Fish Health Newsletter

American Fisheries Society/Fish Health Section

Volume 33 Number 2 May 2005

In this issue:

Call for Program Chair for the International Symposium on Aquatic Animal Health 2006

The Executive Committee of the Fish Health Section of the American Fisheries Society is soliciting applications for a **Program Chairman** to help in organizing the International Symposium on Aquatic Animal Health (ISAAH 2006). Unlike past ISAAH meetings, this one will be coordinated by a professional meeting organizer, John Cooksey, best known for his work with the World Aquaculture Society. Most of the arrangements for the meeting will be handled by Mr. Cooksey but we still need a Program Chairman to handle such duties as assembling a program committee, soliciting plenary speakers, grouping abstracts into the proper sessions, performing some editorial duties on the abstracts, and coordinating publication of the abstract book. The meeting will be held in San Francisco, CA, September 2-7, 2006, but the Program Chairman need not be from the West Coast. Although some work is involved, previous organizers have found this to be a very rewarding experience. If you would like to participate, please contact John Hawke at Louisiana State University, jhawke1@lsu.edu, 225-578-9705.

AFS FHS Blue Book Update

The new version of the AFS FHS Blue Book CD hit the streets a few months ago. If by chance you don't have the current version, it can be ordered on-line at www.fisheries.org. Since coming out in the CD format, the Blue Book has been comprised of two sections. One section contains the diagnostic chapters for many fish, shellfish, and crustacean pathogens—everything you ever wanted to know about how to diagnose these organisms, and many chapters with terrific photos and videos! Diane Elliott is the current chair of the Technical Standards Committee and this Committee has the charge for reviewing modifications to these chapters and soliciting new chapters for emerging pathogens.

The second part of the BB contains the Inspection Manual. This manual is a collaboration between AFS FHS and the USFWS Fish Health Centers and is intended to be the reference document for standard fish health inspection methods. A review team comprised of USFWS and AFS FHS members is responsible for modifying the manual as needed. The review process begins with you!

The actual review process is in an Appendix to the manual and follows a timeline. **This year, the new CD was a little late getting out to everyone, so to get us back on schedule, we will have a shortened comment period from now until June 15, 2005.** If you find that there are significant errors in the methods, or have a comment about the specific techniques used, or have a suggestion for using a different technique, please send your comment in writing to either Phil Hines (Phil_Hines@fws.gov) or Sue Marcquenski (Susan.Marcquenski@dnr.state.wi.us) who are the current co-chairs of the Inspection Manual Oversight Review Team. **June 15** is your deadline!! Otherwise, you will need to wait for the next cycle to have your comment reviewed. Thanks for taking the time to make the Blue Book even better!

Susan V. Marcquenski
Fish Health Specialist
WI Department of Natural Resources
Box 7921
101 S. Webster St.
Madison WI 53707
phone: 608.266.2871
fax: 608.266.2244

Update on Blue Book Revisions in Progress

Seven revised chapters on shellfish (molluscan) diseases are either ready for inclusion in a new version of the Blue Book or are in the final stages of correction. These include chapters on haplosporidiosis, mikrocytosis, marteiliasis, juvenile oyster disease, bonamiasis, hematopoietic neoplasm, and Perkinsus infections of bivalves. A QA/QC chapter has also been reviewed and is being revised. However, there is still a need for new chapters or revised versions of existing chapters. The most pressing need is for additional chapters on important crustacean diseases; Lagenidium of decapod crustaceans (written in 1994) is the only crustacean disease chapter included in the Blue Book. In addition, a number of the chapters on bacterial, viral, parasitic, and fungal diseases of finfish have not been revised since 1994, and some of the molluscan disease chapters are of the same vintage. The development of improved diagnostic methods for some of these diseases during the past 10 years may have made certain chapters obsolete. The FHS Technical Standards Committee is seeking volunteer authors to write or revise chapters for the Blue Book. We know that there are many FHS members out there who can lend their expertise and talents to such a task. We would appreciate your input and assistance to keep the Blue Book current.

Diane Elliott
206-526-6282
diane_elliott@usgs.gov

FISH HEALTH SECTION ELECTION 2005
(biographical sketches of candidates are on following pages)

Ballot of Candidates

_____
Please signify your choice with an **X**

Vice-President: (vote for one)
Pete Taylor _____
Ted Meyers _____
Barbara Nowak _____
Joyce Evans _____

Secretary-treasurer: (vote for one)
Linda Beck _____
Stewart Johnson _____

Technical standards committee: (vote for one)
Drew Mitchell _____
Linda Rhodes _____
Al Camus _____

Professional Standards committee: (vote for one)
Christine Densmore _____
David Powell _____

Nominating and balloting committee: (vote for one)
Gavin Glenney _____
Evi Emmenegger _____

DEADLINE for VOTING is MAY 15th, 2004

Please send completed ballot to Gael Kurath:
Email: "gael_kurath@usgs.gov"
Fax: Gael Kurath at (206) 526-6654
Regular mail: Gael Kurath
Western Fisheries Research Center
6505 NE 65th St.
Seattle, Washington 98115

Any inquiries can be directed to Gael Kurath, phone (206) 526-6583

Biographical Sketches of Candidates for FHS Election 2005

The following colleagues have agreed to serve the FHS in the positions listed.
They have provided the following professional biographical information:

Vice-President:

Peter Taylor

USFWS Abernathy Fish Culture Technology Center, Longview WA.

Dr. Pete Taylor is currently the Section Head for fish health research at the Abernathy Fish Culture Technology Center, USFWS, Longview, WA. Prior to that, he was Head of the fish health research section at the Southeastern Fish Culture Lab, NBS, in Marion, AL. for four years (1992-1996). For six years (1986-1992) he was an Area Fish Health Specialist and Director of the Belzoni Fish Diagnostic Lab with a joint appointment from the Mississippi Cooperative Extension Service and the School of Veterinary Medicine at Mississippi State University. Before entering public service, he worked in private industry at Wildlife Vaccines, Inc. in Denver, CO, developing vaccines for aquaculture.

Dr. Taylor received his undergraduate degree in fisheries and wildlife management from New Mexico State University, Las Cruces, NM. He completed a Master's degree in fish diseases at Auburn University, Auburn, AL and his PhD in fish pathology, also from Auburn University. Pete has been a member of AFS/FHS since 1972. He is an AFS Certified Fisheries Scientist and a FHS Certified Fish Pathologist. He has served the FHS on the Board of Professional Certification, the Awards Committee and the Promotions Committee. He has also served AFS as a member of the Board of Professional Certification. His research focus is on the bacterial diseases of fish, the development and improvement of diagnostic methods and the development of new treatments and vaccines. He is currently working on many aspects of Bacterial Cold Water Disease caused by Flavobactium psychrophilum. He is published in areas of parasitology, bacteriology and epidemiology. He has contributed sections on ciliated protozoan parasites and Bacterial Coldwater Disease in the "Blue Book".

Ted Meyers

Alaska Department of Fish and Game, Juneau, AK.

