and transmitted to managers, stakeholders, decision-makers and the public.

In February of 2004, they lead an effort to host an Upper Klamath Basin Science Conference that provided an open forum for the exchange and discussion of scientific issues. The conference was deemed such a success that DOI officials requested USGS take the lead in planning a similar conference for the Klamath Basin. It was held in June of 2004 and was also viewed as a success.

The main premise behind these conferences was to bring people from various agencies and stakeholder groups together to discuss science issues and help direct and proioritize the information still needed to help resolve resource ussues in the Klamath Basin. Since the conferences were held, there has been a renewed commitment by researchers and managers to hold more such workshops to discuss and review current research.

In an interview with this reporter, Rip emphasized that the award was a reflection of the high quality work of all the staff at KFFS, both past and present, and that the guidance and support given by Lyman Thorsteinson and Frank Shipley had been indispensable.

Habitat Requirements of the Endangered California Freshwater Shrimp *(Syncaris pacifica*)

by *Barbara Martin and Mike Saiki (not pictured), Dixon Field Station*

The California freshwater shrimp (*Syncaris pacifica*) is a federally listed endangered species whose distribution is restricted to low elevation perennial streams in Marin, Sonoma, and Napa counties north of San Francisco Bay, California. Shrimp are typically found within or on undercut stream banks, exposed roots, overhanging woody debris, and overhanging vegetation. Unfortunately, little else is known about the habitat requirements of this freshwater shrimp.

Purpose: Our research was conducted to develop a better understanding of the habitat requirements of California freshwater shrimp populations inhabiting Lagunitas and Olema creeks. Specific objectives were: (1) to determine if shrimp are homogenously distributed throughout these streams or if their distribution is concentrated in certain localities; and (2) to determine if shrimp distribution is associated with selected habitat characteristics (e.g., stream morphometry, water quality, types and amounts of underwater cover, cohabiting fish species).

Our study will allow the National Park Service to better assess the relation between shrimp abundance and selected habitat variables in reaches of Lagunitas and Olema creeks lying within National Park boundaries.

The ecological data will be used to help develop a comprehensive monitoring plan for habitat conditions and shrimp populations, and to better understand characteristics of suitable shrimp habitat and characteristics of viable shrimp populations.

Sampling Sites. A total of 10 glides, five pools, and five riffle/runs were randomly selected from the two combined creeks to serve as fixed sampling sites (one pool, riffle, and two glides were located on Olema Creek). Within each site, there were 15 sampling points. Five sampling points were located on each of the right and left banks and five were located mid-channel. Each sampling point encompassed a 3-m length of stream with a 3-m rest section between each sampling point. Our field work consisted of collecting shrimp and fish, and measuring associated habitat variables at each sampling point.

Habitat Measurements: At each sampling point within a site, water quality (temperature, dissolved oxygen concentration, pH, specific conductance, turbidity, ammonia concentration), point current velocity, types and amounts of in-stream cover/shelter for shrimp (e.g., overhanging banks, submerged roots of riparian vegetation, submerged vines and branches of riparian vegetation, submerged aquatic macrophytes), and bottom substrate composition were recorded.

Results. Data from this study will be summarized in a final report that compares abundance of California freshwater shrimp among various habitat

types occurring in Lagunitas and Olema creeks, and identifies the habitat characteristics (environmental variables) that best explain variations in shrimp abundance. The final report will also include our recommendations for habitat characteristics that foster high shrimp abundance.

PCBs Damage Fish Immune System

(CRRL Arctic Charr Research)

by Gary Wedemeyer

Levels of polychlorinated bipenyl's (PCBs) presently found in remote polar waters are sufficient to harm the economically important Arctic charr (*Salvelinus alpinus*), according to new research by Alec Maule of the WFRC-Columbia River Research Laboratory (CRRL). Alec and his colleagues fed various doses of PCBs to Arctic charr in laboratory tanks. Then, for 4-months, the researchers fed some fish and starved others. The later regimen replicates the fast that charr endure each year during the fresh water phase of their life cycle. At the end of the period, the scientists measured the activity of disease-fighting proteins and enzymes in the test and control fish.

PCBs triggered several adverse immunological changes in the test fish, the scientists report in the January Issue of "Environmental Toxicology and Chemistry." Charr that received PCB's had a less-active form of the protein lectin in their blood than did fish whose diets were free of PCBs. Lectin binds to many microbes and other foreign materials in the blood. For fully fed fish, lectin remained active, regardless of whether the fish received PCB's. On the other hand, PCB's significantly lowered the activity of pathogen-killing enzymes (lysozymes) in fed fish but not in fasting fish.

