Proceedings

4th International Symposium on Aquatic Animal Health

September 1–5, 2002
New Orleans, Louisiana USA

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Proceedings of the Fourth International Symposium on Aquatic Animal Health,
September 2nd – 6th, 2002, Sheraton New Orleans Hotel,
New Orleans, Louisiana USA

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Department of Pathobiological Sciences, School of Veterinary Medicine,
Louisiana State University
and
The ISAAH 2002 Organizing Committee

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A Welcome from the Organizers

The Organizing Committee for the Fourth International Symposium on Aquatic Animal Health, ISAAH2002, is pleased to welcome you to New Orleans. We promise an informative and broad program, comprised of over 300 contributions, including an excellent lineup of plenary speakers each morning, 11 special sessions, 25 contributed sessions, and almost 100 posters. A wide range of topics are covered by premier scientists in each field, presenting the most recent data available. The high quality of the contributions made organizing the sessions a pleasure, and we thank all of the contributors for their effort.

We have also arranged some exciting social events to bring us all together to enjoy some of the flavor and culture of New Orleans. Starting on Sunday, re-establish old friendships and start some new ones as you listen to the music of Warren Clark's French Quarter Jazz Quintet at the Ice Breaker. On Tuesday, enjoy food and drink with the fish at the Aquarium of the Americas. The final act of the program, the banquet, features a terrific New Orleans buffet followed by Cajun dancing and dance instruction to the music of Les Freres Michot. As they say in Louisiana, Laissez le Bon Temps Roulez!

We sincerely thank each and every participant for their attendance at ISAAH2002. We hope your time in New Orleans is both entertaining and professionally rewarding.

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Business Center for Academic Societies Japan
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Tokyo 113, Japan

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National Marine Fisheries Service Laboratory
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http://www.shellfish.org/

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Intervet, Inc.

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United States Department of Interior US Geological Survey (USGS)

USGS Columbia Environmental Research Center

Louisiana Sea Grant College Program

Sea Grant LOUISIANA

This publication was produced by the Louisiana Sea Grant College Program, a part of the National Sea Grant College Program, maintained by NOAA and the U.S. Department of Commerce. Louisiana Sea Grant is also supported by the state of Louisiana.

Mission Statements from some of the symposium supporters:

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ATCC is a nonprofit biological resource center that distributes biological materials to scientists worldwide. We offer viruses, cell lines and hybridomas, bacteria, cloned genes and DNA libraries, filamentous fungi, yeasts, protozoa and algae, and cell culture media and reagents. We also provide services such as a patent depository, culture safe deposit, and mycoplasma detection for cell lines. We are pleased to be a part of this year's aquatic animal health symposium. We invite you to visit our Web site at www.atcc.org to browse our catalogs, where customers with ATCC accounts can order online. ATCC has been serving life scientists since 1925 with high-quality, well-characterized products and we welcome the deposit of useful materials. Contact us at news@atcc.org for information about depositing. http://www.atcc.org/

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The American Veterinary Medical Association's mission is to advance the science and art of veterinary medicine, including its relationship to public health, biological science, and agriculture. The AVMA provides a forum for the discussion and development of official positions on issues important to the veterinary profession. The AVMA is the authorized voice for the profession in presenting its views to government, academia, agriculture, pet owners, the media, and other concerned publics. The AVMA's Aquaculture and Seafood Advisory Committee, with input from, and to, private practitioners, scientists, educators, industry, the public, governmental agencies and organizations, develop solutions and programs dealing with aquatic animal health, medicine, and seafood safety that assist the profession provide optimal services to all. Currently, endemic and emerging diseases in cultured and wild aquatic animals, public health, environmental, educational and regulatory issues are of importance. http://www.avma.org/

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Aqua Health Ltd. was purchased by Novartis in 2000, combining the pioneering fish vaccine company's longstanding service and reputation with the resources and experience of the pharmaceutical giant. Novartis is committed, through Aqua Health, to world leadership in providing aquatic animal health solutions to both the rapidly expanding aquaculture industry, and the global wild stock enhancement efforts. To accomplish this, the company employs a dedicated staff of researchers and support staff, including: fish biologists, microbiologists, immunologists, chemists, veterinarians and fish farmers. It has research and production facilities in Canada, USA, Chile, and the UK, and engages in cooperative research programs with institutions around the world. With a focus on immunoprophylaxis, the company has a complete line of licensed fish vaccines, including an autogenous program to rapidly develop custom vaccines for particular situations. Several pharmaceuticals are also available, or under development, in several countries. For more information call toll free: 1-877-544-4966 or 902-566-4966.
http://www.novartis.com/

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Catfish Farmers of America (CFA), founded in 1968, is the nation's strongest aquaculture organization with membership from forty-one states. CFA is a trade organization that represents the interest of farm-raised catfish producers, suppliers, processors, and academia. With annual production of 600 million pounds, catfish accounts for over 50% of both volume and value of all U.S. aquaculture production. CFA's 1,700 members farm almost 90% of the total water acres within the industry. Major functions are legislative activity, research initiatives, and member services. The *Catfish Journal*, recognized as the industry leader for news and information, is CFA's official publication. http://www.catfishjournal.com/index.htm

COOPERATIVE AQUATIC ANIMAL HEALTH RESEARCH PROGRAM (CAAHRP)

The Cooperative Aquatic Animal Health Research Program (CAAHRP) is an interdisciplinary unit within the School of Veterinary Medicine, Louisiana State University. The mission of CAAHRP is to be a premier program for aquatic animal health research, education, and diagnostics. The program conducts cutting edge nationally and internationally recognized research, educates high quality graduate students, engages in public outreach, and provides state of the art diagnostic services. Faculty members participating in CAAHRP have active research programs concerned with finfish and shellfish pathogens. Research interests include vaccine development, understanding bacterial pathogenesis through the use of molecular techniques, development of state-of-the-art diagnostic procedures, and application of molecular techniques to understand the fish immune system. For more information about CAAHRP and the graduate and contract research opportunities available, please contact Dr. Ron Thune, Louisiana State University Department of Pathobiological Sciences, 3305 Veterinary Medicine Building, Baton Rouge, LA 70803; phone (225) 578-9684; fax (225) 578-9701; e-mail thune@vetmed.lsu.edu

INTERVET, INC.

Intervet International, the world's third largest Animal Health company, is dedicated to the R&D, production and marketing of efficacious and safe aquatic animal health (AAH) products. Intervet has three specialised AAH R&D centres, each located in a major aquaculture production area, plus support facilities at 55 globally-distributed subsidiary companies. In addition to developing key AAH pharmaceuticals such as CHORULON (hCG), Intervet has a range of vaccine combinations for injection, immersion and oral applications to various fish and shrimp species, including those targeted against *Vibrio anguillarum* 01, 02α and 02β, *V. salmonicida*, *V. viscosus*, *V. alginolyticus*, *V. harveyi*, *V. parahaemolyticus*, *V. vulnificus*, *Aeromonas salmonicida*, *Edwardsiella ictaluri*, IPN virus, salmon PD virus and ISA virus. With Intervet's R&D centres, vision and commitment to global aquaculture in place, the future looks bright. http://www.intervet.com/

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The Louisiana Aquaculture Association has been recognized through Joint Resolution of the Louisiana Legislature as the official voice of production aquaculture in this state. The LAA includes the membership of the Louisiana Catfish Farmers' Association, Louisiana Crawfish Farmers' Association, Louisiana Alligator Farmers' and Ranchers' Association, and a number of independent producers of other species. The purposes of LAA are to 1): influence public policy for the advancement of aquaculture, 2) influence the direction and scope of government-funded aquaculture research and extension, 3) promote the exchange of information among members, and 4) promote the sale and use of Louisiana aquaculture products.

LOUISIANA DEPARTMENT OF WILDLIFE AND FISHERIES
The mission of the Louisiana Department of Wildlife and Fisheries is to manage, conserve and promote wise utilization of Louisiana's renewable fish and wildlife resources and their supporting habitats through replenishment, protection, enhancement, research, development and education for the benefit of current and future generations; to provide opportunities for knowledge of and use and enjoyment of these resources; and to provide a safe environment for the users of these resources. http://www.wlf.state.la.us/

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Louisiana Sea Grant develops knowledge, technology, personnel, and public understanding to advance the sustainable and economically viable use of marine and coastal environments and resources. A federal-state partnership based at Louisiana State University, the program is part of a national network of 29 university-based programs committed to addressing a broad range of marine and coastal issues through research, advisory services, and education. A key element of Louisiana Sea Grant activities is the transfer of research-generated technology to the marketplace, thus enhancing existing businesses and creating new enterprises. Sea Grant-supported projects draw upon the expertise of the Louisiana university community in the natural sciences, engineering, sociology, economics, law, and public policy. Extensive cooperation with pertinent federal, state, business, and citizen groups assures that the program addresses current state, regional, and national priorities. Louisiana Sea Grant was established at Louisiana State University in 1968. Ten years later, its high quality and comprehensive scope won the university official designation as the nation's thirteenth Sea Grant College. In 1995, this designation was reaffirmed after a thorough and laudatory review conducted by the National Sea Grant College Program of the National Oceanic and Atmospheric Administration. http://www.laseagrant.org/

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The Society of Environmental Toxicology and Chemistry (SETAC) is an independent, nonprofit professional society that provides a forum for individuals and institutions engaged in the study of environmental issues, management and conservation of natural resources, environmental education, and environmental research and development. In the 1970's, no forum existed for interdisciplinary communication among environmental scientists—biologists, chemists, toxicologists—and others interested in environmental issues such as managers and engineers. The Society of Environmental Toxicology and Chemistry (SETAC) was founded in 1979 to fill the void. A unique strength of SETAC is its commitment to balance the interests of academia, business, and government. SETAC is concerned about global environmental issues. Its members are committed to good science worldwide, to timely and effective communication of research, and to interactions among professionals so that enhanced knowledge and increased personal exchanges occur. http://www.setac.org/

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Agriculture is a knowledge-based, global enterprise, sustained by the innovation of scientists and educators. The mission of CSREES is to advance knowledge for agriculture, the environment, human health and well being, and communities. Our primary functions are (1) program leadership to identify, develop, and manage programs to support university-based and other institutional research, education, and extension services; and (2) fair, effective, and efficient administration of Federal assistance implementing research, education, and extension awards and agreements. http://www.reeusda.gov/

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The Columbia Environmental Research Center (CERC) is a U.S. Geological Survey (USGS) research facility located in Columbia, Missouri. Clean water resources and habitat quality are essential for the health of all living organisms, a fundamental concept in the mission of CERC. The Center provides scientific information and data needed to address national and international environmental contaminant issues, and effects of habitat alterations on aquatic and terrestrial ecosystems. The Center has a unique capability for conducting multidisciplinary research that includes large river floodplains, coastal habitats, wetlands, streams, and lakes. Scientists at CERC form partnerships with national, state, and local agencies, nongovernmental organizations, and universities to enhance scientific information needed for management of the nation's resources.http://www.cerc.cr.usgs.gov/

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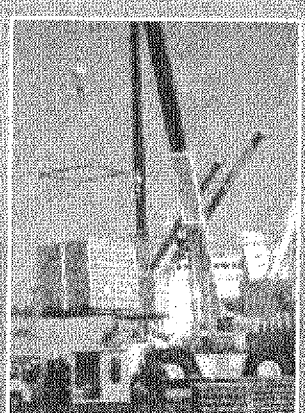
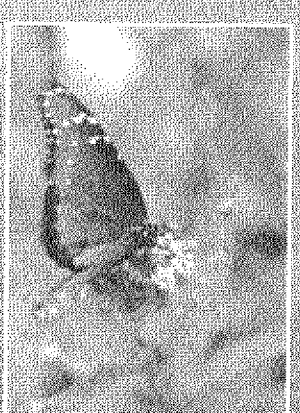
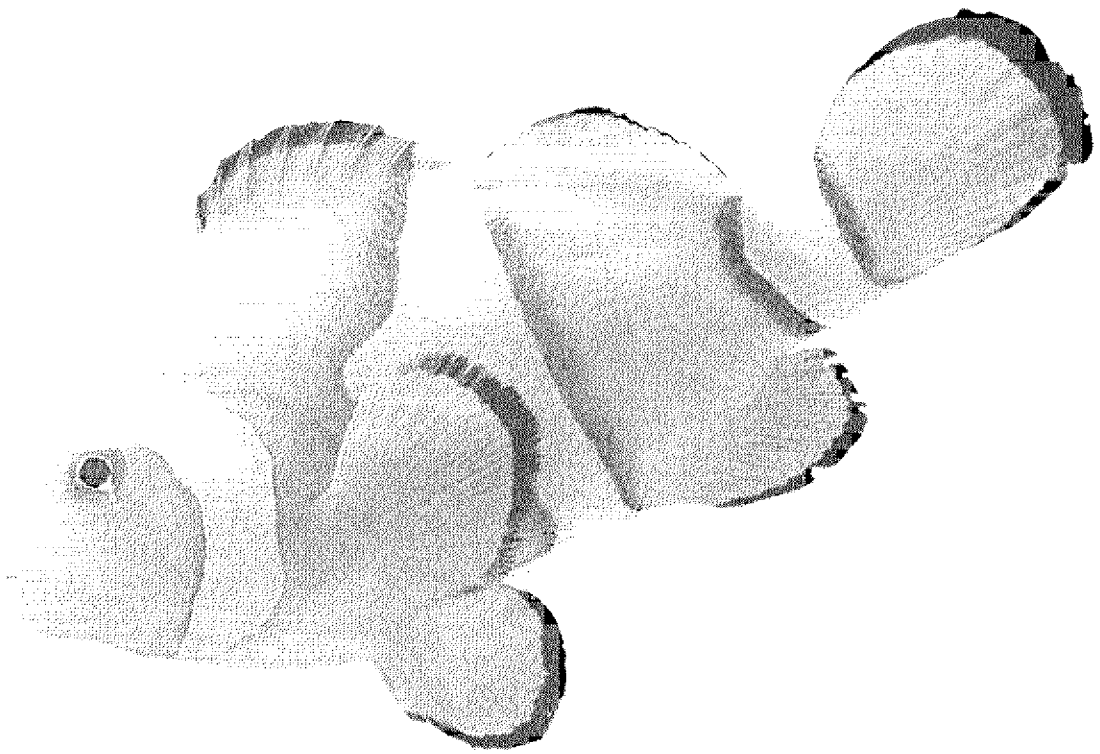
Safeguarding America's Agricultural and Natural Resources







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Floor Plans

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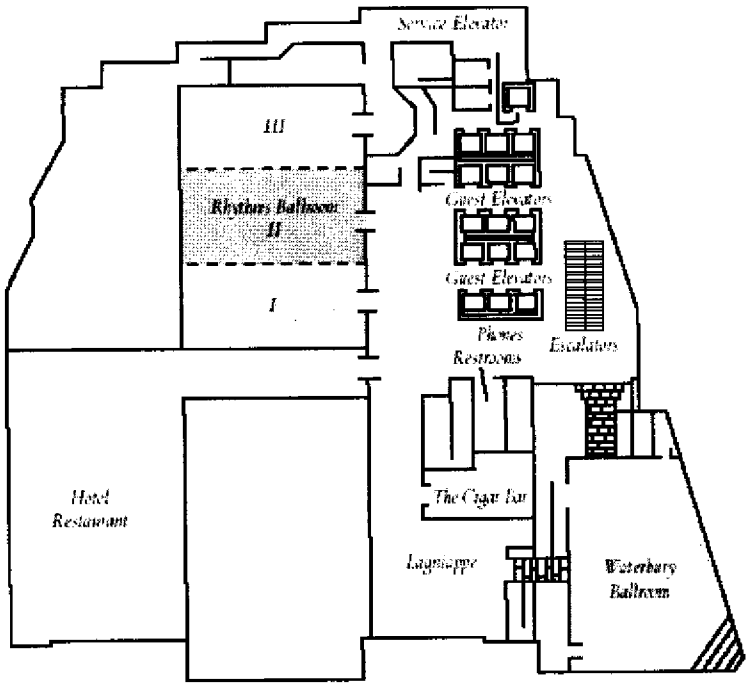

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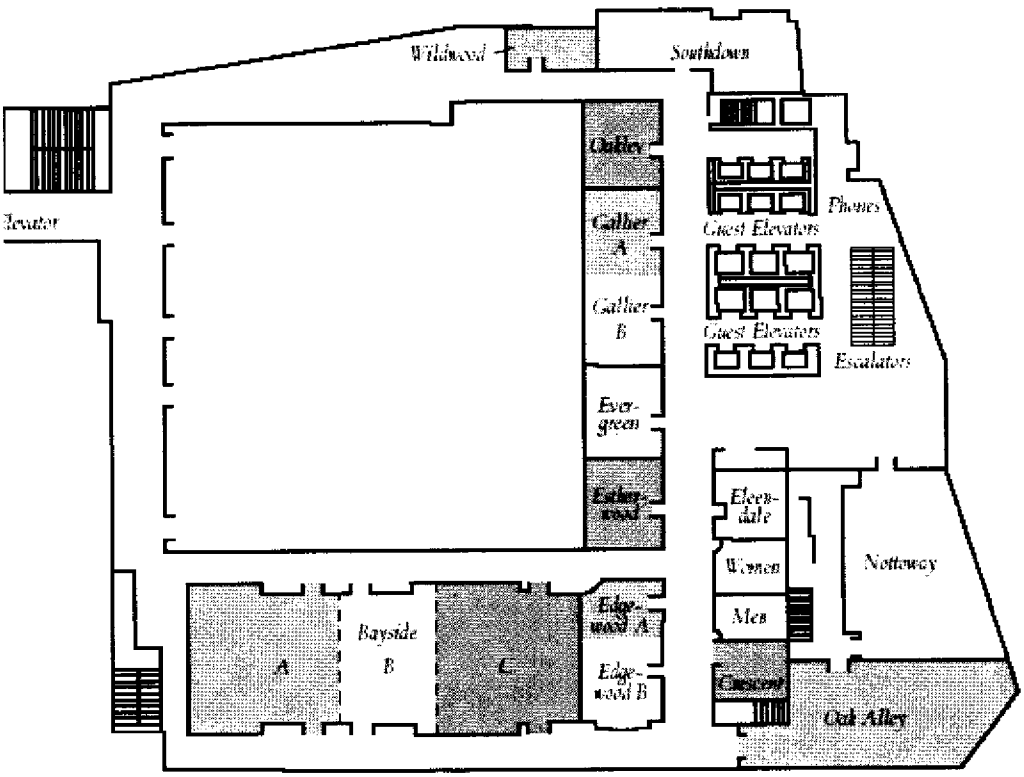

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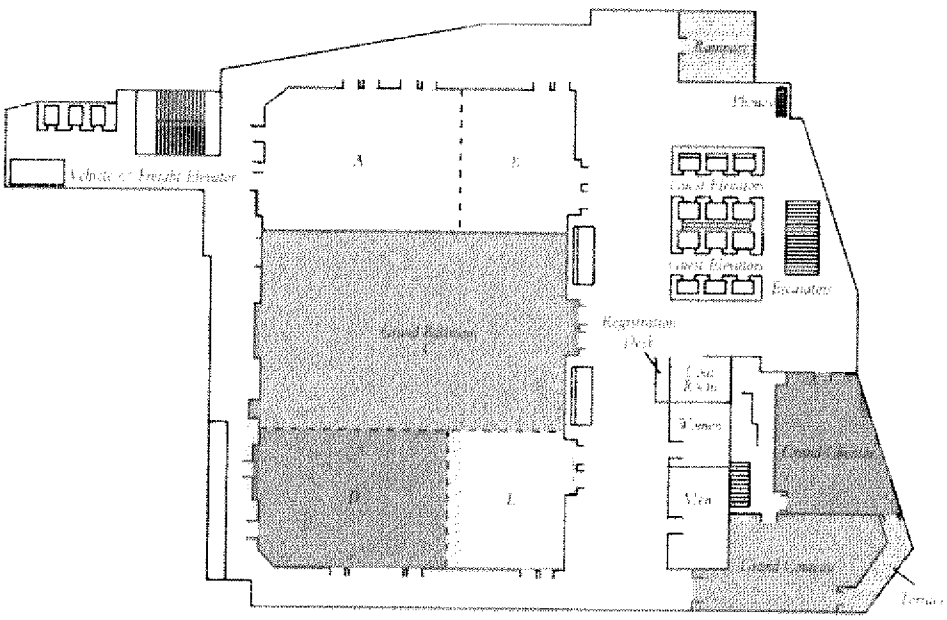

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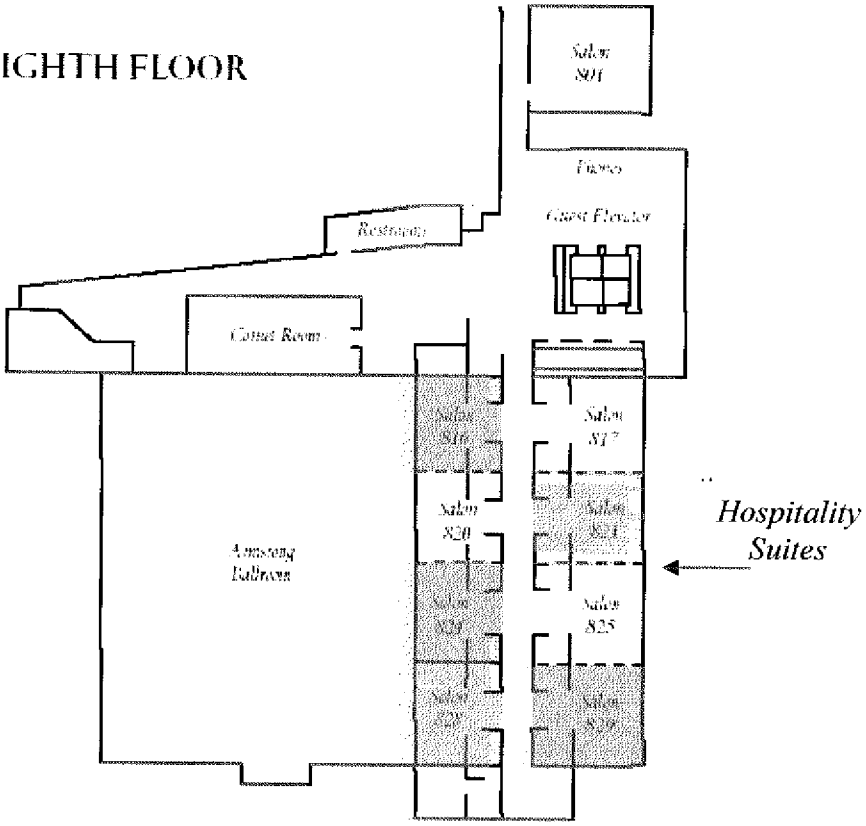

Scientific Sessions

Monday, September 2			
0830-0900	**The U.S. Fish and Wildlife Service's National Wild Fish Health Survey: an aquatic resources management tool** Thomas A. Bell p. 24		
0900-0930	**Environmental contaminant effects on endocrine systems of aquatic species** Timothy S. Gross		
0930-1000	**Global climate and large-scale influences on aquatic animal health** William S. Fisher		
1000-1030	Break		
	Grand Ballrooms A/B	***Grand Ballroom D***	***Grand Ballroom E***
1030-1200	Virology 1 p. 24	Environmental Health Special Session 1 p. 25	Treatments p. 27
1200-1330	Lunch		
1330-1500	Virology 2 p. 28	Environmental Health Special Session 2 p. 29	Quality Assurance Special Session 1 p. 30
1500-1530	Break		
1530-1730	Virology 3 p. 31	Histopathology Special Session p. 32	Quality Assurance Special Session 2 p. 33
Tuesday, September 3			
0830-0900	**Transmission of myxozoan pathogens: interactions between cultured and wild fish** Jerri L. Bartholomew p. 34		
0900-0930	**Introduction of non-indigenous shrimp viruses and their potential impact on farmed and native wild shrimp populations.** Donald V. Lightner		
0930-1000	**Overview of ISA management in Maine and Canada** Daniel D. MacPhee		
1000-1030	Break		
	Grand Ballrooms A/B	***Grand Ballroom D***	***Grand Ballroom E***
1030-1200	Parasitology 1 p. 34	Virology 4 p. 35	Mycobacteria Special Session p. 36
1200-1330	Lunch		
1330-1500	Parasitology 2 p. 38	Infectious Salmon Anemia Special Session 1 p. 39	Crustaceans 1 p. 40
1500-1530	Break		
1530-1700	Parasitology 3 p. 41	Infectious Salmon Anemia Special Session 2 p. 42	Crustaceans 2 p. 43

Wednesday, September 4			
0830-0900	**The application of molecular genetics to aquatic pathogen detection and systematics** Kimberly S. Reece p. 45		
0900-0930	**Molecular epidemiology of aquatic pathogens** Gael Kurath		
0930-1000	**Applied studies on epizootic ulcerative syndrome** Supranee Chinabut		
1000-1030	Break		
	Grand Ballrooms A/B	***Grand Ballroom D***	***Grand Ballroom E***
1030-1200	Bacteriology 1 p. 45	Parasitology 4 p. 46	*Aphanomyces* Special Session p. 47
1200-1330	Lunch		
1330-1500	Bacteriology 2 p. 48	Infectious Salmon Anemia Special Session 3 p. 49	Miscellaneous 1 p. 50
1500-1530	Break		
1530-17:00	Bacteriology 3 p. 51	Infectious Salmon Anemia Special Session 4 p. 52	Miscellaneous 2 p. 53
Thursday, September 5			
0830-0900	**Antimicrobial effectors in marine molluscs and crustaceans** Evelyne Bachère p. 55		
0900-0930	**Immune relevant genes in fish** Ikuo Hirono		
0930-1000	**Recent developments in our knowledge of fish immune responses** Norman W. Miller		
1000-1030	Break		
	Grand Ballrooms A/B	***Grand Ballroom D***	***Grand Ballroom E***
1030-1200	Immunology 1 p. 55	Bacteriology 4 p. 56	Toxicology 1 p. 57
1200-1330	Lunch		
1330-1500	Immunology 2 p. 58	Vaccine 1 p. 59	Molluscs 1 p. 61
1500-1530	Break		
1530-1700	Immunology 3 p. 62	Vaccine 2 p. 63	Molluscs 2 p. 64

Scientific Program
Monday, September 2, 2002

0815-0830 Welcome and Opening Remarks

0830-1000: Plenary Session 1 ***Grand Ballrooms A & B***
Chair: Andrew Kane

0830 The U.S. Fish and Wildlife Service's National Wild Fish Health Survey: an aquatic resources management tool
Thomas A. Bell, U.S. Fish and Wildlife Service, Division of the National Fish Hatchery System, 4401 North Fairfax Drive, Arlington, Virginia 22203 USA
thomas_a_bell@fws.gov

0900 Environmental contaminant effects on endocrine systems of aquatic species
Timothy S. Gross, USGS Biological Resources Division, Florida Caribbean Science Center and The College of Veterinary Medicine, Department of Physiological Sciences, University of Florida, 7920 NW 71st St. Gainesville, FL 32653 T_S_Gross@usgs.gov

0930 Global climate and large-scale influences on aquatic animal health
William S. Fisher, Director, Gulf of Mexico Aquatic Mortality Network, USEPA Office of Research and Development, NHEERL/Gulf Ecology Division, 1 Sabine Island Dr., Gulf Breeze, FL 32561 Fisher.William@epa.mail.epa.gov

1000-1030 Morning Break

Monday
1030-1200 Concurrent Oral Sessions 1, 2, and 3

1030 Session 1: Virology 1 ***Grand Ballrooms A & B***
Moderator: Mamoru Yoshimizu

1030 The prevalence of infectious salmon anemia virus in farmed Atlantic salmon in New Brunswick
Carol A. McClure[1]*, K. Larry Hammell[1], Ian R. Dohoo[1], Pascale Nerette[1], and Leighanne J. Hawkins[2]
[1]Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE C1A 4P3, Canada cmcclure@upei.ca
[2]Maritime Veterinary Services, St. George, NB E5C 3A5, Canada

1045 Genetic changes in infectious pancreatic necrosis virus due to cell culture adaptation
Øystein Evensen[1]*, Nina Santi[2], and Vikram N. Vakharia[3]
[1] Section for Aquatic Biology, Norwegian School of Veterinary Science, P.O.Box 8146 Dep., 0033 Oslo NORWAY oystein.evensen@veths.no
[2] Department of Pathology, National Veterinary Institute, P.O. Box 8156 Dep., 0033 Oslo, NORWAY nina.santi@vetinst.no
[3] Center for Agricultural Biotechnology, University of Maryland Biotechnology Institute and Virginia-Maryland Regional College of Veterinary Medicine, College Park, Maryland 20742, USA vakharia@umbi.umd.edu

1100 (Infectious?) Hemolytic anemia of salmon: an emerging disease occurring in seawater cultured coho salmon (*Oncorhynchus kisutch*) in Chile

Pedro Smith *, Julio Larenas, Jorge Contreras, Jorge Cassigoli, Claudia Venegas, María E. Rojas, Alvaro Guajardo, Oscar Troncoso, and Diana Macías
Faculty of Veterinary Sciences, University of Chile, Santiago, Chile. Casilla 2 Correo 15 Santiago, Chile psmith@uchile.cl

1115 Biological control of fish viral disease with anti-viral substances produced by bacteria

Mamoru Yoshimizu*, Yoshio Ezura, and Takahisa Kimura
Graduate School of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido 041-8511 Japan yosimizu@fish.hokudai.ac.jp

1130 Inhibition of rhabdoviral glycoprotein and nucleoprotein gene transcription by Japanese flounder Mx

Christopher Marlowe, A. Caipang*, Ikuo Hirono, and Takashi Aoki
Laboratory of Genetics and Biochemistry, Department of Aquatic Biosciences, Tokyo University of Fisheries, Konan 4-5-7 Minato, Tokyo 108-8477, Japan

1145 Host and geographic range extensions of the North American strain of viral hemorrhagic septicemia virus (VHSV)

R.P. Hedrick[1], S. Yun[1], W.N. Batts[2], G.S. Traxler[3], J. Kaufman[4], and J.R. Winton[2]
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[3]Pacific Biological Station, Department of Fisheries and Oceans, Nanaimo, British Columbia. V9T 1E1, Canada
[4]Oregon Department of Fish and Wildlife, Department of Microbiology, Oregon State University, Corvallis, Oregon 97331-3804 USA

1200-1330 Lunch on own

1030 Session 2: Environmental Health Special Session 1 ***Grand Ballroom D***

Moderator: Jill Jenkins

1030 Potential impacts of selected contaminants on endocrine biomarkers in carp and lake trout from sites in the Great Lakes

Stephen B. Smith[1]*, Jeffery C. Wolf[2], Clifford L. Rice[3], and Sergei Chernyak[4]
[1]Biological Resources, U.S. Geological Survey, MS 433 National Center, Reston, VA 20192: sbsmith@usgs.gov
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[3]Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705: ricec@ba.ars.usda.gov
[4]Great Lakes Science Center, U.S. Geological Survey, 4850 Green RD, Ann Arbor, MI 48150: sergei_chernyak@usgs.gov

1045 The influence of methyl mercury on reproductive biomarkers in aquatic species

Beverly S. Arnold[1]*, Timothy S. Gross[2], D. Shane Ruessler[3], and Nicola Kernaghan[4]
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[4]USGS Biological Resources Division, Caribbean Science Center and College of Veterinary Medicine, Department of Physiological Sciences, University of Florida, 7920 NW 71st St. Gainesville, FL 32653 nikki_kernaghan@usgs.gov

1100 Abnormal hermaphroditism in shovelnose sturgeon (*Scaphirhynchus platorynchus*) from the Missouri River

Diana M. Papoulias[1]*, Mark L. Wildhaber[1], Aaron J. Delonay[1], Mandy L. Annis[1], Steven Krentz[2], and Donald E. Tillitt[1]
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[2]U.S. Fish and Wildlife Service, 3425 Miriam Avenue, Bismarck, ND 58501 steven_krentz@fws.gov

1115 Environmental contaminants and developmental mortality in fish and alligators

María S. Sepúlveda[1,2]*, John J. Wiebe[1], D. Shane Ruessler[1], and Timothy S. Gross[1,2]
[1]USGS-BRD Florida Caribbean Science Center, 7920 NW 71st St., Gainesville, FL 32653 USA marisol_sepulveda@usgs.gov; jon_wiebe@usgs.gov; shane_ruessler@usgs.gov; tim_s_gross@usgs.gov
[2]Department of Physiological Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610 USA

1130 Effects of urban land-use on stonerollers in the Mobile River Basin

Deborah Cartwright[1,3]*, Vicki Blazer[2], Wade Bryant[3], Humberto Zappia[4], and Marsha Black[1]
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1145 Biomarkers from finfish in the Calcasieu Estuary, Louisiana, USA

Jaquelyn M. Craig[1] and Jill A. Jenkins[2]
[1]Johnson Controls, Inc. at USGS National Wetlands Research Center, 700 Cajundome Blvd., Lafayette, LA 70506 USA jaquelyn_matuszewski@usgs.gov
[2]U.S. Geological Survey, National Wetlands Research Center, 700 Cajundome Blvd., Lafayette, LA 70506 USA jill_jenkins@usgs.gov

1200-1330 Lunch on own

1030 Session 3: Treatments ***Grand Ballroom E***

Moderator: Stephen Smith

1030 Single-dose pharmacokinetics of florfenicol in koi (*Cyprinus carpio*) and gourami (*Trichogaster trichopterus*)

Roy P. E. Yanong[1]*, Eric W. Curtis[1], Venkatesh Atul Bhattaram[2], Mathangi Gopalakrishnan[2], Nahal Ketabi[2], Nelamangala V. Nagaraja[2], Hartmut Derendorf[2], and Robert D. Simmons[3]

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[3]Schering-Plough Animal Health, 1095 Morris Avenue, Union, NJ 07083 USA robert.simmons@spcorp.com

1045 Disinfection of water for aquaculture

Hisae Kasai*, Toyohiko Nishizawa, Takahisa Kimura, and Mamoru Yoshimizu

Graduate School of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido 041-8611 Japan, hisae@fish.hokudai.ac.jp

1100 Insights into the pathophysiology and treatment of amoebic gill disease in Atlantic salmon

Mark Powell[1*], James Harris[1], Melaine Leef[1], Shane Roberts[1], Michael Attard[1], Will Callahan[2], Hamish McWilliam[2], Tes Toop[2], and Barbara Nowak[1]

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[2]School of Biological and Chemical Science, Deakin University, Geelong, Victoria 3217 Australia. Ttoop@deakin.edu.au

1115 Pharmacokinetics of oxytetracycline in summer flounder, *Paralichthys dentatus*: a complete pharmacokinetic study and oral and intramuscular administration in compromised and healthy fish

Kathleen P. Hughes* and Stephen A. Smith

Aquatic Medicine Laboratory, Dept. of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA, 24061 USA. khughes@vt.edu, stsmith7@vt.edu

1130 Dose titration of oxytetracycline against *Streptococcus iniae* infection in blue tilapia

Ahmed M. Darwish*, Steven D. Rawles, and Bill R. Griffin

Harry K. Dupree-Stuttgart National Aquacutlure Research Center, United States Department of Agriculture, Agriculture Research Service, P. O. Box 1050, Stuttgart, Arkansas 72160 USA, Adarwish@spa.ars.usda.gov

1145 Protocol of use of a marine probiotic strain in aquaculture

Susana Prado, Alicia E. Toranzo, and Juan L. Barja*

Dpto. Microbiología y Parasitología, Facultad de Biología e Instituto de Acuicultura, Universidad de Santiago de Compostela, 15782, Spain mpactjlb@usc.es

1200-1330 Lunch on own

Monday
1330-1500 Concurrent Oral Sessions 4, 5, and 6

1330 Session 4: Virology 2 ***Grand Ballrooms A & B***
Moderator: Kimberly Reece

1330 Diagnostic hematological changes in rainbow trout infected with INHV
Scott E. LaPatra[1*], Gerald R. Jones[1], and Charlie E. Smith[2]
[1]Clear Springs Foods, Inc., Research Division, P.O. Box 712, Buhl, Idaho 83316 USA
scottl@clearsprings.com
[2]212 Story Hill, Bozeman, Montana 59715 USA fishdoc1@mci.net

1345 Differentiation of serologically related cyprinid rhabdoviruses by molecular genetic methods
Rose-Marie Le Deuff, David M. Stone, Keith. Way, Paul D. Martin, and Peter F. Dixon*
CEFAS Weymouth Laboratory, The Nothe, Weymouth, Dorset DT4 8UB, UK
p.f.dixon@cefas.co.uk

1400 A real-time nucleic acid sequence based amplification (NASBA) procedure for detection of fish nodaviruses
William G. Starkey*, Rose-Mary Millar, Mary E. Jenkins, Jacqueline H. Ireland, K. Fiona Muir, and Randolph H. Richards
Institute of Aquaculture, University of Stirling, Scotland, UK, FK9 4LA wgs1@stir.ac.uk

1415 Non-lethal detection of VHSV in fish blood by RT-PCR
Carmen López-Vázquez, José G. Olveira, Isabel Bandín, Inés Romero-Brey, Juan L. Barja*, and Carlos P. Dopazo
Dpt. Microbiología y Parasitología, Instituto de Acuicultura, Univ. Santiago de Compostela, Spain. mpdopazo@usc.es

1430 A comparison of white sturgeon herpesvirus types I and II and development of specific diagnostic PCR assays
K.B.Andree, S. Yun, T.S. McDowell, and R.P. Hedrick*
Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, 1 Shields Avenue, Davis, California 95616 U.S.A.