Since 1985, Ted Meyers has been the Principal Fish Pathologist and manager of the statewide fish and shellfish health program for the Alaska Department of Fish and Game (ADF&G), Division of Commercial Fisheries in Juneau, Alaska. His responsibilities include regulatory oversight of state fish/shellfish disease policies while directing two diagnostic laboratories in Anchorage and Juneau with a total complement of 6 staff members. He received an A.A.S. degree in forestry from Paul Smiths College, NY, a B.S. degree in fisheries management from Utah State University, an M.S. degree in fish diseases from Oregon State University and a Ph.D. in aquatic veterinary medicine from Cornell University. Ted has been an Assistant Professor of Fisheries with the University of Alaska (1982-1985), a research Associate Fish Pathologist with Oregon State University (1980-1982), a Sea Grant Trainee (1975-1979) while in the Cornell Aquavet program, an environmental project reviewer for the Maryland Fisheries Administration (1974-1975) and a fish pathologist for a consulting firm in Maryland (1974). Ted is editor of the ADF&G fish pathology section laboratory manual and has published over 70 papers in peer reviewed professional journals and two book chapters on the subjects of finfish and shellfish pathology/toxicology including work with marine crustacean diseases, the North American strain of Viral Hemorrhagic Septicemia Virus in marine fishes, Infectious Hematopoietic Necrosis Virus in sockeye salmon and the enzyme-linked immunosorbent assay (ELISA) for screening and diagnosis of Bacterial Kidney Disease in wild and hatchery salmonids. Ted chaired the Histopathology Technical Group for oil spill assessment studies in Prince William Sound, Alaska

after the Exxon Valdez oil spill of March 1989. In 2002 he chaired a consultant panel for the Montana Water Center regarding review of the 5 year administrative and research goals of the National Whirling Disease Foundation Initiative. He has been a member of the American Fisheries Society (AFS) Fish Health Section (FHS) since 1978, has been a FHS certified Fish Pathologist (# 4) since 1983, has served as a member and Chair of the FHS Board of Certification and Professional Standards Committee, is a past President (1994-1995) of the FHS of the AFS, is a member and past Chair (1994-1995) of the Pacific Northwest Fish Health Protection Committee, is a member of the Phi Kappa Phi and Xi Sigma Pi honor societies and is a current member of the Society for Invertebrate Pathology and the European Association of Fish Pathologists.

Barbara Nowak
Aquafin CRC and University of Tasmania, Australia.
Barbara Nowak is Health Program Leader for Cooperative Research Centre for Sustainable Aquaculture of Finfish (Aquafin CRC). She has MSc in Fish Biology, PhD in Aquatic Toxicology/Pathology and DSc in Fish Diseases. She has a tenured faculty position at School of Aquaculture, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania. Her current research focuses on amoebic gill disease in salmonids and tuna health in captivity. In the last five years she co-authored 67 peer-reviewed journal articles. These papers covered a range of topics, including aquatic toxicology, fish pathology, parasitology, epidemiology and fish immunology. She currently directs research of 7 graduate students and has supervised 10 PhD students to graduation. Her teaching duties include undergraduate courses on aquatic animal health, physiology of aquatic animals and graduate course on histopathology of aquatic animals. She organizes and runs a wide range of fish health courses for aquaculture industry and fish health professionals. Together with Dr Diane Elliott and Dr David Bruno, she has been organizing regular fish histopathology workshops for European Association of Fish Pathologists and co-edited a number of CDROMs on fish histopathology. She is Associate Editor of the Journal of Aquatic Animal Health and is a member of editorial boards for two other journals.

Joyce Evans
USDA, ARS, Chestertown, MD and Auburn, AL.
Joyce Evans is an Aquatic Pathobiologist for the USDA, ARS, Aquatic Animal Health Research Laboratories in Chestertown, Maryland and Auburn, Alabama. Joyce received her Ph.D. in Marine, Estuarine, and Environmental Science from the University of Maryland at Baltimore. She served as the Fish Pathologist, Aquatic Toxicology Coordinator, and Marine Mammal and Sea Turtle Stranding Coordinator for the Maryland Department of Natural Resources from 1987-1998. Joyce has been with USDA for the past 6 years leading and contributing to projects involving aquatic animal vaccinology and immunity, aquatic animal diagnostics, fish disease pathogenesis and applied epidemiology. Her primary research interests include evaluating the effects of environmental stressors on fish disease susceptibility and the development of commercial fish vaccines. She has authored or co-authored over 75 journal and book publications, presented at 70 national and international aquatic animal health meetings, and is co-inventor of 5 patented fish vaccines. Joyce has served as an Associate Editor to Journal of Aquatic Animal Health since 1998. She is also an Affiliate Assistant Professor at both Auburn University (AL) and Washington College (MD) and serves as a co-advisor on numerous graduate committees.

Secretary-treasurer:

Linda Beck

USFWS-Bozeman Fish Health Center, Bozeman, MT.

Linda Beck is a Microbiologist for the USFWS-Bozeman Fish Health Center in Bozeman, Montana. Her educational background includes a B.S. in Microbiology and an M.S. in Biological Sciences from Montana State University. Currently, the majority of her responsibilities are evaluating histology slides for the diagnosis of infectious and non-infectious diseases. Over her eight years with the fish health center, she has conducted research on whirling disease, sturgeon iridovirus, New Zealand mud snails and collaborated with numerous State and Federal agencies, research facilities and graduate students. Since 2002, she has been an AFS Certified Fish Health Inspector. She extremely enjoys meeting new people and interacting with other fisheries professionals. She wants to become more active in the Fish Health Section and welcomes the chance to serve as the Secretary/Treasurer.

Stewart C. Johnson

NRC Institute for Marine Biosciences, Halifax, Nova Scotia.

Stewart Johnson is a Senior Research Officer at the National Research Council of Canada's Institute for Marine Biosciences in Halifax, Nova Scotia. He received a BSc from the University of Victoria, an MSc from Dalhousie University in the Department of Biology and a PhD from the Biology Department of Simon Fraser University. Dr. Johnson completed post-doctoral fellowships at the University of British Columbia, and Stanford University. Dr. Johnson has published over 50 papers on infectious diseases and genomic and molecular mechanisms of host-pathogen interactions in aquatic animals. His primary interest has been on pathogenic copepods, i.e., sealice, and developmental immunological responses to viral and bacterial pathogens. Dr. Johnson's extensive research career has included collaborative programs with national and international partners within academia, industry, and government research labs. He has in-depth experience in participating and leading national and international research and advisory committees.

Technical Standards Committee:

Andrew (Drew) Mitchell

USDA, ARS, Stuttgart, AR.

His education includes: Bachelor of Arts, Biology (1973) - Glassboro State College, New Jersey; Master of Science, Fisheries and Allied Aquacultures (1976) - Auburn University, Alabama; and the 7- month Fish Disease Long Course (1979) - Fishery Academy, Leetown, WV. He spent his whole career working at Stuttgart, AR (1977 - Fishery Biologist, USDI/USFWS, 1993 - Fishery Biologist/Management, USDI/National Biological Survey/Service, and 1997 - Fishery Biologist/ Research, USDA/Agricultural Research Service). He began work at Stuttgart in 1977 as a fish diagnostician servicing state, Federal, and private fish growers. He established protocols for the inspection of the Asian tapeworms (*Bothriocephalus acheilognathi*) and devised successful treatments for this worm and other parasitic diseases. In 1985, as an added duty, he established the triploid grass carp program for the USFWS and continued to lead and develop the program through the early 1990s. In 1997, his diagnostic activities ceased and he became a full time researcher working primarily with fish trematode diseases. He wrote an extensive history of fish health in the United States (2001). He developed a successful pond shoreline treatment for the aquatic snails (*Planorbella spp.*) that vector a serious catfish trematode (*Bolbophorus damnificus*) problem. He worked extensively with the impact of an exotic trematode (*Centrocestus formosanus*) on wild and cultured fish in the USA and sought ways to control the exotic snail (*Melanoides tuberculatus*) that vectors this parasite. He has been active in the FHS since 1988 and has

been an AFS Certified Fish Pathologist since 1983. He authored two FHS Blue Book chapters and was a member of the Nominating Committee, Time and Place Committee, Board of Certification, and Archive Committee.