When Maule and his colleagues exposed the starved charr to infectious bacteria, half of the PCB exposed fish died, yet only 30 % of their PCB-free peers did. There was no survival effect from PCBs among the fish that had been given food. Simply put, PCB's significantly affected the ability of Artic charr to resist disease.

This study was highlighted in Science Daily News, February 26, 2005, www.sciencenews.org

Metadata News

by Vivian Hutchison, NBII/WFRC

Interested in learning about metadata? New NBII Metadata Workshops are scheduled for the spring and summer! The target audience for this training is anyone who is currently creating Federal Geographic Data Committee (FGDC) metadata and using the NBII Biological Data Profile or anyone who anticipates that they may need to create this type metadata in the near future.

Participants usually range in experience from beginners to people who have been doing some metadata work and want to learn more. The workshops are generally 1 ½ days, with the half day

as optional time to practice using the software or ask more in-depth questions of the trainer.

Topics covered in the training sessions will include:

- Introduction to metadata - why is it important?
- Background on the development of the FGDC metadata standard and the Biological Data Profile
- In-depth look at the 7 sections of the standard
- Tips on writing good metadata and how to implement a program in your organization
- Metadata tools / software / resources
- Hands-on time to work with software and practice creating metadata
- NBII and FGDC Clearinghouses – how to contribute metadata records to them

Come join us! The spring and summer tentative calendars are:

- Introduction to Metadata. June 16-17 Arlington, VA (sponsored by NBII and NatureServe, and held at NatureServe headquarters).
- Train the Metadata Trainer. June 27-29 Denver, CO (sponsored by NBII and NOAA and held at the USGS Center for Biological Informatics)

This workshop is for those interested in training others on how to create metadata – participants need a familiarity with metadata such as can be acquired in the Introduction to Metadata workshops.

Additional workshops are pending and will likely be added. Watch the NBII Metadata Training Calendar! (www.nbii.gov/datainfo/metadata).

If you are interested in attending any of these sessions, please email NBII's Metadata Coordinator Viv Hutchison (vhutchison@usgs.gov) or call me at 206.526.6282 ext 329.

The NBII Metadata Program offers more than just training. Check out our Clearinghouse at http://www.nbii.gov/datainfo/metadata/clearinghoue There are now 25 partner nodes contributing records, and we are always interested in talking to new potential partners. The Clearinghouse is managed by NBII partner Oak Ridge National Laboratory in Oak Ridge, TN.

Additionally, any time you upload a record to the Clearinghouse for inclusion on the NBII Principal Node, your record goes through a quality assurance/quality control process directed by Diane Schneider at USGS FORT in Fort Collins, CO. It is carefully reviewed by Diane's team and a metadata parser.

Finally, the NBII Metadata Program offers assistance in creating your metadata records. Just have a few records to create? Looking at a backlog of legacy records in need of metadata? Contact Viv Hutchison (vhutchison@usgs.gov) for information on how NBII's expert metadata creator, Cheryl Solomon (a partner with NASA Global Change Directory) can help. Let's get your data sets described and cataloged!

For more information about NBII's Metadata Activities, visit www.nbii.gov/datainfo/metadata/

WFRC Notables and Notes

Yasutake Retires — Again

by Gary Wedemeyer

After a research career lasting more than 50 years and spanning two centuries, Senior Scientist Emeritus Dr. Tosh Yasutake has decided to retire for the second and (perhaps) final time.

Tosh served as an Army medic during World War Two, earning a Bronze Star for bravery. After discharge, he attended college on the GI bill and began research as a histopathologist at CRRL (then called the Western Fish Nutrition Laboratory) back in 1953 when the Center was under the U.S. Fish and Wildlife Service. In 1960 he transferred to WFRC-Seattle (then, the Western Fish Disease Laboratory). Tosh saw the Center renamed 4 more times (National Fisheries Research Center, Pacific NW Natural Science Center, National Biological Science Center, Western Fisheries Research Center) and transferred into or out of 4 government agencies (FWS, National Biological Survey, National Biological Service, U.S. Geological Survey).

In spite of all the reorganizations, Tosh still managed to do most of the original research in fish histopathology and in 1982, published "The Microscopic Anatomy of Salmonids: An Atlas" which quickly became a standard reference text in ichthyology/fishery biology. It is still in use today and was reprinted by the U.S. Fish and Wildlife Service in 2004 due to the continuing demand.

Tosh was awarded a Ph.D. by the University of Tokyo in 1980. The only American to have been so honored. He was again honored by the American Fisheries Society, receiving the Snieszko Distinguished Service Award in 1987.