1445 An unusual DNA virus responsible for tumor development in damselfish
Michael C. Schmale*, Patrick D.L. Gibbs, Jeff VanWye, and Jennifer J. Rahn
Division of Marine Biology and Fisheries, University of Miami, Miami, FL 33149 USA
mschmale@rsmas.miami.edu

1500-1530 Afternoon Break

1330 Session 5: Environmental Health Special Session 2 ***Grand Ballroom D***

Moderator: Jill Jenkins

1330 Histopathologic and biochemical biomarker responses demonstrate improvement in flatfish health following remediation of a PAH-contaminated site in Eagle Harbor, in Puget Sound, WA

Mark S. Myers*, Bernadita F. Anulacion, Barb L. French, Cathy A. Laetz, William D. Reichert, Jon L. Buzitis, and Tracy K. Collier
Environmental Conservation Division, Northwest Fisheries Science Center, National Marine Fisheries Service/NOAA, 2725 Montlake Blvd. E., Seattle, WA 98112-2013
mark.s.myers@noaa.gov

1345 Use of transgenic medaka for assessing mutagenicity of chemical contaminants in aquatic systems

Richard N. Winn*, Stacy Muller, and Michelle Norris
Aquatic Biotechnology and Environmental Laboratory, University of Georgia, 2580 Devils Ford Road, Athens, Georgia 30605 USA rwinn@smokey.forestry.uga.edu

1400 Gene expression and proteomics: The application of microarray techniques and SELDI mass spectroscopy for the identification of biomarkers/bioindicators

William B. Schill[1]*, Vicki S. Blazer[1], Deborah L. Hodge[2], and Howard A. Young[2]
[1]US Geological Survey, Leetown Science Center, National Fish Health Research Laboratory, 11700 Leetown Road, Kearneysville, West Virginia 25430 USA bane_schill@usgs.gov; vicki_blazer@usgs.gov
[2]NCI-Frederick Building 560, NCI-FCRDC, Frederick, Maryland 21702-1201 USA hodgedl@ncifcrf.gov, youngh@ncifcrf.gov

1415 Male gamete quality assessments as bioindicators

Jill A. Jenkins
U.S. Geological Survey, National Wetlands Research Center, 700 Cajundome Boulevard, Lafayette, LA 70506 USA jill_jenkins@usgs.gov

1430 Modulation of white perch immune function: Investigations on fish health in selected tributaries of the Chesapeake Bay

Chris Ottinger[1]*, Vicki Blazer[1], Christine Densmore[1], Deborah Cartwright[1], Luke Iwanowicz[2], Elizabeth Frankenberry[1], and Larry Pieper[3]
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[2]Department of Environmental Sciences, Virginia Institute of Marine Science, 1208 Greate Rd., Gloucester Point VA 23062 USA luke_iwanowicz@usgs.gov
[3]Maryland Department of Natural Resources, Sarbanes Cooperative Oxford Laboratory, 904 South Morris Street, Oxford MD 21654 USA lpieper@dnr.state.md.us

1445 Cellular response of crustacean hemocytes to lipopolysaccharides

Washington Cardenas[1]* and Jill A. Jenkins[2]
[1]University of New York School of Medicine, Department of Medical and Molecular Parasitology, 341 East 25th Street, New York, New York 10010 USA. cardew01@med.nyu.edu
[2]U.S. Geological Survey, National Wetlands Research Center, Lafayette, Louisiana 70506 USA jill_jenkins@usgs.gov

1500-1530 Afternoon Break

1330-1500 Session 6: Quality Assurance in Fish Disease Diagnostics Special Session 1
Moderator: David Groman ***Grand Ballroom E***

Working toward a unified approach to fish diagnostic laboratory quality assurance

Why QA programs are needed in fish disease diagnostics
David Groman
Aquatic Diagnostic Services, Atlantic Veterinary College, Prince Edward Island, C1A 4P3, Canada
groman@upei.ca

Australian national QA program and adaptation to fish disease diagnostics in Australia
Iain East
Aquatic Animal Health, Agriculture, Fisheries and Forestry, Canberra, Australia Iain.East@affa.gov.au

Quality assurance and the National Aquatic Animal Health Program for Canada
Nellies Gagne
Fish Health Unit, Department of Fisheries & Oceans, Moncton, Canada. GagneNA@dfo-mpo.gc.ca

Current and future status of QA programs for fish disease diagnostic laboratories in the European Union
Trevor Hastings
Fisheries Research Services, Marine Laboratory, Aberdeen, Scotland T.Hastings@marlab.ac.uk

USDA-APHIS quality assurance and laboratory certification programs for diagnostic fish disease laboratories—present and future directions
Ann Wiegers
Quality Assurance Division, NVSL/CVB, APHIS, US Department of Agriculture, Ames, Iowa, USA
Ann.L.Wiegers@aphis.usda.gov

AFS/FHS & USFWS joint efforts in establishing QA/QC program for fish disease diagnostic laboratories
Patricia Barbash
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(Individual abstracts not available; see Groman, D.B., for summary abstract)

1500-1530 Afternoon Break

Monday
1530-1700 Concurrent Oral Sessions 7, 8, and 9

1530 Session 7: Virology 3 ***Grand Ballrooms A & B***
Moderator: Peter Dixon

1530 Comparison of fish-to-fish and waterborne transmission of channel catfish virus in a pond environment
David J. Thompson[1]*, Lester Khoo [2], David J. Wise[3], and Larry A. Hanson[1]
[1]Department of Basic Sciences, CVM, MSU, Mississippi State 39762
hanson@cvm.msstate.edu
[2]School of Veterinary Medicine, University of Pennsylvania, New Bolton Center, PA 19348.
lkhoo@vet.upenn.edu
[3]DREC, MSU, Stoneville, MS 38776. dwise@drec.msstate.edu

1545 Seroprevalence of largemouth bass iridovirus in Florida largemouth bass as determined by immunodiffusion
Woody Fraser[1], Michael J. Howarth[2], Bill Johnson[3], Wes Porak[3], Ruth Francis-Floyd[2], and Jack M. Gaskin[4]*
[1]Veterinary Diagnostic Laboratory, Florida Department of Agriculture and Consumer Services, Kissimmee, FL 34741 fraserw@doacs.state.fl.us
[2]Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610. rff@mail.vetmed.ufl.edu
[3]Eustis Fisheries Research Laboratory, Florida Fish and Wildlife Conservation Commission, Eustis, FL 32726. bill.johnson@fwc.state.fl.us; wes.porak@fwc.state.fl.us
[4]Department of Pathobiology, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610. gaskinj@mail.vetmed.ufl.edu

1600 Quantitative trait locus (QTL) associated with resistance to lymphocystis disease in Japanese flounder (*Paralichthys olivaceus*)
Kanako Fuji[1*],Kazunobu Kobayashi[1],Osamu Hasegawa[2], Akiyuki Ozaki[1], Takashi Sakamoto[1], and Nobuaki Okamoto[1]
[1]Department of Aquatic Biosciences, Tokyo University of Fisheries, Konan 4, Minato, Tokyo 108-8477, Japan nokamoto@tokyo-u-fish.ac.jp
[2]Kanagawa Prefectural Fisheries Research Institute, Misaki-cho, Miura, Kanagawa 238-0237, Japan hasega-o@f5.dion.ne.jp

1615 Effect of water temperatures on the pathogenesis of koi herpesvirus (KHV), and the development of TaqMan PCR and ELISA for KHV detection in previously exposed fish
O. Gilad[1] *, S.C. Yun[1], M.A. Adkison[1], G.D. Marty[1], C.M. Leutenegger[1], H. Bercovier[2], and R.P. Hedrick[1]
[1]University of California, Davis, CA, USA
[2]The Hebrew University, Ein Karem, Jerusalem, Israel

1630 Outbreaks of Koi herpesvirus in koi and common carp in Germany
Rudolf W. Hoffmann*, Hatem Soliman, and Mansour El-Matbouli
Institute for Zoology, Fish Biology and Fish Diseases, University of Munich, Kaulbachstrasse 37, 80539 Munich, Germany.

1645 The epidemiology and proposed prophylaxis of nodavirus related disease in commercially farmed Atlantic halibut (*Hippoglossus hippoglossus*) in Norway

Hogne Bleie[1]*, Audun H. Nerland[2], Ingunn Sommerset[2], Súsanna Húsgarð[2], Geir K. Totland[3], Sindre Grotmol[3], and Leif Berg[4]
[1]National Veterinary Institute Bergen, PO Box 1263, Sentrum, N-5811 Bergen, Norway hogne.bleie@vetinst.no
[2]Institute of Marine Research, PO Box 1870 Nordnes, N-5817 Bergen, Norway audun.nerland@imr.no
[3]University of Bergen, Department of Zoology, Allegaten 41, N-5007 Bergen, Norway geir.totland@zoo.uib.no
[4]Stolt Seafarm, Agapollen, N-5420 Rubbestadneset, Norway leif.berg@stoltseafarm.com

1530 Session 8: Histopathology Special Session ***Grand Ballroom D***

Moderator: John Fournie

1530 Proposed diagnostic criteria for proliferative thyroid lesions in bony fishes

John W. Fournie[1]*, William E. Hawkins[2], Marilyn J. Wolfe[3], and Jeffrey C. Wolf[3]
[1]U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, 1 Sabine Island Drive, Gulf Breeze, Florida 32561 USA fournie.john@epa.gov
[2]Department of Coastal Sciences, The University of Southern Mississippi, Gulf Coast Research Laboratory, P.O. Box 7000, Ocean Springs, Mississippi 39564 USA william.hawkins@usm.edu
[3]Experimental Pathology Laboratories, Inc., P.O. Box 474, Herndon, Virginia 20172 USA mwolfe@epl-inc.com; jwolfepl@aol.com

1545 Unusual findings in guppies (*Poecilia reticulata*) used in toxicological studies

Jeffrey C. Wolf* and Marilyn J. Wolfe
Experimental Pathology Laboratories, Inc. P.O. Box 474, Herndon, Virginia 20172 USA

1600 Unusual findings in medaka used as test animals in toxicologic studies

Marilyn J. Wolfe
Experimental Pathology Laboratories, Inc. P.O. Box 474, Herndon, Virginia 20172 USA
mwolfe@epl-inc.com

1615 Morphometric evaluation of hepatic lesions in killifish, *Fundulus heteroclitus*, exposed to polyaromatic hydrocarbons

Cynthia B. Stine[1], David Smith[2], Wolfgang K. Vogelbein[3], and Andrew S. Kane[1]*
[1]Aquatic Pathobiology Center, University of Maryland, Department of Veterinary Medicine. 8075 Greenmead Drive, College Park, Maryland 20742, USA
[2]Department of Epidemiology and Preventive Medicine, University of Maryland, 10 South Pine Street, Baltimore, MD 21201, USA
[3]Virginia Institute of Marine Sciences, Gloucester Point, Virginia, 23062, USA

1630 Prevalence, histopathology, and ultrastructure of vascular neoplasms in mummichogs (*Fundulus heteroclitus*) from a creosote-contaminated site

Wolfgang K. Vogelbein* and David E. Zwerner
Dept. of Environmental Sciences, Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA 23062 USA wolf@vims.edu, dez@vims.edu

1645 Diseases in zebrafish (*Danio rerio*) from research facilities—histological lesions

Michael L. Kent[1,2]*, Janell K. Bishop-Stewart[1,2], Jennifer L. Matthews[2], and Jan M. Spitsbergen[2,3]

[1]Center for Fish Disease Research, Department of Microbiology, 220 Nash, Oregon State University, Corvallis, Oregon 97331-3804

[2]Zebrafish International Resource Center, 5274 University of Oregon, Eugene, Oregon 97403-5274

[3]Department of Enivronmental and Molecular Toxicology, Marine/Freshwater Biomedical Center, Oregon State University, Corvallis Oregon 97331

1530-1715 Session 9: Quality Assurance in Fish Disease Diagnostics Special Session 2

Moderator: David Groman ***Grand Ballroom E***

Approaches to interlaboratory quality assurance ring testing and quality control protocols for fish diagnostic laboratories

Current status of laboratory certification and ring testing programs for fish disease diagnostic laboratories worldwide

David Groman

Aquatic Diagnostic Services, Atlantic Veterinary College, Charlottetown, Prince Edward Island, Canada. Groman@upei.ca

The Veterinary Laboratory Association QA Program—a model for voluntary interlaboratory ring testing of fish disease laboratories

Dennis Olexson

Diagnostic Services, Atlantic Veterinary College, Charlottetown, Prince Edward Island, C1A 4P3, Canada Olexson@upei.ca

The need for standardization of biologics and diagnostic reagents use in fish disease laboratories

Caroline O'Farrell

Molecular Biology Division, ATCC, Manassas, Virginia, USA CO'Farrell@atcc.org

Development of an NCCLS document for aquaculture

Tracy Dooley.

NCCLS, 940 West Valley Road, Suite 1400, Wayne, PA 19087 USA tdooley@nccls.org

Status report—establishing quality control ranges for disk diffusion susceptibility testing of aquatic bacterial pathogens

Ron Miller

US-FDA Center for Veterinary Medicine, Laurel, Maryland, 20708, USA RMiller@CVM.FDA.GOV

Discussion of a unified minimum standard for QA in fish disease diagnostic laboratories

Chaired by Alasdair McVicar

Ocean & Aquaculture Science Directorate, DFO, 200 Kent St., Station 12W114, Ottawa, K1A 0E6, Canada mcvicara@dfo-mpo.gc.ca

(Individual abstracts [except Miller, Ron] not available; see Groman, D.B., for summary abstract)

Tuesday, September 3, 2002

0830-1000 Plenary Session 2 ***Grand Ballrooms A & B***
Chair: Jim Winton

0830 Transmission of myxozoan pathogens: interactions between cultured and wild fish
Jerri L. Bartholomew, Center for Fish Disease Research, Department of Microbiology, Oregon State University, Nash Hall 220, Corvallis, Oregon 97331 USA
bartholj@orst.edu

0900 Introduction of non-indigenous shrimp viruses and their potential impact on farmed and native wild shrimp populations
Donald V. Lightner, Department of Veterinary Science and Microbiology, University of Arizona, Tucson, AZ 85721 USA dvl@u.arizona.edu

0930 Overview of ISA management in Maine and Canada
Daniel D. MacPhee, Maritime Veterinary Services Ltd., 246 Main Street, PO Box 1105, St. George, New Brunswick, E5C 3S9, Canada mvs@nbnet.nb.ca

1000-1030 Morning Break

Tuesday
1030-1200 Concurrent Oral Sessions 10, 11, and 12

1030 Session 10: Parasitology 1 ***Grand Ballrooms A & B***
Moderator: Jerri Bartholomew

1030 Implications for disease diagnoses of three myxosporean parasites of finfish by *in situ* hybridization
Dolores V. Baxa[1]*, Jonathan D. W. Moran[2] and Ronald P. Hedrick[1]
[1]School of Veterinary Medicine, Department of Medicine and Epidemiology, University of California, Davis, California 95616, USA dbantonio@ucdavis.edu, rphedrick@ucdavis.edu
[2]Microtek International (1998) Ltd., National Research Council of Canada, Institute for Marine Biosciences, 326-1411 Oxford Street Halifax, Nova Scotia, B3H 3Z1 Canada jmoran@microtek-intl.com

1045 Distribution of *Myxobolus cerebralis* in free-ranging fish populations in Idaho: associations with landscape variables
Christine M. Moffitt[1], Alf H. Haukenes[1], and Keith A. Johnson[2]
[1]Department of Fish and Wildlife Resources, University of Idaho, Moscow, ID 83844-1136, USA cmoffitt@uidaho.edu
[2]Eagle Fish Health Laboratory, Idaho Department of Fish and Game, 1800 Trout Road, Eagle, ID 83616, USA kjohnson@idfg.state.id.us

1100 Comparative susceptibility of different rainbow trout strains to infection with *Myxobolus cerebralis*, the causative agent of whirling disease
Mansour El-Matbouli[1]*, Miriam P. Küppers[1], Terry S. McDowell[2] and Ronald P. Hedrick[2]
[1]Institute for Zoology, Fish Biology and Fish Diseases, University of Munich, Kaulbachstrasse 37, 80539 Munich, Germany
[2]Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, California 95616 USA

1115 The characterization and quantification of proteolytic genes expressed by *Myxobolus cerebralis*

G. O. Kelley*, M. A. Adkison, C. Leutenegger, and R. P. Hedrick
Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, California 95616 USA gokelley@udcavis.edu

1130 Relationships among members of marine *Myxobolus* spp. from mullet based on small subunit ribosomal DNA sequences

Sihem Bahri[1], Karl B. Andree[2] and Ronald P. Hedrick[2]
[1]Faculté des Sciences de Tunis, Département de Biologie, Laboratoire de Microbiologie, Campus Universitaire 2092, TUNISIA Sihem.Bahri@fst.rnu.tn
[2]School of Veterinary Medicine, Department of Medicine and Epidemiology, University of California, Davis, California 95616, USA Kbandree@ucdavis.edu

1145 Susceptibility of *Myxobolus cerebralis*-infected rainbow trout fingerlings to infectious pancreatic necrosis virus

Christine L. Densmore*, Philip E. McAllister, Vicki S. Blazer, and Christopher A. Ottinger
U.S. Geological Survey, National Fish Health Research Laboratory, Leetown Science Center, 11700 Leetown Road, Kearneysville, WV 25430
christine_densmore@usgs.gov; phil_mcallister@usgs.gov; vicki_blazer@usgs.gov; chris_ottinger@usgs.gov

1200-1330 Lunch on own

1030 Session 11: Virology 4 ***Grand Ballroom D***

Moderator: Michel Bremont

1030 PCR development and screening of wild and captive-reared pallid (*Scaphirhynchus albus*) and shovelnose sturgeon (*S. platorynchus*) for the presence of iridovirus

Beth MacConnell[1]*, Kevin Kwak[2], Ronald P. Hedrick[2], and Molly Toner[1]
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[2]School Veterinary Medicine, Dept. Medicine & Epidemiology, UC Davis, Davis, CA 95616 USA ktkwak@ucdavis.edu; rphedrick@ucdavis.edu

1045 Biochemical and molecular characterization of a novel ranavirus isolated from diseased grouper, *Epinephelus* spp.

Qiwei Qin[1, 2]*, Chengyin Shi[1], Hui Shen[1] and Toong Jin Lam[1, 2]
[1]Tropical Marine Science Institute, The National University of Singapore, 10 Kent Ridge Crescent, Singapore 119260 tmsqinqw@nus.edu.sg
[2]Department of Biological Sciences, The National University of Singapore, 10 Kent Ridge Crescent, Singapore 119260

1100 **Infectious disease model development using zebrafish (*Danio rerio*) and a viral pathogen**

George E. Sanders* and James R. Winton
U.S. Geological Survey, Western Fisheries Research Center, 6505 N.E. 65th Street Seattle, Washington 98115 USA gsander@u.washington.edu

1115 **Essential role of the NV protein for the pathogenicity of *Novirhabdovirus* in rainbow trout**

Maria-Isabel Thoulouze, Monique Béarzotti, Stéphane Biacchesi, Edwige Bouguyon, Catherine Carpentier, and Michel Brémont*
Unité de Virologie et Immunologie moléculaires, Institut National de la Recherche Agronomique, 78352 Jouy-en-Josas CEDEX, France

1130 **Geographic variations among infectious hypodermal and hematopoetic necrosis virus (IHHNV) isolates and characteristics of their infection**

Kathy F.J. Tang, Bonnie T. Poulos*, Jun Wang, Rita M. Redman, and Donald V. Lightner
University of Arizona, Department of Veterinary Science and Microbiology, 1117 E. Lowell St., Tucson, Arizona 85721 USA

1145 **Induced resistance to white spot syndrome virus (WSSV) infection in *Penaeus stylirostris* through pre-infection with infectious hypodermal and hematopietic necrosis virus (IHHNV)**

Kathy F.J. Tang*, Stephanie V. Durand, Brenda L. White, Rita M. Redman, Leone L. Mohney, and Donald V. Lightner
University of Arizona, Department of Veterinary Science and Microbiology, 1117 E. Lowell St., Tucson, Arizona 85721 USA

1200-1330 **Lunch on own**

1030 **Session 12: Mycobacteria Special Session** ***Grand Ballroom E***

Moderator: Stephen Kaattari

1030 **Mycobacteriosis in wild striped bass (*Morone saxatilis*) of the Chesapeake Bay**

Ilsa Kaattari*, Martha Rhodes, Howard Kator, Wolfgang K. Vogelbein, and Steve Kaattari
Department of Environmental and Aquatic Animal Health, Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA 23062 imkaat@vims.edu

1045 **Experimental mycobacteriosis in striped bass (*Morone saxatilis*): microbiological results**

Martha Rhodes[1]*, Howard Kator[1], David T. Gauthier[1], Wolfgang K. Vogelbein[1], and Christopher A. Ottinger[2]
[1]Department of Environmental Sciences, Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA 23062 martha@vims.edu
[2]U.S. Geological Survey, Leetown Science Center, National Fish Health Research Laboratory, Kearneysville WV 25430

1100 Experimental mycobacteriosis in striped bass *(Morone saxatilis)*: histology

David T. Gauthier[1]*, Wolfgang K. Vogelbein[1], Martha Rhodes[1], Howard Kator[1], Stephen L. Kaattari[1], and Christopher A. Ottinger[2]
[1]Department of Environmental Sciences, Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA 23062 gauthier@vims.edu
[2]U.S. Geological Survey, Leetown Science Center, National Fish Health Research Laboratory, Kearneysville WV 25430 chris_ottinger@usgs.gov

1115 Mycobacteriosis in a collection of frogfish (*Antennarius striatus*): an atypical presentation

Roy P. E. Yanong[1]*, Eric W. Curtis[1], Scott P. Terrell[2], and Gail Case[3]
[1]Tropical Aquaculture Laboratory, Department of Fisheries and Aquatic Sciences, Institute of Food and Agricultural Sciences, University of Florida, 1408 24th St. SE, Ruskin, Florida 33570 USA rpy@mail.ifas.ufl.edu
[2]Walt Disney Animal Programs, Disney's Animal Kingdom, 1200 N. Savannah Circle, Bay Lake, Florida 32830 USA Scott.P.Terrell.-ND@disney.com
[3]Aquarium, Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, Florida 34236 gcase@mote.org

1130 Clinical presentations of *Mycobacterium* sp. in cultured summer flounder (*Paralichthys dentatus*)

Kathleen P. Hughes* and Stephen A. Smith
Aquatic Medicine Laboratory, Dept. of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA, 24061 USA. khughes@vt.edu, stsmith7@vt.edu

1145 Immunomodulatory effects and efficacy of a DNA vaccine for aquatic mycobacteriosis

David J. Pasnik* and Stephen A. Smith
Aquatic Medicine Laboratory, Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061 USA. dpasnik@vt.edu, stsmith7@vt.edu

1200-1330 Lunch on own

Tuesday
1330-1500 Concurrent Oral Sessions 13, 14, and 15

1330 Session 13: Parasitology 2 ***Grand Ballrooms A &B***
Moderator: Michael Kent

1330 Development of *Myxidium* spp. (Myxozoa) in the intestine of tiger puffer experimentally fed with infected gut tissue
Hiroshi Yokoyama[1]*, Tetsuya Yanagida[1], Hiroshi Nasu[2], Mamoru Sameshima[2], and Kazuo Ogawa[1]
[1]Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi 1-1-1, Bunkyo, Tokyo 113-8657, Japan
ayokoh@mail.ecc.u-tokyo.ac.jp
[2]Kumamoto Prefectural Fisheries Experimental Center, Ooyano 2450-2, Amakusa, Kumamoto 869-3603, Japan

1345 Investigations in the life cycle of *Enteromyxum* spp. (Myxozoa). A two-host cycle?
Oswaldo Palenzuela*, María J. Redondo, and Pilar Alvarez-Pellitero
Institute of Aquaculture "Torre la Sal" (CSIC). Ribera de Cabanes, Castellón ES12595 Spain
oswaldo@iats.csic.es

1400 *Parvicapsula minibicornis*: transmission and impact on anadromous salmonids of the Fraser River, British Columbia
S.R.M. Jones[1]*, G. Prosperi Porta[1], S.C. Dawe[1], and D.P. Barnes[2]
[1]Fisheries and Oceans Canada, Pacific Biological Station, 3190 Hammond Bay Road, Nanaimo, British Columbia, V9T 6N7, Canada jonesss@pac.dfo-mpo.gc.ca
[2]Fisheries and Oceans Canada, Cultus Lake Laboratory Cultus Lake, British Columbia, V2R 5B6, Canada

1415 Investigations into the distribution and transmission of *Kudoa thyrsites* to Atlantic salmon in British Columbia
S.R.M. Jones*, G. Prosperi Porta, and S.C. Dawe
Fisheries and Oceans Canada, Pacific Biological Station, 3190 Hammond Bay Road, Nanaimo, British Columbia, V9T 6N7, Canada jonesss@pac.dfo-mpo.gc.ca

1430 Patterns in pathological changes associated with *Kudoa* spp. from the pericardium of fishes in the northern Gulf of Mexico
Reginald B. Blaylock[1]*, Stephen A. Bullard[1], and Chris Whipps[2]
[1]Gulf Coast Research Laboratory, College of Marine Sciences, The University of Southern Mississippi, P. O. Box 7000, Ocean Springs, MS 39566-7000 reg.blaylock@usm.edu
[2]Department of Microbiology, Oregon State University, Corvallis, OR 97331-3804
whippsc@ucs.orst.edu

1445 Mode of transmission of *Glugea plecoglossi* (Microspora) in the experimental infection model using rainbow trout
Sun-Joung Lee*, Hiroshi Yokoyama, and Kazuo Ogawa
Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi 1-1-1, Bunkyo, Tokyo 113-8657, Japan
aa07086@mail.ecc.u-tokyo.ac.jp

1500-1530 Afternoon Break

1330 Session 14 Infectious Salmon Anemia (ISA) 1 (USDA-APHIS Special Session)
Introduction ***Grand Ballroom D***
Moderator: Otis Miller

1330 Welcome, scope, and aims of the session
TBA

1345 Role and function of the OIE Fish Diseases Commission in the field of aquatic animal health
Tore Håstein
National Veterinary Institute, Oslo, Norway tore.hastein@vetinst.no

Applied Research Response
Moderator: TBA

1400 Comparisons of various ISA viral detection assays
Peter Merrill
MicroTechnologies, Inc., 41 Main Street, Richmond, ME 04357, USA wetvet@gwi.net

1415 The development of vaccines/diagnostics/new technologies in Canada in regards to ISAV
Frederick S.B. Kibenge
Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, P.E.I., Canada kibenge@Upei.CA

1430 Epidemiology of ISA in Maine
Stephen K. Ellis[1] and Laura L. Gustafson[2]*
[1]USDA Animal and Plant Health Inspection Service, Veterinary Services, Marine Trades Center-WCTC, 16 Deep Cove Road, Eastport, ME 04631 USA, stephen.k.ellis@usda.gov
[2]Environmental Medicine Consortium, College of Veterinary Medicine, North Carolina State University, Raleigh, NC; USA laura_gustafson@ncsu.edu

1445 The epidemiology of ISA in Scotland
Alexander G. Murray
FRS Marine Laboratory, PO Box 101, Victoria Road, Aberdeen AB11 9DB, UK
murrays@marlab.ac.uk

1500-1530 Afternoon Break

1330 Session 15: Crustaceans 1 ***Grand Ballroom E***

Moderator: Jeff Lotz

1330 Histological characteristics of abnormalities and diseases in crustacean zooplankton from the Great Lakes region

Gretchen A. Messick[1]*, Thomas F. Nalepa[2], Henry A. Vanderploeg[2], Suzanne S. Tyler[1], and Joann F. Cavaletto[2]

[1]NOAA, Cooperative Oxford Laboratory, 904 S. Morris Street, Oxford, Maryland 21654 USA Gretchen.Messick@noaa.gov

[2]NOAA, Great Lakes Environmental Research Laboratory, 205 Commonwealth Boulevard, Ann Arbor, Michigan 48105 USA Henry.A.Vanderploeg@noaa.gov

1345 A national survey to demonstrate freedom from white spot virus and yellow head virus in Australian crustaceans

Iain J. East[1]*, Peter F. Black[2], Vanessa Findlay[3], and Eva-Maria Bernoth[1]

[1]Aquatic Animal Health, Office of the Chief Veterinary Officer Agriculture, Fisheries and Forestry—Australia GPO Box 858, CANBERRA ACT 2601 iain.east@affa.gov.au

[2]Animal Health Sciences, Office of the Chief Veterinary Officer Agriculture, Fisheries and Forestry—Australia GPO Box 858, CANBERRA ACT 2601 peter.black@affa.gov.au

[3]Biosecurity Australia, Agriculture, Fisheries and Forestry—Australia GPO Box 858, CANBERRA ACT 2601 vanessa.findlay@affa.gov.au

1400 Modeling shrimp viral epidemics

Jeffrey M. Lotz

Department of Coastal Sciences, University of Southern Mississippi, Gulf Coast Research Laboratory, P.O. Box 7000, Ocean Springs, Mississippi, 39566-7000 USA jeff.lotz@usm.edu.