Linda D. Rhodes
NMFS Northwest Fisheries Science Center, Seattle, WA.
I have over 25 years of research experience, including the areas of aquatic toxicology, molecular immunology, and microbial pathogenesis. In my earlier years, I worked as a histopathologist/zoologist examining relationships between anthropogenic contaminants in marine sediments and fish diseases. My Ph.D. research (molecular and cellular biology, University of Washington) focused on the transcriptional regulation of immunoglobulin expression in mammals. My postdoctoral research (University of Maine at Orono) examined the molecular effects and disposition of the contaminant dioxin (TCDD) in soft-shell clam (*Mya arenaria*) and oncogene alterations in a model fish for fresh-water toxicology, medaka (*Oryzias latipes*). Since 1997, I have worked at the Northwest Fisheries Science Center (Seattle), studying *Renibacterium salmoninarum*, the etiological agent of bacterial kidney disease. My research includes bacterial detection, molecular genetics, vaccine development, and epidemiology of *R. salmoninarum* using molecular, cellular, and statistical techniques.

Methods for diagnosing diseases and detecting pathogens are typically derived from research findings. However, knowledge of the realities of field and rearing facilities is required to appropriately adapt and refine research methods for application. I am a hands-on researcher with extensive laboratory and field experience. I understand real-world limitations and demands as well as the need for rigor and reproducibility in diagnostic and detection methods, and hope to utilize my background as a technical standards committee member.

Al Camus
Mississippi State University, Stoneville, MS.
Al Camus is veterinary pathologist and Assistant Professor of Aquatic Animal Health in the Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University. Since January 2002, he has served as Director of the Aquatic Diagnostic Laboratory, at the National Warmwater Aquaculture Center located in Stoneville, Mississippi. The laboratory serves as the primary diagnostic facility for Mississippi's approximately 400-farm commercial catfish industry. Al received his D.V.M from Louisiana State University in 1984 and after four years in private practice, began studies in fish health and pathology at the University of Rhode Island in 1988. Returning to Louisiana State University in 1991, he completed his pathology residency training three years later. As an Instructor at LSU, Al served as a diagnostician for the Louisiana Aquatic Animal Diagnostic Laboratory until receiving his Ph.D. in 2001. Professional interests include the histologic and molecular diagnosis of aquatic animal diseases and the pathobiology of infectious diseases of aquatic animals, with emphasis on diseases of finfish aquaculture and their prevention. For the past 10 years, Al has helped organize and teach "Aquamed," An Aquatic Animal Pathobiology Course, sponsored by the Gulf States Consortium for Aquatic Animal Medicine and regularly lectures on fish physiology and disease. Al has been active in the investigation of diseases of warmwater aquaculture and has published on a variety of disease issues, including enteric septicemia of catfish, *Bolbophorus damnificus* trematode infections, *Aphanomyces invadens* infections in catfish and bluegill, *Providencia rettgeri* meningoencephalitis in farmed alligators, gastric cryptosporidiosis in red drum, lead poisoning in alligators, effects of T-2 toxin in catfish feeds, hepatic vacuolated cell lesions in white perch, and the molecular pathogenesis of *Streptococcus iniae*

infections. Current research interests center on the pathogenesis of channel catfish anemia, an emerging streptococcal disease of channel catfish, and proliferative of gill disease. Most recently, Al co-authored a book chapter on health management in channel catfish.

Professional Standards Committee:

Christine Densmore

USGS National Fish Health Research Laboratory, Kearneysville, WV.

Christine received her B.A. with a major in biology and minor in history from Gettysburg College in 1986. She received her D.V.M from the Virginia-Maryland Regional College of Veterinary Medicine at Virginia Tech in 1990. Following three years of clinical practice in small animal/exotic animal medicine, Christine returned to Virginia Tech and received her Ph.D. in aquatic animal pathobiology in 1997. Since, she has been employed as a research fisheries biologist and staff veterinarian at the US Geological Survey's National Fish Health Research Laboratory/ Leetown Science Center in Kearneysville, WV. Christine conducts and participates in a variety of research related to aquatic animal pathobiology. Her research involves a number of disciplines including histopathology, clinical pathology, immunology, microbiology, and cell culture. Currently, her major research interests include piscine and amphibian ranaviruses, development of cell culture methods and other diagnostic tools for tropical marine invertebrates, salmonid whirling disease, and health issues pertaining to populations of fishes in the Chesapeake Bay watershed. Besides the Fish Health Section of AFS, Christine is also a member of the American Veterinary Medical Association, the International Association for Aquatic Animal Medicine, and Partners in Reptile and Amphibian Conservation. Christine is running for election to the Professional Standards Committee as she would welcome the opportunity to contribute to the Fish Health Section's administration and maintenance of its high professional values and principles.

David Powell

ProFishent, Inc., Redmond, WA

Ph.D., M.S., and B.S. credentials, Certified Fish Pathologist

Vice President of Research at ProFishent, Inc.

Scientist and project/program manager with an extensive record of accomplishments in fish vaccine development, independent research and collaborative project leadership. My career has helped me become an expert in aquatic animal health and fisheries science. I have an international background focusing on Sweden, Norway, and Chile. I serve on the board of directors for ProFishent, Inc. and the Lake Washington chapter of the Puget Sound Anglers in Washington State.

I received my masters and doctoral degrees at the UW Fisheries Program under Dr. Richard Kocan and Marsha Landolt. I worked 5 years at the Rangen Aquaculture Research Center in Hagerman, Idaho. I was then recruited by Bob Busch to work for the highly successful BioMed, Inc. and later Alpharma, Inc. as Research and Development Manager for 5 years. I have completed multiple short courses in technology management, human resources, basic accounting and project management. I currently conduct federally funded research in the areas of: salmonid and catfish immune function, risk management, nanotechnology, and cryopreservation.

Nominating and Balloting Committee:

Gavin Glenney, PhD.

USDA, National Center for Cool and Cold Water Aquaculture, Kearneysville, WV.

I recently graduated with a PhD from Mississippi State University. Before returning to school, my work experience was in commercial aquaculture. I have worked with a variety of aquaculture species and culture systems- from A. salmon (net pens) to tilapia (Recirc. Systems). I am currently working in the area of rainbow trout immunology with the USDA at The Center for Cool and Cold Water Aquaculture. A short time ago I was certified as a Fish Health Inspector, and aspire to become a Certified Fish Pathologist. I am interested in becoming an active member with the AFS/FHS. I have chosen the nominating and balloting committee to become familiar with how the section operates and its members.

Evi Emmenegger

USGS Western Fisheries Research Center, Seattle, WA.

I am currently a Microbiologist with U.S. Geological Survey, Western Fisheries Research Center (WFRC) in Seattle, WA, and collectively through education and employment have over 19 years of experience in the field of fish pathology. My educational background includes Bachelor's of Science degrees in Fisheries and Microbiology from Oregon State University (OSU) and a Master's of Science degree from the School of Fisheries at the University of Washington. After receiving my baccalaureate I returned to my home state and worked for a few years as a microbiologist for the Alaska Department of Fish & Game in the Fish Pathology section. After I completed my graduate studies, I became a research scientist with the molecular biology research team, of the fish health section at the WFRC, where I work with Gael Kurath and Jim Winton. The primary focus of my research is studying viral fish pathogens. Specifically, I work on improving detection and diagnosis of these viral pathogens, utilizing methodologies that genetically distinguish different virus isolates, and developing products that reduce losses in fish populations due to viral disease. I have published a number of peer reviewed papers and I have presented my research at numerous scientific conferences. Throughout my career I have attended AFS fish health meetings, workshops, and continuing education classes that not only foster my goals as a fish health professional, but also provide an enthusiastic forum for discussing fish disease issues. The successes of the fish health section are due largely to the work of previous officers and committee members. I hope to continue the ongoing success of the section by participating in the nomination of these dedicated fish health section members.