Tosh retired from the Center (for the first time) in 1988. However, he soon returned as Senior Scientist Emeritus and continued coming to his office at the lab 2 days a week for the next 16 years ─ working on a variety of projects.

Tosh requested that there be no going-away party and when asked about his plans for the future, he just smiled and made no reply. As he walked out to the parking lot for the last time, he could be heard humming to himself *"I'm gonna ease on down, just ease on down the road..."*

Dr. Julio Harvey: Invasive Species Research

Greetings Everyone! Having been raised in southern California in an environment that can essentially be described as coastal chaparral and desert, my interest in microbial biology and the world of fungi in particular was not piqued until later in my undergraduate studies at the University of California Berkeley (UCB) where I met mycologists Dr. Scott Kroken and Dr. Greg Saenz who, at the time, were graduate students of Dr. John Taylor, a fungal phylogeneticist who would later become instrumental as an invaluable thesis committee member during the completion of my doctoral

research at the University of California Santa Cruz (UCSC).

After spending a year studying abroad at Lunds Universitette in the southern province of Skane, Sweden, where my foci varied from film, drama and socioeconomic history to microbial biology and plant genetics, I returned to Berkeley for a final year and received my bachelor's degree in Integrative Biology in 1996.

I then worked 2 years for the Simpson Timber Company laboratories in Blue Lake, CA, as a laboratory technician responsible for propagating tissue cultures of various redwood and eucalyptus tree species. After this, I was lucky enough to be accepted to the doctoral program offered by the Department of Ecology and Evolutionary Biology at University of California -Santa Cruz (USCSC). My thesis advisor was Dr. Lynda Goff and I was awarded a doctoral degree in spring of 2004. During my studies at Santa Cruz, my academic interest in marine ecology and symbioses between marine algae and fungi took me from southern Baja California, Mexico to as far north as Coos Bay, Oregon. It was during these excursions that the interest in and love for the marine environment that I had begun to develop in my childhood along the coast of Santa Barbara, CA, was solidified.

In order to collect data for ongoing subtidal ecology research while simultaneously collecting marine brown algal specimens of the family *Sargassaceae* for my thesis research, it was prerequisite, as well as my great pleasure, to become involved in the world of research SCUBA diving.

Seeing the subtidal world first hand was one of the most amazing things I had yet experienced as a graduate student. Nevertheless, the symbiotic association between the marine endophytic fungi in the genus *Haloguignardia* and the brown algae they infect proved to be so bizarre as to eclipse even the experience of underwater research with regards to my level of interest at the time. Not surprisingly, these relationships became the subject of my thesis work, leading to publications addressing the phylogenetic history and taxonomy of the host algae, the coevolutionary history of the hosts and their fungal endosymbionts in addition to ecological aspects of these marine fungi.

Having accomplished both the academic goal of studying fungal biology in the marine environment as well as spending a good portion of my studies investigating amazing marine environments first hand, I left Santa Cruz with great feelings of completion and personal satisfaction.

After meeting with and discussing the ongoing research of Dr. Rusty Rodriguez at his laboratory here at the WFRC, I realized our interests in the world of science were aligned in many ways. I readily agreed to work with his group toward the development of peptide nucleic acid beacons (PNAs) for use in screening ballast waters foreign to the Puget Sound area for the presence of microscopic, planktonic life history stages of invasive marine species.

Currently, our central goals are to accomplish efficient screening of invasives in commercial ballast water as well as in the local waters of Puget Sound via the development of microarrays featuring species specific PNAs. We've begun to develop this technology using organisms known to have planktonic larval stages post reproduction, such as bivalves and crustaceans that are readily available for collection locally. Additionally, we will include

phytoplankton, other zooplankton and even species of marine macroalgae in our study as there is virtually no limitation to either the number or kinds of species to which these techniques are applicable. Furthermore, once having fully developed this technology, it will undoubtedly have great utility in our other ongoing studies regarding how fungal endosymbionts confer ecological advantages to their host plants, including invasive species.

It is presently my good fortune to conduct this work here at the WFRC and it will be my pleasure to meet and become acquainted with you all.

Ranson's Husband Named U.S. Air Force Chaplin of the Year

by Gary Wedemeyer

Judy Ranson (WFRC Seattle) and her husband Colonel Gary Ranson, have recently returned from Washington, DC where Gary was named Air Force Chaplain of the Year for his service to US troops in the Middle East. Having already served 4 tours in Iraq and Kuwait (including 90 days in Kirkut), Col. Ranson is now in Kuwait and will be retiring from the USAF on his birthday in November. Says wife Judy "Yeah!"