1415 Pathogenicity of white spot syndrome virus to the ornamental shrimp, *Lysmata wurdemanni*

Susan E. Laramore

Aquatic Animal Health Laboratory, Harbor Branch Oceanographic Institution, Inc., 5600 U.S. Hwy. 1 North, Fort Pierce, Florida 34946 USA SAllen@hboi.edu

1430 Effects of temperature on WSSV and TSV in *Litopenaeus vannamei* in controlled experiments

Robin M. Overstreet* and Tershara Matthews

Department of Coastal Sciences, The University of Southern Mississippi, PO Box 7000, Ocean Springs, Mississippi 39566 USA robin.overstreet@usm.edu

1445 The low molecular weight gill proteome of WSSV-challenged *Litopenaeus vannamei*

Ryan B. Carnegie[1]*, Severine A. Patat[2], and Kevin L. Schey[2]

[1]Program in Marine Biomedicine and Environmental Sciences, Medical University of South Carolina, 171 Ashley Avenue, Charleston, South Carolina 29425 USA carnegie@musc.edu

[2]Department of Pharmacology, Medical University of South Carolina, 171 Ashley Avenue, Charleston, South Carolina 29425 USA patatsa@musc.edu, schevkl@musc.edu

1500-1530 Afternoon Break

Tuesday
1530-1700 Concurrent Oral Sessions 16, 17, and 18

1530 Session 16: Parasitology 3 ***Grand Ballrooms A & B***
Moderator: Margaret Ewing

1530 Comparison of culture conditions on development, survival and growth of *Ichthyophthirius multifiliis in vitro*
D.A. Pugovkin*, H.E. Segner, and T. Wahli
Centre for Fish & Wildlife Health Institute of Animal Pathology Laengassstrasse 122 Berne 3012 Switzerland dmitri.pugovkin@itpa.unibe.ch

1545 Pathology associated with a *Tetrahymena*-like organism in southern flounder, *Paralichthys lethostigma*
Stephen A. Smith* and Kathleen P. Hughes
Aquatic Medicine Laboratory, Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA, 24061 USA stsmith7@vt.edu, khughes@vt.edu

1600 Amoebic gill disease in cultured salmonids in Australia
Barbara F. Nowak*, Mark D. Powell, Marianne Douglas-Helders, Mark Adams, Phil Crosbie, and Jeremy Carson
Tasmanian Aquaculture and Fisheries Institute, Aquafin CRC, University of Tasmania, Locked Bag 1-370 Launceston, Tasmania 7250 Australia B.Nowak@utas.edu.au

1615 Pathogenic diplomonad flagellates from fish: recent advances in species recognition, epizootiology, and pathology
Sarah L. Poynton[1,2]*, John Jenkins[3], Mohammad R. Saghari Fard[2,4], and Erik Sterud [5]
[1]Department of Comparative Medicine, Johns Hopkins University School of Medicine, Room 1-127 Jefferson, Johns Hopkins Hospital, 600 North Wolfe Street, Baltimore, MD 21287, USA, spoynton@jhmi.edu
[2]Department of Inland Fisheries, Institute for Freshwater Ecology and Inland Fisheries, Müggelseedamm 310, Berlin, D 12587, Germany, spoynton@igb-berlin.de
[3]Department of Veterinary Pathology, Armed Forces Institute of Pathology, 14th Street and Alaska NW, Washington, DC 20306, USA, jenkinsj@afip.osd.mil
[4]M.Sc. Program in International Agricultural Sciences, Humboldt University, Berlin, Germany, fardreza@yahoo.com
[5]National Veterinary Institute, P.O. Box 8156 Dep, N-0033 Oslo, Norway
erik.sterud@vetinst.no

1630 Observations on the life stages of *Sphaerothecum destruens* gen. and sp. nov., a mesomycetozoean fish pathogen formerly referred to as the rosette agent
Kristen D. Arkush[1]*, Leonel Mendoza[2], Mark A. Adkison[3], and Ronald P. Hedrick[3]
[1]Bodega Marine Laboratory, University of California, P.O. Box 247, Bodega Bay, California 94923 USA kdarkush@ucdavis.edu
[2]Medical Technology Program, Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, Michigan 48824-1030 USA mendoza9@pilot.msu.edu
[3]Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California at Davis, Davis, California 95616 USA maadkison@ucdavis.edu; rphedrick@ucdavis.edu

1645 **Genetic evidence for more than one species of costia (*Ichthyobodo necator*)**
Heather A. Callahan[1]*, R. Wayne Litaker[2], and Edward J. Noga[1]
[1]Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, North Carolina 27606 USA
hacallah@unity.ncsu.edu, ed_noga@ncsu.edu
[2]NOAA, 101 Pivers Island Road, Beaufort, North Carolina 28516 USA
wayne_litaker@med.unc.edu

1530 **Session 17: Infectious Salmon Anemia (ISA) 2 (USDA-APHIS Special Session)**
Grand Ballroom D

Diagnostic and Laboratory Response
Moderator: TBA

1530 **The ASK cell line: an improved diagnostic tool for isolation, propagation, and titration of the infectious salmon anemia virus (ISAV)**
Jill B. Rolland[1]*, Deborah A. Bouchard[2], and James R. Winton[1]
[1] Western Fisheries Research Center, Biological Resources Discipline, United States Geological Survey, 6505 NE 65th St., Seattle, Washington 98115 USA Jill_Rolland@usgs.gov
[2] Micro Technologies, Inc., 41 Main St., Richmond, Maine 04357 USA microtech@wiscasset.net

1545 **Evaluation of infectious salmon anemia diagnostic tests**
Carol A. McClure[1]*, K. Larry Hammell[1], Ian R. Dohoo[1], Henrik Stryhn[1], and Leighanne J. Hawkins[2]
[1]Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE C1A 4P3, Canada cmcclure@upei.ca
[2]Maritime Veterinary Services, St. George, NB E5C 3A5, Canada

1600 **Development of a strain typing assay for ISAV**
Marcia Cook*, Sherry Vincent, and Steve Griffiths
Research and Productivity Council (RPC), 921 College Hill Rd., Fredericton, N.B., Canada, E3B 6Z9; mcook@rpc.unb.ca

1615 **The genetics of ISAV**
Carey O. Cunningham
FRS Marine Laboratory, PO Box 101, 375 Victoria Road, Aberdeen AB11 9DB, UK;
c.cunningham@marlab.ac.uk

Management Response
Moderator: TBA

1630 **ISAV in Norway and the Faroe Islands**
Cato Lyngøy
Quality Assurance Director, Pan Fish ASA, St. Olavs plass 1 N-6002 Ålesund, Norway
Cato.lyngoy@panfish.no

1645 **The eradication of an epidemic of ISA in Scotland**
Ronald M. Stagg
FRS Marine Laboratory, PO Box 101, Victoria Road, Aberdeen AB11 9DB, UK
staggr@marlab.ac.uk

1700 Practical grower experiences in the proactive prevention and control of infectious salmon anemia

Sebastian Belle
Maine Aquaculture Association. Box 148, Hallowell. Me. USA 04347 Futureseas@aol.com

1530 Session 18: Crustaceans 2 ***Grand Ballroom E***

Moderator: Robin Overstreet

1530 Characterizing immune response to viral challenge in litopenaeid shrimp using functional genomics approaches

Paul Gross[1]*, Javier Robalino[1], Brandon Cuthbertson[1], Thomas Bartlett[1], Eleanor Shepard[2], Robert Chapman[2], Gregory Warr[1], and Craig Browdy[2]
[1]Marine Biomedicine and Environmental Sciences Program, Medical University of South Carolina, Charleston, SC 29425, USA, grossp@musc.edu
[2]Marine Resources Research Institute, South Carolina Department of Natural Resources, Charleston, SC 29412, USA

1545 Effect of extracellular products from a *Vibrio* isolate on the haemocytes of the edible crab, *Cancer pagurus*

Carolina Costa-Ramos* and Andrew F. Rowley
School of Biological Sciences, University of Wales Swansea, Singleton Park, Swansea, SA2 8PP, U.K. a.f.rowley@swansea.ac.uk

1600 Experimental infection of necrotizing hepatopancreatitis (NHP) in *Litopenaeus vannamei* by injection and ingestion

Amanda G. Vincent*, Verlee M. Breland, and Jeffrey M. Lotz
College of Marine Sciences, University of Southern Mississippi, Gulf Coast Research Laboratory, P.O. Box 7000, Ocean Springs, Mississippi 39566-7000, USA amanda.vincent@usm.edu, verlee.breland@usm.edu, jeff.lotz@usm.edu

1615 Chitinolytic activities of bacteria associated with shell disease syndrome in the edible crab, *Cancer pagurus*

Claire L. Vogan* and Andrew F. Rowley
School of Biological Sciences, University of Wales Swansea, Singleton Park, Swansea, UK SA2 8PP, c.l.vogan@swansea.ac.uk

1630 Diagnosis of *Hematodinium* infection in the Norway lobster *Nephrops norvegicus* by ELISA, PCR, dot blot, and *in situ* hybridization

Hamish J. Small[1]*, Douglas M. Neil[1], and Graham H. Coombs[2]
[1]Division of Environmental and Evolutionary Biology, Graham Kerr Building, University of Glasgow, Glasgow, G12 8QQ, Scotland, United Kingdom D.Neil@bio.gla.ac.uk
[2]Division of Infection & Immunity, Joseph Black Building, University of Glasgow, Glasgow, G12 8QQ, Scotland, United Kingdom g.coombs@bio.gla.ac.uk

1645 **Aspects of the pathology of *Hematodinium* sp. infections in snow crabs (*Chionoecetes opilio*) from Newfoundland, Canada**

Jeffrey D. Shields[1]* and David M. Taylor[2]

[1]Virginia Institute of Marine Science, The College of William and Mary, School of Marine Science, Gloucester Point, Virginia 23062, USA.

[2]Department of Fisheries and Oceans, St. John s, Newfoundland A1C 5X1 CANADA

Wednesday, September 4, 2002

0830-1000 Plenary Session 3 ***Grand Ballrooms A & B***

Chair: Ron Hedrick

0830 The application of molecular genetics to aquatic pathogen detection and systematics
Kimberly S. Reece, School of Marine Science, Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA 23062 kreece@vims.edu

0900 Molecular epidemiology of aquatic pathogens
Gael Kurath, USGS Western Fisheries Research Center 6505 NE 65th St., Seattle, WA 98115 USA gael_kurath@usgs.gov

0930 Applied studies on epizootic ulcerative syndrome
Supranee Chinabut, Aquatic Animal health Research Institute, Department of Fisheries, Kasetsart University Campus, Jatujak, Bangkok 10900, Thailand
Supranee@fisheries.go.th

1000-1030 Morning Break

Wednesday
1030-1200 Concurrent Oral Sessions 19, 20, and 21

1030 Session 19: Bacteriology 1 ***Grand Ballrooms A & B***

Moderator: Rocco Cipriano

1030 Susceptibility of passively immunized rainbow trout and challenge survivors to *Flavobacterium psychrophilum*
S.E. LaPatra[1]*, B.R. LaFrentz[2], G.R. Jones[1], A.W. Morton[1], M. Higgins[1], and K.D. Cain[2]
[1]Clear Springs Foods, Inc., Research Division, P.O. Box 712, Buhl, ID 83316 USA
scottl@clearsprings.com
[2]Department of Fish and Wildlife Resources, University of Idaho, Moscow ID 83844-1136 USA
kcain@uidaho.edu

1045 *Flavobacterium psychrophilum* infection of Atlantic salmon eggs among from the northeastern United States
Rocco C. Cipriano*
U.S. Geological Survey, National Fish Health Research Laboratory, 11700 Leetown Road, Kearneysville, WV 25430 USA rocco_cipriano@usgs.gov

1100 Starvation of *Flavobacterium psychrophilum* in stream water, broth and distilled water
Ioannis N. Vatsos, Kim D. Thompson*, and Alexandra Adams
Institute of Aquaculture, Stirling University, Stirling, FK9 4LA, UK. kdt1@stir.ac.uk

1115 Presence of *Flavobacterium psychrophilum* on eggs of rainbow trout (*Oncorhynchus mykiss*)
Inger Dalsgaard* and Lone Madsen
Danish Institute for Fisheries Research, Fish Disease Laboratory, Stigbøjlen 4, DK-1870 Frederiksberg C, Denmark, id@dfu.min.dk

1130 Pathogenicity of morphologically and genetically characterized *Flavobacterium columnare* strains in Channel catfish

Swapna Thomas* and Andrew. E. Goodwin
Aquaculture/Fisheries Center, University of Arkansas at Pine Bluff,1200 N University Drive, Pine Bluff, AR 71601, USA sthomas@uaex.edu, agoodwin@uaex.edu

1145 Fish models for signature-tagged mutagenesis identification of virulence-associated genes in *Yersinia ruckeri*

Alicia Gallaccio[1], Wook Kim[2], Margaret Thorburn[3], and Roselynn Stevenson[1]*
[1]Department of Microbiology, University of Guelph, Guelph, Ontario N1G 2W1, Canada agallacc@uoguelph.ca; rstevens@uoguelph.ca
[2]Department of Microbiology and Infectious Diseases, University of Calgary, Calgary, Alberta, Canada T2N 4N1 wkim@ucalgary.ca
[3]Department of Population Medicine, Ontario Veterinary College, University of Guelph Guelph, Ontario, Canada N1G 2W1 mthornur@ovc.uoguelph.ca

1200-1330 Lunch on own

1030 Session 20: Parasitology 4 ***Grand Ballroom D***

Moderator: Linda Pote

1030 The population dynamics of *Planorbella trivolvis*, the intermediate snail host in the *Bolbophorus* spp. life cycle

Barbara A George[1]* and Linda M. Pote[2]
[1]Mississippi State University, College of Veterinary Medicine, Department of Basic Science, Mississippi State, Mississippi 39762 USA wer4animals@aol.com
[2]Mississippi State University, College of Veterinary Medicine, Department of Basic Science, Mississippi State, Mississippi 39762 USA lpote@cvm.msstate.edu

1045 The preference of mollusk-eating fish for three aquatic snails that vector fish trematodes

Andrew J. Mitchell
Harry K. Dupree Stuttgart National Aquaculture Research Center, P. O. Box 1050, 2955 Hwy. 130 East, Stuttgart, Arkansas 72160 USA

1100 Pathological effects of blood flukes (Sanguinicolidae) on marine fishes relative to aquaculture

Stephen A. Bullard* and Robin M. Overstreet
College of Marine Science, The University of Southern Mississippi, P.O. Box 7000, Ocean Springs, MS 39566-7000 USA

1115 The function of antibodies in the digestive tract of sea lice *Lepeophtheirus salmonis*

Ian R. Bricknell[1]* Rob S. Raynard[1], Katy Adamson[1], Paul Cook[1], and James E. Bron[2]
[1]FRS Marine Laboratory, Aquaculture & Aquatic Animals health 375 Victoria Road, Torry Aberdeen AB11 9DB, UK
[2]Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, UK

1130 Ecology and faunistic characteristic of sturgeon parasites from the Azov sea basin

A.V. Kazarnikova* and H.V. Shestakovskaya
RosrubNIIproject Ichthyopathological Lab., Oborona st., 49, Rostov-on-Don, Russia, 3440008, kazarnikova@aaanet.ru

1145 Parasitic diseases of North Pacific wild fish and shellfish: Do life history patterns of host mortalities exist?

J. Frank Morado
National Oceanic & Atmospheric Administration, National Marine Fisheries Service, Alaska Fisheries Science Center, Resource Assessment & Conservation Engineering Division, 7600 Sand Point Way NE, Seattle, WA 98115-0070 USA. Frank.Morado@noaa.gov

1200-1330 Lunch on own

1030 Session 21: *Aphanomyces* Special Session ***Grand Ballroom E***

Moderator: Vicki Blazer

1030 Overview of *Aphanomyces invadans* in menhaden along the East Coast of the United States

Vicki S. Blazer[1]*, W.B. Schill[1], C.L. Densmore[1], L. Pieper[2], Y. Kiryu[3] and S. Page[1]
[1]National Fish Health Research Laboratory, U.S. Geological Survey, 11700 Leetown Road, Kearneysville, WV 25430 USA vicki_blazer@usgs.gov; bane_schill@usgs.gov; christine_densmore@usgs.gov; Sandra_page@usgs.gov
[2]Maryland Department of Natural Resources, Stevensville, MD USA lpieper@dnr.state.md.us
[3]Department of Environmental Sciences, Virginia Institute of Marine Science, Gloucester Point, VA 23062 USA yasu@vims.edu

1045 Environmental factors associated with the occurrence of ulcerous lesions in Atlantic menhaden, *Brevoortia tyrannus*, in a small coastal embayment

Howard Kator*, Larry Haas, Iris Andersen, David Zwerner and Wolfgang K. Vogelbein
Department of Environmental Sciences, Virginia Institute of Marine Science, Gloucester Point, Virginia 23062 USA. kator@vims.edu, lhaas@vims.edu, iris@vims.edu, dez@vims.edu wolf@vims.edu

1100 Pathogenicity of the oomycete, *Aphanomyces invadans*, in Atlantic menhaden, *Brevoortia tyrannus*

Yasunari Kiryu[1]*, Jeffrey D. Shields[1], Wolfgang K. Vogelbein[1], Howard Kator[1], and Vicki S. Blazer[2]
[1]Department of Environmental Sciences, Virginia Institute of Marine Science, Gloucester Point, Virginia 23062 USA yasu@vims.edu, jeff@vims.edu, wolf@vims.edu, kator@vims.edu
[2]National Fish Health Research Laboratory, Biological Resources Division, U.S. Geological Survey, 1700 Leetown Road, Kearneysville, West Virginia 25430 USA vicki_blazer@usgs.gov

1115 The antibody response of wild striped snakehead *Channa striata* to the epizootic ulcerative syndrome pathogen, *Aphanomyces invadans*

David Miles[1], Kim D. Thompson[1]*, Juan D. Albaladejo[2], Suppalak Puttinaowarat[3], James H. Lilley[1], and Alexandra Adams[1]
[1]Institute of Aquaculture, Stirling University, Stirling, FK9 4LA, UK
[2]Fish Health Section, Bureau of Fisheries and Aquatic Resources (BFAR), 860 Quezon Avenue, Quezon City 1193, Manila, Philippines
[3]Aquatic Animal Health Research Institute (AAHRI), Department of Fisheries, Kasetsart University Campus, Jatujak, Bangkok 10900, Thailand

1130 Mycotic granulomatosis in ayu caused by *Aphanomyces piscicida* in Japan

Kishio Hatai*, Shinpei Wada, and Osamu Kurata
Division of Fish Diseases, Nippon Veterinary and Animal Science University, 1-7-1 Kyonan-cho, Musashino, Tokyo 180-8602, Japan

1145 Ulcerative mycosis in channel catfish, *Ictalurus punctatus*, black bullhead, *Ameiurus melas*, and bluegill, *Lepomis macrochirus* caused by *Aphanomyces invadans* from recreational ponds in Louisiana, USA

John P. Hawke[1*], Amy M. Grooters[2] and Alvin C. Camus[3]
[1]Cooperative Aquatic Animal Health Research Program, Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803 USA
[2]Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803 USA
[3]Thad Cochran National Warmwater Aquaculture Center, Mississippi State University, College of Veterinary Medicine, P.O. Box 197, Stoneville, MS 38776 USA

1200-1330 Lunch on own

Wednesday
1330-1500 Concurrent Oral Sessions 22, 23, and 24

1330 Session 22: Bacteriology 2 ***Grand Ballrooms A & B***
Moderator: Bill Wolters

1330 Comparison of bacterial loads in ESC-susceptible and resistant channel catfish with real-time PCR

A. Lelania Bilodeau*[1], Brian C. Small[1], William R. Wolters[1], and David J. Wise[2]
[1]USDA-ARS, Catfish Genetics Research Unit, Thad Cochran Warmwater Aquaculture Center, 141 Experiment Station Road, Box 38, Stoneville, Mississippi 38776 USA
[2]Mississippi State University, Delta Research and Extension Center, Thad Cochran National Warmwater Aquaculture Center, P.O. Box 197, Stoneville, Mississippi 38776 USA

1345 Channel catfish families resistant to ESC are different from ESC-susceptible families in both constitutive and inducible complement activity

Lazendra L. Hairston [1]*, Andrew E. Goodwin [1], and W.R. Wolters [2]
[1]University of Arkansas at Pine Bluff, Department of Aquaculture and Fisheries, 1200 North University Drive, Pine Bluff, Arkansas 71611 USA lhairston@uaex.edu
[2]USDA, ARS Catfish Genetics Research Unit, 141 Experiment Station Road, P. O. Box 38, Stoneville, Mississippi 38776 USA Bwolters@msa-stoneville.ars.gov

1400 **Assessing cortisol responsiveness between strains and *Edwardsiella ictaluri* susceptible and resistant families of channel catfish, *Ictalurus punctatus***
Brian C. Small*, A. Lelania Bilodeau, and William R. Wolters
USDA/ARS Catfish Genetics Research Unit, Thad Cochran National Warmwater Aquaculture Center, P.O. Box 38, Stoneville, MS 38776, USA bsmall@ars.usda.gov

1415 **Identification of the gene responsible for hemolysis in *Edwardsiella ictaluri***
Michele L. Williams* and Mark L. Lawrence
College of Veterinary Medicine, Mississippi State University, Box 6100, Mississippi State, MS 39762 USA, mlw12@msstate.edu

1430 ***Edwardsiella ictaluri* O polysaccharide biosynthesis gene cluster and O polysaccharide composition**
Mark L. Lawrence[1]*, Michelle M. Banes[1], and Parastoo Azadi[2]
[1]College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762 USA
lawrence@cvm.msstate.edu, banes@cvm.msstate.edu
[2]Complex Carbohydrate Research Center, 220 Riverbend Road, Athens, Georgia 30602-7229 USA azadi@ccrc.uga.edu

1445 **The use of signature tagged mutagenesis to identify factors involved in the pathogenesis of *Edwardsiella ictaluri***
Ronald L. Thune[1,2]*, Denise Fernandez[1], Maria Kelly-Smith[1], and Jennifer Benoit[1]
Departments of [1]Veterinary Science, Louisiana State University Agricultural Center and [2]Pathobiological Sciences, Cooperative Aquatic Animal Health Research Program (CAAHRP), Louisiana State University School of Veterinary Medicine, Baton Rouge, Louisiana 70803
thune@mail.vetmed.lsu.edu

1500-1530 **Afternoon Break**

1330 **Session 23: Infectious Salmon Anemia (ISA) 3 (USDA-APHIS Special Session)**
Grand Ballroom D

Management Response, continued
Moderator: TBA

1330 **ISA in the Faroe Islands**
Peter Østergård[1]* and Bjorn Harlou[2]
[1]Food and Environmental Agency, Department of Fish Diseases, Falkavegur 6, FO-100 Tórshavn, Faroe Islands; pso@hfs.fo
[2]Ministery of Industry and Agriculture, Veterinary Department, P.O.Box 139, FO-100 Tórshavn, Faroe Islands; cvo@vms.fo

1345 **Management and control of ISA in New Brunswick, Canada**
Mark Moore[1], DVM and Sandi McGeachy[2]*
[1] New Brunswick Department of Agriculture Fisheries and Aquaculture, P.O. Box 1037, St. George, NB E5C 3S9 Canada
[2] New Brunswick Department of Agriculture, Fisheries and Aquaculture, Agriculture Research Station, PO Box 6000, Fredericton, New Brunswick E3B 5H1 Canada;
Sandi.MCGEACHY@gnb.ca

Regulatory Response
Moderator: TBA

1400 Experience with regulatory responses to infectious salmon anemia (ISA) in Norway
Kristin Thorud[1]* and Tore Håstein[2]
[1]Norwegian Animal Health Authority, P.O. Box 8147 Dep., N-0033 Oslo, Norway
ket@dyrehelsetilsynet.no
[2]National Veterinary Institute, P.O. Box 8156 Dep., N-0033 Oslo, Norway,
tore.hastein@vetinst.no

1415 Regulatory aspects of ISA management in Scotland based on risk assessment principles
Alasdair H McVicar
Fisheries Research Services, Marine Laboratory, Aberdeen, Scotland, AB11 9DB
Current address: Department of Fisheries and Oceans, 200 Kent Street, Ottawa, Canada K1A 0E6.
mcvicara@dfo-mpo.gc.ca

1430 ISA surveillance and regulatory actions at Atlantic salmon farms in New Brunswick, Canada
K. Larry Hammell
Dept of Health Management, Atlantic Veterinary College, University of Prince Edward Island, PEI, Canada lhammell@upei.ca

1445 APHIS Veterinary Services implements an infectious salmon anemia (ISA) program
Otis Miller, Jr.
USDA, APHIS, 4700 River Road, Unit 46, Riverdale, MD, USA 20737; Otis.miller@aphis.usda.gov

1500-1530 Afternoon Break

1330 Session 24: Miscellaneous 1 ***Grand Ballroom E***
Moderator: Barbara Nowak

1330 Role of the veterinary profession and the American Veterinary Medical Association in aquatic animal health
A. David Scarfe
American Veterinary Medical Association, 1931 N. Meacham Rd., Suite 100, Schaumburg, IL 60173, USA. DScarfe@avma.org

1345 Undertaking an import risk analysis—the influence of knowns and unknowns in science
Sarah N. Kleeman
Aquatic Animal Biosecurity, Biosecurity Australia, Agriculture Fisheries Forestry Australia, GPO Box 858, Canberra ACT 2601 Australia Sarah.Kleeman@affa.gov.au

1400 Evaluation of health risks to the farmed southern bluefin tuna
Barbara F. Nowak
School of Aquaculture, Tasmanian Aquaculture and Fisheries Institute, Aquafin CRC, University of Tasmania, Locked Bag 1-370 Launceston, Tasmania 7250 Australia B.Nowak@utas.edu.au

1415 Natural history of Atlantic surgeonfish and implications for nutritional management in captivity

Ruth T. Francis-Floyd[1,2]*, G. Christopher Tilghman[2], RuthEllen C. Klinger[1], Ilze K. Berzins[3], M. Andrew Stamper[4]

[1]Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, P.O. Box 100136, Gainesville, Florida, 32611, USA. RFF@mail.ifas.ufl.edu

[2]The Department of Fisheries and Aquatic Sciences, University of Florida, 7922 NW 71 Street, Gainesville, Florida, 32653, USA. RFF@mail.ifas.ufl.edu

[3]The Florida Aquarium, 701 Channelside Drive, Tampa, Florida 33602. Iberzins@Flaquarium.org

[4]The Living Seas, Walt Disney World Resorts, P.O. Box 10,000, Lake Buena Vista, Florida, 32830. andy.m.stamper@disney.com

1430 Experimental production of gastric dilation and its association with serum osmolality and biogenic amines in chinook salmon (*Oncorhynchus tshawytscha*)

J.S. Lumsden[1]*, S. Marshall[2], M. Gillard[2], B. Wybourne[2], and M. Minamikawa[1]

[1]Institute of Animal, Veterinary, and Biomedical Sciences, College of Sciences, Massey University, PB 11-222, Palmerston North, New Zealand. jslumsde@uoguelph.ca; M.Minamikawa@massey.ac.nz

[2]New Zealand King Salmon, 10-18 Bullen St., Tahunanui, PB 1180, Nelson, New Zealand. snm@kingsalmon.co.nz; mjg@kingsalmon.co.nz; b.wybourne@xtra.co.nz

1445 Possible cataract preventative effect of histidine related to ionic balance and water regulation in fish lens

Olav Breck[1,2]*, Rune Waagbø[1], Patrick Campbell[3], Ellen Bjerkås[4], Jeremy Rhodes[5] and Julie Sanderson[5]

[1]Institute of Nutrition, Directorate of Fisheries, P.O.Box 185, Sentrum, N-5804 Bergen, olav.breck@nutr.fiskeridir.no, rune.waagbo@nutr.fiskeridir.no

[2]Marine Harvest Norway Ltd. P.O.Box 4102,Dreggen, N-5835 Bergen, olav.breck@marineharvest.com

[3]Biomar Ltd., FK3 8UL Grangemouth, United Kingdom, pcampbell@biomar.co.uk

[4]Norwegian School of Veterinary Science, P.O.Box 8146 Dep. N-0033 Oslo, ellen.bjerkaas@veths.no

[5] University of East Anglia, NR47TJ Norwich, United Kingdom, j.sanderson@uea.ac.uk; j.rhodes@uea.ac.uk

1500-1530 Afternoon Break

Wednesday

1530-1700 Concurrent Oral Sessions 25, 26, and 27

1530 Session 25: Bacteriology 3 ***Grand Ballrooms A & B***

Moderator: Alicia Toranzo

1530 *Edwardsiella ictaluri* encodes a putative Type III secretion system

Jennifer L. Benoit[1]*, Maria Kelly-Smith[1], Denise H. Fernandez[1], and Ronald L. Thune[1,2]

[1]Department of Veterinary Science, Louisiana State University Agricultural Center, Baton Rouge, Louisiana 70803, USA jbenoit6@lsu.edu

[2]Department of Pathobiological Sciences, Cooperative Aquatic Animal Health Research Program (CAAHRP), School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana 70803, USA thune@mail.vetmed.lsu.edu

1545 **Identification of virulence genes in the aquaculture pathogen *Streptococcus iniae***
John T. Buchanan 1*, Jason A. Stannard2, Mark E. Westerman2, Vaughn E. Ostland2, Jon C. Van Olst2, James M. Carlberg2, and Victor Nizet1
[1]University of California San Diego, School of Medicine: Department of Pediatrics, Division of Infectious Diseases, 9500 Gilman Drive, La Jolla, CA 92093-0672, jtbuchan@ucsd.edu; vnizet@ucsd.edu
[2]Kent SeaTech Corporation, 11125 Flintkote Ave Suite J, San Diego, CA 92121, mwesterman@kentseatech.com

1600 **Case Report: unique pathology linked to infection by *Aeromonas salmonicida* subsp. *achromogenes* in pen reared wild cod from Newfoundland.**
David B. Groman[1]*, Daryl S. Whelan[2], Mada Coles[1] and Ann-Margaret MacKinnon[3]
[1]Aquatic Diagnostic Services, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island, C1A 4P3, Canada groman@upei.ca
[2]Fish Health Section, Newfoundland Department of Fisheries & Aquaculture, St. Johns, Newfoundland, A1B 4J6 Canada DarylSWhelan@mail.gov.nf.ca
[3]Fish Health Unit, Department of Fisheries & Oceans, Science Branch, Moncton, New Brunswick, E1C 9B6, Canada MacKinnonA@dfo-mpo.gc.ca

1615 **Genetic analysis of fish isolates of *Lactococcus garvieae* by RAPD analysis**
Carmen Ravelo, Beatriz Magariños, Sonia López-Romalde, Alicia E. Toranzo and Jesús L. Romalde*
Dpto. Microbiología y Parasitología. Facultad de Biología. Campus Sur. Universidad de Santiago de Compostela. 15782. Spain. mpromald@usc.es

1630 **Experimental transmission and pathogenesis of *Piscirickettsia salmonis* isolated from white seabass (*Atractoscion nobilis*)**
Kristen D. Arkush[1,3*], Mark S. Okihiro[2], and Ronald P. Hedrick[3]
[1]Bodega Marine Laboratory, University of California, P.O. Box 247, Bodega Bay, California 94923 USA kdarkush@ucdavis.edu
[2]California Department of Fish and Game, 4065 Oceanside Blvd, Suite G, Oceanside, California 92056 USA ms.okihiro@att.net
[3]Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California at Davis, Davis, California 95616 USA rphedrick@ucdavis.edu

1645 **Molecular detection methods developed for a systemic rickettsia-like organism in *Penaeus monodon* (Decopoda: Crustacea)**
Linda M. Nunan[1]*, Bonnie T. Poulos[1], Rita M. Redman[1], Marc Le Groumellec[2], and Donald V. Lightner[1]
[1]University of Arizona, Department of Veterinary Science and Microbiology, 1117 E. Lowell St., Tucson, Arizona 85721 USA
[2]AQUALMA, Aquaculture de la Mahajamba, Mahajanga 401 Madagascar

1530-1730 **Session 26: Infectious Salmon Anemia (ISA) 4 (USDA-APHIS Special Session)** ***Grand Ballroom D***
Group Discussion
Moderator: TBA

1530-1715 **Introductory Remarks**
A. David Scarfe

Group Discussion—Practical Future Considerations for possible ISA prevention, control, and eradication
Short presentations by Sharon MacLean and Jill Rolland

1715-1730 **Next Steps/Closing Remarks**
Otis Miller, Jr. (USDA/APHIS)

1530 Session 27: Miscellaneous 2 ***Grand Ballroom E***
Moderator: Paul Bowser

1530 Effects of *Ichthyophonus* on survival of Yukon River chinook salmon
Richard Kocan[1], Paul Hershberger[1] and James Winton[2]
[1]Aquatic and Fishery Sciences, University of Washington, Seattle, WA 98195
[2]Western Fisheries Research Center, USGS, 6505 NE 65th St., Seattle, WA 98115

1545 Do fish have prions? Results of a one year study
Birgit Oidtmann[1]*, Michael Baier[2] and Rudolf W Hoffmann[1]
[1]Institute of Zoology, Fish Biology and Fish Diseases, Veterinary Faculty, University Munich, Kaulbachstr. 37, 80539 Munich, Germany B.Oidtmann@zoofisch.vetmed.uni-muenchen.de
[2]Transmissible Spongioform Encephalopathies, Robert-Koch-Institut, Nordufer 20, 13353 Berlin, BaierM@rki.de

1600 Catfish anemia: New theories, no answers
Andrew E. Goodwin*
Aquaculture/ Fisheries Center, University of Arkansas at Pine Bluff, Department of Aquaculture/ Fisheries, 1200 N University Drive, Pine Bluff, AR-71601, USA agoodwin@uaex.edu

1615 A glomerulopathy of chinook salmon (*Oncorhynchus tshawytscha*) in New Zealand
John S. Lumsden[1]*, Miho Minamikawa[1], and Ben Wybourne[2]
[1]Institute of Animal, Veterinary, and Biomedical Sciences, Massey University, PB 11-222, Palmerston North, New Zealand. jslumsde@uoguelph.ca; M.Minamikawa@massey.ac.nz
[2] New Zealand King Salmon, 10-18 Bullen St., Tahunanui, PB 1180, Nelson, New Zealand. b.wybourne@xtra.co.nz

1630 Lymphosarcoma in hatchery reared yearling tiger muskellunge *Esox masquinongy* X *E. niger*
Paul R. Bowser[1]*, James W. Casey[1], Gregory A. Wooster[1], Rodman G. Getchell[1], Chun-Yao Chen[1] and Linda A. Chittum[2]
[1]Aquatic Animal Health Program, Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853-6401 USA prb4@cornell.edu, jwc3@cornell.edu, gaw5@cornell.edu, cc157@cornell.edu
[2]Aquatic Animal Health Laboratory, Division of Wildlife State of Colorado Department of Natural Resources, P. O. Box 128, Brush, Colorado 80723-0128
Current address: Fisheries Experiment Station, 1465 West 200 North, Utah Department of Wildlife Resources, Logan, UT 84321 USA lchittum@udwrfes.org

1645 Microbial concerns for cryopreserved larvae and gametes of aquatic species

Jill A. Jenkins[1] and Terrence R. Tiersch[2]*

[1]U.S. Geological Survey, National Wetlands Research Center, Lafayette, Louisiana 70506 USA jill_jenkins@usgs.gov

[2]Aquaculture Research Station, Louisiana Agricultural Experimental Station, Louisiana State University Agricultural Center, Baton Rouge, Louisiana 70803 ttiersch@agctr.lsu.edu

Thursday, September 5, 2002

0830-1000 Plenary Session 4 ***Grand Ballrooms A & B***

Chair: Sarah Poynton

0830 Antimicrobial effectors in marine molluscs and crustaceans
Evelyne Bachère, DRIM, UMR 5098, IFREMER/CNRS/Univ., Montpellier 2, Place E. Bataillon, 34095 Montpellier cedex 5 France Evelyn.Bachere@ifremer.fr

0900 Immune relevant genes in fish
Ikuo Hirono, Laboratory of Genetics and Biochemistry, Department of Aquatic Biosciences, Tokyo University of Fisheries, Konan 4-5-7, Minato-ku, Tokyo 108-8477, Japan hirono@tokyo-u-fish.ac.jp

0930 Recent developments in our knowledge of fish immune responses
Norman W. Miller, Department of Microbiology, University of Mississippi Medical Center, Jackson, MS 39216 USA nmiller@microbio@umsmed.edu