FHS Continuing Education Program: Spring Viremia of Carp Virus
June 17, 2005, Shepherdstown, West Virginia

The continuing education program at the 30th Annual Eastern Fish Health Workshop will be on Spring Viremia of Carp (SVC). Dr. Andy Goodwin (University of Arkansas at Pine Bluff) will organize and conduct the program on Friday, June 17. It will concentrate on the impact of SVCV on wild and cultured fish with special emphasis on the detection, identification, and epidemiology of the new Asian strains that have now been found in North America. Experts from Eastern and Western Europe will describe the history of the disease in their regions providing useful insights to compare with reports from North American scientists that have dealt with SVC in the US. In addition, speakers will describe the success of regulatory efforts to control the disease overseas and APHIS will discuss current and future regulation of SVCV in the US. The primary goal of the session is to provide a realistic assessment of SVC disease so that resource managers, regulators, diagnosticians and the aquaculture industry can make informed decisions about how the disease should be dealt with in North America and cover the taxonomy of the virus, its impact, plus control and management strategies among cultured and wild fishes.

The program includes:

- Andy Goodwin, University of Arkansas at Pine Bluff
  An overview of SVCV in the United States and an introduction to some of the important questions without answers.
- Barry Hill, CEFAS, Weymouth, UK
  Impacts, control and regulation of SVCV in wild and cultured fish in western Europe with a history of SVCV strains from China.
- Igor Shchelkunov, Russia
  Impacts, regulation and control of SVCV on wild and cultured fish in Eastern Europe.
- David Stone, CEFAS, Weymouth, UK.
  Molecular biology and taxonomy of SVCV: relationships between SVCV (European and Asian), PFRV, and other similar rhabdoviruses.
- Andy Goodwin, University of Arkansas at Pine Bluff.
  Diagnosis of SVCV, PCR and serological tests. What they tell us and what they don't.
- Sue Marcquenski, Wisconsin, DNR.
  SVCV and the wild fish of Wisconsin.
- Robert Bakal, USFWS, Warm Springs, GA.
  SVCV and the wild fish of North Carolina and Virginia.
- Jill Rolland, APHIS.
  The current and future regulation of SVCV in the United States.

Upon completion of this course, attendees shall be awarded 7 continuing education credits by the FHS of the American Fisheries Society. There is a $35 registration fee, which includes breakfast and lunch. Reservations for the course may be made by completing the appropriate sections of the Eastern Fish Health Workshop registration form (http://www.fisheries.org/fhs/eastern.htm). Registrations must be made by May 23, 2005.

FHS Continuing Education
APPLYING RISK ASSESSMENT PRINCIPLES TO FISH HEALTH SITUATIONS

8:30 – 4:30 Monday June 27, 2005
DoubleTree Riverside Hotel, Boise, Idaho
In conjunction with the **Western Fish Disease Workshop**
Cost: $40 (Submit with workshop registration)
http://www.fisheries.org/fhs/western_fish_dis_workshop.htm

Fish health professionals are often required to contribute to the assessment of risks to fish health due to management decisions. This class is being designed to make understandable and useful to fish health minds the often confusing process of assessing fish health risks. Those participating should come away with a clear grasp of the concepts and processes involved in a quality assessment, and have materials and notes to guide them when called upon.

The day will consist of:

- An overview of the definitions, concepts and processes of risk analysis, including hazard identification, risk assessment, risk management, and risk communication.
  Taught by USDA/APHIS.

- Risk analysis and our international obligations
  Dr. Ken Stepushyn, Fisheries and Oceans, Canada.

- Group exercises to practice applying the principles to real fish health situations
  Kevin Amos, NOAA Fisheries.

- Case studies of risk assessments: how they were approached, what was learned
  Scott Foott,
  Warren Groberg,
  Jerri Bartholomew.

Significant take home reference materials will be supplied.

If you have a risk scenario that would make a good group exercise, please tell Craig Olson at colson@nwifc.org (360-528-4343). Any questions you have can be directed to Craig also.

This class is worth 6 CE hours for the AFS – Fish Health Section,
7 CE hours approved for Idaho Veterinarian CE credit.
Other veterinary recognition is being sought.

FHS Continuing Education: Current Topics in Aquatic Toxicology
Minneapolis, MN
July 30, 2005

This day-long session follows the AFS-Fish Health Section Annual Meeting. Lectures will cover all of the major areas of interest including hot topics like estrogenic compounds, algal toxins, mycotoxins, and botulism. The goal of the session is to provide a broad overview of fish toxicology with practical information of interest not only to scientists but also to diagnosticians responsible for the investigation of fish kills.

For more information on the FHS meeting and registration materials, visit the FHS web site at http://www.fisheries.org/fhs/2005_fhs.htm

- Intro to Fish Toxicology and Session Overview
  Andy Goodwin, University of Arkansas at Pine Bluff
- Algal Toxins in Freshwater Systems
  Andy Goodwin, University of Arkansas at Pine Bluff
  Freshwater algal toxins; their mode of action, their sources, and their effects on fish and how to investigate a suspect fish kill. Cyanobacteria, golden algae, anatoxins, microcystins, nodularins, cylindrospermopsin
- Marine Algal Toxins
  Wolfgang K. Vogelbein, Department of Environmental Sciences, The College of William and Mary
  Marine algal toxins: Red tides, Pfisteria, other toxic blooms, and bioaccumulation of toxins in marine animals. What happens to fish and how to recognize toxic bloom problems
- Mycotoxins
  Patricia S. Gaunt, Mississippi State University College of Veterinary Medicine
  Fungal toxins: their mode of action, their sources, and their effects on fish: Ochratoxin, aflatoxin, and a bunch of other “o-toxins”
- Botulism
  Rod Getchell, Cornell University, Department of Microbiology and Immunology
  Botulism in farmed and wild fish: Aquaculture, the Great Lakes, and more.
- Pesticides and Herbicides
  Andy Goodwin, University of Arkansas at Pine Bluff
  Drift and runoff of pesticides, what are the real risks to aquatic animals? How do you investigate a suspect pesticide kill?
- Heavy Metals and Acid Rain
  John M. Besser, USGS Columbia Environmental Research Center
  Metal ecotoxicology: Metal sources, routes of exposure, and toxic effects on fish.
- Pollution and Neoplasia
  Jack Fournie, EPA, Gulf Breeze, FL
  Toxicant induced neoplasia in fish. Sources, metabolism, and typical lesions.

- Methods To Assess Sub-Lethal Exposure
  Donald Tillitt, USGS Columbia Environmental Research Center
  Biological markers for sub-lethal exposure to natural and man-made toxicants: Induction of hepatic enzymes, endocrine disruption, and other biological markers of chemical exposure and effect.

Upon completion of this course, attendees shall be awarded 7 continuing education credits by the FHS of the American Fisheries Society. There is a $45 registration fee. Reservations for the course may be made by completing the appropriate sections of the general FHS Meeting registration form. Registrations must be made by June 15, 2005.