Middle School Girls' Science Club Visits CRRL

by T. Craig Robinson, CRRL

On February 9, 2005, the Columbia River Research Laboratory (CRRL) hosted a site visit by an Advocates for Women in Science, Engineering and Mathematics (AWSEM) science club from Gardiner Middle School in Oregon City, Oregon. The purpose of AWSEM is to provide girls (grades 6-12) with fun and educational science, engineering and math activities. Girls do hands-on science and math activities with a group leader after school. Girls also go on site visits to meet and learn about women in science, math and engineering careers (www.awsem.org).

Six girls and their AWSEM advisors participated in hands-on activities designed by CRRL biologists to engage them in the scientific techniques of fisheries biology. Activities included demonstrations of fish marking techniques and fish physiology and morphology. The visit concluded with a lunchtime Jeopardy game to reinforce skills that the girls learned in the demonstrations as well as a question and answer period for the girls to learn more about career experiences of staff biologists.

WFRC Bests RO in Lake Chelan Fishing Derby!

by Gary Wedemeyer

In an exclusive interview with this reporter, Center Director Lyman Thorsteinson reports that he easily bested WRO Dr. Fank Shipley in all categories in an informal fishing derby held recently at Lake Chelan, Washington. The categories were First, Biggest, and Most lake trout, kokanee, and rainbow trout caught.

Normally dominant in fishing derbies of all kinds, Dr. Shipley was no match for Lyman in this particular event. "I think Frank was just having a bad day" said Lyman.

Selected WFRC Research Publications/Presentations

Freilich, J.E., G. Pess, J.J. Duda, J. Allaway, and B. Eaton. 2005. Steps in the Creation of an Elwha Research Consortium. *Paper presented at the Society for Ecological Restoration, Northwest Chapter Conference, Sustainability and Restoration: A Practical Partnership for the 21st Century, April 4-8, 2005, Seattle.*

ABSTRACT: The removal of two dams on the Elwha River now scheduled for 2008, will be the largest dam removal ever conducted. Five species of salmon will return to waters they have not occupied for 90 years. Although the Elwha Restoration Act of 1992 provides funds to remove the dams, restore the fish, and protect the water (which is drinking water for the City of Port Angeles), no funds are provided for research. The Elwha represents an extraordinary opportunity for research in a wide range of fields from hydrology to marine-derived nutrients, to bears and eagles. The research is particularly valuable because all of the river above the dams is in Olympic National Park and is thus free from anthropogenic disturbance that could confound results. We will present the steps we've taken in forming a Research Consortium to do the research, and discuss the many obstacles we've faced in gaining support.

Morley, S., Coe, H., Duda, J.J., Kloehn, K., Pess, G., McHenry, M., Earnest, Liermann, S., Kiffney, P., and T. Beechie. 2005. Pre-Dam Removal Monitoring in the Elwha River Basin: Establishing Baseline Conditions for Primary and Secondary Productivity. *Paper presented at the Puget Sound Georgia Basin Research Conference, March 29-31, 2005. Seattle, Washington.*

ABSTRACT: In order to effectively evaluate the potential effects of dam removal on primary and secondary productivity in the Elwha River Basin, a coordinated data-collection effort is needed for establishing pre-dam removal conditions. Building on earlier monitoring work conducted by the Lower Elwha Tribe and the USGS in the mid-90's, we began collecting baseline data in the summer of 2004. In order to sample mainstem, tributary, and side channel habitats below, between, and above the dams, data collection was coordinated amongst NOAA, the USGS, and the Lower Elwha Klallam Tribe. The focus of this first year of data collection was on standardizing data collection protocols, collecting a representative number of samples from varied habitats, and on establishing long-term monitoring locations. Along with physical habitat characterization, at each of our monitoring sites we collected benthic invertebrates, periphyton, and water chemistry samples. Based on this collaborative research effort, we will determine adequate sample size, appropriate spatial distribution of samples sites (lateral and longitudinal), and refine sampling protocols as necessary for ongoing dam removal monitoring.

Rodriguez, Rusty, U.S. Geological Survey, Harvey, Julio, Universtiy of Washington, Hoy, Marshal, University of Washington, Redman, Regina, Montana State University, Elder, Nancy**,** U.S. Geological Survey. 2005. Development of Molecular Diagnostic Tools to Assess the Introduction, Establishment and Ecology of Invasive Species in Puget Sound. *Paper presented at the Puget sound Georgia Basin Research Conference, March 29-31, 2005, Seattle, Washington.*

ABSTRACT: Non-native marine species have been and continue to be introduced into Puget Sound via several vectors including ship ballast water. Some non-native species become invasive and negatively impact native species or near shore habitats. Currently, there are no predictive capabilities to determine if a non-native species will become invasive. We have begun a project to develop DNA-based diagnostic tools to rapidly assess ship

ballast samples for the presence of native and non-native species. Integrating microarray technology and novel nucleic acid chemistry the diagnostic systems will have the capacity to analyze up to 18,000 different species. This will allow us to analyze the presence and abundance of species representing near shore communities. In addition, these tools will allow us to monitor the temporal and biogeography of organisms that have planktonic larval stages.