1000-1030 Morning Break

Thursday
1030-1200 Concurrent Oral Sessions 28, 29, and 30

1030 Session 28: Immunology 1 ***Grand Ballrooms A & B***

Moderator: Ikuo Hirono

1030 Role of somatic mutation and immunoglobulin structural diversity in the teleost immune function: A critical problem in addressing fish disease resistance
Stephen L. Kaattari
Dept. of Environmental and Aquatic Animal Health, School of Marine Science, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062 USA kaattari@vims.edu

1045 Isolation and characterization of a novel CXC chemokine in common carp (*Cyprinus carpio* L.)
Ram Savan[1]*, Tomoya Kono[2], Azumi Aman[1] and Masahiro Sakai[2]
[1]United Graduate School of Agricultural Sciences, Kagoshima University, Kagoshima 890-0065, Japan kgd103u@student.miyazaki-u.ac.jp
[2]Faculty of Agriculture, Miyazaki University, Miyazaki, 889-2192, Japan m.sakai@cc.miyazaki-u.ac.jp

1100 Immuno-related genes expressed in fish stimulated by immunostimulant
Tomoya Kono[1]* and Masahiro Sakai[2]
[1]United Graduate School of Agricultural Sciences, Kagoshima University, Kagoshima 890-0065, Japan tomoya-kono@ma.neweb.ne.jp
[2]Faculty of Agriculture, Miyazaki University, Miyazaki, 889-2192, Japan m.sakai@cc.miyazaki-u.ac.jp

1115 Cloning and characterisation of a channel catfish (*Ictalurus punctatus*) Mx gene

Karen P. Plant[1,2]* and Ronald L. Thune[1,2]
[1]Department of Veterinary Science, Louisiana State University Agricultural Center
[2]Department of Pathobiological Sciences, Cooperative Aquatic Animal Health Research Program (CAAHRP), School of Veterinary Medicine, Louisiana State University, Skip Bertman Drive, Baton Rouge, Louisiana, 70803, USA kplant1@lsu.edu; thune@mail.vetmed.lsu.edu

1130 Atlantic salmon major histocompatibility complex II α and β promoters—expression studies

Olav Vestrheim[1], Maria Lundin[3], Jacob Torgersen[1], Espen Rimstad[2], and Mohasina Syed[1]*
[1]Dept. of Morphology, Genetics and Aquatic Biology, Norwegian School of Veterinary Science, Post Box 8146 Dep, N-0033 Oslo, Norway. olav.vestrheim@veths.no, mohasina.syed@veths.no; Jacob.torgersen@veths.no
[2]Dept. of Pharmacology, Microbiology and Foodhygiene, Norwegian School of Veterinary Science, Post Box 8146 Dep, N-0033 Oslo, Norway. espen.rimstad@veths.no
[3]Natural Sciences, Södertörns University College, Box 4101, 141 04 Huddinge, Sweden maria.lundin@sh.se

1145 New peptide binding domain lineages found in MHC class I of rainbow trout (*Oncorhynchus mykiss*) shared with cyprinid species; conservation of variation

Ikunari Kiryu[1]*, Johannes M. Dijkstra[1], Yasutoshi Yoshiura[1], Keiichiro Hashimoto[2], and Mitsuru Ototake[1]
[1]Inland Station / National Research Institute of Aquaculture, Tamaki, Mie 519-0423, Japan ikunari@fra.affrc.go.jp
[2]Institute for Comprehensive Medical Science, Fujita Health University, Toyoake, Aichi 470-1192, Japan keihashi@fujita-hu.ac.jp

1200-1330 Lunch on own

1030 Session 29: Bacteriology 4 ***Grand Ballroom D***

Moderator: Jesús Romalde

1030 Molecular genetics of major soluble antigen (MSA) in the salmonid pathogen, *Renibacterium salmoninarum*

Linda D. Rhodes*, Alison M. Coady, and Mark S. Strom
Northwest Fisheries Science Center, REUT Division, 2725 Montlake Boulevard East, Seattle, WA 98112, linda.rhodes@noaa.gov

1045 Injury and potential for *Renibacterium salmoninarum* transmission in chinook salmon *Oncorhynchus tshawytscha* marked with coded wire tags by conventional and automated methods

Diane G. Elliott[1]*, Geraldine Vander Haegen[2], Carla M. Conway[1], Connie L. McKibben[1], LynnMarie J. Applegate[1], Kyong Yi[2], and Joan Thomas[2]
[1]Western Fisheries Research Center, Biological Resources Discipline, U.S. Geological Survey, 6505 Northeast 65th Street, Seattle, Washington 98115 USA diane_elliott@usgs.gov
[2]Washington Department of Fish and Wildlife, 600 Capitol Way North, Olympia, Washington 98501 USA vandegev@dfw.wa.gov

1100 ***Photobacterium damselae*** **subspecies** ***piscicida*** **is capable of replicating in hybrid striped bass macrophages**

Ahmad A. Elkamel[1] and Ronald L. Thune[1,2]*
[1]Department of Veterinary Science, Louisiana State University Agricultural Center, Baton Rouge, Louisiana 70803, USA
[2]Department of Pathobiological Sciences, Cooperative Aquatic Animal Health Research Program (CAAHRP), School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana 70803, USA thune@mail.vetmed.lsu.edu

1115 **Virulence of siderophore deficient and** ***aroA*** **deletion mutants of** ***Photobacterium damselae*** **subsp.** ***piscicida*** **in a hybrid striped bass (*Morone saxatilis* x *M. chrysops*) infection model**

John P. Hawke[1]*, Ron A. Miller[2], and Ronald L. Thune[1]
[1]Cooperative Aquatic Animal Health Research Program, Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, 70803
[2]Division of Food and Animal Microbiology, Food and Drug Administration-Office of Research, 8401 Muirkirk Road, Laurel, MD 20708

1130 **Characterization of** ***Photobacterium damselae*** **subsp.** ***piscicida*** **strains isolated from cultured sole in Spain**

Beatriz Magariños, Jesús L. Romalde, Sonia López-Romalde, and Alicia E. Toranzo*
Dpto. Microbiología y Parasitología. Facultad de Biología. Campus Sur. Universidad de Santiago de Compostela 15782. Spain. mpaetjlb@usc.es

1145 **Development of a PCR-based procedure for the rapid detection of** ***Pseudomonas anguilliseptica***

Sonia López-Romalde, Beatriz Magariños, Carmen Ravelo, Alicia E. Toranzo and Jesús L. Romalde*
Dpto. Microbiología y Parasitología. Facultad de Biología. Campus Sur. Universidad de Santiago de Compostela 15782. Spain mpromald@usc.es

1200-1330 **Lunch on own**

1030 **Session 30: Toxicology** ***Grand Ballroom E***

Moderator: Wolfgang Vogelbein

1030 ***Pfiesteria piscicida*****: Molecular analysis of the life cycle does not support the presence of toxic amoeboid stages**

R. Wayne Litaker[1], Mark W. Vandersea[1], Steven R. Kibler[1], Edward Noga[2], and Patricia A. Tester[1]
[1]Center for Coastal Fisheries and Habitat Research, National Ocean Service, NOAA, 101 Pivers Island Road, Beaufort, North Carolina 28516-9722
[2]College of Veterinary Medicine, Department of Clinical Sciences, Box 8401, 4700 Hillsborough St. Raleigh, NC 27606

1045 Characterization of fish mortalities caused by *Pfiesteria shumwayae* (Strain CCMP 2089)

Jeffrey D. Shields*, Yasunari Kiryu, Wolfgang K. Vogelbein, Leonard Haas, and Kimberly S. Reece
Virginia Institute of Marine Science, The College of William and Mary, School of Marine Science, Gloucester Point, Virginia 23062, USA

1100 *Pfiesteria shumwayae* kills fish by myzocytosis not exotoxin secretion

Wolfgang K. Vogelbein[1]*, Vincent J. Lovko[1], Jeffrey D. Shields[1], Kimberly S. Reece[1], Leonard W. Haas[1], Patrice L. Mason[1], and Calvin C. Walker[2]
[1]Dept. of Environmental Sciences, Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA 23062 USA wolf@vims.edu; vlovko@vims.edu; jeff@vims.edu; kreece@vims.edu, ptrice@vims.edu; lhaas@vims.edu
[2]United States Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Gulf Ecology division, 1 Sabine Island Dr., Gulf Breeze, FL 32561 USA walker.calvin@epa.gov

1115 Innate and acquired disease resistance of chinook salmon to *L. anguillarum* following dietary exposure to PCBS or PAHS

David B. Powell[1*], Roger C. Palm, Jr.[1], Ann Skillman[2], and Kathy Godtfredsen[3]
[1]ProFishent Inc., 17806 NE 26th Street, Redmond, Washington, 98052-5848, davidp@profishent.com, rogerp@profishent.com
[2] Battelle Marine Science Lab, 1529 West Sequim Bay Road, Sequim, Washington, 98382, ann.skillman@pnl.gov
[3] Windward Environmental LLC, 200 W Mercer Street, Suite 401, Seattle, Washington, 98119, kathyg@windwardenv.com

1130 Toxicity of aerially applied pesticides to fish and shrimp: Identification of compounds likely to cause mortality in aquaculture

Kelly R. Winningham* and Andrew E. Goodwin
Department of Fisheries, University of Arkansas at Pine Bluff, 1200 North University Drive, Pine Bluff, Arkansas 71601 USA Kwinningham@uaex.edu

1145 TBA

1200-1330 Lunch on own

Thursday

1330-1500 Concurrent Oral Sessions 31, 32, and 33

1330 Session 31: Immunology 2 ***Grand Ballrooms A & B***

Moderator: Norm Miller

1330 Expression level of immunological factors from rainbow trout (*Oncorhynchus mykiss*) after infection with either bacterial or viral pathogens

Ken Overturf[1]*, Scott LaPatra[2], and Dan Bullock[3]
[1]USDA/ARS, Hagerman Fish Culture Experiment Station, 3059-F National Fish Hatchery Road, Hagerman, ID 83332 U.S.A. kennetho@uidaho.edu
[2]Clear Springs Foods, Inc., Research Division, PO Box 712, Buhl, Idaho 83316 U.S.A. scottl@clearsprings.com
[3]USDA/ARS, Hagerman Fish Culture Experiment Station, 3059-F National Fish Hatchery Road, Hagerman, ID 83332 U.S.A. dbullock@uidaho.edu

1345 The lack of a specific antibody response in haddock (*Melanogrammus aeglefinus*); immunoglobulin quaternary structure and proteomic diversity

B.E. Bentley[1,2]*, A. Dacanay[1], L.L. Brown[1], and S.C. Johnson[1]
[1]National Research Council Canada, Institute for Marine Biosciences, 1411 Oxford St., Halifax, Nova Scotia, Canada B3H 3Z1 Andrew.Dacanay@nrc.ca; Laura.Brown@nrc.ca; Stewart.Johnson@nrc.ca
[2]Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1 Erin.Bentley@nrc.ca

1400 Endogenous antibiotic defenses in hybrid striped bass and other fish

Umaporn Silphaduang*, Zhiqin Fan, and Edward J. Noga
Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh 27606 USA ucsilpha@unity.ncsu.edu

1415 Neutralization of a salmonid rhabdovirus by single-chain antibodies

Niels Lorenzen[1]*, Pauline M. Cupit[2], Ellen Lorenzen[1], Katja Einer-Jensen[1], Jesper S. Rasmussen[1], Christopher J. Secombes[2], and Charlie Cunningham[2]
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1430 Role of complement C3b in adaptive immunity in teleost fish

J. Oriol Sunyer*, N. Bosch, A. Gelman, and G. Lorenzo
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1445 Ontogeny of the lymphoid tissues of the paddlefish, *Polyodon spathula*

Beth Peterman and Lora Petrie-Hanson*
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1500-1530 Afternoon Break

1330 Session 32: Vaccine 1 ***Grand Ballroom D***

Moderator: Scott LaPatra

1330 DNA vaccines against rhabdoviral disease of finfish: Tools for understanding virus defense mechanisms

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1345 A dose response study of three potential DNA vaccines against channel catfish virus (CCV) in channel catfish

Heather C. Harbottle[1,2]*, Karen P. Plant[1,2], and Ronald L. Thune[1,2]
[1]Departments of Veterinary Science, Louisiana State University Agricultural Center, and
[2]Pathobiological Sciences, Cooperative Aquatic Animal Health Research Program (CAAHRP), School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803 USA
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1400 Adenovirus as a method for the delivery and expression of foreign genes in rainbow trout (*Oncorhynchus mykiss*)

Ken Overturf[1]*, Scott LaPatra[2], and Paul N. Reynolds[3]
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Paul.Reynolds@ccc.uab.edu

1415 A subunit vaccine for infectious pancreatic necrosis virus (IPNV) using a baculovirus/insect larvae system

Raghunath B. Shivappa[1]* Philip E. McAllister[2] and Vikram N. Vakharia[1]
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[2]USGS/LSC, National Fish Health Research Laboratory, 11700 Leetown Road, Kearneysville, WV 25430 USA phil_mcallister@usgs.gov

1430 Efficacy of an ISAV vaccine in Atlantic salmon in freshwater and seawater

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1445 Protective effect of cutaneous antibody against *Ichthyophthirius* on channel catfish

De-Hai Xu* and Phillip H. Klesius
U.S. Department of Agriculture, Agricultural Research Service, Aquatic Animal Health Research Laboratory, P.O. Box 952, Auburn, AL 36831

1500-1530 Afternoon Break

1330 Session 33: Molluscs 1 ***Grand Ballroom E***

Moderator: Eugene Burreson

1330 Withering syndrome, an epidemic rickettsial disease of wild and cultured abalone in California

James D. Moore[1,2]*, Carolyn Friedman[3], Thea Robbins[2], and Ronald P. Hedrick[1]
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[3]School of Aquatic and Fisheries Sciences, University of Washington, Seattle WA 98105 USA carolynf@u.washington.edu

1345 Antimicrobial activity of oyster lysozyme against *Vibrio species* and *Perkinsus marinus*

Kim-Lien T. Nguyen*, Chwan-Hong Foo, Casey Barocco, Qing-Gang Xue and Jerome F. La Peyre
Cooperative Aquatic Animal Health Research Program, Department of Veterinary Science, Louisiana State University Agricultural Center, Baton Rouge, Louisiana 70803 USA knguye5@lsu.edu; cfoo1@lsu.edu; cbarocco@lsu.edu; qxue@lsu.edu; jlapeyre@agctr.lsu.edu

1400 *Paramoeba* sp. as a potential pathogen in seed clam (*Mercenaria* sp.) hatcheries in the Indian River Lagoon, Florida

Jan H. Landsberg*, Ruth O. Reese, and William S. Arnold
Florida Marine Research Institute, Florida Fish and Wildlife Conservation Commission, 100 Eighth Avenue Southeast, St. Petersburg, Florida 33701-5095 USA jan.landsberg@fwc.state.fl.us; ruth.reese@fwc.state.fl.us; bill.arnold@fwc.state.fl.us

1415 European flat oyster pathogen *Marteilia refringens* life cycle investigations: How we found a needle in a haystack

Corinne Audemard[1]*, Frédérique Le Roux[2], Benoît Sautour[3], Pierre-Guy Sauriau[4], and Franck Berthe[2]
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1430 Life history of an exotic sabellid polychaete, *Terebrasabella heterouncinata*: Fertilization strategy and influence of temperature on reproduction

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[2]School of Aquatic and Fishery Sciences, University of Washington, Box 355020, Seattle, WA 98195 USA carolynf@u.washington.edu

1445 Current eradication status of the exotic sabellid worm in the state of California

Thea T. Robbins[1], Carl A. Finley[2], Fred Wendell[3], and Carolyn S. Friedman[4]
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1500-1530 Afternoon Break

Thursday
1530-1700 Concurrent Oral Sessions 34, 35, and 36

1530 Session 34: Immunology 3 ***Grand Ballrooms A & B***
Moderator: Mike Belosevic

1530 Phagocytic responses of *Ictalurus punctatus* fry to intraperitoneally injected particulate material; a light microscopy and cytochemical study

Gavin W. Glenney*, Lora Petrie-Hanson
College of Veterinary Medicine, P.O. Box 6100, Mississippi State University, Starkville, MS. 39762-6100 gwg2@ra.msstate.edu, lora@cvm.msstate.edu

1545 Respiratory burst activity (= phagocytosis) of rainbow trout (*Oncorhynchus mykiss*) phagocytes induced by *Aeromonas salmonicida* and various opsonins can be measured from highly diluted blood

Sami Nikoskelainen[1], Esa-Matti Lilius[2]*
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[2]Department of Biochemistry and Food Chemistry, University of Turku, 20014 Turku, Finland esa-matti.lilius@utu.fi

1600 Transferrin and the innate immune response of fish: Identification of a novel and highly conserved mechanism of macrophage activation

James L. Stafford[1]* and Miodrag Belosevic[1, 2]
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[2]Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta, mike.belosevic@ualberta.ca

1615 The effect of seasonality on the immune responses of rainbow trout (*Oncorhynchus mykiss*)

Alison L. Morgan[1]*, Mark J.R. Porter[1], Charlie Burrells[2], and Kim D. Thompson[1]
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1630 Innate immunity of rainbow trout (*Oncorhynchus mykiss*) is extensively affected by the environmental temperature

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1645 The effect of CpG oligodeoxynucleotides on the innate immune response of common carp, *Cyprinus carpio* L.

Asmi Citra Malina AR. Tassakka[1], and Masahiro Sakai[2]*
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1530 Session 35: Vaccine 2 ***Grand Ballroom D***

Moderator: Kira Salonius

1530 Specificity of developing channel catfish immune response to heterotypic bacterial challenge

Rebecca Mackey* and Lora Petrie-Hanson
College of Veterinary Medicine, P.O. Box 6100, Mississippi State, MS 39762-6100
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1545 Selected immunogens of *Piscirickettsia salmonis*: Molecular analysis to evaluate vaccine development

Vitalia Henríquez, Pablo Conejeros, Patricio Cataldo, Lorena Valladares, M. Verónica Rojas, and Sergio H. Marshall
Laboratory of Molecular Genetics and Immunology, Institute of Biology, Faaculty of Basic Sciencias, Universidad Católica de Valkparaíso, Valparaíso—Chile

1600 Persistence of vaccine components at the injection site of Atlantic salmon (*Salmon salar* L.) following intraperitoneal injection with oil-based vaccines against furunculosis

Stephen Mutoloki[1]*, Svein Alexandersen[2], and Øystein Evensen[1]
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1615 Characterization of serum and mucosal antibody responses of rainbow trout (*Oncorhynchus mykiss*) to *Flavobacterium psychrophilum*

Benjamin R. LaFrentz[1*], Scott E. LaPatra[2], Gerald R. Jones[2], James L. Congleton[3], Boling Sun[1], and Kenneth D. Cain[1]

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[3] U.S. Geological Survey, Idaho Cooperative Fish and Wildlife Research Unit, Department of Fish and Wildlife Resources, University of Idaho, Moscow, ID 83844-1141, USA

1630 Antigenic and immunogenic properties of *Flavobacterium psychrophilum*

Kenneth D. Cain[1]*, Ben LaFrentz[1], Leslie Grabowski[1], and Scott E. LaPatra[2]

[1]Department of Fish and Wildlife, University of Idaho, Moscow, Idaho 83844-1136
[2]Clear Springs Foods, Inc. Research Division, P.O. Box 712, Buhl, Idaho 83316

1645 Evaluation of different oral vaccines against rainbow trout lactococcosis

Asteria Luzardo-Alvarez[1], Carmen Ravelo[2], Alicia E. Toranzo[2], Jesús L. Romalde[2]*, and José Blanco-Méndez[1]

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1530 Session 36: Molluscs 2 ***Grand Ballroom E***

Moderator: Carolyn Friedman

1530 Re-evaluating SSO disease: *Haplosporidium costale* infections of the Eastern oyster in Virginia, Connecticut, and Massachusetts

Nancy A. Stokes[1]*, Lisa M. Ragone Calvo[1], Eugene M. Burreson[1], Inke Sunila[2], and Roxanna Smolowitz[3]

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[3]Marine Biological Laboratory,7 MBL Street, Woods Hole, MA 02543 USA, rsmol@mbl.edu

1545 A disease of cultured paua (*Haliotis iris*) in New Zealand caused by a novel haplosporidian

Benjamin K. Diggles[1]*, Joan Nichol[2], Mike Hine[1,3], St John Wakefield[2], Nathalie Cochennec-Laureau[4], Rodney Roberts[5], and Carolyn S. Friedman[6]

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[6]School of Aquatic and Fishery Science, University of Washington, PO Box 355020 Seattle, WA 98195, USA, carolynf@u.washington.edu

1600 **Development of real time PCR assays for detection of the oyster pathogen *Perkinsus marinus* in environmental water samples**

Corinne Audemard*, Kimberly Reece, Lisa Ragone Calvo, Nancy Stokes, and Eugene M. Burreson
Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, Virginia, 23062 USA audemard@vims.edu

1615 **Comparative pathogenicity of dermo (*Perkinsus marinus*) between the Caribbean and American oyster**

John Scarpa[1]*, Dave Bushek[2], and Susan E. Laramore[1]
[1]Harbor Branch Oceanographic Institution, Inc., 5600 U.S. Hwy. 1 North, Fort Pierce, Florida 34946 USA jscarpa@hboi.edu. sallen@hboi.edu
[2]Baruch Marine Field Laboratory, PO Box 1630, University of South Carolina, Georgetown, South Carolina 29442 USA bushek@sc.edu

1630 **Investigations of an *Isonema*-like flagellate causing mortality in larval hard clams, *Mercenaria mercenaria***

Lisa M. Ragone Calvo[1], Ralph Elston[2], Kimberly S. Reece[1], and Eugene M. Burreson[1]*
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[2] AquaTechnics, Carlsburg, WA 98324, aquatech@olypen.com

1645 **Haemocyte-parasite interactions in the edible cockle, *Cerastoderma edule***

Emma C. Wootton*, Elisabeth A. Dyrynda, and Norman A. Ratcliffe
School of Biological Sciences, University of Wales Swansea, Singleton Park, Swansea, SA2 8PP, UK., e.c.wootton@swansea.ac.uk

Posters

1. **Infectious disease models for the zebrafish *(Danio rerio)***
Meagan E. Pressley[1]*, Sharon Blake[1], Nicholas Stasulis[1], Eckhard Witten[2], Bruce Nicholson[1], and Carol H. Kim[1]
[1]Department of Biochemistry, Molecular Biology, and Microbiology, University of Maine, Hitchner Hall, Orono, Maine 04469 USA carolkim@maine.edu
[2] Zoologisches Institut und Zoologisches Museum der Universitaet Hamburg, Martin-Luther-King-Pl. 3, Hamburg, Germany 20146 PEWitten@aol.com

2. **Development of novel multiplex PCR detection systems for economical important viral diseases of fish**
M Arias[1]; M. Agüero[1]; J. Fernández[1]; E. Blanco[1]; A. Gibello[2]; M. Blanco[2]; L. Domínguez[2]; and J.M. Sánchez-Vizcaíno[1]
[1]Centro de Investigación en Sanidad Animal, CISA-INIA, Valdeolmos,28130, Madrid, Spain. arias@inia.es
[2]Dpto. de Sanidad Animal, Facultad de Veterinaria, UCM, Avda. Puerta de Hierro s/n, 28040, Madrid, Spain.mmblanco@vet.ucm.es

3. **Tissue distribution kinetics of grouper iridovirus in Taiwan (TGIV) during infection: an *in situ* hybridization study**
Chia-Ben Chao[1], Chun-Yao Chen[2]*, and Hon-Tu Huang[3]
[1]Institute for Animal Disease Prevention and Control, Kaohsiung, Taiwan
cbc0613@ksts.seed.net.tw
[2]Aquatic Animal Health Program, Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853 USA cc157@cornell.edu
[3]Department of Biological Sciences, National Sun Yat-Sen University, Kaohsiung, Taiwan

4. **Evaluation of methods of extraction of viral RNA from infected cell monolayers**
José G.Olveira, Sandra Juiz-Rio, Susana Castro, Juan L.Barja, and Carlos P. Dopazo
Dpt. Microbiología y Parasitología, Instituto de Acuicultura, Univ. Santiago de Compostela, Spain. mpdopazo@usc.es

5. **The effect of stocking density and other parameters of the susceptibility of Atlantic salmon (*Salmo salar* l.) to infectious pancreatic necrosis virus**
Tim Bowden*, Keith Lockhart, David Smail, and Tony Ellis.
FRS Marine Laboratory, Aquaculture and Aquatic Animal Health, 375 Victoria Road, Aberdeen, AB11 9DB, Scotland, UK. t.j.bowden@marlab.ac.uk, d.smail@marlab.ac.uk, t.ellis@marlab.ac.uk

6. **Interference of the life cycle of fish nodavirus with fish retrovirus**
S.C. Chi*, K. W. Lee, T. M. Cheng and J. R. Shieh
Department of Zoology, National Taiwan University, Taiwan, R.O.C.

7. **Molecular diagnosis of fish diseases: The way forward**
Carey O. Cunningham* and Mike Snow
FRS Marine Laboratory, PO Box 101, 375 Victoria Road, Aberdeen AB11 9DB, UK
c.cunningham@marlab.ac.uk

8. **Fingerprinting of *Flavobacterium psychrophilum* isolates by plasmid profile**
Shotaro Izumi[1]* and Hisatsugu Wakabayashi[2]
[1]Physiology and Molecular Biology Division, National Research Institute of Fisheries Science, Yokohama 236-8648, Japan sizumi@affrc.go.jp
[2]HW Fish Health Laboratory, Sendagi 3-22-11-717, Bunkyo-ku, Tokyo 113-0022, Japan hisa718@alpha.ocn.ne.jp

9. **Genotyping of *Flavobacterium psychrophilum* by PCR-RFLP analysis**
Shotaro Izumi[1] and Hisatsugu Wakabayashi[2]*
[1]Physiology and Molecular Biology Division, National Research Institute of Fisheries Science, Yokohama 236-8648, Japan sizumi@affrc.go.jp
2 HW Fish Health Laboratory, 3-22-11-717, Sendagi, Bunkyo-ku, Tokyo 113-0022, Japan hisa718@alpha.ocn.ne.jp

10. **Studying mycobacterial infection using small fish models and *M. marinum* expressing GFP**
Gregory W. Broussard*, Lisa S. Granados, and Don G. Ennis
Department of Biology, University of Louisiana, P.O. Box 42451, Lafayette, LA 70504-2451 USA gwb6707@louisiana.edu

11. **A microtiter plate assay method for mucosal adhesion of *Aeromonas hydrophila* using a water-soluble tetrazolium salt (WST-1)**
Nobuhiro Mano*, Aki Namba, and Haruo Sugita
[1]Department of Marine Science and Resources, Nihon University Kameino 1866, Fujisawa, Kanagawa 252-8510, Japan nmano1@brs.nihon-u.ac.jp

12. **First isolation of *Vibrio anguillarum* serotype O1 from sole (*Solea solea*) in Italy**
Amedeo Manfrin[1]*, Mauro Doimi[2], Paolo Antonetti[3], Lourdes Delgado Montero[1], Katia Qualtieri[1], Lucia Selli[1], and Giuseppe Bovo[1]
[1]Fish Pathology Department, Istituto Zooprofilattico, Via Romea 14/a, 35020 Legnaro (Padova), Italy, manfrin@izsvenezie.it
[2]D. & D. Consulting, Mestre (Venezia), maurdoimi@tin.it
[3]Local Veterinary Service n.12 - Tronchetto 30125 Venezia

13. **Cold shock response of *Vibrio vulnificus*: With and without acclimation periods**
Kristi L. Huels[1]*, Shin Hee Kim[2], Haejung An[2], and Yolanda J. Brady[1]
[1]Auburn University, Department of Fisheries and Allied Aquacultures, 203 Swingle Hall, Auburn, AL 36849-5419 USA huelskl@acesag.auburn.edu, ybrady@acesag.auburn.edu
[2]Auburn University, Department of Nutrition and Food Science, 362 Spidle Hall, Auburn, AL 36849 USA kimshin@auburn.edu, haejung.an@auburn.edu

14. **Discriminant analysis on the blood chemistry of *Streptococcus iniae* and *Vibrio vulnificus*-infected tilapia, with references to histopathology**
Chun-Yao Chen*[1] and Paul R. Bowser[2]
[1]Aquatic Animal Health Program, Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853-6401 cc157@cornell.edu
[2]Aquatic Animal Health Program, Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853-6401 prb4@cornell.edu

15. **Antimicrobial profiles of members of genus *Serratia* considered typical fish pathogens**

José A. González, Carmen S. Gallardo, Ariana Pombar, Aldara R. Gallego, and Luis A. Rodríguez*
Facultad de Ciencias, Campus Ourense. Universidad de Vigo. Dpto. Biología Funcional y Ciencias de la Salud. As Lagoas s/n, Ourense 32004, Spain. lalopez@uvigo.es

16. **Prevalence of Type E botulism in fish in the Lower Great Lakes**

Rodman G. Getchell[1]*, Gregory A. Wooster[1], Natalija Topic-Popovic[2], William J. Culligan[3], and Paul R. Bowser[1]
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[3]Dunkirk Fisheries Research Station, New York State Department of Environmental Conservation, Dunkirk, New York, 14048 USA wjcullig@gw.dec.state.ny.us

17. **Mucus adhesion of bacteria isolated from intestinal tract of carp, *Cyprinus carpio***

Aki Namba*, Nobuhiro Mano, and Haruo Sugita
[1]Department of Marine Science and Resources, Nihon University Kameino 1866, Fujisawa, Kanagawa 252-8510, Japan gr36021n@st.brs.nihon-u.ac.jp

18. **Screening of tuna's microbiota captured in different fishing-ground (Pacific, Indian and Atlantic oceans)**

Pablo Rego, Carmen S. Gallardo, Teresa M. Sánchez, Belén Araujo, and Luis A. Rodríguez*
Facultad de Ciencias, Campus Ourense. Universidad de Vigo. Dpto. Biología Funcional y Ciencias de la Salud. Facultad de Ciencias. As Lagoas s/n, Ourense 32004, Spain.
lalopez@uvigo.es

19. **Effect of lactic acid and the supernatants from different lactic-acid bacteria strains against *Vibrio* strain**

Ariana Pombar, Maria J. Pérez, José A. González, Francisco J. García, and Luis A. Rodríguez*
Facultad de Ciencias, Campus Ourense. Universidad de Vigo. Dpto. Biología Funcional y Ciencias de la Salud. Facultad de Ciencias. As Lagoas s/n, Ourense 32004, Spain.
lalopez@uvigo.es

20. **Interaction between different populations of lactic-acid bacteria and typical virulent *Vibrio* strain**

Ariana Pombar, Maria J. Pérez, Carmen S. Gallardo, Francisco J. García, and Luis A. Rodríguez*
Facultad de Ciencias, Campus Ourense. Universidad de Vigo. Dpto. Biología Funcional y Ciencias de la Salud. Facultad de Ciencias. As Lagoas s/n, Ourense 32004, Spain.
lalopez@uvigo.es

21. **Cloning and characterization of a phospholipase gene of *Pasteurella piscicida*: This enzyme shows hemolytic activity**

Hiroaki Naka, Ikuo Hirono* and Takashi Aoki
Laboratory of Genetics and Biochemistry, Department of Aquatic Biosciences, Tokyo University of Fisheries, Konan 4-5-7, Minato-ku, Tokyo 108-8477, Japan hirono@tokyo-u-fish.ac.jp

22. **PCR detection of *Pseudomonas anguilliseptica* from winter disease outbreaks in sea bream**

Mar Blanco[1]*, Alicia Gibello[1], Marisa Arias[2], Montserrat Agüero[2], Lucas Domínguez[1] and José F. Fernández-Garayzábal[1]
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Centro de Investigación en Sanidad Animal. Carretera de Valdeolmos a El Casar s/n. 28130 Valdeolmos, Madrid, Spain arias@inia.es

23. **Evaluation of API Staph for identification of different species of genus *Staphylococcus* isolated from diseased rainbow trout**

Carmen S. Gallardo*, Belén Araujo, Teresa M. Sánchez, Ariana Pombar, and Luis A. Rodríguez
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24. **Survival of virulent and avirulent *Hafnia alvei* strains in fresh water microcosms**

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25. **Hydrophobicity assays using virulent and avirulent *Hafnia alvei* strains**

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26. **Siderochrome production by virulent and avirulent strains of *Hafnia alvei***

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27. **Effect of mainteinance at refrigerated temperatures of *Hafnia alvei* strains on their biochemical profiles**

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28. ***Hafnia alvei:* Determination of time and temperature correlation when using API 20E**

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29. **Early events of the infection of *Piscirickettsia salmonis* of CHSE-214 cells by confocal microscopy**

Jorge Contreras, Julio Larenas, Claudia Venegas, Paula Vera, Alvaro Guajardo and Pedro Smith*

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30. **Vertical transmission of *Piscirickettsia salmonis* and a study of the mode of entrance into the ovum**

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31. **Infectivity study of *Piscirickettsia salmonis* in CHSE-214 cells by immunogold and standard transmission electron microscopy**

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32. **Experimental infection of coho salmon (*Oncorhynchus kisutch*) exposing the surface of skin, gills and intestine with *Piscirickettsia salmonis***

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33. **Characterization of a piscirickettsiosis-like disease in Hawaiian tilapia**

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34. **Investigation of fish to fish variation in oxolinic acid concentrations in commercially farmed fish and laboratory held fish following commercial oral therapy**

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35. ***In vitro* metabolic profiles to characterize and predict drug residues in aquacultured finfish**

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36. **Resistance to oxytetracycline in bacteria from salmon farming in Chile**

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37. **Metomidate anaesthesia in turbot**

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38. **Effectiveness of commercial disinfectants against typical bacterial fish pathogens**

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39. **Lesions of estuarine fish in Florida: Are they caused by the same pathogen?**

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40. **Mixed infection of fungal disease in snakehead, *Channa striatus* (Bloch) in Thailand**
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41. **Ulcerative mycosis, salinity, and granuloma formation in Florida fish**
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42. **Sanitary/epizootic state of sturgeon fish plants (SFP) from the Azov sea basin**
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43. **Parasitological and bacteriological findings in freshwater ornamental fish imported in Italy**
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44. **Health management for offshore aquaculture of red drum**
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45. **SDS-PAGE and Western blot analysis of the *Triactinomyxon* spores, the cause of whirling disease in salmonid fish**
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46. **First report of subsidiary protrusions on the caudal processes of two novel types of hexactinomyxon spores (Myxozoa)**
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47. **Coccidiosis in bluegill**
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48. Tricaine dramatically reduces the ability to diagnose protozoan ectoparasite (*Ichthyobodo necator*) infections

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49. Inhibition of reproduction in the roach (*Rutilus rutilus*) by the tapeworm *Ligula intestinalis*

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50. Susceptibility and inflammatory response to *Aeromonas hydrophila* in 17α-methyltestosterone-induced sterile common carp, *Cyprinus carpio* (Linn.)