Meetings and Workshops:

30th Annual Eastern Fish Health Workshop
Shepherdstown, West Virginia
June 13-17, 2005

For more information, see the announcement on the FHS website (http://www.fisheries.org/fhs/eastern.htm) or contact:
Rocco C. Cipriano
United States Geological Survey
National Fish Health Research Laboratory
11700 Leetown Road
Kearneysville, WV 25430
P: 304/724-4432
F: 304/724-4435
E: rocco_cipriano@usgs.gov

46TH WESTERN FISH DISEASE WORKSHOP
BOISE, IDAHO
JUNE 27-29, 2005

The meeting is co-hosted by IDFG Eagle Fish Health Laboratory, Ken Cain (U of Idaho) and Scott LaPatra (Clear Springs Foods, Inc.). Craig Olson has organized the continuing education program for the first day. Abstract are due May 25. For more information, see the FHS website (http://www.fisheries.org/fhs/western_fish_dis_workshop.htm). For information about registration contact:
Eagle Fish Health Laboratory
Idaho Department of Fish and Game
1800 Trout Road
Eagle, ID 83616
Telephone: (208) 939-2413
e-mail: kjohnson@idfg.idaho.gov

SECOND CALL FOR ABSTRACTS
2005 AFS FISH HEALTH SECTION MEETING

The 2005 Annual Meeting of the Fish Health Section/American Fisheries Society will be held in Minneapolis, MN, during 27-29 July 2005. Registration will be held on the evening of Tuesday 26 July 2005. A Continuing Education session is being planned for Saturday 30 July 2005. The meeting will be held at the Ramada Inn Airport at the Mall of America. This location is 2 miles from the Minneapolis/St. Paul International Airport. Complementary shuttle service to the motel is available as is complementary shuttle service to the Mall of the America, located approximately 0.5 miles from the motel. A block of rooms has been set aside until July 10. Please indicate you are with the American Fisheries Society conference. Room rates per night are as follows: $95.00 Single/double occupancy with $6.00 for each additional adult. Parlor suites are also available at 155.00.
Additional information on the meeting location may be found at the motel web site: http://www.ramada.com/Ramada/control/Booking/property_info?propertyId=14208&brandInfo=RA.

The host for the meeting is Joe Marcino (joe.marcino@dnr.state.mn.us). The scientific program is being organized by Paul Bowser (prb4@cornell.edu). Information and instructions for abstract submissions are posted on the Fish Health Section Web Site.

A continuing education course "**Current Topics in Aquatic Toxicology**" is being planned for Saturday July 30th. This will be organized by Dr. Andrew Goodwin. The session will provide an overview of contemporary topics associated with aquatic toxicology. The target audience will be those individuals who want an in depth coverage of current issues in that area. Individuals participating in this program will earn 7 CE credit hours from the Fish Health Section of the American Fisheries Society. Contact person is Andrew Goodwin (agoodwin@uaex.edu). The class size is limited to 50 people, based on time when registration is received.

Registration for the 2005 Fish Health Section Meeting will be as follows:

Early registration - FHS Members	$ 75.00
Student FHS Members	$ 70.00
- non-Members	$ 85.00
After 15 June 2005 (receipt date)	
FHS Members	$ 85.00
Student FHS Members	$ 70.00
- non-Members	$ 95.00
Banquet fee:	$ 33.00 per person
CE Registration Fee	$ 45.00

Important Deadlines:
May 15, 2005 (See attached form) - Preliminary submission that includes the following: 1) Intent to attend, 2) title of presentation, 3) whether poster or oral is preferred. Oral presentation will be made electronically using PowerPoint. This preliminary submission will allow the organizing committee to prepare a preliminary agenda prior to the meeting.
June 15, 2005 - Early Registration Fees receipt date.
June 15, 2005 - Formal abstracts and electronic version of oral presentations receipt date.

2005 AFS FISH HEALTH SECTION ANNUAL MEETING
Ramada Inn Airport at the Mall of America
Minneapolis, Minnesota
July 26 - 29, 2005
CE Session on July 30, 2005

Please return electronically before: 15 May 2005; use one form per participant

Returning this form will help us in our planning of the meeting and to set up the various sessions in the Scientific Program.

I intend to attend the 2005 Fish Health Section Meeting
Name:
Affiliation:
Mailing Address:
City:
State:
Zip Code:

Phone:
Fax:
Email:

I plan to submit an abstract for an oral presentation(s): YES_____ NO _____
I plan to submit an abstract for a poster presentation(s): YES_____ NO _____

Tentative title(s):

I plan to attend the CE Session: YES_____ NO_____
(**Please note: attendance at the CE session can only be reserved following receipt of the registration fee for the CE session. Some of the most recent CE Sessions have been "overbooked," so you are encouraged to submit your fee for CE participation early**)

Please forward this form via e-mail to:
Paul Bowser: prb4@cornell.edu
and
Joe Marcino: joe.marcino@dnr.state.mn.us

REGISTRATION FORM
2005 AFS FISH HEALTH SECTION ANNUAL MEETING
Ramada Inn Airport at the Mall of America
Minneapolis, Minnesota
July 26 - 29, 2005
CE Session on July 30, 2005

Return before: 1 June 2005 for Early Registration Fees; use one form per participant

Name:

Affiliation:

Mailing Address:

City:
State:
Zip Code:

Phone:
Fax:
Email:

I plan to attend the 2005 Fish Health Section Annual meeting and will_____, will not _____ give an oral/poster (circle one) presentation.

Make checks to Fish Health Section c/o Joe Marcino. Mail to: Joe Marcino, MN Department of Natural Resources, Box 25, 500 Lafayette Road St. Paul, MN 55155. Credit cards are not accepted.

Early registration (received before 15 June 2005)	
- FHS Members	$ 75.00 ($85.00 after 15 June)
- Student FHS members	$ 70.00
- non-affiliated	$ 85.00 ($95.00 after 15 June)
CE Registration Fee	$ 50.00
Extra Banquet Ticket ($33.00 per person)	$_____
Total Enclosed:	**$_____**

Guide for Presentations and Abstract Preparation

Please prepare and submit your abstract electronically before 15 June 2005, following these directions:

1. Limit abstract to a single side of an 8.5x11" page maintaining 1.5 inch margins on the top, bottom and sides of the page.

2. Provide a concise title. Capitalize the first letter of every word in the title using bold 14 pitch Times Roman font.

3. Type the remainder of the material (authors, affiliations, and text) in plain Times Roman 12 pitch font.

4. Provide a double-space between the title and authors (use first name, middle initial, and last name) and underline the name of the presenting author.

5. Provide a single space between the authors and their affiliations and denote each of the author's affiliation using numerical superscripts. The superscript should be placed after an author's name and follow his/her affiliation. Affiliations should include complete mailing addresses, without abbreviations. Do not include phone numbers or email addresses in the affiliation.

7. Provide a double space between the affiliation and main body of text. Spell all words completely upon their first use. Abbreviations are acceptable thereafter, but do not begin a sentence with an abbreviation. Do not use figures, tables, or references in the abstract. Denote genus and species with italic script.

Power Point Presentations

Power Point will be used for all oral presentations. Ensure that all fonts on the presentation are equal to or greater than a 28 pitch (some gene sequencing excepted). Your presentation should be forwarded to Dr. Paul Bowser no later than **15 June 2005** so that master CD's for the session can be prepared.