Duda, J.J., Morley, S., Coe, H., Liermann, M., Kloehn, K., Reisenbichler, R.R., Freilich, J.E., Pess, G., Winter, B., Connolly, P., Jezorek, I., McHenry, M., and Kiffney, O. 2005. Pre-Dam Removal Monitoring on the Elwha River Basin: Establishing Baseline Levels of Stable Isotopes in Fish and Benthic Communities. *Paper presented at the Puget Sound Georgia Basin Research Conference, March 29-31 2005. Seattle.*

ABSTRACT*:* The removal of two dams blocking the Elwha River to migrating salmon represents an unequalled opportunity to study large-scale river restoration. Salmon contribute a significant flux of marine derived nutrients, through spawning and carcass deposition, into relatively nutrient poor freshwaters. Because salmon migration has been blocked for over 90 years, the middle and upper reaches of the Elwha River have lacked this significant nutrient subsidy. As part of a larger research program designed to track the ecological effects of dam removal, we collected baseline data on marine derived nutrient levels above, between, and below the Elwha River dams from mainstem, side channel, and tributary habitats. We sampled benthic macroinvertebrates, algae, and fish tissues, which will be analyzed for stable isotope ratios of carbon and nitrogen. Documenting baseline values of stable isotopes in the years prior to dam removal will be an invaluable tool in understanding the ecological effects of salmon populations returning into the Elwha River ecosystem.

Duda J.J., Black, R., Cochrine, G., Connolly P., Gelfenbaum, G., Jenkins, K., Konrad, C., Melis, T., Munn, M., Reisenbichler, R., Rubin, S. D., Ruggiero, P., Shafroth, P., Schreiner, E., and Warrick J. 2005. Investigations of the Ecological, Fluvial, and Nearshore Impacts of the Elwha River Dam Removal.*Poster Presentation: Puget Sound Georgia Basin Research Conference, March 29-31, 2005. Seattle.*

ABSTRACT: The U.S. Department of Interior will remove two dams from the Elwha River of Washington's Olympic Peninsula to restore the physical and biological integrity of the river ecosystem. The dams have interrupted coarse-sediment and organic-debris transport as well as anadromous and fluvial fish migration since the early 20th century profoundly impacting ecological processes, channel morphology, lotic communities, and nutrient dynamics. As lead research agency for Interior, the USGS is actively involved in the research and monitoring of the Elwha River restoration project. This poster presents ongoing USGS research regarding ecological, fluvial, and nearshore components of the restoration project. Documentation of baseline status of fish, aquatic macroinvertebrate, periphyton, and wildlife communities and marine-derived nutrient levels from multiple taxa prior to dam removal is an important first step in documenting both short- and long-term effects of dam removal and restoration. The sediment analysis integrates fluvial, estuarine, and marine processes and includes marine sediment mapping, monitoring fluvial and marine sediment transport. New sediment monitoring technologies including laser diffraction and acoustic backscatter will be tested and used to monitor the effects of delta erosion and channel re-stabilization on water and habitat quality. Nearshore research to date includes process-based numerical modeling of sediment dispersal and accumulation.

Rodriguez, R. 2005. Life In The Stress Zone: The Role of Fungal Symbiosis in The Distribution and Survival of Plants in Puget Sound. *Paper presented at the Puget Sound Georgia Basin Research Conference, March 29-31. Seattle, Washington.*

ABSTRACT: All plants in natural ecosystems are thought to be symbiotic with endophytic fungi that reside entirely within plant tissues. These fungi are known to be important to the structure, function, and health of plant communities. In fact, without fungal symbioses, plant communities would not survive many environmental stresses. Fungal symbionts express a variety of symbiotic lifestyles including mutualism, commensalism, and parasitism. Mutualistic fungi have been shown to increase plant growth and productivity, and confer stress tolerance against drought, salt, temperature, disease, herbivory. We propose that fungal endophytes provide a mechanism for the habitat expansion of native and invasive plants species including Dunegrass and Spartina spp. We assessed the endophytic fungi in several plant species and found that the host changes it's symbiotic partner in response to mircohabitat stresses (a phenomenon we describe a Symbiotic Modulation). We are determining the biogeographic distribution of Spartina endophytes and thier significance in the invasiveness of this plant species. We have found that one fungal endophyte confers salt tolerance to plants and may be required for the inavsion of Spartina spp. The role of symbiosis in the invasion of invasive species and the evolution of plants in high stress habitats will be discussed.