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51. The development of expressed sequence tags and a cDNA microarray for studying the immune responses of Atlantic halibut (*Hippoglossus hippoglossus* L.)

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52. Identification of a single major QTL controlling nonspecific cytotoxic cell (NCC) activity in an OSU x HC rainbow trout model

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53. Isolation and partial characterisation of serum immunoglobulins (IgM) from sterlet (*Acipenser ruthenus*), Russian (*A. güldenstadti*) and Siberian (*A. baeri*) sturgeon

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54. Serum immunoglobulin of hybrid striped bass (*Morone chrysops* × *M. saxatilis*).

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55. Passive immunization of rainbow trout (*Oncorhynchus mykiss*) against *Flavobacterium psychrophilum*

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56. Some medicinal plants as immunostimulant for fish

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57. Nitric oxide production in the culture of head kidney leukocytes of carp (*Cyprinus carpio*) and synergistic effect of recombinant human cytokines

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58. Hypoxia induces HSP 70 production in juvenile Nile tilapia, *Oreochromis niloticus* (L.)

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59. The effect of dexamethason on crucian carp (*Carassius carassius*, L) leukocytes

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60. Pro-opiomelanocortin (POMC) related hormones effect of carp *Cyprinus carpio* phagocytic cells

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61. Oral immunization of salmonids with biodegradable microparticle-based vaccines

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62. Distribution, persistence, and pathological analysis of a DNA vaccine against infectious hematopoietic necrosis virus

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63. **Comparative study on inflammatory changes in different species of salmonids vaccinated intraperitoneally with oil-adjuvanted vaccines**
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64. **Pathology in weedy sea dragons (*Phyllopteryx taeniolatus*) and leafy sea dragons (*Phycodurus eques*) associated with a mixed bacterial infection**
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65. **Comparative histopathology of *Streptococcus iniae* and *S. difficile*-infected tilapia**
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66. **The spread of pikes (*Esox lucius*) with tumors in the Chernobyl zone (Kyiv Reservoir, Dnieper River, Ukraine)**
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67. **Physiological indices of pikes (*Esox Lucius*) affected by tumors within Chernobyl Catastrophic (Kyiv Reservoir, Dnieper River, Ukraine)**
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68. **Methods of diagnostics of fishes illnesses**
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69. **Sanitary condition of marine mollusks cultured in México**
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70. **An East Coast estuary without dermo or MSX? The presence of *Perkinsus* or *Haplosporidium* pathogens is undetectable in bivalves from Delaware's inland bays**
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71. **A novel lysozyme purified from the plasma of the eastern oyster, *Crassostrea virginica***
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[2]Florida Gulf Coast University, Fort Myers, Florida 33965 USA avolety@fgcu.edu
[3]Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062 USA chu@vims.edu

72. **Extracellular enzyme activities of Perkinsus atlanticus in culture and comparison with *Perkinsus marinus***
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73. **Effects of growth factors, hormones and lectins on Eastern oyster cells in primary cultures**
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74. **Development of serological techniques to study host induced proteins of the oyster parasite *Perkinsus marinus***
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75. **Post capture muscle necrosis in the Norway lobster, *Nephrops norvegicus*, and its physiological consequences**
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76. **Proteomic analysis of hemolymph from *Litopenaeus vannamei* infected with white spot syndrome virus**

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77. **Putative attenuated infectious hypodermanal and hematopoetic necrosis (IHHN) virus: Case study with *Litopenaeus vannamei***

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78. **Horseshoe crab (*Limulus polyphemus)* hemolymph biochemical and immunological parameters**

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79. **Histological techniques and manifestations of abnormal protrusions in copepods from Michigan lakes**

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80. **Detection of white spot syndrome virus (WSSV) genome in frozen commodity shrimp sold at Massachusetts supermarkets**

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81. **Heavy metals in wild and cultured marine shrimp from different geographic regions and in frozen commodity shrimp sold in Massachusetts supermarkets: Preliminary results**

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82. Estrogenic potential and vitellogenin induction in fish related to sewage plants effluents

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83. Chronic effects of bromodichloromethane on medaka

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84. Hepatic and gonadal lesions in medaka (*Oryzias latipes*) exposed to trichloroacetic acid as embryos

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85. The use of immunological parameters of water animals as a criterion of environment condition

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86. White cachama (*Piaractus brachypomus*) as a bioindicator of cadmium-polluted waters

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87. Waterborne nitrite exposure in white cachama (*Piaractus brachypomus*)

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88. **Quantitative analysis of fish swimming and startle behaviors in response to low level stressors**

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90. **Initial assessment of sediment quality and benthic condition within the Lower St. Johns River estuary**

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91. **Pathogenesis of the Acute Ulceration Response (AUR)**

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94. Evidence for immune-regulated transgene expression in channel catfish, *Ictalurus punctatus*

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95. Development of methods to genetically sterilize transgenic fish

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96. Fish diseases education: use of a DVD-ROM for teaching producers, students, and colleagues about fish pathogens

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97. Fishdisease.net—an online home for aquatic animal health professionals

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98. Interdisciplinary training in aquatic animal health at the University of Florida

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ABSTRACTS

Plenary Sessions

The U.S. Fish and Wildlife Service's National Wild Fish Health Survey: an aquatic resources management tool

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The U.S. Fish and Wildlife Service (Service) has completed the research, development and pilot-testing of a national fish pathogen distribution database, and is now in an implementation stage, including public access to the database. Work began on the project in 1996, and we intend to continue it for as long as it provides aquatic resource agencies with an effective resource management tool. The project is referred to as the National Wild Fish Health Survey (Survey), and comprises three components: 1) fish collection, 2) sample examination/analysis and 3) a searchable database. The Service's nine regionally-located Fish Health Centers (FHCs) initiate, coordinate, and in many cases actually collect samples of free-ranging fish. A larger portion of fish collected for the Survey is accomplished by State, Tribal and other Federal agency partners. All collection, preservation and transportation of the fish follow uniform Survey-specific techniques, including defining sample location by GPS-technology. All examinations and analyses of the fish are conducted at the Service's FHCs according to standardized protocols published in the Survey's *Laboratory Procedures Manual*, which is planned for public availability via the Web. The last component is a publicly-accessible, Web-based, searchable database. The database includes an advanced query system permitting users to search for any combination of pathogens (19 listed), fish species (171 species) and time-frame (1995 to and including 2002). The results of any query document the number of samples that match the selected query parameters. The results may be viewed in tabular or graphical form. Tabular results are summarized by State or by USGS watershed units. Graphical results can be displayed as well by either State boundary or watershed maps. All results may be examined at several levels of detail dependent upon the user's needs. Available details range from a simple examination of how many and where samples have been collected all the way to actual laboratory case-sheets showing exact pathogen-specific methods used for each fish (or pool of fish) and the results of that unique fish/pathogen/method analysis. The current database includes information generated from over 48,000 individual fish collected from nearly 1,400 distinct locations in 44 States.

Environmental contaminant effects on endocrine systems of aquatic species

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Since the early 1990s, it has been established that a wide variety of anthropogenic (man-made) chemicals in the environment are capable of modulating and/or adversely affecting or disrupting endocrine function in vertebrate organisms. The physiological effects of exposure to these chemicals have been termed "endocrine disruption" or "endocrine modulation" and the active compounds labeled as "endocrine-disruptors" or "endocrine-active-agents." Endocrine disruption has been defined as the action of "an exogenous agent that interferes with the production, release, transport, metabolism, binding, action, or elimination of natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes." Exposure to these chemicals and/or contaminants in the environment is often highest for aquatic species as urban run-off, industrial or water-treatment discharge and agricultural applications. Effects on aquatic species may be physiological with or without adverse population or community effects and may involve a wide assortment of endocrine mediated functions and potential receptor mediated events. Indeed, effects may involve the steroid receptor superfamily, including the sex steroids, thyroid hormones and adrenal hormones, as well as hypothalamic-pituitary and other protein hormones. The physiological processes regulated by the endocrine system in aquatic species are diverse and numerous. Likewise, the mechanisms of action and effects of potential endocrine disrupting chemicals (EDCs) are equally diverse. Receptor-mediated events involve EDCs acting as hormone mimics (agonists and/or antagonists) and adversely impacting hormone synthesis, catabolism, secretion, transport, and/or signal transduction. Examples of non-receptor mediated modes of EDC action include altered enzyme function and selective toxicities for endocrine active or target tissues whereas altered gene expression and induction of oxidative stress are types of receptor mediated events. EDCs may also act by altering developmental processes often producing multigenerational effects. Endocrine-active anthropogenic chemicals are numerous and diverse. Evidence for endocrine-disrupting effects due to these chemicals comes from a diverse array of reports involving multiple aquatic vertebrate taxonomic groups, limited invertebrate taxa, and results from both *in vitro* and *in vivo* studies. Reported effects of EDCs have included effects at multiple levels of biological organization, including molecular, biochemical, cellular, tissue, organism, community and population. However, few reports have documented effects at the community level and higher. In addition, most studies have focused upon reproductive effects; however, effects on growth, metabolism, and thyroid and immune function have also been noted. Today most evidence has also been derived from studies of wildlife and the ecotoxicology of potential EDCs and/or endocrine-modulating chemicals. A review of the current evidence for the endocrine-disrupting effects in aquatic wildlife will be presented and current examples for fish, reptiles, amphibians and invertebrates compared.

Global climate and large-scale influences on aquatic animal health

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The last 3 decades have witnessed numerous large-scale mortality events of aquatic organisms in North America. Affected species range from ecologically-important sea urchins to commercially-valuable American lobsters and protected marine mammals. Short-term forensic investigations of these events have sometimes characterized a causative agent or condition, but have rarely provided sufficient insight to predict and manage animal health. Traditionally, we apply the tools of microbiology, parasitology and pathology to investigate causality. More recently, satellite imagery and remote sensing have provided physical information on broad temporal and spatial scales that can be compared with local biological phenomena. These tools provide a means to develop epizootiological models that include large-scale influences, such as global climate and land use change, on aquatic diseases. A prime example is worldwide bleaching of corals in relation to water temperatures driven by El Niño-Southern Oscillation (ENSO) conditions. Bleaching can be exacerbated by increased exposure of corals to ultraviolet light, a situation that is linked to loss of stratospheric ozone and local doldrum conditions that sometimes accompany ENSO. Another example is a sea fan coral disease stemming from the long-range transport of a nonindigenous fungal pathogen; its transport is linked to increased global dust and easterly trade winds coincident with decadal variability in the North Atlantic Oscillation. Transport of essential nutrients, particularly iron, under this same scenario has been hypothesized to account for the increased number and severity of coral diseases in the Caribbean and to indirectly affect red tide blooms in the Gulf of Mexico. Sea-level rise, a large-scale change that is predicted to vary with location, will affect temperature, salinity and estuarine flushing. Health consequences for coastal organisms, such as the economically-important eastern oyster, can be expected. Oyster population success is dependent on fluctuating estuarine salinity and temperature and their population dynamic is heavily influenced by a salinity- and temperature-dependent protozoan disease. Local land use decisions, although sometimes seemingly inconsequential, accumulate into watershed land-use patterns that can greatly influence the quality of coastal waters. Elevated nutrients from watershed effluent are suspected in increased hypoxia in the Gulf of Mexico, as well as the increase in the number and severity of harmful algal blooms across North America. Toxic algae have been implicated not only in frequent fish and shellfish mortalities, but in bivalve neoplasia and sea turtle fibropapillomatosis. Regional observing systems that employ satellite imagery and real-time remote sensors are being developed to forecast conditions conducive to blooms of harmful algae. Our investigation of existing and emergent diseases will be greatly enhanced by tools that provide additional information on large-scale factors. However, these tools are still being applied on a retroactive, case-by-case basis. The greatest benefits will accrue when large-scale physical trends can be applied to predict health risks to multiple species.

Transmission of myxozoan pathogens: interactions between cultured and wild fish

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The myxozoa are one of the largest and most diverse groups of fish pathogens, causing infections that result in a range of effects. With the increasing movement of fish worldwide and the diversification of aquaculture, some familiar myxozoans have reemerged as problems, and at the same time the effects of new species are becoming recognized. In some cases, it is clear that problems have occurred as a result of exotic parasites introduced into areas where existing fish are naïve. Alternatively, introduction of naïve fish into parasite-endemic areas has also had disastrous effects.

In salmonid aquaculture, several pathogenic myxozoans illustrate these scenarios. The most familiar, *Myxobolus cerebralis,* was originally detected when susceptible rainbow trout were introduced into Germany, where the parasite occurred naturally. The cosmopolitan distribution of the alternate host plays a role in the parasite's ability to establish, but its dissemination has largely been a result of transfers of infected fish in stocking or enhancement programs, by natural migration or even as a food product. It is recognized that birds, baitfish and anglers may also play a role, but the extent of transfer by these routes is unknown. In contrast, mortalities caused by *Ceratomyxa shasta* have largely resulted from transfers of naïve fish into enzootic waters, with little evidence that fish movement has further disseminated the pathogen. Although many transfers were made unknowingly, as a result of enhancement programs, other transfers were part of management strategies that employed the parasite to limit interactions between hatchery and wild populations. An unexpected outcome of these introductions was increased mortality in the wild populations.

Examples of how the expansion of aquaculture has resulted in exposure to naturally occurring parasite species can be drawn from the growing mariculture industry. Economic loss attributed to post-harvest soft flesh, caused by *Kudoa thrysites*, has continually been a problem for commercial fisheries. With the increase in net pen culture of salmonids in the Pacific NW, this parasite is now of concern to that industry. In the Mediterranean, serious losses of cultured sea bass and sea bream have been attributed to *Myxidium leei*. Because of its broad host range and apparent ability to transmit directly between fish hosts, this emerging myxozoan pathogen now represents a challenge to culture of a number of species.
For each of these cases, protection of cultured species will require enhanced diagnostics to limit introductions, identification of resistant strains for culture in enzootic areas and more information on pathogen distribution. The challenge for protection of wild species will be to determine the primary routes of transmission and identify those that can be controlled and to develop effective intervention methods.

Introduction of non-indigenous shrimp viruses and their potential impact on farmed and native wild shrimp populations

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At least four virus-caused pandemics have adversely affected the global penaeid shrimp farming industry since 1980. These viruses in the approximate order of their discovery are Infectious hypodermal and hematopoietic necrosis virus (IHHNV), Yellow head virus (YHV), Taura syndrome virus (TSV), and White spot syndrome virus (WSSV). The socioeconomic impact of the diseases caused by these viruses have been so severe in some shrimp producing countries of Asia and the western hemisphere (Americas) that three (TSV, WSSV, and YHV) of the four have been designated as "Notifiable" by the Office International des Epizootics and the fourth (IHHNV) is listed with "Other Significant Diseases." IHHNV, TSV, and WSSV have had major impacts on cultured and wild shrimp in the Americas. While YHV is considered to pose a significant threat to wild and cultured shrimp in the Americas, it will not be discussed further here.

After its initial discovery in cultured shrimp in Hawaii in 1981, IHHNV was subsequently found to be widely distributed in cultured shrimp in the Americas and in wild shrimp collected along the Pacific coast of the Americas. The impact of the introduction of IHHNV on shrimp farming and on wild shrimp stocks on Mexico's Gulf of California during the late 1980's and early 1990's is well documented. Recently, IHHNV has been found to be widely distributed in wild and cultured *Penaeus monodon* in east and SE Asia where it does not seem to cause production losses. Molecular studies show considerable variation among Asian isolates of the virus, while little variation was found in Americas isolates. All isolates of IHHNV from the Americas are nearly identical with IHHNV from the Philippines, suggesting, along with other aspects of its history and epidemiology in the Americas, its introduction from the Philippines, perhaps with live *P. monodon* that were imported as a candidate aquaculture species during the very early development of shrimp farming in the Americas.

TSV appears to have emerged from an unknown source in Ecuador in 1991. The disease was recognized as a major new disease of farmed *Penaeus vannamei* by early 1992 and it was named Taura Syndrome. By 1994, when the viral etiology of TS had been established, the virus had been moved with live shrimp movements to many of the shrimp growing countries of the Americas. While studies of wild shrimp stocks are few, TSV has been found in wild shrimp in some countries where TSV has caused significant epizootics. Where present, its impact on wild shrimp remains undocumented. By 1999, TSV reached Asia with infected stocks of *P. vannamei*, introduced for aquaculture purposes. Physiochemical and more recent molecular studies of TSV indicate that a single strain of the virus was present, but that new strains are emerging, which differ in host range and virulence.

WSSV emerged in East Asia in 1992-93 and it was quickly dispersed with infected seed and brood stock across the Asian continent to SE Asia and India where it caused and continues to cause significant losses. WSSV even reached shrimp farms in southeastern Europe (1997) and the Middle East (1999) via live shrimp movements. Despite the absence of evidence of live shrimp introductions from Asia to the Americas, WSSV was diagnosed in captive wild shrimp or crayfish and in cultured domesticated shrimp stocks in the eastern and southeastern U.S. at several sites in 1995-1997. Early in 1999, WSSV was diagnosed as the cause of serious epizootics in Central American shrimp farms. By mid to late 1999, WSSV was causing major losses in Ecuador (then among the world's top producers of farmed shrimp), and by 2000-01, export of shrimp from Ecuador was down nearly 70% from pre-WSSV levels. Although the documentation is sketchy, WSSV has been found in wild shrimp stocks in the Americas. In the US, the virus was successfully eradicated from shrimp farms and it has not been reported from farmed shrimp stocks since 1997. However, its sporadic detection in wild shrimp stocks (Gulf of Mexico and SE Atlantic states) suggests that it has become established there or that it continues to be introduced. It has been proposed that the introductions of WSSV to the Americas were the result of importation of frozen shrimp products from WSSV-affected areas of Asia and the value-added reprocessing of those frozen shrimp for the US market in coastal processing plants. WSSV also reached Spain and Australia in 2000-2001. In both cases successful containment and eradication was reported, and for both events the importation and use of infected frozen shrimp as a fresh feed for broodstock was implicated as the route of introduction.

Regardless of where they were obtained, isolates of WSSV have shown little genetic or biological variation, suggesting that the virus emerged and was spread from a single source.

Until the WSSV pandemic, the penaeid shrimp farming industry in Asia and the Americas remained largely dependent on wild shrimp stocks for stocking its farms. This dependence included the collection and use of wild seed for stocking of its farms directly or the use of captive wild broodstock for the production of seed stock in hatcheries. This dependence has fostered the intensification and spread of the viral diseases in shrimp aquaculture and in wild populations. The shrimp farming industry as a whole has recognized this fact and it has begun to change its farming practices in order to continue to develop, if not survive. Biosecure production systems (that are designed to exclude potentially infected wild shrimp seed) stocked with shrimp stocks known to be free of the major shrimp pathogens have become a common practice in many shrimp growing regions. While many of the shrimp stocks currently used to stock farms are produced from captive wild broodstock, only those that test negative for WSSV in Asia and WSSV and TSV in the Americas are used to stock biosecure farms. A further sign of a maturing industry is its movement towards the expanded development and use of specific pathogen-free domesticated shrimp stocks of each of the most important penaeid shrimp species.

Overview of ISA Management in Maine and Canada

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The salmon aquaculture industry in eastern North America is concentrated in the lower Bay of Fundy and the upper Gulf of Maine. The industry is divided by the international border separating New Brunswick (Canada) and Maine (USA.). Within 50 kilometers of the border there are more than 120 marine sites producing Atlantic salmon. The high density of farms facilitates the spread of diseases such as Infectious Salmon Anemia (ISA). However, the blend of state, provincial and national jurisdictions creates a complex regulatory environment for the control of fish diseases. The first local outbreaks of ISA were on salmon farms in New Brunswick in 1996. Producers adopted a variety of strategies, both at the farm and industry level, in an attempt to control the problem. In the absence of success it became apparent that regulatory intervention was required. Since a plan for the control of ISA did not exist in federal or provincial regulations, a program evolved in the midst of the epizootic. Key areas to address included the nature of appropriate regulatory action, diagnostic criteria, the threshold for action, indemnification, and compliance. In spite of the efforts to control the disease in New Brunswick, ISA spread to salmon farms in Maine. A program for the control of ISA in Maine has been adopted which is based on the success and failure of efforts in New Brunswick. Currently the cornerstone of ISA control in the Bay of Fundy is the early identification and removal of infected salmon.

The application of molecular genetics to aquatic pathogen detection and systematics

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The advances in molecular genetic technology during the past twenty years have facilitated progress on many fronts of aquatic disease research including pathogen detection and identification, host/parasite interactions, mechanisms of virulence and our understanding of the taxonomic relationships among pathogenic organisms and related taxa. There is now a relatively large database of molecular information available for microorganisms, which has been used for development of sensitive and specific detection assays for aquatic pathogens. In addition, DNA sequences, particularly small subunit (SSU) ribosomal RNA gene data, are used for molecular phylogenetic analyses.

Molecular diagnostics are now routinely developed for pathogenic microorganisms. DNA probes for in situ hybridizations and primers for use in the polymerase chain reaction (PCR) are available for a wide a variety of pathogens found in the aquatic environment. Molecular detection methods can greatly facilitate disease diagnoses, especially where it is difficult and/or time-consuming to isolate and identify pathogens and in situations where pathogens cannot be distinguished based on morphological characters. In addition, molecular diagnostics are invaluable when a particular species within a genus or even certain strains of the same species may be pathogenic, while closely related species or strains are harmless. However, in such cases, it is essential that molecular probes and primers are reliable, accurate and sensitive. For development of genus-specific, species-specific and strain-specific probes, therefore, it is important to obtain sequence data from as many different strains and species within a genus as possible. In addition, molecular data are needed from closely related taxa. Intra- as well as inter-specific sequence variation needs to be examined and adequately characterized. This optimizes the chance of developing a genus-specific probe that works for all members of the genus and of developing species-specific or strain-specific probes that do not cross-react with other species or strains. To minimize the chances of a species-specific probe failing to detect a particular strain of a species, as many strains as possible from a wide geographic range should be examined. Finally, in order to confidently employ molecular diagnostics, the assays should undergo rigorous validation against established diagnostic protocols.

Molecular data is often used for examining phylogenetic relationships among pathogens and related taxa, and has been particularly helpful with taxa whose morphological and ultrastructural characters are equivocal. DNA sequence differences are also being used more frequently to support and validate descriptions of new species. For assessing taxonomic affinities, as for development of molecular diagnostics, it is important to carefully select the targeted genes or genomic regions and to assess inter- and intra-specific DNA sequence variation at that locus. Sequence information, at the same gene, for a number of related taxa is required for phylogenetic analyses to be informative. In addition, there must be an appropriate amount of sequence variation present to allow for discrimination among taxa, but not so much variation that alignment and identification of positional homology become problematic. In conclusion, although molecular techniques can be very powerful, caution must be employed to ensure that assays are developed based on adequate molecular information and that the information and techniques are used appropriately.

Molecular epidemiology of aquatic pathogens

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At present very few aquatic pathogens have been investigated from a comprehensive epidemiological and evolutionary viewpoint. Infectious hematopoietic necrosis virus (IHNV) is a rhabdoviral pathogen that infects both wild and cultured salmonid fish throughout the Pacific Northwest of North America. IHNV causes severe epidemics in young fish and can also cause disease or occur asymptomatically in adults. In a broad survey of 323 IHNV field isolates, sequence analysis of a 303 nt variable region within the glycoprotein gene revealed a maximum nucleotide diversity of 8.6%, indicating unusually low genetic diversity overall for this virus. The virus isolates analyzed were obtained from both wild and cultured fish hosts, from juvenile and adult life stages, and from asymptomatic fish as well as fish with clinical disease signs or epidemic mortality. The isolates represented 106 different collection sites throughout the geographic range of IHNV and they were isolated over a period of 36 years from 1966-2001. Phylogenetic analysis revealed three major virus clades, designated U, M, and L, that varied in topography and genetic diversity. Segregation into phylogenetic clades correlated most strongly with geographic origin. The U clade had the largest geographic range, from Alaska through Oregon. It also contained the largest number of virus isolates, but it had the lowest intraclade diversity, suggesting genetic stasis. The L clade contained IHNV isolates from California and the southern Oregon coast, and it also had relatively low genetic diversity. In contrast, several measures of intraclade genetic diversity indicated that the M clade, which was focused in the rainbow trout farming region of south central Idaho, had 3-4 fold more diversity than the U or L clades, and exhibited relatively rapid evolution. In interpreting these epidemiological and phylogenetic patterns we hypothesize that factors influencing IHNV evolution may have included the ocean migration ranges of their salmonid host populations, host jumps between salmonid species, and anthropogenic effects associated with fish culture.

Applied studies on epizootic ulcerative syndrome

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The results of a three-year joint project between the Aquatic Animal Health Research Institute, Bangkok and University of Stirling, UK are described. The project aimed to develop improved methods for diagnosis and control of EUS in Asia. Researches was conducted in collaboration with a number of regional institutes to encourage contact between researchers, and help build a consensus among fisheries workers regarding EUS causation and control.

Diagnosis

A diagnostic PCR technique was developed using DNA extracts from cultures of *Aphanomyces invadans* isolated from affected fish. Attempts to use the PCR to identify *A. invadans* DNA in water from affected ponds, or in tissues of affected fish, proved unreliable. A comparative study of histological techniques identified a Uvitex-H&E staining technique (Hatai, pers. comm.) as the most rapid and reliable means of visualising *A. invadans* granulomas. Histological samples supplied by international scientists allowed verification of the presence of EUS in previously unconfirmed areas, namely Nepal, Vietnam, and Pakistan.

Control

In vitro studies on the fungus were also used to screen 50 compounds for antifungal activity. Candidate treatments were then tested in tank and pond trials. Bath challenge models were developed for use in pond trials to test preventative treatments. These trials were conducted in Thailand, Bangladesh and India. A variety of inputs were found to reduce the number of fish affected and/or the severity of infections, but none were able to prevent infection completely. Farmer-based studies in collaboration with the CARE-LIFE project showed that Bangladeshi farmers were keen to adopt treatments, and believed them to be successful.

Epidemiological studies in Bangladesh and Nepal identified a range of factors that increased the risk of EUS infection. Most significant of these was connection of ponds to natural water bodies and presence of wild fish in the pond. This was supported by prevalence data, which showed that EUS is endemic in natural water bodies in most of the areas examined. A range of management strategies to reduce the risk of EUS outbreaks was formulated.

Immunological studies demonstrated differences in the immune reaction of EUS-susceptible and EUS-resistant fish. Snakehead macrophages were found to be less inhibitory of *A. invadans* than macrophages from other fish. An immunostimulant trial indicated that Salar-bec (a vitamin supplement) is effective in boosting this non-specific response in susceptible snakeheads. Trials also showed that snakeheads from EUS-endemic areas have a strong serum response *to A. invadans* infection, and a passive immunisation trial showed that the antibodies involved are protective. This shows that there is potential for vaccine development, although this was not pursued further on this project.

Information dissemination

Collaborative research, seminar tours and production and dissemination of manuals and leaflets increased awareness of the project and its research outputs in South and Southeast Asia.

Antimicrobial effectors in marine molluscs and crustaceans

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Antimicrobial peptides are key elements of innate immunity, and their role in the defence against pathogens appears to be as important as antibodies or immune cell reactions. These molecules are ubiquitous, found in the entire living kingdom, ranging from plants to animals including mammals, fishes, amphibians, invertebrates such as insects, chelicerates, tunicates and recently molluscs and crustaceans. Of the 400 antimicrobial peptides reported, at least about 50% were mainly identified in insects.

Antimicrobial peptides are gene-encoded molecules containing 15 to 100 amino acids. Most of the peptides are amphipathic, with a net positive charge and presenting around 50% hydrophobic amino acids. These immune effectors share common characteristics and similarities in structural patterns and motifs: they are (i) cyclic peptides stabilized by intramolecular disulfide bonds, represented by the defensin peptides; (ii) linear peptides; or (iii) peptides characterized by a high content of proline and/or glycine residues. In general, the antimicrobial peptides have a broad range of activity and a weak cytotoxicity. They show a great diversity in modes of action. Antimicrobial peptides can be lytic or can block bacterial membrane biosynthesis, resulting in cell death.

In mollusc bivalves, to date, antimicrobial peptides have only been fully characterised in mussel, *Mytilus edulis* and *M. galloprovincialis*. They are all cysteine-rich peptides belonging to four classes : defensin-like or MGD1, mytilins, mytimicins and myticins. They present broad range of antimicrobial activity against Gram-positive and Gram-negative bacteria and against filamentous fungi. In oyster, the presence of such effector has only been reported. In crustaceans, antimicrobial peptides have been first evidenced and partially characterized in crabs, a 6.5 kDa and a 11.5 kDa peptide in *Carcinus maenas* and the 3.7-kDa callinectin in *Callinectes sapidus*, whereas in penaeid shrimps, a peptide sequence has been recently identified, with homologies with anti-LPS factor from Limulus. In the shrimp *Penaeus vannamei*, an original family of antimicrobial peptides named penaeidin has been fully characterized at the level of amino acid and cDNA sequences. These peptides present a chimeric structure consisting of two domains, a proline-rich N-terminal sequence and a cyclic C-terminal domain presenting six cysteine residues and a chitin-binding motif. The penaeidins display antimicrobial activity against Gram-positive bacteria and filamentous fungi and agglutinating properties for Gram-negative bacteria. Finally, in crustaceans, beside gene-encoded cationic peptides, peptides with antimicrobial activity were evidenced to derive from the processing or cleavage of pre-existing circulating molecule, as observed for hemocyanin, respiratory plasmatic protein.

Regarding production in organisms, antimicrobial peptide expression appears to be regulated by different tissue-specific pathways, and these effectors may consequently participate in either a local or a systemic reaction. Antimicrobial peptides are produced in phagocytic cells of vertebrates and invertebrates, and in various tissues such as epithelia of mammals and insects or insect fat body. Peptides are produced constitutively and stored in circulating cells, where they can act intracellularly against phagocytosed micro-organisms. Peptides can also be released by exocytosis upon microbial stimulation. In various epithelia of invertebrates and vertebrates, antimicrobial peptides are either produced constitutively or induced in response to infection or inflammation, and participate to a local antimicrobial reaction. Finally, antimicrobial peptide expression in fat body cells is induced in response to infection and peptides are secreted into body fluids, which characterises the acute or systemic reaction in insects.

In mussel, the antimicrobial peptides are constitutively synthesized as precursors in circulating hemocytes and the different classes of peptides are stored as active forms in distinct granules of different hemocyte populations. The expression and involvement of mussel peptides in immune response resemble that seen in mammals' neutrophils using antimicrobial peptides in phagolysosomes.

In crustaceans to date, the regulation of expression and distribution of antimicrobial peptides have been solely studied through the penaeidins in penaeid shrimps. The peptides are constitutively synthesized and stored in circulating hemocytes. Acquired data permit to distinct different phases in the shrimp response following microbial challenge or infections, where the hemocytes play a central role in the regulation and distribution of the peptides. Through their expression and immune functions, penaeidin represent a novel model of antimicrobial-mediated immune responses, which would be ubiquitous in crustaceans.

Antimicrobial peptides appear to be multifunctional molecules. A vast domain remains to be explored to determine their role and their interaction with other components of the defence mechanisms in bivalves and crustaceans.

Immune relevant genes in fish

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The fish immune system comprises of the non-specific and specific immune defences—having both humoral and cellular mechanisms—to resist against infectious diseases. However, the biodefence and immune related system of fish are poorly understood at the molecular level. Characterization of immune system of fish is important for development of vaccines and other infectious diseases prevention methods. In view of the above reasons, we used Japanese flounder for characterization of immune system in the molecular level. Expressed sequence tag (EST) analysis has been conducted on Japanese flounder, *Paralichthys olivaceus* liver, spleen, skin, rhabdo virus infected Japanese flounder leukocytes and Ig positive cells, and ConA/PMA or LPS treated leukocytes and kidney cells cDNA libraries. We sequenced more than 3,500 clones of these cDNA libraries. Many putatively identified immune relevant genes, such as cytokines, cytokine receptors, cell surface molecules, transcription factors, complement component, anti-microbial proteins, proteases and protease inhibitors were identified. We also cloned and characterized several immune relevant genes (TCRs, Igs, TNF, CD3, chemokines, and so on) from the BAC library. Currently the expression pattern for most of immune and defense related genes, from LPS or ConA/PMA treated peripheral blood leukocytes, after DNA vaccination study and after viral infection are characterized by quantitative real-time fluorescence RT-PCR and microarray which was spotted about 1,000 different Japanese flounder cDNAs. The expression patterns and the amount of expressed mRNAs of these genes were different in the various genes examined, stimulations and time points.

Recent developments in our knowledge of fish immune responses

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Early *in vivo* and *in vitro* functional studies have demonstrated that teleosts exhibit a wide variety of adaptive and innate immune responses, which, for the most part, are similar to those found in higher vertebrates. The recent identification of immunologically relevant genes orthologous to those in mammals confirms the notion that the basic aspects of immunity have been highly conserved during evolution. However, there are a number of novel immune related genes identified in fish that are either quite different from their mammalian counterparts, or have no recognizable mammalian equivalents, suggesting that certain aspects of teleost immune responses are quite different from those of mammals. Such genes include IgD, a second TCR ß gene, and Novel Immune Type Receptors (NITRS). For most fish species the lack of cell surface markers and immunologically relevant *in vitro* culture systems has severely limited critical functional assessments of not only novel but also recognized immune gene products. In light of this, *Ictalurus punctatus* (channel catfish) has proven to be a quite useful bony fish model for studying immunological functions. The *Ictalurus* culture system has allowed for the *in vitro* characterization of interactions between different cell lineages, and the currently unique availability of distinct clonal leukocyte cell lines has permitted the assignment of specific functions to cells possessing defined cell surface phenotypes in ways not currently possible in any other fish species.