Your oral presentation should not exceed **12 minutes**. During the sessions, a timekeeper will monitor the length of your talk and a ring a bell after 12 minutes has expired. Moderators will be instructed to close the talks at that time.

Abstracts and electronic version of oral presentations should be forwarded to:

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Aquatic Animal Health Program
Department of Microbiology and Immunology
College of Veterinary Medicine
Cornell University
Ithaca, New York 14853-6401
Phone: (607) 253-4029
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FHS PARTICIPATION IN THE AAVLD / USAHA ANNUAL MEETINGS

Scott LaPatra

As you know, the Fish Health Section (FHS) has committed to becoming more involved on issues of importance to the membership. For the last seven years I attended and participated in the United States Animal Health Association (USAHA) and the American Association of Veterinary Laboratory Diagnosticians (AAVLD) annual meeting. Last year the meeting was held in San Diego, California, and this year it was held in Greensboro, North Carolina. For background information, the USAHA is the most well established animal health organization that has approximately 1,400 members and works with a variety animal health entities both nationally, including the United States Department of Agriculture Animal Plant Health Inspection Service (USDA/APHIS), and internationally. The purpose of the AAVLD, which works closely with the USAHA, is the dissemination of information relating to the diagnosis of animal disease, the coordination of the diagnostic activities of regulatory, research and service laboratories, the establishment of accepted guides for the improvement of diagnostic laboratory organizations relative to facilities, equipment and personal qualifications.

The FHS objectives, interests and goals regarding animal health are very similar to the USAHA. One of the reasons we were in attendance was to offer our expertise and established programs in aquatic animal health and maintain visibility with other groups also interested in aquatic animal medicine. The AAVLD and the USAHA Aquaculture Committees met jointly and was chaired by myself representing USAHA. My co-chair is Dr. Tom Baldwin who represents the AAVLD, however, he was unable to attend this years meeting. As in past years, I updated the committee about the Sections activities. Additionally, the committee has been very successful at passing resolutions which are then forwarded to the Executive Committee of the USAHA. This year two resolutions were supported by the Committee. The minutes from the meeting along with the two resolutions that were forwarded to the USAHA Executive Committee are included below. If you have any questions or need for additional information, please don't hesitate to contact me or one of the FHS Executive Committee members. I would like to gratefully acknowledge the efforts of Dr. Jerry Heidel who recorded the minutes of the meeting.

Minutes

Opening comments - Scott LaPatra

The meeting began at 12:30 pm. Attendees were welcomed and asked to introduce themselves.

Update from the National Aquaculture Association - Betsy Hart

A short review of the NAA, a producer organization, was provided. The diverse nature of the membership was emphasized, including representation of all aquacultured species. The NAA provides a unified voice for aquaculture, helping to assure the vitality of the various aquaculture industries. Committees represent various components of aquaculture, and through their governing board, assure a united stand on issues. The NAA offers a strong informational web site. Current issues facing organized aquaculture were reviewed, including the National Animal Identification Program and environmental issues.

Update from the National Animal Identification Program - Valerie Ragan

NAIP is being developed for disease eradication, and is applicable to any disease and all livestock. The program is currently assessing applicability to aquaculture and how to best implement an effective program; i.e. the program will be tailored to the animals in question. An industry working group has been formed that is working with USDA on an acceptable plan for the use of the NAIP in the aquaculture arena.

Update from USDA-APHIS - John Clifford and Jill Rolland

USDA has found it important to work closely with the aquaculture industries in establishing programs and protocols related to aquatic animal diseases that could threaten the aquatic industry. The National Aquatic Animal Health Plan (NAAHP) is being developed, which is a guidance document, with three federal agencies involved: Commerce, Interior, and USDA. A partnership of these agencies with industry and professional representatives has been created to develop a transparent plan based upon consensus.

A presentation summarizing the NAAHP as well as updates on the response to recent outbreaks of infectious salmon anemia (ISA), spring viremia of carp (SVC), and white spot disease of shrimp. EU-generated directives related to export of fish, fish products, and mollusks to the EU were reviewed.

The presentations generated lively audience discussion related to USDA interactions with and impact upon producer groups and aquaculture-related commerce.

Update from AVMA Aquatic Veterinary Medicine Committee - David Scarfe

The background and activities of the AVMA Aquatic Veterinary Medicine Committee (formerly known as the Aquaculture and Seafood Advisory Committee) were presented. The committee has addressed a wide variety of topics related to aquatic animal health, regulatory issues, and environmental concerns. These include national aquatic animal health programs, diagnostics, therapeutic agents, effluents, seafood safety, and promotion of the important role of veterinarians in the aquaculture industry.

The Whitney Laboratory for Marine Bioscience - Bob Kahrs

The Whitney Laboratory in St. Augustine, Florida, affiliated with the University of Florida, is developing a program in marine animal health that includes development of a Center for Marine Animal Health. Training and funding are available for graduate students and post-doctorates. Attendees were urged to contact the laboratory director for more information.

Update from the Fish Health Section/American Fisheries Society – Scott LaPatra

The FHS/AFS has continued its active involvement in fish health issues at all levels. The organization provides expertise to a variety of stakeholders, both public and private, in the aquaculture industries. The FHS provides professional certification, continuing education and regional and national meetings. They have recently developed a Standard Inspection Manual in collaboration with the US Fish and Wildlife Service that is reviewed annually and has been provided to the National Aquatic Animal Health Taskforce.

Forecasting Disease Emergence in the Aquaculture Industry - Victoria Bridges

A presentation from the Center for Emerging Issues summarized their overall activities related to analysis of emerging animal diseases, surveillance systems for emerging animal health events, and tracking and trending of health events. A current project is focused on forecasting disease in the aquaculture industry. The goal is to develop a "disease emergence profile" for the food fish industry. This includes describing

characteristics of disease emergence factors through analysis of current situations and the forces for change. Predictive, decision-making tools are the anticipated result of this work.

Old Business
Last year's resolutions, their fate, and USDA response were reviewed and discussed.

New Business
Stan Bruntz presented a request on behalf of the Committee on Animal Health Information Systems with respect to the National Animal Health Reporting System (NAHRS). This group is requesting appointment of a chair for the Aquaculture group.
Motion: Jerry Heidel will assume the chair of the NAHRS Aquaculture Commodity Working Group and that he will contact existing members to assess their willingness in continuing their membership; and in the absence of such willingness will fill the vacant positions with appropriate members. Motion carried.

Resolution #1: introduced by Jerry Heidel on behalf of Ralph Elston and the Pacific Coast Shellfish Growers Association.
The USAHA requests USDA-APHIS to promote listing of the paramyxean protozoan parasite *Marteiliodes chungmuensis*, known to infect oyster species including the Pacific oyster, *Crassostrea gigas*, and the Iwagake oyster, *Crassostrea nippona*, and possibly other bivalve species, as a Notifiable Disease in the Office Internationale Epizooties (OIE) International Aquatic Animal Health Code.

The motion in support of this resolution was defeated. The committee recommended that Dr. Elston directly contact Dr. Jill Rolland, USDA/APHIS, with a request for USDA/APHIS to consider listing of this parasitic disease; this would initiate a thorough review of the condition to determine if there is sufficient data to support this listing. Additionally, Dr. Elston should seek further producer support for diverse geographical areas of the United States.

Resolution #2: introduced by Don Hoenig.
The USAHA requests the United States Department of Agriculture Animal Plan Health Inspection Service (USDA/APHIS) to begin to work immediately to establish sufficient, annual funding for the long-term maintenance of the USDA/APHIS/Veterinary Services ISA program including indemnification for loss incurred by US salmonid growers in the implementation of the program.