Hershberger, P.K., N.E. Elder**,** J. Wittouck, K. Stick, and R.M. Kocan. 2005. Abnormalities in Larvae from the Once-Largest Pacific Herring Population in Washington State Result Primarily from Factors Independent of the Spawning Location. *Transactions of the American Fisheries Society 134: 326-337*.

ABSTRACT: Among larvae from Pacific herring *Clupea pallasii* populations in Washington State, those from Cherry Point consistently demonstrated abnormalities indicative of distress, including low weights and lengths at hatch, increased prevalences of skeletal abnormalities, and shorter survival times in food-deprivation studies. Biomass of adult, pre-spawn Pacific herring at Cherry Point declined from 13,606 mt in 1973 to a record low 733 mt in 2000, but correlation of larval abnormalities with adult recruitment was weak, indicating that the larval abnormalities did not directly cause the decline. Larval abnormalities originated primarily from factors independent of conditions at the spawning location because they were not reproduced by incubation of foreign zygotes along the Cherry Point shoreline, but they were reproduced after development of indigenous zygotes in controlled laboratory conditions. Precise cause(s) of the abnormalities were not determined; however, recent zoographic trends in elevated natural mortality among adult Pacific herring and resulting reduced age structures may be involved.

Garver, K.A., LaPatra, S.E., and Kurath, G. 2005. Efficacy of an IHN Virus DNA Vaccine in Chinook (*Oncorhynchus tshawytscha*) and Sockeye (*Oncorhyhchus nerka*) Salmon. *Diseases of Aquatic Organisms (in press).*

ABSTRACT: The level of protective immunity was determined for chinook (*Oncorhynchus tshawytscha*) and sockeye/kokanee salmon (anadromous and landlocked *Oncorhynchus nerka*) following intramuscular vaccination with a DNA vaccine against the aquatic rhabdovirus, infectious hematopoietic necrosis virus (IHNV). A DNA vaccine containing the glycoprotein gene of IHNV protected Chinook and sockeye/kokanee salmon against waterborne or injection challenge with IHNV and relative percent survival (RPS) values of 23-86% were obtained under a variety of lethal challenge conditions. Although this is significant protection, it is less than RPS values obtained in previous studies with rainbow trout. In addition to the variability in the severity of the challenge and inherent host susceptibility differences, it appears that use of a cross-genogroup

challenge virus strain may lead to reduced efficacy of the DNA vaccine. Neutralizing antibody titers were detected in both chinook and sockeye that had been vaccinated with 1.0 and 0.1 mg doses of the DNA vaccine and vaccinated fish responded to viral challenges with higher antibody titers than mock-vaccinated control fish.

Garver, Kyle A., Conway, Carla M., Elliott, Diane G., and Gael Kurath. 2005. Analysis of DNA vaccinated fish reveals viral antigen in muscle, kidney and thymus, and transient histopathological changes. *Marine Biotechnology (in press).*

ABSTRACT: The safety and high efficacy of a DNA vaccine against infectious hematopoietic necrosis virus (IHNV) were analyzed by investigating tissue distribution, persistence, expression patterns, and histopathological effects of the vaccine. Plasmid pIHNw-G, containing the gene for the viral glycoprotein, was detected immediately after intramuscular injection in all tissues analyzed, including the blood, but at later timepoints was found primarily in the muscle tissue where it persisted to 90 days. Glycoprotein expression was detected in muscle, kidney and thymus tissues with levels peaking at 14 days and becoming undetectable by 28 days. Histological examination of vaccinated fish revealed transient damage that was localized in muscle tissue and was associated with needle injection. At 90 days post vaccination no damage was detected in any tissues indicating the vaccine to be safe and well tolerated. Our results suggest that antigen biodistribution and muscle regeneration may be factors in the exceptionally high efficacy of the IHNV DNA vaccine in fish.