Both autonomous and non-autonomous clonal lymphoid cell lines have been developed in channel catfish. The autonomous cell lines continuously proliferate without the need for stimulation or exogenous factors, and are represented by functionally active macrophage, T, and B cell lines. In contrast, the non-autonomous cell lines require periodic stimulation with antigen, and are represented by alloantigen specific cytotoxic T cells, and NK-like cells. The non-autonomous cytotoxic T cells lines have proven useful for identifying the signal transduction events operative during antigen and cytokine stimulation, as well as the cytotoxic mechanisms employed by these cells. Some, but not all, catfish NK-like cell lines are armed with IgM via a putative FcμR, enabling antibody dependent cell cytotoxic responses. Catfish NK-like clones have also been shown to express some of the recently described NITR genes. The NITR genes that have been identified in *Ictalurus* reflect an extraordinary continuum of structural variation, including certain NITRs that lack Immune Tyrosine Inhibitory Motifis (ITIMs) but encode a positive charged residue in the transmembrane region, reminiscent of activating receptors of the mammalian leukocyte receptor complex (LRC). Representative catfish NITRs exhibit both tissue- and apparent lineage-specific expression as well as regulated expression during the course of *in vitro* immune stimulation. It is anticipated that the development of additional important lymphocyte surface and functional markers will be facilitated though the use of such clonal cell lines and recombinant proteins.

Oral & Special Sessions
ABSTRACTS

A comparison of white sturgeon herpesvirus types I and II and development of specific diagnostic PCR assays

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The two members of the family Herpesviridae described from sturgeon are the white sturgeon herpesvirus type 1 (WSHV-1) and white sturgeon herpesvirus type 2 (WSHV-2). WSHV-2 is the more commonly encountered and most serious of the two viruses and leads to significant losses among cultured juvenile white sturgeon each year. Both WSHV-1 and 2 are similar morphologically and both attack the integument of white sturgeon. The viruses however, clearly differ from each other as shown by comparisons of the genome by RFLP analyses and DNA sequencing. Sequence similarities found in both viruses suggest an affinity to the alpha herpesviridae, which is a group of herpesviruses found solely in aquatic hosts. Additionally, as there are no treatments available for these viral agents in sturgeon, detection and isolation of infected individuals in a population is the best method of control. For this reason we have developed type-specific PCR assays for rapid, specific and sensitive diagnosis for both WSHV-1 and WSHV-2 in white sturgeon. In a recent study, we compared virus isolation to the newly developed PCR assay for the diagnosis of WSHV-2 infections in juvenile white sturgeon.

Experimental transmission and pathogenesis of *Piscirickettsia salmonis* isolated from white seabass (*Atractoscion nobilis*)

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Piscirickettsia salmonis is a significant pathogen of salmonids that has been reported in Chile, Europe, and Canada. While coho salmon (*Oncorhynchus kisutch*) are the principal hosts for *P. salmonis* infections, the disease has also been detected in rainbow trout (*O. mykiss*), chinook salmon (*O. tshawytscha*), and Atlantic salmon (*Salmo salar*) in Chile. Strains of *P. salmonis* isolated from Chile, British Columbia and Norway show similar ribosomal DNA sequences although they differed in virulence when compared in experimental laboratory trials with coho salmon. The origin of the initial infections with *P. salmonis* in Chile, where salmon are introduced species, is unknown although a marine fish reservoir has been suspected. During the course of examinations into losses of juvenile white seabass (*Atractoscion nobilis*) in southern California USA a *P. salmonis*-like organism was isolated and identified. This isolate shows rDNA sequences and antigenic characteristics that clearly indicate it is *P. salmonis*. This is the first isolation of *P. salmonis* from a nonsalmonid fish. Intraperitoneal injections of the white seabass isolate into both coho salmon and white seabass demonstrated its virulence for both fish species. The disease in both salmon and white seabass is similar, involving primarily liver (massive necrosis, granulomatous inflammation, macrophage aggregate and granuloma formation) with secondary spread and granulomatous inflammation in spleen, kidney, intestine, and gill. These studies clearly indicate that *P. salmonis* can occur in nonsalmonid fish species, and cause a disease similar to salmonid piscirickettsiosis.

Observations on the life stages of *Sphaerothecum destruens* gen. and sp. nov., a mesomycetozoean fish pathogen formerly referred to as the rosette agent

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The rosette agent is an obligate intracellular parasite initially identified in 1984 as the cause of a disease and the subsequent mortality of chinook salmon (*Oncorhynchus tshawytscha*) held in seawater net pens in the state of Washington USA and was detected in chinook salmon in California USA in 1994. The agents from both Washington and California states have been propagated continuously in the CHSE-214 line. The parasite from these cultures has been shown to induce experimental infection in both salmon and trout species that is clinically identical to the naturally occurring disease as originally observed in chinook salmon. The rosette agent shares similar morphologic characteristics to members of the class Mesomycetozoea, a novel group of parasites of fish, shellfish, mammals, birds and other animals near the choanoflagellates in the divergence between animals and fungi. Studies of the rosette agent both from infected fish tissues and as propagated in cell cultures have revealed previously unrecognized life stages of the parasite and modes of its transmission. These new observations combined with the available phylogenetic data on the other mesomycetozoeans have led us to conclude that the organism previously known as "the rosette agent" should be classified as a new genus and species: *Sphaerothecum destruens*.

The influence of methyl mercury on reproductive biomarkers in aquatic species

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A variety of anthropogenic (man-made) and natural chemicals in the environment are capable of modulating and/or adversely affecting or disrupting endocrine function in vertebrate organisms. Environmental pollutants such as o,p'-DDT, Bisphenol-A, Kepone, PCBs, and dioxins are some of the best studied endocrine active agents. Other commonly found compounds are not so well known in this capacity. Mercury contamination is a major concern in the southeastern United States, but little is known about effects of low level exposure on health and reproduction in wildlife. Mercury levels for wildlife are, in general, high and exposure assessments have indicated significant exposure and potential risks for multiple taxonomic groups including: birds, fish, reptiles, mammals and invertebrates. Mercury, in its most toxic form—methyl mercury—is a major contaminant in many aquatic ecosystems in the U.S. and biomagnifies easily up the food chain. Although commonly found in the environment, only a few studies have examined endocrine or reproductive function in aquatic organisms exposed at environmentally significant concentrations. Also, laboratory studies with largemouth bass, Nile tilapia, and freshwater mussels have showed that environmentally relevant levels of methyl mercury lowered plasma concentrations of reproductive hormones. Although not commonly considered as an endocrine active agent mercury may depress hormone production in fish by either interfering with gonadal development or with the pituitary-hypothalmic-gonadal axis. These results demonstrate that methyl mercury can affect plasma hormone concentrations and could potentially affect feral populations.

Development of real time PCR assays for detection of the oyster pathogen *Perkinsus marinus* in environmental water samples

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Perkinsiosis caused by the protozoan *Perkinsus marinus* is currently the most widespread and lethal infectious disease of the eastern oyster, *Crassostrea virginica*. Understanding transmission dynamics is critical for development of effective oyster resource management strategies that are predicated on pathogen avoidance. This requires examination of environmental parasite abundance in relation to host oyster mortality, infection acquisition rate and environmental factors. Recent advances in molecular techniques for the detection of *P. marinus*, combined with a real-time PCR system (Light Cycler, Roche Molecular Biochemicals) for DNA quantitation, constitute the required tools for sensitive, specific and quantitative detection of *P. marinus* in environmental samples. Strategies for optimizing this technique will be presented, as well as preliminary results of the application to environmental samples collected from several Chesapeake Bay sites.

European flat oyster pathogen *Marteilia refringens* life cycle investigations: how we found a needle in a haystack

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During the last thirty years, the European flat oyster *Ostrea edulis* underwent a severe decrease in production; from 30,000 tons per year in 1960 to 1,500 tons today. The Paramyxean protozoan parasite, *Marteilia refringens* is partly responsible for this decrease. The existence of an indirect life cycle, i.e. involving other host species, has been postulated for this parasite. However, the lack of appropriate tools to specifically detect all parasite developmental stages, as well as the high number of potential host species living in the vicinity of oyster beds, limited the investigation of this life cycle. This research recently became feasible with the development of molecular detection tools, specific and sensitive to any *M. refringens* developmental stage, which could be used to examine the fauna of a model system (the "claires" ponds) characterized by low biodiversity. Our strategy to demonstrate the existence of *M. refringens* host(s) species, involved sampling of the pond fauna, searching by PCR for *M. refringens* DNA among all the sampled species, localization of the parasite DNA by *in situ* hybridization, and confirmation of the involvement of a species in the life cycle by experimental transmission. This strategy identified the copepod *Paracartia grani* as a host of *M.refringens*.

Relationships among members of marine *Myxobolus* spp. from mullet based on small subunit ribosomal DNA sequences

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Six marine species of *Myxobolus* collected from species of mullet (*Mugil cephalus* and *Liza ramada*) in Ichkeul Lake, Tunisia, were studied to evaluate traditional criteria used in the identification and the taxonomy of myxozoan species, including spore morphology, host specificity and tissue tropism. Sequences representing 1600-1700 base pairs of the 18S rDNA from the examined marine species were characterized and distance analysis showed them as a monophyletic group that form a separate clade from freshwater *Myxobolus* spp. Sequence analysis confirms that these marine myxozoan isolates infecting mullet, which are morphologically different from each other are in fact different species. Furthermore, the molecular sequences showed that closely related species often infect the same host and tissue. These results demonstrate the utility of DNA sequence data in providing more insight into evolutionary relationships among the *Myxobolus* species. However, until the life cycle of myxosporean species has been elucidated, we should continue the use of spore morphology, with host and tissue specificity playing a secondary role, in the identification of species.

Implications for disease diagnoses of three Myxosporean parasites of finfish by *in situ* hybridization

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The use of *in situ* hybridization techniques for diagnosing various pathogens has increased dramatically over the past years. Non-radioactive protocols have been developed in our laboratory for detecting several myxosporean parasites including *Myxobolus cerebralis*, *Kudoa thyrsites* and *Tetracapsula bryosalmonae*, previously known as PKX, the organism causing proliferative kidney disease. The importance of this technique is based upon its ability to detect and localize the earliest stages of these organisms in their respective hosts or among suspect hosts, which is critical for the development of effective management strategies for these diseases. Recent findings on these parasites demonstrating the application of *in situ* hybridization will be presented.

Practical grower experiences in the proactive prevention and control of infectious salmon anemia

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Clinical ISA was first reported in Maine waters in 2001. Maine salmon growers have developed and implemented both proactive and reactive responses to the emergence of clinical ISA. Annual third party biosecurity audits were initiated in 1997. These audits proved to be a valuable learning tool for farm managers and greatly assisted in the identification of higher risk farm practices. In 1998 an ISA action plan was developed and implemented by the growers with the help of a number of fish health professionals. This plan was revised annually based on the evolving state of knowledge about ISA epidemiology, improving ISAV detection methodologies and experience in ISA management from other salmon producing areas facing ISA outbreaks. In 2001 the Maine Aquaculture Association developed and implemented a cooperative bay management program designed to increase grower coordination and reduce risks associated with fish health and environmental impact. The MAA Bay Management Agreement was based on the most recent MAA ISA Action Plan and a systematic review of cooperative bay management plans and biosecurity protocols from around the world. The MAA Bay Management Agreement establishes minimum standards, procedures and protocols designed to minimize disease risk and improve grower communications and cooperation. MAA has worked closely with state and federal authorities to ensure that grower practices and responses are consistent with the best available scientific guidance in aquatic disease prevention and response. The USDA ISA Program has been critical to the rapid and aggressive response to the emergence of clinical ISA in US waters. Continued close cooperation between federal and state authorities and the growers will significantly increase the effectiveness of any ISAV control and eradication efforts.

***Edwardsiella ictaluri* encodes a putative Type III secretion system**

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Type III secretion systems are utilized by numerous Gram-negative bacteria to translocate virulence proteins directly into eukaryotic cells. Assembly of the needle complex of type III secretion systems, which spans the entire Gram-negative cell wall, requires the coordinated expression of between 20 and 30 proteins. The assembled apparatus allows for direct translocation of effector proteins from the bacteria to the cytoplasm of the host cell, and is essential for virulence. A virulence-attenuated strain of *Edwardsiella ictaluri*, the causative agent of enteric septicemia of catfish, was identified using signature-tagged mutagenesis. Genetic analysis of the region surrounding the mutation reveals high similarity to several genes encoding inner membrane proteins of the needle complex of a type III secretion system. This finding indicates that a type III secretion system is involved in the pathogenesis of *E. ictaluri*.

The lack of a specific antibody response in haddock (*Melanogrammus aeglefinus*); immunoglobulin quaternary structure and proteomic diversity

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The development of non-salmonid marine finfish aquaculture requires effective disease management strategies for these species. Recent immunological studies on members of the family Gadidae, including Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*), indicate that these fish may respond differently to immunization than other vertebrates. We have investigated the effects of immunization of haddock on the specific antibody response, immunoglobulin (Ig) quaternary structure and Ig diversity. Specific antibody levels and immunoglobulin diversity were determined for groups of haddock maintained at 12°C and immunized with either a commercial vaccine against *Vibrio anguillarum* and *Aeromonas salmonicida*, trinitrophenyl coupled to keyhole limpet hemocyanin (TNP-KLH) and adjuvant, or saline and adjuvant. A non-injected group served as a control. Using ELISA we were unable to detect antibody responses to antigens in any of the groups. We were not able to detect any differences in Ig diversity between groups using two-dimensional electrophoresis and immunoblotting. We have found that the basic architectural structure of haddock Ig is similar to that of other vertebrates comprising of two 85 kDa heavy chains and two 25 kDa light chains. Data from non-reducing, denaturing agarose/acrylamide electrophoresis and analytical FPLC suggest that haddock Ig is tetrameric. However, with respect to levels of disulphide cross-linking, haddock Ig appears to exist in its highest cross-linked form as a trimer. The apparent absence of a specific antibody response in haddock suggests that other facets of immunity are responsible for immunocompetency in this species.

Comparison of bacterial loads in ESC-susceptible and resistant channel catfish with real-time PCR

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Enteric septicemia of catfish (ESC) is the most prevalent disease affecting commercial catfish farms. Susceptibility to the causative agent of ESC, the bacterium *Edwardsiella ictaluri*, appears to be consistent within each family of channel catfish. Real-time PCR technology was utilized to measure differences in bacterial loads and clearance rates for ESC-susceptible and resistant families of channel catfish during immersion challenge. Only surviving fish at each time point were included. The quantity of *E. ictaluri* DNA present in blood, kidney, and spleen samples from individual fish was compared for 2 families of catfish that exhibited strong susceptibility or resistance to ESC in previous challenges. No family differences ($p > 0.05$) in bacterial levels were determined in the blood, kidney, and spleen 2 hours post-exposure. Significant differences ($p < 0.05$) in the quantity of bacterial DNA between resistant and susceptible families were evident for blood and kidney tissue 24 hours following exposure to *E. ictaluri*, as well as in the blood 48 hours and 5 days post-exposure. However, differences ($p < 0.05$) were not observed in spleen tissue until 5 days post-exposure. Mean quantities of bacterial cell equivalents per 100 µL of blood at 24 hours post-exposure were $9.2 \times 10^4 \pm 6938$ for the susceptible family and $5.87 \times 10^4 \pm 4358$ for the resistant family. Overall, families that are susceptible to ESC carried higher levels of bacterial DNA in their blood than resistant families; however the clearance rate from blood for surviving fish in both groups was similar.

Patterns in pathological changes associated with *Kudoa* spp. from the pericardium of fishes in the northern Gulf of Mexico

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During examinations of about 1,300 fishes belonging to 130 species in 50 families we found 5 new host records for *Kudoa* spp., at least some of which represent new species. We report for the first time species from the Gulf of Mexico that infect and cause lesions in the heart/pericardium. Results based on gross and histological analysis suggest that the pericardium is the primary site of infection. Pathological changes in the pericardium associated with the infection include inflammatory infiltrate and granulomatous lesions that potentially could affect the health of the host. Of the approximately 50 known species of *Kudoa*, about half are typically found in the somatic musculature. Only 2 species are reported to infect primarily the pericardium. All isolates of *Kudoa* spp. from the pericardium of fishes from the northern Gulf of Mexico are similar molecularly and different from those sympatric species that typically infect the somatic musculature, but pathological responses to the *Kudoa* spp. from the pericardium vary among host species suggesting differences in host susceptibility. Comparing life history characteristics among the hosts with patterns in pathological responses may elucidate host-parasite relationships. This study was supported through NOAA Award No. NA06FL0501.

Overview of *Aphanomyces invadans* in menhaden along the East Coast of the United States

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Deep penetrating ulcers with histological evidence of penetrating fungal (oomycete) hyphae and chronic granulomatous inflammation have been observed in tributaries along the eastern seaboard of the U.S. since at least the early 1980's. This syndrome was termed ulcerative mycosis and the cause(s) somewhat controversial. In 1998, we were able to isolate *A. invadans* from the lesions of menhaden captured in the Wicomico River, MD. Molecular studies suggest this isolate is identical to *A. invadans* isolates from EUS (epizootic ulcerative syndrome) outbreaks in Southeast Asia and Australia. PCR analyses on tissue sections indicate the same pathogen is responsible for ulcerative lesions of menhaden from Delaware Bay to Georgia. Infectivity studies indicate this is a primary pathogen of menhaden. Evaluation of lesion incidence data and precipitation information for selected areas suggests that rainfall/runoff is an important factor in disease incidence. In laboratory studies we have shown that salinity significantly influences hyphal growth and sporulation. It is not known if other factors present in freshwater runoff will also influence oomycete growth. Current studies are directed toward 1) understanding the source(s) and transport of the pathogen in Maryland tributaries; 2) environmental factors that favor both hyphal growth and sporulation; and 3) correlation between lesion incidence in menhaden and concentration of fungal zoospores in selected tributaries.

The epidemiology and proposed prophylaxis of nodavirus related disease in commercially farmed Atlantic halibut (*Hippoglossus hippoglossus*) in Norway

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One of the greatest obstacles to culturing of Atlantic halibut (*Hippoglossus hippoglossus*), as for several other marine fish species worldwide, has been recurring mass moralities of larvae and juveniles due to nodaviruses causing viral encephalopathy and retinopathy (VER). To be able to explore any prophylactic measures against the virus, a thorough understanding of its epidemiology is needed. Nodaviruses consist of an icosahedral capsid 25-35 nm in diameter, containing two single stranded positive-sense RNA-molecules: RNA1 (3100 nt) and RNA2 (1400 nt), where the former encodes the RNA-dependent RNA polymerase and the latter encodes the capsid protein. A specific and highly sensitive RT-PCR method for the detection of nodavirus in tissue has previously been established. A semi-quantitative method based on ultra-centrifugation followed by RT-PCR has recently been developed to detect nodaviruses present in seawater. These methods, together with traditional histology and immunohistochemistry, were utilized on samples from commercial rearing facilities in order to enhance our understanding of the entry, shedding, and subequent spread of the pathogen. Latent carriers were identified. Recombinant capsid protein expressed in *E. coli* bacteria has also been produced following the sub cloning of RNA2 into a plasmid vector. The protein has been used to immunize rabbits in order to obtain specific polyclonal antibodies, as well as for an ELISA-test to detect serum antibodies in fish blood. The recombinant protein has further been the backbone in one of two attempted vaccine strategies for halibut broodstock.

Lymphosarcoma in hatchery reared yearling tiger muskellunge *Esox masquinongy* X *E. niger*

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Yearling hybrid tiger muskellunge (*Esox lucius* X *E. masquinongy*) being cultured within the Colorado Division of Wildlife fish hatchery system were found to have external lesions that were grossly and microscopically consistent with descriptions of esocid lymphosarcoma. Esocid lymphosarcoma has been described as a tumor of adult northern pike (*E. lucius*) and muskellunge (*E. masquinongy*) that has a proposed retroviral etiology. Due to concerns regarding the potential infectious nature of the condition in a hatchery environment, an experiment was conducted to determine if the lesion could be transmitted in the laboratory with cell-free filtrates to naive young-of-the-year (YOY) tiger muskellunge. At 32 weeks post challenge, grossly visible lesions were observed on the challenged fish. Histological evaluation of these lesions confirmed that they were esocid lymphosarcoma. We believe this is the first report of an epizootic of esocid lymphosarcoma in tiger muskellunge and in any esocid other than an adult fish.

Possible cataract preventative effect of histidine related to ionic balance and water regulation in fish lens

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Cataracts have been described in wild and farmed salmonid populations. The condition has caused significant losses in European aquaculture in the last 10-15 years, mainly affecting Atlantic salmon (*Salmo salar*) in seawater. Cataract causative factors have been related to environmental, physiological, genetic and nutritional conditions. The role of nutrition received special attention after the omission of mammalian bloodmeal in salmonid diets seemed to coincide with occurrences of outbreaks of cataracts in salmon farms in the 1990's. Our feeding trials show that histidine, an amino acid especially high in bloodmeal, has a cataract mitigating effect. A high percentage of senile cataracts in humans have osmotic involvement. Experiences from the salmon industry have shown that sub optimal timing of smolt transfer to seawater, as well as fluctuations in salinity in sea farms induce osmotic cataracts. Although such salmonid cataracts are considered reversible, it is postulated that severe or repeated osmotic changes may lead to irreversible lens opacifications. At the University of East Anglia, Norwich, lens culturing systems have been developed, to investigate osmoregulation in the salmon lens. The response to hyperosmotic incubation conditions have been tested, measured as changes in lens transparency, volume and Na^+ influx. Results of feeding trials, partly combined with *in vitro* studies will be presented and a possible cataract-preventing role of histidine related to lens osmoregulation will be discussed.

The function of antibodies in the digestive tract of sea lice *Lepeophtheirus salmonis*

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The gut of the sea lice *Lepeophtheirus salmonis* is considered a suitable immunological target for vaccines, modelled on the successful vaccine against the tropical cattle tick *Boophilus microplus*. No studies have been carried out into the physiology of the gut of *L. salmonis* or the function of the Atlantic salmons' (*Salmo salar*) antibodies *in vivo*. This study characterises the environment of the digestive tract of *L. salmonis* and the ability of Atlantic salmon antibodies to function under these conditions. Fed and starved sea lice were investigated; starved (48 hours), fed, fed (with blood visible in the gut). The osmolarity and salmon haemoglobin level of the gut contents were measured to determine the osmolarity, as well as allowing for the calculation of the dilution of salmon blood in the gut. The ability of salmon antibodies to function under these conditions was established by an ELISA against HGG using buffers that mimic the conditions found *in vivo*. Starved lice gut contents had an osmolarity of 968±31mOsmol/l^{-1}, fed lice 789±38 mOsmol/l^{-1} and blood fed lice 684±11mOsmol/l^{-1}. This was isotonic with the haemolymph in starved animals and hypo-osmotic in both groups of fed animals. Salmon antibodies were found to function in osmolarities up to 400mOsmol/l^{-1} but lost 55-64% of it binding ability above this. The dilution of haemoglobin with the gut contents was undetectable (starved), 1:41,600 (fed), and 1:2,936 (blood). The gut of sea lice remains osmotically high, suggesting that seawater is ingested when feeding. The osmotic environment in the gut does not allow antibody function. The low level of haemoglobin in the gut suggests that blood is not a major component of the sea louse diet and the considerable dilution of blood would seriously impeded the function of antibodies in the gut of sea lice.

Identification of virulence genes in the aquaculture pathogen *Streptococcus iniae*

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Disease associated with the pathogen *Streptococcus iniae* represents a serious health and economic problem in cultured fish species worldwide. In North America, the tilapia and hybrid striped bass (HSB) industries have been particularly impacted. Understanding the genes associated with virulence in this bacterium can provide targets for disease treatment and vaccine development. To this end, we sought to (1) characterize strains of *S. iniae* associated with invasive infection in HSB, (2) perform random transposon mutagenesis of a suitably virulent strain, (3) develop a HSB challenge model to screen mutants for reduced virulence, and (4) identify the specific genes disrupted in attenuated mutants. Evaluation of eight *S. iniae* strains isolated from a commercial HSB production facility led to the identification of reproducibly virulent J289. A challenge model was developed using ~25-gram HSB injected intraperitoneally with 100-µl of bacterial suspension, and observed for 6 days at 26°C for mortality. The LD_{90} of J289 was determined experimentally in these conditions to be ~$4x10^6$ CFU of bacteria. Wild-type strain J289 was rendered competent and transformed with temperature-sensitive plasmid pTV_1OK containing transposon Tn917. Chromosomal transposition events were selected by erythromycin resistance at the nonpermissive temperature (37°C), and a library of highly random, single insertion mutants confirmed by Southern analysis. Mutants were screened and confirmed in the HSB challenge model at the wild-type LD_{90}. To date we have identified one completely avirulent and five significantly attenuated *S. iniae* transposon mutants. Detailed genetic analysis of the chromosomal loci affected by transposon integration is in progress.

Pathological effects of blood flukes (Sanguinicolidae) on marine fishes relative to aquaculture

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Fish blood flukes (Sanguinicolidae) infect the vascular system of fishes, and some are pathogens of fishes in aquaculture systems. Cultured hosts associated with appropriate snail, bivalve, or polychaete intermediate hosts may accumulate heavy infections of adult worms and their eggs. The resulting disease, sanguinicoliasis, has caused mass mortalities of fishes in ponds and cages in North America, Europe, and Asia. In the sanguinicolid life cycle, the cercaria emerges from the intermediate host and then penetrates into and matures in the definitive fish host. The resulting adult worm releases eggs into the fish's vascular system. These eggs may be sequestered in gill, heart, kidney, liver, spleen, pancreas, or other organs where they cause inflammation and infarcts as well as decrease organ efficiency. Infections by particular species under appropriate environmental conditions kill the host. Treatment of debilitated fishes is difficult, and the combination of stock destruction and facility disinfection is a realistic option for managing cases in freshwater systems. However, early detection of the parasite, careful site selection and construction of culture facilities and elimination of infected hosts (definitive or intermediate) are suggested for marine systems. Histological evaluation of four wild fishes demonstrates that the adult flukes and their eggs affect these hosts differently. For example, the heart of different species exhibits different types of inflammation in different degrees, some harmful. The response to the egg also differs according to host and site and perhaps also to intensity and season. Study supported by NOAA/NMFS Nos. NA06FL0501, NA16RG1646, and NA17FF2010.

Antigenic and immunogenic properties of *Flavobacterium psychrophilum*

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Identification of distinct properties and specific antigens important in conferring protective immunity against bacterial coldwater disease is highly desirable. Differential characteristics between virulent and non-virulent strains of *Flavobacterium psychrophilum* have been investigated in an attempt to identify potential virulence factors. The antigenic nature of these strains and their ability to confer protective immunity to rainbow trout fry was evaluated. Single and 2-dimensional PAGE comparisons of the virulent (CSF-259-93) and non-virulent (ATCC 49418) strain has shown that differential gene expression does occur. Preliminary analysis shows that at least two major proteins (p15 and p53) appear to be represented in the virulent (CSF) strain, while at least one (p37) protein is differentially expressed in the ATCC strain. Western blot analysis of trout immune serum shows that antibodies are directed to distinct antigenic components of *F. psychrophilum*. Trout fry challenged with $1.25x10^6$cfu/ml of the CSF and ATCC strains exhibited a cumulative mortality of 91.8% and 30.7%, respectively. An earlier study showed that fish surviving challenge with the virulent (CSF) strain exhibited a relative percent survival (RPS) of 92.8% when re-challenged with this strain. However, RPS of fish surviving challenge with the non-virulent (ATCC) strain was only 31.3% when re-challenged with the CSF strain. Taken together, it appears that the ATCC strain lacks the ability to confer a cross-protective immune response due to an absence of key immunogenic components.

Genetic evidence for more than one species of costia (*Ichthyobodo necator*)

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Ichthyobodo necator ("costia") is a common and important parasite that is found on the skin and gills of a wide variety of freshwater and marine fish. Whether costia isolates from different hosts are separate species has been a matter of debate. To address this question, genomic DNA was isolated from costia trophonts collected from rainbow trout (*Oncorhynchus mykiss*), koi (*Cyprinus carpio*), mirror carp (*Cyprinus carpio*), goldfish (*Carassius auratus*), channel catfish (*Ictalurus punctatus*), swordtail (*Xiphophorus helleri*), and Japanese flounder (*Paralichthys olivaceus*). The small-subunit ribosomal RNA (SSU rRNA) gene was amplified and cloned. The resulting sequences were aligned relative to each other based on a previously sequenced costia isolate from hybrid striped bass (*Morone saxatilis* male x *M. chrysops* female). The phylogenetic relationships among isolates were determined using maximum-likelihood statistics. Our phylogenetic data suggested that rather than being a single species, costia is actually a complex of at least four groups that probably represent different species. One group consisted of the hybrid striped bass and swordtail isolates. The second and third groups consisted of the rainbow trout and mirror carp isolates, respectively. The fourth group consisted of the koi, goldfish, catfish, and flounder isolates. The four groups differed among each other in their SSU rRNA sequence by 2-5 %. The significance of these findings to our understanding of the epidemiology of costia will be discussed.

Investigations of an *Isonema*-like flagellate causing mortality in larval hard clams, *Mercenaria mercenaria*

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In February 2001 a commercial hatchery in Virginia experienced severe losses of larval hard clams. Larval mortalities occurred repeatedly during a two-month period with larval cohorts generally dying between day 3 and 7 post-spawn. During this period larvae were concentrated on the bottom of the culture tanks and did not clear food. Clinical observations of fresh material collected following separate spawns revealed high abundances of empty shells or high prevalences of abnormal live animals, which exhibited velar hyperextension, velar cell rounding and sloughing, and velar cell deciliation. A protistan organism was observed emerging from the shells of moribund individuals. This organism exhibited a slow undulating motion and possessed two polar flagella. Histological evaluation demonstrated a prevalence of 40-50%; infections ranged from light to very heavy in intensity. In section, the protist cells were round to ovoid, contained an eccentric vacuole, and were found in the extrapallial space. The protist was successfully isolated and cultured. Culture of the organism facilitated electron microscopic studies, molecular analysis, and transmission investigations. Electron microscopy of the cultured organism revealed ultrastructural characteristics consistent with *Isonema*-like flagellates. Small subunit ribosomal DNA of the protist was sequenced and a BLAST search indicated high sequence similarity with marine euglenozoa including *Diplonema* sp. Challenge experiments demonstrated that the cultured *Isonema*-like organism is both invasive and pathogenic to larval hard clams.

Cellular response of crustacean hemocytes to lipopolysaccharides

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Aquatic organisms, having developed various defense strategies, respond immunologically to both abiotic and biotic factors. "Disease," being defined as a "demonstrable negative deviation from the normal state (health) of a living organism," can be delineated only after establishing baseline values for normality. In freshwater crayfish, the primary biotic agents responsible for disease include viruses, bacteria, rickettsia-like organisms, fungi, protists and metazoans. The immune response is mediated by cellular and humoral components. The primary objective of this study was to assess the *in vitro* immune response of crayfish *Procambarus zonangulus* against microbial molecules (lipopolysaccharides (LPS) of Gram-negative bacteria). As measured by flow cytometry, the response of circulating hemocytes to LPS was a decrease in cell size and a reduction of viability measured after staining with calcein-AM and ethidium homodimer dyes. Hemocytes incubated with detoxified LPS (LPSdex) resulted in decreased cell size, but viability was maintained. The pattern of cellular activation by LPS indicated that the polysaccharide moiety was stimulatory and the Lipid A moiety was noxious. Phenoloxidase specific activity from incubation mixtures using control and LPSdex treatments were significantly lower than from LPS treatments ($P \leq 0.05$). An animal exposure assay to the pesticide fipronil resulted in hemocyte cell responses not unlike those observed with LPS. These multiparametric and kinetic analyses of response mechanisms in crustaceans document baseline information useful for assessments of crustacean health in aquatic environments.

The low molecular weight gill proteome of WSSV-challenged *Litopenaeus vannamei*

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Viruses abound in marine and estuarine environments, and several have emerged as important pathogens of aquacultured penaeid shrimp. While shrimp responses to bacterial and fungal disease challenges have been increasingly well documented, shrimp immune and physiological responses to viral challenges remain largely unknown. We are using 2D chromatography and mass spectrometry to examine the low molecular weight (< 20 kDa) proteomic response of *Litopenaeus vannamei* gill to White Spot Syndrome Virus (WSSV) challenge. WSSV-infected shrimp homogenate was injected into the tail muscle of *Litopenaeus vannamei* maintained in aquaria. Gills were collected from challenged animals at T = 0, 4, 6, 12, and 24 hr (N = 8 / time interval) and from unchallenged controls, and homogenized in anticoagulant buffer. Tissue debris was pelleted by centrifugation, and the supernatant retained for analysis of soluble proteins. Direct MALDI mass spectrometry of the supernatant revealed that the most prominent changes with time after challenge were an increase in expression of 8.2 and 9.3 kDa molecules, and the decrease in expression of an 8.5 kDa molecule. First (gel filtration) and second (reverse phase HPLC) dimensional chromatographic separation, followed by MALDI-MS, revealed numerous other expression changes, including the downregulation of 2.3 and 2.5 kDa molecules and the upregulation of 3.5, 5.7, and 7.3 kDa molecules. Characterization of protein expression changes continues, and proteins of interest are being sequenced by tandem mass spectrometry.

Effects of urban land-use on stonerollers in the Mobile River Basin

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As part of the National Water Quality Assessment Program (NAWQA), nine sites of different urban intensities were utilized to investigate potential impacts to biological endpoints associated with water quality. This study is part of a larger NAWQA study to investigate effects on urban land-use on small streams in the Mobile River Basin. Our data, in cooperation with the USGS, will be used to evaluate fish health via histopathology and biomarkers measured in a small, relatively short-lived species. Central stonerollers (*Campostoma anomalum*) were collected from nine sites located around Birmingham, AL. Twenty stonerollers were collected at each site by electrofishing and examined for external and internal lesions. Livers were removed and frozen in liquid nitrogen for analysis of glutathione concentrations (GSH), and DNA strand-breaks. Whole fish were either preserved in 10% buffered formalin or frozen, and sent to the USGS National Fish Health Lab, Kearneysville, WV, for histopathological examination or spore enumeration, respectively. Concentrations of GSH from sites with high urban intensities were statistically higher from those from low urban land-use intensities. Histopathological examination found a parasite within the connective tissue. The parasite appears to proliferate in the fish from areas with high land-use. These results indicate that fish health biomarkers do respond to urban land-use intensities, although significant differences may only occur between the highest and lowest stations in the urban gradient.