The motion in support of this resolution was passed (Appendix 1).

Resolution #3: introduced by Scott LaPatra
The United States Animal Health Association (USAHA) requests that the United States Department of Agriculture (USDA), Animal Plant Health Inspection Service (APHIS), Veterinary Services (VS) to determine if the data needed to perform credible risk assessments exists and identify information gaps. Appropriate steps should be taken to fill in these gaps for the prevention of the introduction and the potential establishment of viruses of finfish (as identified in the National Aquatic Animal Health Plan) of economic significance into the US commercial farmed fish industry sectors.

The motion in support of this resolution was passed (Appendix 2).

The meeting adjourned at 5:30 pm.

Presence of an IHNV DNA vaccine during water hardening of rainbow trout eggs does not result in either immune tolerance or protective immunity

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The use of DNA vaccines is a promising new approach for the management of many important fish rhadoviral diseases in aquaculture and natural resource salmonid hatcheries. Recent reports provide compelling evidence that DNA vaccines provide exceptionally high levels of protection against infectious hematopoietic necrosis virus (IHNV), viral hemorrhagic septicemia virus (VHSV), or hirame rhabdovirus (HIRRV) (reviewed in Kurath, 2005). Unfortunately, the current requirement for these vaccines to be administered to fish individually by intramuscular injection is a major obstacle to immunizing large numbers of fish. The current study investigated the use of the water hardening step in fish egg fertilization as a quick and economical method for mass immunization of rainbow trout with a DNA vaccine. It has been reported that a salmonid egg takes in approximately 20% of its volume from the surrounding water during water hardening (Leitritz and Lewis, 1976), and we speculated that this influx of water might also provide a simple route for the delivery of a DNA vaccine into several hundred eggs simultaneously. Whereas it was impossible to predict what effects the presence of a DNA vaccine during the earliest stages of egg maturation would have on the fry after hatch, we based our study design on testing three possible outcomes: (1) no effect, (2) development of immune tolerance (lack of reactivity) to the G-protein, or (3) stimulation of protective immunity. Although the last possibility was considered the least likely in theory, the great value of a mass delivery method for DNA vaccines made it worth investigating.

The IHNV DNA vaccine pIHNw-G is a circular plasmid DNA that contains the gene for the viral glycoprotein (G), from the WRAC strain of IHNV (Corbeil et al., 2000). Upon intramuscular (i.m.) injection into rainbow trout fry the G protein is expressed and elicits rapid innate immunity, followed by long-term specific immunity. This vaccine has been shown to be highly efficacious in a variety of conditions (reviewed in Kurath 2005). A single low dose of 0.1-1.0 µg DNA in 1-3 g fry provides strong protection against subsequent lethal IHNV challenge, with relative percent survival (RPS) values of 80-100% (summarized in Garver et al., in press).

The study design was as follows (see Figure 1 and Table 1):
1. Freshly fertilized rainbow trout eggs from four females were pooled, divided into three groups and water hardened in either: a) water containing the pIHNw-G vaccine, b) water containing an equal amount of a mock vaccine called pLuc that has no viral gene (Corbeil et al., 2000), or c) water alone. The concentration of vaccine or mock vaccine in the water was adjusted so that uptake of a 10-µl volume (approximately 20% of the average egg volume) would deliver 0.1 µg of DNA to the inside of the egg.
2. Following water hardening eggs were held in separate batches, hatched, and progeny were reared until they reached an average weight of 1.5 g. Water temperature throughout the study was 15C.
3. At average weight 1.5 g, fish in each of the three water-hardening groups were assigned to three subgroups of 100 fish each. A subgroup was then vaccinated by i.m. injection with 50 µl of phosphate-buffered saline containing either (i) no DNA, (ii) 0.1 µg pIHNVw-G vaccine, or (iii) 0.1 µg pLuc mock vaccine.
4. At 30 days post-vaccination duplicate groups of 25 fry from each of the nine vaccination subgroups were moved into 4 L tanks and exposed to a standard one hour static immersion virus challenge with a dose of 2×10^4 pfu/ml of the rainbow trout virulent IHNV strain 220-90. At the same time, duplicate groups from each vaccination subgroup were mock-challenged by immersion in water with no virus.

5. Each of the 36 challenge tanks (18 virus challenged and 18 mock-challenged) was monitored daily for mortality for a period of 30 days. Dead fish were removed and frozen at -80C.
6. At the end of the 30 day challenge period survivors were counted and euthanized, and data was analyzed in terms of final cumulative percent mortality (CPM) and relative percent survival (RPS) calculated as RPS = [1-(average CPM of duplicate experimental groups/average CPM of duplicate respective PBS-vaccinated groups)].

The goal was to describe the effects of water hardening in the presence of the pIHNw-G DNA vaccine on the immune status of the fry. If this delivery method stimulated protective immunity, the progeny fish would survive a lethal virus challenge even if they had no later vaccination. Alternatively, if exposure of the eggs to the DNA vaccine resulted in immune unresponsiveness (tolerance) to the IHNV G protein, then the subsequent vaccination with pIHNw-G would not elicit a protective immunity against viral challenge

A summary of the experimental treatment groups and the final CPM 30 days post-challenge is shown in Table 2,. Treatment groups D-R were all controls to verify that the system functioned as expected when eggs were not water hardened in the presence of the pIHNw-G vaccine. Cumulative mortality in all mock-challenged groups (J-R) was 0-8%, with an average of 3.3% CPM. Groups D, E, G, and H were never immunized with pIHNw-G at any time during the study, and served as positive controls that characterized the severity of the viral challenge. Mortality in these groups started at day 5-6 and was epidemic during days 7-16, reaching final CPM ranging from 64-92%, with an average of 80%. Controls for the activity of the vaccine were groups F and I, where pIHNw-G was delivered in the standard manner to fish from eggs that were water hardened either in water or pLuc. The low mortality of 0-20% in these groups, with an average of 6%, resulted in calculated RPS values of 88-97% and confirmed that the vaccine elicited strong protection.

The critical treatment groups for assessing any biological effect of water hardening eggs in the pIHNVw-G vaccine were groups A, B, and C (Table 2). The kinetics of mortality in these groups is in Figure 2. Both groups A and B, which were water hardened in pIHNw-G and later mock vaccinated with buffer or pLuc, had high mortality averaging 82-86%. This level is the same as the mortality experienced in groups D, E, G, and H, proving conclusively that water hardening in pIHNw-G did not elicit any protective immunity in progeny fry. Group C, which was from the same egg group but was subsequently vaccinated with pIHNw-G, had low mortality averaging only 2%, for an RPS of 98%. This active protection proves that exposure to pIHNw-G at water hardening did not stimulate the development of immune tolerance to the G protein, since that would have rendered the vaccine ineffective when administered to fry. Thus, the CPM in groups A, B, and C match the prediction for no effect of water hardening in the presence of pIHNw-G (Table 1).

These results, while disappointing, are clear in demonstrating that water hardening will not be an effective method for mass delivery of the DNA vaccine, and it will also not be useful in its current form as a method for stimulation of immune tolerance as a research model. The lack of effect could be due to some selective process that does not allow entry of the DNA into the egg, degradation of the vaccine inside the egg, lack of expression of the G protein in the egg, or possibly, an inability of the maturing embryo to produce or respond to the G protein because the appropriate cells or tissues had not yet developed when the vaccine was present. None-the-less, this study clearly suggests that other methods of DNA vaccine delivery should be pursued.