Enzmann, P.J., Kurath, G., Fichtner, D., and Bergmann, S.M. 2005. Infectious hematopoietic necrosis virus: Monophyletic origin of European IHNV isolates from North-American genogroup M. *Diseases of Aquatic Organisms (in press). (Ed. note: this paper is the product of a collaboration with Dr. Peter Enzmann, who came for a week's visit to WFRC last summer to learn viral genotyping technology)*

ABSTRACT: *Infectious hematopoietic necrosis virus* (IHNV) was first detected in Europe in the year 1987 in France and Italy, and later, 1992, in Germany . The source of the virus and the route of introduction are unknown. The molecular epidemiology of IHNV outbreaks in Germany since the first introduction of IHNV was investigated here. The complete nucleotide sequences of the glycoprotein (G) and non-virion (NV) genes from nine IHNV isolates from Germany have been determined, which has allowed the identification of characteristic differences between these isolates. Phylogenetic analysis of partial G gene sequences (mid-G, 303 nucleotides) from North American IHNV isolates (Kurath et al., 2003) has revealed three major genogroups, designated U, M and L. Using this gene region with two different North American IHNV data sets it was possible to group the European IHNV strains within the M-genogroup, but not in any previously defined sub-group. Analysis of the full length G gene sequences indicated that an independent evolution of IHNV had occurred in Europe. IHNV in Europe seem to be of a monophyletic origin, again most closely related to North American isolates in the M genogroup. Analysis of the NV gene sequences also showed the European isolates to be monophyletic, but resolution of the three genogroups was poor with this gene region. As a result of comparative sequence analyses several different genotypes have been identified circulating in Europe.

Gadomski, Dena M., and Michael J. Parsley. 2005. Effects of Turbidity, Light Level, and Cover on Predation of White Sturgeon Larvae by Prickly Sculpins. *Transactions of the American Fisheries Society 134:369–374.*

ABSTRACT: White sturgeon *Acipenser transmontanus* occur in rivers of the western United States and southwestern Canada, but some populations are in decline because of recruitment failure. Many river systems in this area have been altered as a result of development that has caused major environmental changes. Our goal

was to examine how three changes—lower turbidity levels, higher light levels, and altered substrates—might affect predation by prickly sculpin *Cottus aspe*r on white sturgeon larvae. We experimentally investigated predation at various turbidity levels and found that significantly more white sturgeon yolk sac larvae were eaten at lower turbidity levels.The effects of light level (1–4 and 7–15 lx), the presence or absence of rocks as cover, and prey size (14–17 mm and 20–24 mm total length) on the outcome of predator–prey interactions were also examined. Significantly fewer white sturgeon were eaten during trials that combined the lowest light level, cover, and the smallest larvae. Our results suggest that altered river conditionscaused by impoundment and other factors have increased predation on white sturgeon larvae.

Hershberger, Paul, Gregg, Jacob, Traxler, Garth, and Jonathon Richard. 2005. Larval Herring Acquire Resistance after Challenge with Viral Hemorrhagic Septicemia Virus. *Paper presented at the Puget Sound Georgia Basin Research Conference, March 29-31, 2005. Seattle, Washington.*

ABSTRACT: Post-metamorphosed juvenile herring, exposed to viral hemorrhagic septicemia virus (VHSV) as larvae, demonstrated limited resistance to subsequent challenge with VHSV. Among juvenile herring that were exposed to VHSV as larvae, subsequent challenge with VHSV resulted in 53-77% mortality, significantly less than that of immunologically naïve cohorts (93-97%) that were exposed for the first time as juveniles. Furthermore, protection from a second exposure to VHSV was directly proportional to larval age at the time of first exposure, with 77% cumulative mortality occurring among juveniles initially exposed at 44 days post-hatch and only 53% cumulative mortality occurring among groups initially exposed at 89 days post-hatch. These results provide insight into natural mechanisms of acquired resistance to VHSV, and may begin to provide reasons why epidemics of VHSV appear sporadically among herring populations in regions where the pathogen is endemic.

Rodriguez, Rusty and Regina Redman (Department of Biology, University of Washington, Seattle, and Department of Microbiology, Montana State University, Bozeman, Montana). 2005. Balancing the Generation and Elimination of Reactive Oxygen Species. 2005. Proceedings of the National Academy of Science. 102(9): 3175–3176. *(Ed. note: this paper was published by PNAS as Invited Commentary on a paper by Chen and Dickman in the same issue).*