***Flavobacterium psychrophilum* infection of Atlantic salmon eggs among from the northeastern United States**

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Restoration of Atlantic salmon in the northeastern United States concentrates on stocks in the Connecticut, Merrimack, Penobscot and several smaller rivers in Maine. River-specific brood stocks conserve the genetic integrity of salmon within each river. Gametes produced from limited numbers of adult salmon returning to each of these rivers are extremely valuable and must provide a sufficient number of offspring to continue restoration. In the course of study, chronic symptoms of Bacterial Coldwater Disease, caused by *Flavobacterium psychrophilum*, often affected F_1 generation offspring of sea-run adults. Further study showed that the pathogen also caused mortality among yolk-sac fry in Heath incubators and reductions in egg viability were apparently associated with intra-ovum infection. Evidence for vertical transmission of *F. psychrophilum* was widespread throughout the range of populations encompassed by the restoration program. The pathogen, therefore, has a significant role in the production of quality gametes and may be an important factor in the post-stocking survival of salmon fry and smolts.

Development of a strain typing assay for ISAV

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In 1997, ISAV was first identified within aquaculture cages of the Bay of Fundy, Canada. Initial focus of surveillance and fallowing of infected sites has expanded to include characterization of ISAV isolates within Atlantic Canada so as to identify strains that might be associated with high host mortality and to enable farmers and industry to track the disease. To achieve this characterization, we are developing a diagnostic assay for use in surveillance programs capable of providing quick, reliable results for ISAV detection and concurrently type ISAV isolates. In this assay, RT-PCR, which has proven useful in surveillance efforts, has been combined with DGGE technology to identify and type ISAV based on nucleotide sequence variability within a specific gene segment. Following initial standardisation with known isolates, DGGE may be performed on RT-PCR products to rapidly determine the strain of each sample without the need for additional manipulations such as sequencing or restriction enzyme digestion.

Effect of extracellular products from a *Vibrio* isolate on the haemocytes of the edible crab, *Cancer pagurus*

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Previous studies have shown that the extracellular products (ECP) of a *Vibrio* sp. isolated from shell disease-infected edible crabs (*Cancer pagurus*) cause rapid death when injected intrahaemocoelically (Vogan et al., *Microbiology*, 148, 743-54, 2002). The present study examined the effect of ECP on the haemocytes of *C. pagurus*. Injection of ECP into crabs caused a rapid decline in the total number of circulating haemocytes. Heat treatment (100°C for 30 min) of the ECP had no effect on the ability of these preparations to cause mortality of the crabs and the decline in circulating haemocytes. Histological examination of ECP-injected crabs showed that the haemocytes normally found in circulation formed large clumps in the branchial septa and lamellae of the gills. Incubation of ECP with haemocytes *in vitro* for >90 min caused a rapid clumping reaction. Long-term (72 hr) incubation of Rose Bengal-stained live crab haemocytes embedded in Marine Agar 2216 gel streaked with the *Vibrio* isolate, resulted in the lysis of these cells at the margins of the bacterial colonies. Overall, these results show that the ECPs of this isolate cause a rapid clumping reaction of the haemocytes without any apparent loss in viability in short-term (>90 min) incubation. Longer-term incubation (72 hr) leads to lysis of the haemocytes. Whether the haemocyte activating factor(s) are the same of the toxic factors that cause death of the crabs is discussed as is the chemical nature of the active principle(s).

Biomarkers from finfish in the Calcasieu Estuary, Louisiana, USA

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Biomarkers from organismal, cellular, and molecular levels of biological organization were collected from fish in April and October of 2000 as part of a U.S. Fish and Wildlife Service effort to assess natural resources at the Calcasieu Estuary in southwest Louisiana. Since the 1920s, petrochemical and agrochemical activities have impacted the estuary at which the U.S. Environmental Protection Agency is currently conducting human health and ecological risk assessments. Although 13 species were collected for the risk assessments from 11 designated contaminated sites and 3 reference sites, sample sizes for black drum (*Pogonias cromis*), red drum (*Sciaenops ocellatus*), and spotted seatrout (*Cynoscion nebulosus*) allowed for the most robust analyses. Data were collected on relative condition factor (Kn), organosomatic indices (liver, spleen, and gonads), splenic macrophage aggregate (MA) area and frequency, blood DNA integrity, the hepatic enzyme 7-ethoxyresorufin o-deethylase activity (EROD), and ages were estimated from otoliths. For all three species, EROD activity and DNA abnormalities were significantly higher ($p \leq 0.05$ for both) at contaminated sites. For red drum and speckled trout, Kn was significantly lower ($p \leq 0.0001$ for both) at contaminated sites. Unvalidated fillet contaminants values were not useful for analyses. When collected from adequate numbers of more than one fish species exhibiting different life histories and physiologies, data from such biomarker analyses reliably reflect environmental condition. Results and interpretations for the biomarker studies will be presented.

The genetics of ISAV

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Molecular analysis of infectious salmon anaemia virus (ISAV) has provided the complete genome sequence and enabled identification of major genes and their products. Although ISAV is an orthomyxovirus, it has a genome organisation that is strikingly different to that found in influenza viruses. The work of major groups to elucidate the genome of the virus and analyse its genes will be reviewed. Analysis of nucleotide sequences has proved extremely valuable in epizootiological investigations and the relative value of different genes in this work will be discussed. Detection of ISAV and diagnosis of ISA has come to rely heavily on molecular tests such as PCR. The available methods and the interpretation of results will be presented.

Presence of *Flavobacterium psychrophilum* on eggs of rainbow trout (*Oncorhynchus mykiss*)

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In a previous Danish study *Flavobacterium psychrophilum* was found in ovarian fluid and milt as well as outside fertilized eggs but not inside the eggs. The possibility that *F. psychrophilum* is vertically transmitted via the egg has been raised. A rainbow trout hatchery with outbreaks of bacterial cold-water disease (rainbow trout fry syndrome), caused by *F. psychrophilum*, was included in the study. The following samples were examined bacteriologically: milt, ovarian fluid and unfertilized eggs at the time of stripping, fertilized eggs, and at the eyed-egg stage. The unfertilized and fertilized eggs were examined both before and after disinfection with an aqueous iodine solution. The samples were inoculated onto agar plates and into enrichment media: tryptone yeast extract salts (TYES) agar/broth, Blood agar, and TYES agar/broth added sulfadiazin and trimethoprim. The bacteriological examination of the different samples will be presented in details. Furthermore, some of the samples and enrichment media were examined using nested PCR with DNA probes against a sequence of the 16S rRNA genes. The route by which the bacterium might gain access to the egg was studied in an experiment by adding a strain of *F. psychrophilum* to the milt or to both the milt and eggs before fertilization and water-hardening. Totally, more than 2000 eggs were examined. The results showed that *F. psychrophilum* was present on the surface of eggs. However, the observations obtained by other authors of *F. psychrophilum* being inside eggs were not confirmed in this study, neither in naturally nor experimentally infected eggs.

Dose titration of oxytetracycline against *Streptococcus iniae* infection in blue tilapia

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Experimental trials were performed to evaluate the efficacy of oxytetracycline (OTC) in controlling *Streptococcus iniae* infection in blue tilapia, *Tilapia aurea*. Minimum-inhibitory-concentration studies of OTC against multiple *S. iniae* isolates indicated general sensitivity at concentrations of 0.25-0.5 µg/ml.. Oxytetracycline dose levels tested were 25, 50, 75 and 100 mg active ingredient per kilogram of fish body weight per day. Administration of medicated feed started within 4-5 h after infection by waterborne exposure to *S. iniae* (after skin scraping) and continued for 14 consecutive days, followed by a 21 d post-treatment observation. The 50 mg OTC treatment significantly increased the survival of the infected tilapia from 7 % in the infected non-medicated group to 45 %. The 75 and 100 mg OTC treatments had survival rates (85 and 98 %, respectively) significantly higher than the 50 mg treatment. There was no significant difference between the 75 and 100 mg treatments and the uninfected non-medicated treatment (100 % survival). Survivors of the 100 mg OTC treatment were not detected to be carriers of the infection (negative bacterial isolation) whereas the bacterium was recovered from 10 % of the survivors receiving the 75 mg OTC level.

Susceptibility of *Myxobolus cerebralis*-infected rainbow trout fingerlings to infectious pancreatic necrosis virus

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Myxobolus cerebralis, the etiologic agent of whirling disease, can have numerous deleterious effects on its salmonid host, including potential impacts on immune function. We examined by laboratory trials the effects of *M. cerebralis* infection on the susceptibility of rainbow trout to a viral pathogen, infectious pancreatic necrosis virus (IPNV). Rainbow trout fry, 45 days post-hatch, were bath exposed to triactinomyxon spores (TAMs) of *M. cerebralis* (660 TAMs/fish). At 90 days post-hatch, TAM-exposed fish that were developing clinical signs of whirling disease and unexposed control fish were challenged with 10^5 PFU/ml of IPNV. Mortality levels were monitored for 28 days post-challenge. Individual dead fish and survivors were tested for IPNV by plaque titration of visceral organ homogenates. All fish dying during the 28 day trial tested positive for IPNV (titer $\leq 10^7$ PFU/ml). Overall numbers of fish dying with IPNV were similar between the TAM-exposed and unexposed groups (4.0% and 3.0%, respectively), yet the mortality began and ended 4-5 days earlier for the TAM-exposed fish. No mortalities occurred among control tanks of either TAM-exposed or unexposed fish that were not challenged with virus. At abstract submission, we are currently determining the prevalence and titer of IPNV infection, as well as the prevalence of *M. cerebralis* infection among survivor fish from both TAM-exposed and control groups.

A disease of cultured paua (*Haliotis iris*) in New Zealand caused by a novel haplosporidian

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Mortalities of juvenile paua (*Haliotis iris*) in a commercial culture facility were reported during the Austral summers of 1999/2000 and 2000/2001. Histology of moribund paua showed heavy infections of a novel organism confirmed by TEM and molecular studies to be a haplosporidian. The haplosporidian was associated with chronic mortalities of 82.5–90% in affected raceways. Heavily infected paua exhibited behavioural abnormalities including lethargy, loss of righting reflex, and easy detachment from surfaces. Early infections were characterised by low numbers of multinucleate plasmodia in the connective tissue surrounding the gut, amongst glial cells adjacent to nerves in the mantle and foot, and within gill lamellae. In heavy infections large numbers of plasmodia were present in the hemolymph, gills, heart, kidneys, mantle, foot, epipodium and connective tissue of the digestive gland. Infections could not be transmitted horizontally to healthy juvenile paua by cohabitiation with heavily infected paua, or by injection of healthy paua with hemolymph containing haplosporidian plasmodia. This may indicate that disease is not expressed below 20°C, or that an intermediate host is required for transmission. Spore formation was not observed in juveniles, but sporocyst-like bodies containing acid-fast putative spores were observed in the right kidney of poorly performing adult paua collected from the wild. A survey of 1094 paua collected from 5 spat producing farms and 3 grow out farms during the Austral summer of 2001/2002 failed to detect the haplosporidian. A number of other disease syndromes were detected, however these are considered to be of minimal regulatory significance.

A national survey to demonstrate freedom from white spot virus and yellow head virus in Australian crustaceans

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In November 2000, mud crabs within the Darwin Aquaculture Centre and prawns within the Aquaculture School at the Northern Territory University reacted positively in polymerase chain reaction (PCR) tests specific for white spot virus (WSV). The facilities were immediately destocked and disinfected. In response to concerns that the WSV may have spread beyond the aquaculture facilities, a national survey was designed and conducted to assess the WSV status of Australia's wild crustacean populations. Over 3000 crabs, prawns and other crustaceans were collected from over 60 sites throughout Australia and tested by PCR for WSV. Simultaneously, samples were also examined for the presence of yellow head virus by PCR. No sample contained WSV or YHV, and Australia was shown to be free of these viruses.

Injury and potential for *Renibacterium salmoninarum* transmission in chinook salmon *Oncorhynchus tshawytscha* marked with coded wire tags by conventional and automated methods

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Research has shown that the salmonid pathogen *Renibacterium salmoninarum* (Rs) and other bacteria can become concentrated in the recirculating water of fish marking facilities. In addition, Rs infections can become established in sites of coded-wire-tag (CWT) implantation or in sites of incidental integumental injury occurring during handling. This study compared injuries and the potential for Rs transmission among groups of juvenile chinook salmon marked with adipose fin clips and CWT in a conventional marking trailer and in a new automated trailer. The conventional trailer uses recirculating water to deliver anesthetic, and fish are handled by humans for fin clipping and placement in CWT machines. The automated trailer uses specially designed trays that encourage fish to enter marking lines volitionally, a gating system, and gentle mechanical holding, to accomplish fin clipping and tagging of fish without anesthetic in single-pass water, and with minimal handling by humans. Preliminary analyses of skin and fin injuries by cell viability staining and digital photography showed no differences ($p>0.05$) between the two trailers in proportions of fish with incidental head, body or fin injuries at a given stage of the marking process. However, both procedures were associated with significant incidental injuries, beginning with initial netting of fish from raceways into holding tanks. Bacterial cultures of water samples showed the highest counts of total bacteria in the recirculating water of the conventional marking trailer. Analyses of Rs in water and fish, and possible methods for reducing fish health risks of marking procedures, will be presented.

The status of epidemiologic studies of ISA in Maine

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Infectious Salmon Anemia Virus (ISAV) was first confirmed in February 2001 in Maine. It has since crippled Maine's salmon aquaculture industry, leading to the depopulation of 1.5 million fish in the winter of 2001-2002. We report on preliminary findings from our efforts to track the spread of the virus and identify risk factors associated with this epidemic. We focus on several points of discussion including: (1) a review of the use and impacts of ISA vaccines; (2) the roles of sea lice and subsequent mitigation strategies during and following the outbreak; (3) the roles of fomites such as boats, cage flotation, nets, water and other equipment at cage sites in Cobscook Bay; and (4) the impacts of the use of moist feeds in the early marine grow out phase. We conclude with a discussion of possible implications of these findings for United States/Canadian efforts to manage and prevent the disease.

Photobacterium damselae subspecies _piscicida_ is capable of replicating in hybrid striped bass macrophages

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The purpose of this study was to evaluate the ability of a virulent *Photobacterium damselae* subspecies *piscicida* strain to survive/replicate within macrophages obtained from hybrid striped bass using an *in vitro* assay. Results indicated that the number of bacteria recovered from macrophages at 3, 6, 12 and 18 hours of incubation increased significantly 105, 510, 1091 and 1385%, respectively, from time 0. Growth and multiplication of the intracellular bacteria was observed at a very low multiplicity of infection (1 bacterium to 100 macrophages). In contrast, the numbers of *Escherichia coli* control strain recovered from macrophages declined 47, 80, 91 and 96%, respectively, at 3, 6, 12 and 18 hours. Light and electron microscopy confirmed internalization, uptake, and multiplication of bacteria within spacious, clear vacuoles in the macrophages. Using acid phosphatase as a lysosomal marker, we provide evidence that *P. damselae*, by an unknown mechanism, inhibits phagolysosomal fusion. This study demonstrates that *P. damselae* is a very successful facultative, intracellular pathogen that can survive and multiply within hybrid striped bass macrophages.

Comparative susceptibility of different rainbow trout strains to infection with _Myxobolus cerebralis_, the causative agent of whirling disease

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The susceptibility of four different Bavarian rainbow trout strains and one brown trout strain, as well as one rainbow trout strain from the United States, to *Myxobolus cerebralis* was assessed following dosed exposures in controlled laboratory trials to the infectious stages, triactinomyxons, as well as in field exposure. Both studies were conducted with age-matched rainbow trout of each strain. In the laboratory experiments, fish were exposed to 0, 10, 100, 1000, 5000 triactinomyxons per fish for 3h. The fish were then removed and held in individual 50 l aquaria receiving single pass of 12°C well water for the duration of the experiment. Severity of infection was evaluated 5 mo after exposure by presence of clinical signs (whirling and or black tail), prevalence of infection, severity of microscopic lesions, and spore counts. In the field study, a metal net cage with four sections was prepared and placed in a trout farm fish pond in which *M. cerebralis* was known to be endemic. Five hundred rainbow trout each of one German strain and one American strain were placed separately, each in one section of the cage. Fish were observed twice a week for clinical signs. Five months post exposure the experiment was terminated and sampled as described under the laboratory exposure experiments. Results from both trials verify that one German strain of rainbow trout reared in Bavaria is highly resistant to whirling disease in comparison with two other strains from Germany and the TL-strain from the U.S. The mechanisms underlying the greater resistance of the GR strain of trout to *M. cerebralis* infections are under investigation.

Genetic changes in infectious pancreatic necrosis virus due to cell culture adaptation

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Challenge studies with infectious pancreatic necrosis virus (IPNV) have often been problematic due to the rapid loss of virulence after a few passages in cell culture. The molecular basis for this attenuation is not fully understood. We have tested the virulence of ten different Norwegian strains of IPNV serotype Sp by challenging Atlantic salmon fry. The full-length sequence of segment A has been determined for all the ten isolates after two cell culture passages. For three of the isolates, the virus genome was also sequenced directly from diseased fish. The sequencing data showed a shift from alanine to threonine at amino acid position 221 of VP2 protein after one or two cell culture passages. This amino acid change is not the only virulence determinant of IPNV, but may be involved in cell culture adaptation and attenuation of the virus. It is important to be aware of this alteration when performing IPNV challenge studies or if making recombinant viruses using reverse genetics technique.

Life history of an exotic sabellid polychaete, *Terebrasabella heterouncinata*: fertilization strategy and influence of temperature on reproduction

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Abalone culture facilities have been devastated by an exotic sabellid, *Terebrasabella heterouncinata*, following its introduction from South Africa in the late 1980s. Infestations are associated with shell deformities, increased mortality and financial losses. In addition, the potential introduction and establishment of this exotic pest into the natural environment was unknown. The development of an effective management strategy is dependent upon understanding the life history of this sabellid, including its fertilization strategy and generation time. In the present study, red abalone, *Haliotis rufescens*, with single sabellid infestations were isolated in containers at 18°C. This first, parental generation was held in isolation until individuals produced F_1 larvae. These larvae were subsequently isolated until individuals produced a second, F_2, generation. In a separate study, uninfested abalone were exposed to infested abalone at three temperatures typically encountered in California. Transmitted larvae were observed as they developed to specific life stages: initiation of feeding, development of sexual maturation and production of motile, infestive, larvae. This research demonstrated that isolated individuals do pose the threat of producing fully functional offspring and that the generation time of *T. heterouncinata* is significantly temperature dependent.

Proposed diagnostic criteria for proliferative thyroid lesions in bony fishes

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Distinguishing hyperplastic lesions from neoplasia in the thyroid of bony fishes has been debated by scientists for about one hundred years. As early as the first decade of the last century, the histological interpretation of some of the striking proliferative lesions observed in the thyroid of a number of species has been controversial. The confusion is partly due to the fact that thyroid tissue in bony fishes is unencapsulated, is capable of widespread ectopic growth in many species, and is frequently predisposed to extensive hyperplastic proliferation. Suggested factors that may cause proliferation of fish thyroid include iodine deficiency, poor water quality, genetics, abnormal nutrition, and seasonal factors. In some cases, the resulting hyperplasia can be extensive and has been reported to infiltrate and destroy normal tissues (e.g., Lake Erie coho salmon). In order to make the diagnosis of proliferative thyroid lesions in fishes simpler and to end the continuing controversy, we propose specific criteria for distinguishing hyperplastic from neoplastic lesions in teleosts. The primary lesion categories are follicular cell hyperplasia, adenoma, and carcinoma. Specific diagnostic criteria will be provided for these categories as well as any required sub-categories. For example, follicular cell hyperplasia may be focal, nodular, or ectopic. Illustrated examples of each type of proliferative lesion will be presented to demonstrate the key diagnostic features of each lesion type.

Natural history of Atlantic surgeonfish and implications for nutritional management in captivity

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In a pilot study run during the fall of 2001, Atlantic surgeonfish were fed one of three diets. The first group was fed a diet consisting exclusively of the green alga, *Ulva*, the second was fed a premium commercial pelletized diet, and the third was fed a premium commercial flake. Blue tangs, *Acanthurus coeruleus*, have a digestive system characterized by the presence of a tubular stomach, which presumably breaks down algal cells using acid secretions. Doctorfish (*A. chirugus*) and ocean surgeon fish (*A. bahianus*) have a muscular gizzard-like stomach and ingest sand and substrate as part of their normal feeding behavior. None of these three species performed well on the *Ulva* diet, however, the growth and survival of blue tangs was superior to that of ocean surgeonfish and doctorfish. Surgeonfish fed the flake diet developed lesions consistent with Head and Lateral Line Erosion Syndrome (HLLES) (27%), and eye lesions (16%), characterized by exopthalmia and apparent blindness. Gross evidence of nutritional disease was not observed in fish fed the premium pellet food, with the exception of transient eye lesions in one individual. Weight gain and survival were best in this group. An extensive analysis was run on the nutritional composition of the three foods and will be presented. Vitamin A was of particular interest as it was present in very high concentrations in the pelletized feed (32,001 IU/kg dry matter) compared to the flake food.(3,318 IU/kg dry matter). Vitamin A deficiency has been associated with development of eye lesions in other teleost species, and some aquarists have speculated that it may play a role in development of HLLES lesions.

Seroprevalence of largemouth bass iridovirus in Florida largemouth bass as determined by immunodiffusion

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Investigation of sporadic fish kills in several Florida lakes in 1989 resulted in the recovery of an iridovirus from affected largemouth bass (*Micropterus salmoides)*. This virus has subsequently been reported to be identical with Santee-Cooper ranavirus. Antigen harvested from virus-infected Fathead Minnow (FHM) cell cultures was found to react with precipitating antibody from wild-caught largemouth bass in an agar gel immunodiffusion (AGID) assay. Serum samples were obtained from largemouth bass in four central Florida lakes in 1992, 1993, and 2001, and were tested for precipitating antibody. Forty-five to 73% of the largemouth bass that had been collected from Lakes Harris, Holly, Newnans and Weir tested seropositive. There was a decreasing trend in percent seropositivity from 1993 to 2001. In 2001 the largemouth bass (n = 91) from which serum samples had been collected were also processed for virus isolation at the Warm Springs Fish Health Center (USFWS) in Warm Springs, GA. Results were as follows: 1) Ab +, virus +: n = 14; 2) Ab +, virus -: n = 31; 3) Ab -, virus +: n = 4; and 4) Ab -, virus -: n = 42. The simple AGID assay can greatly facilitate sero-epidemiologic surveys for the Santee-Cooper ranavirus in freshwater fish species.

Quantitative trait locus (QTL) associated with resistance to lymphocystis disease in Japanese flounder (*Paralichthys olivaceus*)

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We used 72 microsatellite markers to search for quantitative trait locus (QTL) associated with resistance to Lymphocystis disease (LCD) in Japanese flounder (*Paralichthys olivaceus*). Linkage tests have been conducted in a backcross family produced by crossing between susceptible male X (susuceptible X resistant) female. One of the microsatellite markers, tightly linked to the LCD resistant locus, was identified. Almost every year, LCD occurred in the fish cultured in Japan and the farmers suffered economically from this disease. Because the causative agent, lymphocystis disease virus (LCDV), induces characteristic ugly nodules on the body and/or fins of affected fish, we think that our results will provide an opportunity for marker-assisted selection to reduce the economic loss by this disease.

Fish models for signature-tagged mutagenesis identification of virulence-associated genes in *Yersinia ruckeri*

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The bacterium *Yersinia ruckeri* infects rainbow trout and other fish, moving from the aquatic environment into the host animal through gills, skin and digestive tract. Host damage may not be immediate, but the bacterium reproduces and survives in the fish organs, avoiding removal by immune defenses. A competitive challenge model allows fewer fish to be used in testing mutants, while accommodating fish variability in responses. Rainbow trout were challenged by immersion in mixtures of a parental strain of serovar 1 strain *Y. ruckeri*, and strain WK4, a constructed mutant in the *flaA* flagellin gene. Motility provided a slight advantage at early stages of infection and in transmission to other fish, but by day 6 no significant difference ($P>0.1$) was found in the numbers of the non-motile mutant (Kan^R) and parent strain recovered from organs. To obtain mutants reduced longer-term survival in the host, we screened libraries of signature-tagged mutants by immersion challenging rainbow trout at 10^8-10^9 bacteria/mL. The challenges were pools of 11 mutants with unique 21-mer nucleotide tags, prepared using pUTmini-Tn5Km2 suicide plasmids. Genomic DNA was extracted from bacteria recovered from fish kidneys after 1, 3 and 7 days. PCR tests detected the distinctive oligomeric tags, to indicate mutants that failed to survive. The pattern of tags missing varied with sampling time and in replicate fish. Competitive challenges with the parental strain were needed to confirm mutants affected in genes contributing to the invasion of the host and the maintenance of the bacterium over prolonged periods.

Experimental mycobacteriosis in striped bass (*Morone saxatilis*): histology

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Mycobacteriosis is a serious emerging disease threat to striped bass (*Morone saxatilis*) in the Chesapeake Bay, USA. Infected fish may present granulomatous inflammation in the viscera, as well as hemorrhagic skin lesions. Grossly normal fish may also have culturable levels of mycobacteria in internal organs. In addition to the classic fish pathogen *M. marinum*, we have isolated several other species of mycobacteria from internal organs of Chesapeake Bay striped bass. These include a new species, *M. shottsii* (proposed), which is the most commonly isolated mycobacterium from striped bass sampled by our group to date. In this study, we examined the temporal progression of disease in striped bass experimentally infected with three species of mycobacteria. Fish (mean 113g) were maintained in fresh water at 18-21°C. Approximately $\sim 10^5$ CFU of *M. shottsii*, *M. marinum*, or the environmental saprophyte *M. gordonae* were injected intracoelomically, and fish were sampled for histology and bacteriology over 42 weeks. Severe granulomatous inflammation of the internal organs was produced by infection with *M. marinum*. A consistent progression of lesion morphology was observed, which included formation, maturation, and eventual disintegration of granulomas. The latter stage was associated with production of disease more severe than that observed after primary infection. Although both *M. shottsii* and *M. gordonae* established persistent culturable infections in the spleen, granulomatous inflammation was mild and restricted to the mesenteries. No pathology attributable to mycobacteria was observed in PBS-injected control fish. External signs of disease were minimal in all groups.

The population dynamics of *Planorbella trivolvis*, the intermediate snail host in the *Bolbophorus* spp. life cycle

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A digenetic trematodes, tentatively identified as *Bolbophorus* spp., is a serious threat to the commercial catfish (*Ictalurus punctatus*) industry. Studies indicate that the American White Pelican (*Pelecanus erythrorhynchos*) is introducing this parasite into catfish ponds. Large populations of snails, *Planorbella trivolvis*, inhabit these ponds and are the intermediate host for *Bolbophorus* spp. Currently, there is no known method to eradicate this parasite. Elimination of the snail appears to be the best method of control: however, there is no information available on the population dynamics of *P. trivolvis* in catfish ponds. Catfish ponds (n=4) were selected to study the *P. trivolvis* population. The ponds were sampled monthly for two years. Preliminary data indicates the highest density of *P. trivolvis* occurred during June, July, August, and October, with greatest abundance in August. Snail spawning took place in May, July, August, and October, with peak spawning in October. Egg masses were found in water temperatures of 9° C in February 2002. The number of eggs varied from 1 to 46 in 944 masses examined. In winter months, *P. trivolvis* were observed burrowing into the mud with dropping temperatures. Four cercarial types were found in the population: strigea, armatae, amphistome, and clinostomoid. Snails positive for *Bolbophorus* spp. were found most prevalent in June and August. Based on the complexity of the life cycle of *Bolbophorus* spp., simply treating ponds when snails are visible may not be adequate for the prevention of infection.

Phagocytic responses of *Ictalurus punctatus* fry to intraperitoneally injected particulate material: A light microscopy and cytochemical study

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Because non-specific immune defenses may be more important for developing fry than acquired immunity, more knowledge is needed in the area of channel catfish non-specific immune system development. We evaluated phagocytic responses as fry developed. Two forms of particulate material, fluorescent microspheres (FMS) and colloidal carbon (CC), were intraperitoneally injected into channel catfish fry at 1, 2, 3, and 4 weeks post hatch (wph). Non-phagocytized FMS and CC were observed within the vasculature in very low amounts as early as the time it took to process the fish at 0-hours post injection (hpi). FMS and CC were more commonly observed within mononuclear phagocytes within the vasculature at 4-hpi. Phagocytes were observed to phagocytize FMS and CC within the coelomic cavity as early as 0-hpi, while the majority of coelomic FMS were phagocytized between 4 and 12-hpi. Enzyme cytochemical staining indicates that a mononuclear phagocytic response predominates in FMS injected fish, while the CC injected fish were observed to elicit a mixed response of polymorphonuclear and mononuclear phagocytes. The predominant organs associated with the observed cellular responses were the spleen, posterior kidney, and anterior kidney. Trafficking and organization was more pronounced within the spleen as fish developed. The only difference observed in particulate clearance rate among the specific ages was that 1 and 2-wph fish seem to clear both FMS and CC faster than the two older ages.

Effect of water temperatures on the pathogenesis of koi herpesvirus (KHV), and the development of TaqMan PCR and ELISA for KHV detection in previously exposed fish

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The koi herpesvirus (KHV) can be the cause of significant mortality among both common (*Cyprinus carpio carpio)* and fancy carp or koi *(Cyprinus carpio koi)*. Replicate groups of koi were held in the laboratory at different water temperatures (e.g. 13, 18, 23, and 28°C) for an appropriate acclimation period and were then exposed to either 12 or 1.2 $TCID_{50}$/ml of KHV as propagated in the KF-1 cell line for 1 h. Fish were observed twice daily and dead fish collected. At selected intervals, koi from one of three replicate groups at each temperature were sampled to evaluate progression of the disease and the remaining two replicate groups evaluated for cumulative mortality. Virus isolation using the KF-1 cell line, PCR (single round), Taqman PCR and histopathology were used to examine tissues as collected from both sampled live fish and dead fish. The greatest mortality, 95.2%, occurred among KHV-exposed fish at 23°C. A more rapid onset, but with less cumulative mortality, occurred at 28°C. Mortality also occurred at 18°C, but not 13°C. Initial results from the pathogenesis studies suggest that the gill is a primary target for infection with subsequent involvement of the kidney, spleen, gut, liver, and the brain. Both Taqman PCR for viral DNA and detection of anti-viral antibodies by Enzyme Linked Immunosorbent Assay (ELISA) provided evidence of prior exposure of koi to KHV in the exposed group only.

Catfish anemia: new theories, no answers

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Channel catfish suffer from a profound anemia that produces hematocrits as low as 1%. Publications beginning in 1986 have convincingly correlated catfish anemia with feed, variously attributing the cause to fungal toxins, folic acid deficiency, or feed contamination by pseudomonads or bacilli. However, anemia typically occurs during the peak of the feeding season when the time elapsed between feed manufacture and consumption is only 2-3 days. In addition, many ponds are fed from the same storage bins, but only sporadic ponds on a farm will develop anemia. Difficulties in applying feed-based theories to outbreaks of this disease have led us to search for other causes of anemia. Our work has involved five commercial catfish ponds that have a history of fall outbreaks of anemia and 3 ponds on the same farm that have not experienced anemia. Hematocrits were measured weekly from August through October, algae samples were examined for potentially toxic species, and fish were cultured for infectious disease. No infectious agents or algae species were found to correlate with anemia. On October 9, mean hematocrits dropped precipitously from 28 to 17 percent in the anemia-prone ponds and in all except one of the control ponds. Losses occurred in several of the ponds during this period. Mean hematocrits returned to normal values one week later. The precipitous nature of the decline provides evidence that some cases of catfish anemia may result from an acute hemolytic crisis.

Case Report: unique pathology linked to infection by *Aeromonas salmonicida* subsp. *achromogenes* in pen reared wild cod from Newfoundland

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In August 2000 Atlantic Cod (*Gadus morhua*) were captured by a modified Cod-trap, acclimatised for one week and then transferred to 2 on-rearing sea-cages. Environmental parameters at the grow-out site were 17.1° C, 85% oxygen saturation and a 28 ppt salinity. Stocking densities were 2.50 and 2.25 Kg/cubic metre respectively. Cod were placed on a moist diet of frozen forage fish 2 weeks post transfer. Sixteen days following first feeding affected individuals showed clinical signs of disease, including lethargy, hyperaemic dorsally located skin "sores," opacity of the eye and darkening of the integumental pigmentation. Cumulative mortality over a 3 week period amounted to 19%. Diagnostic investigations confirmed *Aeromonas salmonicida* subsp. *achromogenes* (atypical furunculosis) as the causative agent. This isolate varied commonly isolated atypical forms by growing readily at 22° C and producing a diffuse brown pigment. Similarly, the morphologic pathology presentation was unique from that seen for fin-fish infected by atypical forms of *Aeromonas salmonicida*. Grossly, fish presented with marked ulceration of the dorsal fin and multiple adjacent subcutaneous granulomata at the fin base. Internally the digestive tract was empty, the kidney and spleen showed marked multifocal granuloma formation, and serosanguinous ascites fluid was present within the pericardial sack. Histology confirmed a severe, chronic, multifocal to coalescing necrotizing granulomatous bacterial splenitis, interstitial nephritis, and subcutaneous dermatitis. The pathology identified in this epizootic differs from the more acute presentation normally seen for atypical furunculosis in salmonids and/or cyprinids, and may represent an evolutionary adaptation unique to the Cod.

Quality assurance in fish disease diagnostics

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Session 1: *Working toward a unified approach to fish diagnostic laboratory Quality Assurance*. This session will encompass detailing the current status and approaches being undertaken in North & South America, Europe and Asia. The session will work toward a joint unified proposal for minimum laboratory QA protocols that fish health associations worldwide could sign-off on and provide to OIE as a recommended standard. **Session 2**: *Approaches to interlaboratory quality assurance ring testing and quality control protocols for fish diagnostic laboratories*. This session will provide examples and discussion on practical approached to establishing a voluntary interlaboratory quality assurance scheme. In addition, discussion on evaluating the current quality control project being undertaken by the USFDA to standardize MIC determination for aquatic bacterial pathogens of fish, and the need for standardization of biologics and diagnostic reagents will be presented.