Acknowledgments

The authors very much appreciate the essential contribution of Scott LaPatra in providing rainbow trout eggs and sperm (twice!) and advice on fertilization, and Kyle Garver and LynnMarie Applegate for helping with wetlab work.

References

Corbeil, S., LaPatra S.E., Anderson, E.D., and Kurath, G. 2000. Nanogram quantities of a DNA vaccine protect rainbow trout fry against heterologous strains of infectious hematopoietic necrosis virus. Vaccine 18:2817-2824.Garver, K.A., LaPatra, S.E., and Kurath, G. Efficacy of an IHN virus DNA vaccine in chinook (*Oncorhynchus tshawytscha*) and sockeye (*Oncorhynchus nerka*) salmon. Dis. Aquat. Org., in press.

Kurath, G. Overview of recent DNA vaccine development for fish. *In*, P.J. Midtlyng ed., Fish Vaccinology, Developments in Biologicals, Basel, Karger Vol 121:201-213, in press.

Leitritz, E., and Lewis, R.C. 1976. Trout and salmon culture (hatchery methods). State of California Dept. of Fish and Game, Fish Bulletin 164.

Table 1. Treatment groups for fry hatched from eggs water hardened in the pIHNw-G DNA vaccine, and predicted mortality levels that distinguish the three phenomena that theoretically could occur.

	Experimental treatments			Predicted mortality		
Group	Water-harden	Vaccine at fry stage	Viral Challenge	No effect	Tolerance	Protective immunity
A	pIHNw-G	PBS	IHNV	High	High	Low
B	pIHNw-G	pLuc	IHNV	High	High	Low
C	pIHNw-G	pIHNw-G	IHNV	Low	High	Low

Table 2. Treatment groups and final cumulative percent mortality (CPM) in duplicate tanks

Group	Water harden	Vaccination	Challenge	Final CPM
A	pIHNw-G	PBS	IHNV	76, 92
B	pIHNw-G	pLuc	IHNV	74, 56
C	pIHNw-G	pIHNw-G	IHNV	0, 4
D	pLuc	PBS	IHNV	64, 92
E	pLuc	pLuc	IHNV	69, 76
F	pLuc	pIHNw-G	IHNV	4, 0
G	water	PBS	IHNV	80, 84
H	water	pLuc	IHNV	80, 92
I	water	pIHNw-G	IHNV	20, 0
J-R	same as A-I	same as A-I	mock	all 0-8

Rainbow trout eggs were water hardened in the presence of the IHNV DNA vaccine pIHNw-G, mock vaccine, or water alone. Progeny fry (average weight 1.5 g) were vaccinated or mock-vaccinated, and 30 days later duplicate tanks of 25 fry per group were challenged or mock-challenged by immersion in 2 x 10^4 pfu/ml of IHNV strain 220-90.

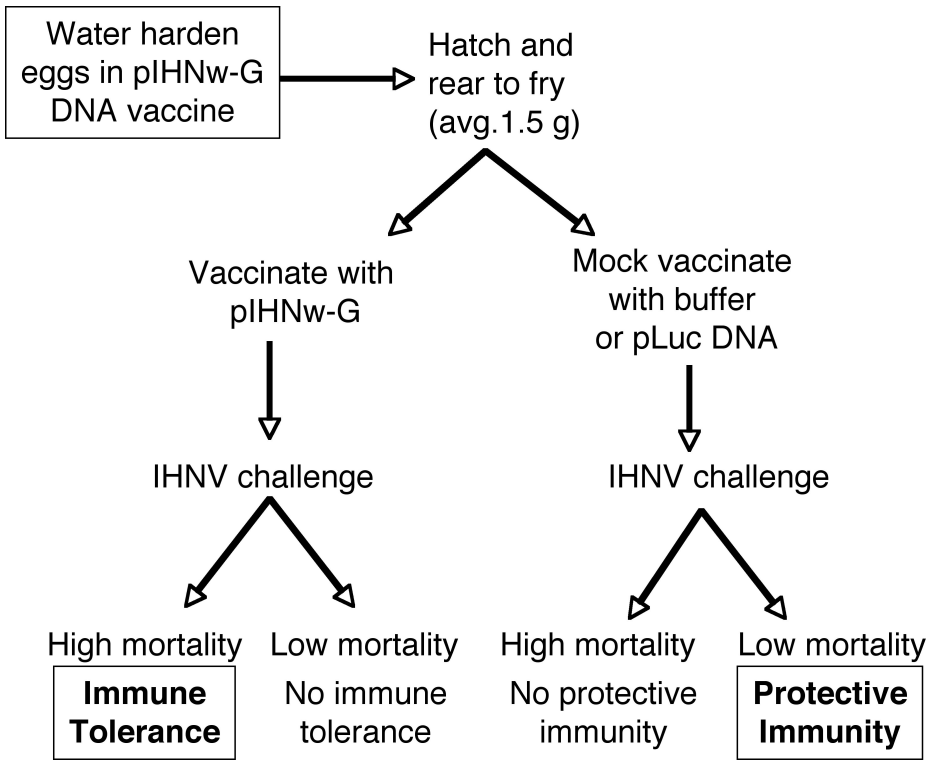

Fig. 1. Experimental strategy for assessing development of either protective immunity or immune tolerance in rainbow trout fry hatched from eggs that were water hardened in the presence of the pIHNw-G DNA vaccine.

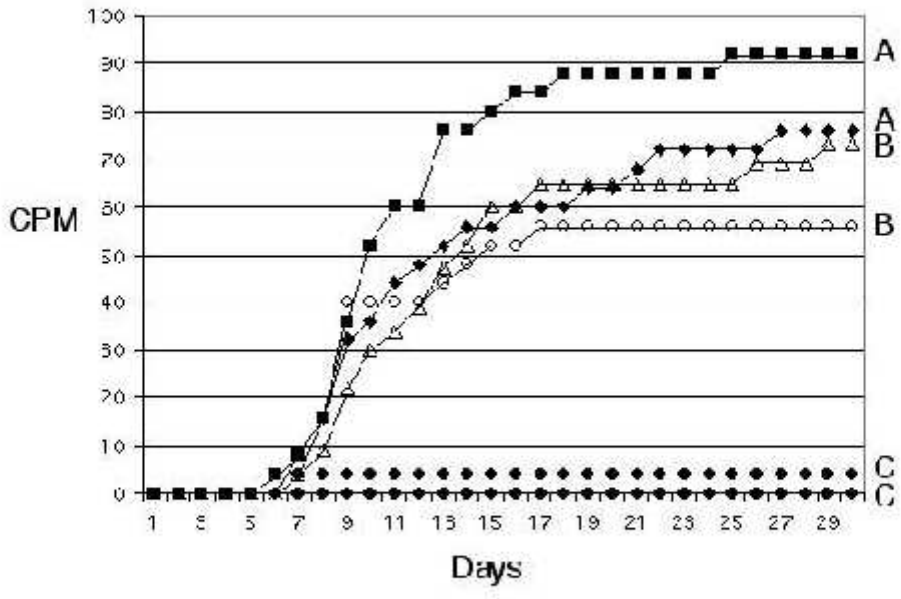

Fig. 2. Cumulative percent mortality among duplicate groups of fry from eggs that had been water hardened in the presence of the IHNV DNA vaccine pIHNw-G and then vaccinated with (A) PBS buffer, (B) pLuc mock vaccine, or (C) pIHNw-G 30 days prior to challenge with IHNV.