OXYGEN: A GAS TO LOVE AND FEAR

Fossil records suggest that bacteria developed the ability to photosynthesize 3,500 million years ago (mya), initiating a very slow accumulation of atmospheric oxygen (1). Recent geochemical models suggest that atmospheric oxygen did not accumulate to levels conducive for aerobic life until 500–1,000 mya. The oxygenation of Earth's atmosphere resulted in the emergence of aerobic organisms followed by a great diversification of biological species and the eventual evolution of humans. However, there are two sides to this molecule. Oxygen in the air we breathe is a relatively nonreactive chemical. However, when oxygen is exposed to electron-transferring chemical reactions, it can be converted to various highly reactive chemical forms collectively designated ''reactive oxygen species (ROS).'' ROS are toxic to biological organisms because they oxidize lipids, proteins, DNA, and carbohydrates, resulting in the breakdown of normal cellular, membrane, and reproductive functions. Ultimately, ROS cause a chain reaction of cellular oxidation, resulting in disease and lethality. ROS are unavoidable byproducts of biochemical pathways, such as glycolysis and photosynthesis, that are central to energy production and storage strategies of aerobic microbes, animals, and plants. As a result, aerobic organisms have evolved enzymatic and nonenzymatic antioxidation mechanisms to eliminate ROS. The growth and reproduction of all aerobic prokaryotes and eukaryotes require a balance between the generation of ROS and the capacity of antioxidation systems to eliminate them. In this issue of PNAS, Chen and Dickman

demonstrate that the amino acid proline is a potent scavenger of ROS. Unlike other amino acids, proline has a cyclized amino nitrogen that has significant influence on the conformation of polypeptides. Proline is also a major component of structural proteins in animals and plants and a known osmoprotectant capable of mitigating the impacts of drought, salt, and temperature stress in plants. Based on the work of Chen and Dickman, proline can now be added to an elite list of nonenzymatic antioxidants that microbes, animals, and plants require to mitigate the impacts of ROS. The ROS story is complicated by the fact that plants and animals also have evolved mechanisms that capitalize on the toxic properties of ROS to combat pathogens. For example, when plants are exposed to microbial pathogens, they produce ROS that induce programmed cell death in the plant cells surrounding the infection site to effectively ‘‘wall off’’ the pathogen and terminate the disease process. ROS may also be transmitted through the phloem to distant plant tissues signaling a pathogen attack. In these examples, ROS act locally as toxin and distantly as signaling molecules. However, it appears that ROS have a number of other potential biochemical functions such as biochemical signaling, gene expression, protein inhibition, environmental sensing, and activation of transcription factors. When organisms are exposed to abiotic stresses such as temperature extremes, dehydration, salt, UV light, ozone, and heavy metals, ROS are produced (11). In fact, the generation of ROS is the only event known to be common among such divergent stresses. When an abiotic stress induces an oxidative environment, organisms produce antioxidation systems to decrease the concentration of toxic intracellular ROS. Chen and Dickman demonstrate that the ROS scavenging property of praline prevents the induction of programmed cell death by ROS generated during nutritional stress. In addition, proline protects fungal cells against other abiotic stresses such as UV light, heat, salt, and hydrogen peroxide. There may be functional roles such as signaling or sensing for ROS during exposure to abiotic stress, but none have been confirmed.

INSIGHT INTO THE EVOLUTION OF SYMBIOSIS

One of the more interesting aspects of the work by Chen and Dickman is the possibility that it may reveal an elusive mechanism responsible for the ability of symbiotic fungi to protect plants against abiotic and biotic stress. Fossil records indicate that fungi have been associated with plants for 400 mya, and it is theorized that these recordings represent early symbiotic interactions that were responsible for the establishment of land plants. Since the first description of plant-fungal symbiosis, all plants studied in natural ecosystems have been found to be symbiotic with fungi. Fungal symbionts may express a variety of lifestyles, including parasitism, mutualism, or commensalism that decrease, increase, or have no effect on host fitness, respectively. However, in natural ecosystems, pathogenic symbioses are the exception, and nonpathogenic symbioses are the rule. Fitness benefits conferred to host plants by fungal mutualists include tolerance to abiotic and biotic stresses such as temperature extremes, dehydration, salt, UV light, heavy metals, and pathogen attack. However, the mechanism(s) responsible for symbiotically conferred stress tolerance are poorly defined, and none focus on the antioxidation property of proline. All of the abiotic stresses listed above result in the production of ROS, and Chen and Dickman demonstrate that proline protects fungi against these stresses (dehydration and pathogen attack were not tested). Therefore, symbiotically conferred stress tolerance may be based on ROS generation, the one common aspect of stress. Mutualistic fungi allow symbiotic plants to perceive stress more quickly than nonsymbiotic plants, resulting in the rapid and strong activation of plant biochemical reactions that mitigate the impacts of stress. It is possible that symbiotic fungi prompt plants to activate the biosynthesis of proline to scavenge ROS generated by stress. The ramifications of the work by Chen and Dickman go beyond the realm of fungi, because it addresses a fundamental aspect of evolution, how cells balance the generation, and elimination of ROS. Based on this work, the role of proline as a ROS scavenger, and its ability to mitigate the impacts of abiotic stress should be evaluated across all evolutionary lineages.