Characterizing immune response to viral challenge in litopenaeid shrimp using functional genomics approaches

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Pathogens in the marine environment (especially the viruses) have caused catastrophic losses to shrimp aquaculture. The manner in which marine crustaceans respond to pathogens is complex and largely unknown. In addition, the role of anthropogenic and natural factors in the etiology of shrimp disease and their effects on immunity is, at best, poorly understood. This is particularly true in the case of antiviral immunity for which there is virtually nothing known about host resistance. We have taken a functional genomics approach to gain an understanding of the underlying genetics of disease response and immunity in crustaceans, using the Pacific white shrimp, *Litopenaeus vannamei* and White Spot Syndrome Virus (WSSV) as models. The Pacific white shrimp is a commonly aquacultured species in many parts of the world and WSSV has had a devastating effect on farms growing the Pacific white shrimp. The strategy employed relies on the discovery of novel genes with putative roles in antiviral defense and/or other aspects of immune response using of ESTs derived from standard and normalized cDNA libraries, and by enrichment of virus-responsive genes by subtractive hybridization. We hypothesize that viral infection triggers changes in gene expression that are at least partially reflective of an immune response. Using this approach, we have identified a suite of genes that appear to be regulated in response to WSSV infection. By characterizing these virus-responsive genes, we aim to gain an understanding about the kinds of pathways involved in the invertebrate antiviral defense.

Channel catfish families resistant to ESC are different from ESC-susceptible families in both constitutive and inducible complement activity

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Enteric septicemia (ESC) is one of the most important diseases of channel catfish. The USDA-ARS Catfish Genetics Research Unit is selectively breeding channel catfish for multiple economically important traits including resistance to ESC. Bath challenges of catfish reveal significant phenotypic variation in mortality between families. These differences are consistent whenever fish from the same families are challenged and potentially controlled in part by genetic variation. In order to discover which aspects of immune function are most important in protection against ESC, replicate tanks with resistant and susceptible catfish families were exposed to *E. ictaluri*. Blood samples were collected before and 4-5 days post exposure. Plasma complement activity was measured by exposing rabbit erythrocytes to serial dilutions of catfish serum and measuring changes in turbidity as blood cells were lysed. At 4-5 days post-exposure, clinical signs of ESC were evident in sensitive and resistant families. Mortality was greater in sensitive families, typically 3 individuals in a 10-fish replicate (the fish were bled and the experiment terminated prior to the time that massive mortality would be expected). In pre-exposure samples, complement activity was significantly higher in families resistant to ESC. After *E. ictaluri* exposure, complement activity remained the same in sensitive families, but was significantly elevated in resistant families. While the actual importance of complement activity related to ESC etiology is unknown, destruction of bacterial cells by the complement system is a recognized component of resistance to bacterial infection.

ISA surveillance and regulatory actions at Atlantic salmon farms in New Brunswick, Canada

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ISAV was first associated with clinical disease in New Brunswick salmon farms in 1996. A fish health surveillance program was established to detect ISAV infections utilizing industry veterinarians and government officials to sample moribund fish during regular visits to each site. Components of the industry support early depopulation of groups of fish based on positive test (IFAT and RT-PCR) results alone, while others wish to delay depopulation until clinical disease (i.e., increasing mortality rates) is confirmed. Depopulation is ordered by the provincial government but compensation for these actions is derived from an industry-sponsored fund. The compensation fund has a pre-set limit and the amount compensated for each fish is pro-rated based on total claims for the year class of fish. As claims increase, the compensation becomes less meaningful to the point where the cooperation of the farms may be compromised. Critical facets of the depopulation actions include the communication of locations and procedures for movement of fish to appropriate processing facilities. Farms want knowledge about positive cases for risk management but also expect confidentiality about disease outbreaks. Surveillance summaries and challenges in evaluating competing economic concerns for disease control actions will be discussed.

A dose response study of three potential DNA vaccines against channel catfish virus (CCV) in channel catfish

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In order to evaluate procedures and methodologies necessary to produce DNA vaccines for CCV proteins three genes were selected for vaccine testing, ORF 59 (the putative major envelope protein), ORF 46 (membrane protein), and ORF 6 (membrane protein). To create the vaccines, ORFs were PCR amplified from a pHC-79 cosmid library of CCV. The amplified ORF PCR product was then T/A cloned into pcDNA3.1/V5/His-TOPO (Invitrogen, Carlsbad, CA). Construction of DNA vaccines were confirmed by DNA sequencing. Each vaccine was transfected into cell culture and western blotted to screen for protein production, which were visualized at predicted molecular weights. A trial to test each gene's potential as a vaccine and the dose required to provide protection was conducted. Treatments consisted of triplicate tanks of 25 fingerling channel catfish testing each of the three vaccines (pORF59, pORF46, pORF6) in three different dosages (50, 25, and 5µg per fish in 50µl TE). Negative controls consisted of fish injected with the non-gene encoding backbone plasmid, pcDNA3.1/V5/His-TOPO, in three doses and one treatment of fish injected with TE. Fish were challenged five weeks post-vaccination at 30°C with CCV, morbid and moribund fish were collected twice daily. Results from this study will be presented.

Role and function of the OIE Fish Diseases Commission in the field of aquatic animal health

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The Office International des Epizooties (OIE) is the World Organisation for Animal Health; it currently comprises 162 member countries. Whilst representation is usually through the Member Countries' Chief Veterinary Officers, Competent Authorities other than the National Veterinary Services may be responsible for aquatic animal health in some Member Countries. In 1960, the OIE established the Fish Diseases Commission because of the increasing awareness of the importance of international trade in fish and other aquatic animals. In 1988, the scope of the Fish Diseases Commission was extended to include diseases and pathogens of molluscs and crustaceans. The expansion of international trade in aquatic animals and their products has called for appropriate rules to avoid the risk of spread of communicable diseases. Standardisation of aquatic animal health requirements for trade and harmonisation of international aquatic animal health regulations are critically important to enable trade to continue whilst maintaining effective national disease control. The international aquatic animal health standards prepared by the FDC are laid down in two important documents, OIE *International Aquatic Animal Health Code* and the *Diagnostic Manual for Aquatic Animal Diseases*. Currently, the Code and *Manual* specifically deal with thirteen "Diseases notifiable to the OIE" and sixteen "Other significant diseases" of aquatic animals. The diseases are classified into one of these two lists on the basis of their socio-economic importance, geographic range and aetiology. Infectious salmon anaemia (ISA) is currently listed under the list of "Other significant diseases," and both the *Code* and *Manual* have chapters dealing with the disease.

Mycotic granulomatosis in ayu caused by *Aphanomyces piscicida* in Japan

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A fungus infection attributed to *Aphanomyces* on the basis of the fungal morphology was first reported in Japanese pond-cultured ayu *Plecoglossus altivelis* by Egusa and Masuda (1971). Later, the infection was also found in other fishes in Japan, and named mycotic granulomatosis based on histopathological findings (Miyazaki and Egusa, 1972, 1973). Hatai *et al.* (1977) first succeeded in isolating the fungus from a lesion using FME agar. They demonstrated its pathogenicity to ayu and other fishes by artificial infection, but carp, *Cyprinus carpio*, was not susceptible to the fungus. Among several media tested, a medium consisting of 1% glucose, 0.25% yeast extract and 1.5% agar was found to be best for the growth of the fungus. The medium was named GY agar (Hatai and Egusa, 1978). Finally Hatai (1980) named the fungus *Aphanomyces piscicida* based on its asexual reproduction characteristics. Infections of this fungus are still a serious problem in cultured ayu. In this paper we will also describe the present immunological and molecular studies on this fungus in our laboratory.

Ulcerative mycosis in channel catfish, *Ictalurus punctatus*, black bullhead, *Ameiurus melas*, and bluegill, *Lepomis macrochirus* caused by *Aphanomyces invadans* from recreational ponds in Louisiana, USA

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Seven cases of chronic ulcerative mycosis affecting populations of channel catfish, black bullhead, and bluegill cultured in recreational ponds were submitted to the Louisiana Aquatic Diagnostic Laboratory between January 2000 and December 2001. Diseased clinical specimens presented with multiple foci of skin ulceration typically overlaying more extensive areas of granulomatous myositis that extended to the vertebral column in advanced cases. Lesions were predominated by fields of plump macrophages and multinucleated giant cells surrounding non-septate, thick walled hyphae with non-parallel cell walls suggestive of an oomycete. Because deep ulcerative mycoses in other fish species in the Western Atlantic USA, Australia, and Southeast Asia have been attributed to *Aphanomyces* spp., diagnostic methods were employed to isolate and identify this oomycete along with other potential pathogens. The fungus was isolated from uncontaminated sites deep in the musculature using modified peptone-yeast-glucose (PYG) medium containing 200 µg/ml streptomycin and 0.1 g/l ampicillin. DNA was extracted from mycelium harvested from PYG broth cultures of representative isolates by grinding in liquid nitrogen followed by chloroform:phenol extraction and isopropanol precipitation. The entire ITS1 region of the rRNA gene was amplified and sequenced using primers ITS1 and ITS2. Comparison of this data with ITS1 sequences for 4 isolates of *Aphanomyces invadans* deposited in the GenBank database indicated 100% homology. Koch's postulates were fulfilled in juvenile (40–50g) channel catfish. This is the first report of ulcerative mycosis caused by *Aphanomyces invadans* in catfish from recreational freshwater ponds in the southeastern United States and represents new host records for channel catfish and black bullhead.

Virulence of siderophore deficient and *aroA* deletion mutants of *Photobacterium damselae* subsp. *piscicida* in a hybrid striped bass (*Morone saxatilis* x *M. chrysops*) infection model

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Two genetically altered strains of *Photobacterium damselae* subsp. *piscicida* were evaluated in different trials to assess the level of virulence reduction when compared to the wild type parent strain LA91-197 in a hybrid striped bass (*Morone saxatilis* x *M. chrysops*) infection model. LA91-197, isolated from diseased hybrid striped bass cultured at a coastal Louisiana fish farm, was chosen as a representative strain of this highly virulent fish pathogen for challenge studies and as the parent strain for generating mutants. The virulence of the wildtype has been determined by LD_{50} calculation to be 540 cfu/ml by immersion and < 1 cfu/g by injection. Mutant strain LSU-P1 is a siderophore deficient mutant produced by transposon mutagenesis and mutant LSU-P2 is an *aroA* defective mutant produced by homologous recombination and integration of a defective *aroA* gene into LA91-197. Specific pathogen free hybrid striped bass, mean weight 48–65 g, were used in virulence attenuation challenge studies. Trials were run in 60L recirculating systems in brackish water (10 ppt) at 20-22°C stocked with 5 fish per system. Challenge trials were done in triplicate by either immersion or injection with negative controls, conducted in triplicate. In the LSU-P1 trial injection doses ranged from 10^2 to 10^6 cfu/g of fish and immersion doses ranged from 10^4 to 10^8 cfu/ml. In the LSU-P2 trial injection doses ranged from 10^3 to 10^6 cfu/g of fish and immersion doses ranged from 10^5 to 10^8 cfu/ml. The LD_{50} was calculated to be 5.6×10^4 cfu/g by injection and 3.6×10^7 cfu/ml by immersion for LSU-P1 and 1.1×10^5 cfu/g by injection and $> 1.0 \times 10^8$ for LSU-P2.

Host and geographic range extensions of the North American strain of viral hemorrhagic septicemia virus (VHSV)

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Viral hemorrhagic septicemia virus (VHSV) has recently been isolated from Pacific sardines (*Sardinops sagax*) from coastal waters of Vancouver Island, British Columbia, Canada, from sardines and mackerel (*Scomber japonicus*) from central and southern California USA and from euchalon or smelt (*Thaleichthys pacificus*) and surf smelt (*Hypomesus pretiosis pretiosis*) from two separate locations in Oregon USA. Mortality and evidence of typical skin lesions of viral hemorrhagic septicemia (VHS) were observed among sardines in Canada but all other fish were healthy in appearance. Prevalence estimates among sardines and smelt ranged from 4–14% among fish in California and Oregon. The mid-G (glycoprotein) gene sequence of these 9 new VHSV isolates demonstrated that they all belong to the North American group of VHSV forming a closely related subgroup. The VHSV isolates from sardines in Canada and California were closely related and differed from VHSV isolates obtained from other species of marine fish and salmonids in Canada. This suggests that populations of sardines, which originate in the coastal waters of California, presumably carry this virus upon migration to more northern waters (e.g. Canada), particularly during periods of ocean warming. These new virus isolations extend both the know hosts (sardines and smelt) and geographic range (Oregon and California, USA) of VHSV. Sardines are currently one of many marine species used as bait fish involved in international commerce. Understanding the impacts of VHSV and other pathogens on the health of these fish populations and the risks associated with subsequent movements of these fish should be addressed by further research.

Selected immunogens of *Piscirickettsia salmonis*: molecular analysis to evaluate vaccine development

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Analysis of salmonid tissues naturally infected with the fastidious intracellular bacterial pathogen *Piscirickettsia salmonis* allowed the identification of a number of protein as putative immunogens for potential vaccine development. A novel purification procedure for *in vitro* grown bacteria as well as a the generation of a battery of polyclonal antibodies against the bacteria allowed to directly correlate the specificity of the *in vivo* selected inmunogens. Two-D gel electrophoresis and westerns blot analysis yielded pure bacterial antigenes prone to be characterized, and the sequence of two of these inmunogenes as well as the evaluation of their potential to generate a classical protein vaccine that might help to control the spread of the disease in netpens in southern Chile.

Outbreaks of Koi herpesvirus in koi and common carp in Germany

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In 1998 mass mortality among koi and common carp in Europe, USA and Israel was associated with the presence of koi herpesvirus (KHV). Since that time, especially in the summer time, outbreaks of the KHV among koi populations in Germany and some other European countries still cause enormous problems for the koi lover and also in carp aquaculture. The most prominent clinical signs of the disease were gill discolouration and necrosis. Other signs included sunken eyes, pale patches on the skin and increase in mucus production on the skin and gills. Microscopic examination revealed severe hyperplasia and necrosis of the gill epithelium and nuclear hypertrophy and margination of chromatin was observed in some epithelial cells. These cells with intranuclear inclusion were often difficult to detect in gill tissue, particularly when the specific pathology was obscured by secondary infections. The disease could be experimentally transmitted to koi and common carp of different ages, but not to gold fish and grass carp. A polymerase chain reaction (PCR) assay to detect KHV in tissues of koi and common carp was developed in the lab of Dr. Hedrick at the University of California in Davis. Beside isolation of the virus in cell culture, we have been using the PCR for routine diagnostics in our lab. In the present work, we will demonstrate an in situ PCR for detection of KHV in histological sections and detail the annual situation of the outbreaks in Germany in the last three years.

Clinical presentations of *Mycobacterium* sp. in cultured summer flounder (*Paralichthys dentatus*)

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A population of 1000 commercially-reared juvenile summer flounder was housed in recirculating systems for approximately one year. After six months, fish began to develop oral masses on the lower mandible. These discrete masses were generally white to yellow in color and encompassed the rostral portion of the mandible. In addition, other fish developed head swelling, exophthalmia, coelomic distention and opercular masses. It was estimated that at least 40% of the entire population displayed one or more of these clinical signs. Impression smears and histopathology of lesions (stained with Ziehl Neelsen acid-fast stain) revealed a dense population of acid-fast bacilli, consistent with *Mycobacterium* sp. All affected tissues had marked effacing and coalescing granulomatous inflammation primarily composed of epithelioid macrophages. This tissue reaction was not the typical teleost granulomatous response to *Mycobacterium* sp. Bacterial cultures from the affected tissues grew on Lowenstein-Jensen and Middlebrook media and were acid-fast positive with Ziehl Neelsen staining.

Pharmacokinetics of oxytetracycline in summer flounder, *Paralichthys dentatus*: a complete pharmacokinetic study and oral and intramuscular administration in compromised and healthy fish

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Two experiments were conducted in commercially-reared juvenile summer flounder held in recirculating aquaculture systems maintained at 28 ppt salinity and 20°C. The first experiment was a complete pharmacokinetic study where oxytetracycline (OTC, 50 mg/kg) was administered via intravenous (IV), intraperitoneal (IP), intramuscular (IM) and per os (PO) to four separate groups of 66 individually tagged summer flounder. Six fish were bled at each time point and each fish was bled 3 times over the course of 47 days post-injection. Results from this experiment gave the pharmacokinetic profile of OTC distribution and elimination in summer flounder. In the second experiment, two separate groups of clinically diseased fish and clinically healthy fish were compared. Clinical signs of disease included coelomic swelling, head swelling, oral masses, exophthalmia, and emaciation. Fish were administered OTC (50mg/kg) via either IM or PO routes, which were considered the most practical routes for use in industry. Six fish from each group were bled at each time point for 30 days. Results from this experiment, indicated that fish with clinical disease when compared to clinically unaffected fish have similar OTC pharmacokinetic profiles when OTC is administered either IM or PO despite evidence of systemic bacterial disease. Bacteriology results and MIC levels from clinically diseased and clinically healthy fish were also examined.

Male gamete quality assessments as bioindicators

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Biomarkers are indicators that respond measurably at several levels of biological organization. Reliable, ecologically relevant bioindicators permit detection of injurious effects before population declines, and can provide insight into pollutant type. Typical biomarkers used to assess reproductive abnormalities in fish have included vitellogenin, plasma sex hormones (i.e. 17β-estradiol and 11-ketotestosterone), and organ histopathology. Gonadotropins, Leydig and Sertoli cell function, and genetic inheritance influence spermatogenesis. Sperm cell function, directly reflective of reproductive function, can be affected by exposure to xenobiotics. Gamete quality was measured in studies of potential biological effects of endocrine-disrupting compounds on common carp (*Cyprinus carpio*) in Nevada and Arizona, and on green swordtails (*Xiphophorus helleri*) in Hawaii. Sperm quality assays for aquatic species can include cell morphology and count, motility, viability, mitochondrial function, acrosome status, DNA integrity, and fertilization rate. During spawning seasons in Arizona, carp sperm viability and mitochondrial function were significantly lower ($p = 0.000$), than at the reference sites. Biological effects were more apparent in males than in females. Power analyses indicated a preferred sample size of 13–17 male fish per site. Results from these studies demonstrate that sperm quality assays are useful bioindicators for assessing potential adverse effects of environmental contaminants on reproductive and endocrine systems in fish. Sperm biomarker assays can be tailored for particular species, and when used in conjunction with such data as contaminant levels, animal health assessments, and reproductive biomarkers, assessments can be made on how ecosystem structure and function respond to anthropogenic stressors.

Microbial concerns for cryopreserved larvae and gametes of aquatic species

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An expansion is occurring in the practice of cryopreservation of male gametes and larvae of aquatic species for both commercial applications and conservation of genetic resources. In domestic species, infective organisms associated with low fertility can be transmissible with frozen milt; therefore, several industry control mechanisms are in place. While mycoplasmas, viruses, bacteria, parasites, and rickettsia have been detected in semen from mammals, rickettsia, viruses, and bacteria have been associated with gonads of fish. A well-designed program for cryopreserving aquatic species sperm should include a comprehensive strategy for collection and storage of high-quality gametes free of pathogenic organisms. Infectious disease testing for fish sperm and indiscriminant disease screening of broodstock to determine which gametes should undergo freezing would be unrealistic; therefore, stored gametes should be handled as if contaminated until definitive testing is performed. Accurate source tracking and site history are requisites. Guidelines for transfer of aquatic animals and aquaculture products are provided within regulatory frameworks and agreements through the USDA's Animal and Plant Health Inspection Service, USDI's Fish and Wildlife Service, and the Office International des Epizooties. To prevent the unintended transfer of microorganisms with frozen samples and the introduction of exotic microbes into ecosystems, these guidelines and appropriate diagnostic manuals can be updated to cover cryopreserved materials. Responsible cryopreservation programs and protocols will not allow the diseases of today to be disseminated throughout the world, and the diseases of yesterday to re-emerge in the future.

Investigations into the distribution and transmission of *Kudoa thyrsites* to Atlantic salmon in British Columbia

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The myxozoan parasite *Kudoa thyrsites* is associated with post-mortem myoliquefaction in farmed Atlantic salmon. Histological analysis, polymerase chain reaction (PCR) and non-radioactive in situ hybridization (ISH) were used to better understand the factors affecting risk of infection with this parasite. Prevalence and intensity of infections were compared among salmon exposed to sea-water drawn from three depths. In three separate experiments, the prevalence and intensity of infection were significantly higher among salmon held in deep versus shallow water, suggesting a benthic source of infection. Over 5000 polychaete annelids representing 18 species were collected from sediments below salmonid net-pens in Departure Bay, Vancouver Island and from the fouling fauna associated with pen netting. Pools and individuals from each species were analyzed by polymerase chain reaction (PCR). No evidence of *K. thyrsites* was observed in 2311 of *Capitella capitata*, the most abundant benthic organism, in 158 *Chone caudata*, 105 *Armandia brevis*, nor in 190 specimens of 14 other species. However, a diagnostic PCR product was amplified from several pools, representing 1337 specimens of *Platynereis bicanaliculata*, the most abundant polychaete among the net-fouling fauna. Prevalence in the species was as high as 4% in some samples. The nucleotide sequence of the amplified PCR product was 100% homologous to the GenBank sequence. An *in situ* hytbridization (ISH) protocol was developed using a digoxygenin-labelled DNA probe homologous to *K. thysites* SSU rDNA. The results of ISH analysis of potential invertebrate hosts in the context of risk of infection will be discussed.

***Parvicapsula minibicornis*: transmission and impact on anadromous salmonids of the Fraser River, British Columbia**

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Prematurely migrating, Late-Run stocks of Fraser River sockeye have experienced significant pre-spawning mortality since 1995. Kidney pathology associated with severe *Parvicapsula minibicornis* infections in these fish suggested that the parasite contributed to the mortality. This study sought to document the distribution and severity of infections of *P. minibicornis* among anadromous salmonids of Fraser River origin. Adult sockeye salmon from several spawning populations were examined for *P. minibicornis* infection using histology and polymerase chain reaction (PCR1). The parasite was first detected in two stocks following in-river migrations of 440km and 619km, respectively. The parasite was later detected in greater than 95% of samples collected at or near the spawning grounds. Severity of infections increased with distance migrated. The parasite occurred in kidney samples from 21 of 22 other sockeye stocks. In addition, kidney samples from spawning pink, coho and chinook salmon were found to be infected with the parasite. Infections in the latter species were less severe than in sockeye. The parasite was detected in naïve juvenile salmonids following caged exposure to lower Fraser River water. Approximately 60% of sockeye smolt collected from the marine environment near Vancouver Island had histological and PCR evidence of infection. Implications regarding the impact of infection with *P. minibicornis* on the health of juvenile and adult anadromous Fraser River salmonids will be discussed.

Disinfection of water for aquaculture

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A pathogen free water source is essential for success in aquaculture. Surface waters commonly used in aquaculture come from coastal waters or rivers and may contain some fish pathogens. Such open water supplies should not be used without treatment. Disinfection of wastewater before discharge is necessary to avoid pathogen contamination into the environment. In this study, we examine the cidal effect of ultraviolet (UV)-produced oxidant by ozonization of seawater and hypochlorite by electrolization of seawater. Additionally, the disinfectant effects of the three methods for a hatchery water supply and wastewater were compared, and the survival rate was assessed for cultured fish that were reared using water treated with these methods. Gram negative bacteria and fish rhabdoviruses, herpesviruses and iridoviruses were killed when UV irradiated at the dose of 10^4 $\mu W \cdot sec/cm^2$. Standard, inexpensive UV lamps can irradiate at that dosage and may be suitable for hatcheries or culturing stations that have problems caused by these microorganisms. This would be the best method for disinfection of UV susceptible pathogens. Water contaminated with Gram-positive bacteria, fish birnaviruses, fish reoviruses, fish nodaviruses and aquatic fungi, having low susceptibilities, should be disinfected with ozonization, electrolization or high quality UV lamps. Disinfection of wastewater is necessary to prevent pathogenic contamination of the environment. Electrolization is easy to scale up and can be used to treat a large volume of water, thus making it a suitable method for disinfecting wastewater.

Mycobacteriosis in wild striped bass (*Morone saxatilis*) of the Chesapeake Bay

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In the mid-1990's, investigators at the Virginia Institute of Marine Science (VIMS) began their studies of wild striped bass exhibiting various degrees of hemorrhagic and/or ulcerative skin lesions. Histological examination of these lesions revealed granulomatous inflammation, often with acid-fast bacilli, which were typical of mycobacteriosis. This diagnosis was confirmed by microbiological tests, including standard culture methods and phenotypic characterization. In addition molecular tests, such as polymerase chain reaction (PCR) for the genus-specific16S rRNA gene, nested PCR, RFLP tests, and genetic sequencing, were conducted. In 1999, aseptic collection and processing of spleen tissues from both healthy-appearing and symptomatic fish was initiated. Microbiological techniques were refined to include serial dilution and culturing of tissue samples. Incubation at room temperature was initiated when the predominant mycobacterial isolate, a new species just named *M. shottsii*, was determined to require this temperature (Rhodes et al., in press). Interestingly, *M. shottsii* belongs to the *M. tuberculosis* clade, with 99.2% homology with both *M. marinum* and *M. ulcerans*. One cohort of fish (N = 118) was collected at regional striped bass tournaments, from recreational fishermen, and seine surveys in the York River, VA. All fish were kept on ice after capture and necropsied within 24 h under aseptic conditions in a biosafety laminar flow hood. Spleens from each fish were weighed and divided into two subsamples. One subsample was fixed for histological examination. The second sample was homogenized in sterile phosphate buffer and divided into two portions: one for microbiological identification and determination of the number of mycobacteria present per gram of tissue, and the second for molecular investigations leading to mycobacterial genus-specific PCR/nested PCR diagnosis. Since such analyses began, a total of 196 striped bass have been aseptically necropsied and quantitatively cultured. Seventy-six percent (76%) of such fish have been found to be infected with mycobacteria, typically producing culture counts of greater than 10^{4-6} colony forming units per gram (CFU/g) of tissue.

Role of somatic mutation and immunoglobulin structural diversity in the teleost immune function: a critical problem in addressing fish disease resistance

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Past work has portrayed teleost humoral immune systems as relatively simplistic versions of mammalian systems. In this context, two of the most critical immunological functions in mammals, affinity maturation (with concomitant somatic mutation) and isotypic diversity, have largely been considered as ancillary or of minimal importance to routine piscine immune function. However, work in our laboratory has demonstrated that affinity maturation does occur in the trout. Currently we have evidence that this affinity maturation can occur without employment of somatic mutational processes in some cases, while in other situations, somatic mutation may be extensively employed. Our work and that of others also indicates that structural diversity of a non-isotypic nature exists within teleost fish. Lack of recognition of the primacy of these functions in teleost immunity has primarily been due to preconceptions based on mammalian function and the use of inappropriate technologies. It is essential to understand the mechanics of these comparable yet unique teleost functions and how they work to provide teleosts effective disease resistance. Methodologies which can accurately assess these immune parameters and their application toward evaluation of fish immunity and resistance are posed.

Environmental factors associated with the occurrence of ulcerous lesions in Atlantic menhaden, *Brevoortia tyrannus*, in a small coastal embayment

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The tidal creeks of Chesapeake Bay are a primary nursery for juvenile menhaden (*Brevoortia tyrannus*). Since the mid-1980's episodes of deeply penetrating ulcerative skin lesions (UDS) have occurred in juvenile menhaden. A field program in the Great Wicomico River (GWR) estuary, VA, was begun in 1998 to investigate the cause of these skin ulcers and to identify environmental parameters associated with this disease. The GWR is a small, productive, highly stratified shallow and poorly flushed estuary whose salinity distribution in the headwaters is subject to large variation, primarily by storm events. During the study we collected approximately 20,000 juvenile menhaden from spring through fall to assess lesion prevalence. Menhaden with UDS consistently yielded cultures of an aseptate water mold resembling *Aphanomyces invadans*. Lesion prevalence tended to be highest in menhaden caught at salinities ≤10-12 psu and was low or undetectable during warm summers with little or no precipitation. Lesion prevalence tended to increase following intense, episodic thunderstorms. In 2001, after a dry summer, lesions first appeared approximately two weeks after a significant rainfall event in mid-August. After another rain event, a fish kill occurred in September, 2001 with lesion prevalence rates as high as 97%. With the exception of episodic precipitation events that lowered headwater salinities, examination of the biological, chemical and physical characteristics of the GWR did not reveal obvious factors that might explain interannual differences in lesion prevalence. Based on field observations and laboratory studies we propose a conceptual model describing the environmental factors involved in menhaden UDS.

Ecology and faunistic characteristic of sturgeon parasites from the Azov sea basin

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From 68 detected parasite species in the Azov sea basin 17 (25 %) are specific for sturgeon. Beluga (*Huso huso*) has 31 species, 6 (19.3%) species are specific. Parasite fauna of Russian sturgeon (*Acipenser guldenstadti*) include 37 parasite species, 13 (35.1%) are specific for sturgeon. Parasite fauna of starred sturgeon (*A. stellatus*) consists of 32 parasite species and 12 (375%) are specific for sturgeon. Sterlad (*A. ruthenus*) has 17 parasite species 8 (47.1 %) are specific for sturgeon. 32 parasite species with 4 (12.5 %) specific species described from bester (*Huso huso* x *A. ruthenus*). There are 6 species common to all sturgeons from the Azov sea basin, and only 1, *Capillaspirura argumentosa*, is specific for sturgeon. The highest losses for sturgeon being reared were caused by representatives of Trichodinidae (*Trichodina nigra*, *T. rectangli*, etc.) and also *Polipodium hydriforme*, *Nitzschia sturionis*, *Diclybothrium armatum*, *Diplostomum spathaceum*, *D. paracaudum*, *Piscicola geometra*, *Ergasilus sieboldi*,and *Argulus foliaceus*. The faunistic analyses of sturgeon parasites show some heterogencity in species distribution. Character of parasite-host relations between sturgeons from the Azov sea basin show that the highest level of specificity belongs to coelenterates (100%), amphilinas (100%), nematodes (57.1%), and leeches (50%). For monogeneas and cestodas it was 40 % accordingly. Protozoans have the lowest level of specificity (5.2 %). Level of specificity for monogeneas, cestodes, trematodes, acanthocephalans and crustaceans was not higher than 30%. The analysis of the parasite fauna in artificial reproduction and commercial rearing shows that the majority belong to protozans (37.5%). These, as a rule, are widely specific parasites whose invasion stages are carried with water to tanks and ponds.

The characterization and quantification of proteolytic genes expressed by *Myxobolus cerebralis*

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Myxobolus cerebralis, a metazoan parasite, is a major fish pathogen that causes whirling disease in feral and captive rainbow trout (*Oncorhynchus mykiss*). The pathogenesis of disease suggests that proteolytic enzymes may be involved during initial tissue invasion of the sporoplasm and the lysis of cartilage during the later stages of infection. Based on parasite specific cDNA sequence data obtained in our laboratory, the expression of serine and cysteine protease gene homologues have been detected from the triactinomyxon stage of *M. cerebralis* and within infected fish tissue. The amino acid sequence data is consistent with the chymotrypsin family of serine protease and the papain family of cysteine proteases. Specifically, the amino acid sequence indicates the expression of a lysosomal cysteine protease (i.e., cathepsin B). We have incorporated the use of real-time TaqMan PCR to quantify the transcription of these protease genes produced by *M. cerebralis* both temporally and spatially in experimentally infected rainbow trout. Rainbow trout (7 weeks post-hatch) were exposed to 2.0×10^4 triactinomyxons/fish with appropriate controls. Six tissues were collected: caudal and dorsal fins, gill, muscle, spinal column, and cranium. Tissues (25 mg) were sampled at 5 minutes post-exposure, 2 hour post-exposure, and 4, 10, 24, 54 days post-exposure. The results show an increase in relative expression of the serine and cathepsin B-like cysteine protease both temporally and spatially. There is a relative increase during the invasion of the fish epidermis and during development in the cartilage suggesting involvement with tissue degradation and possibly sporogenesis.

Diseases in zebrafish (*Danio rerio*) from research facilities—histological lesions

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The use of zebrafish (*Danio rerio*) as a laboratory animal model, particularly in genetic research, has expanded dramatically over the last decade. Interest in diseases and other health issues related to maintaining zebrafish has thus increased accordingly. We provide a diagnostic service to researchers through the Zebrafish International Resource Center. The following are the most common non-infectious, idiopathic or and neoplastic diseases that we have encountered in the some 110 cases, representing over 500 individual fishes from 28 different facilities. We have frequently observed a severe, chronic inflammation and fibroplasia of the ovaries associated with degenerated eggs (thought to be caused by inadequate spawning), pericardial effusion, dilated cardiomyopathy, and hepatic megalocytosis. The latter is often severe, but the causes (either anthropogenic or natural agents) have not been determined. Nephrocalcinosis and gas bubble disease has occasionally been observed. The most common neoplasms observed in fish not deliberately exposed to carcinogens include seminomas, spindle cell sarcomas of the viscera and eye, intestinal carcinomas, thyroid tumors, and liver neoplasms. Studies are ongoing to determine if certain strains or mutants are predisposed to these tumors. In regard to intestinal tumors, a contributing factor may be infections by the nematode *Pseudocapillaria tomentosa*. Other infectious diseases that we have frequently seen include microsporidiosis of the central nervous system, caused by *Pseudoloma neurophilia*, and mycobacteriosis. Supported in part by NIH P40 RR12546

The development of vaccines in Canada in regards to ISAV

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Infectious salmon anaemia virus (ISAV)) is currently the most important viral pathogen threatening commercial aquaculture in the northern hemisphere. Morphological, biochemical and replication properties indicate ISAV is a member of the *Orthomyxoviridae*, similar to influenza viruses. Comparison of ISAV proteins with other orthomyxoviruses revealed low amino acid identity values, between <13% and <25%, supporting the proposal to assign ISAV to a new, fifth genus *Isavirus* within the *Orthomyxoviridae*. Infectious salmon anaemia in the Bay of Fundy, New Brunswick, is now a managed disease following the compensation scheme agreed to by the federal government in 1999, and the various steps taken by the industry including stringent husbandry practices, an ISA surveillance program depopulation of affected sites, and vaccination. However, the ISAV vaccines currently used are not 100% protective. Immunized fish do not clear the virus and may become carriers, which makes control by vaccination incompatible with depopulation control methods. There is still an absolute need to match the ISAV vaccine composition to current viruses, and to improve on the immunogenicity of ISAV vaccines. Studies on over 160 ISAV isolates established the existence of two haemagglutinin (HA) subtypes of ISAV, one American and one European, and four distinct neuraminidase (NA) subtypes, indicating the existence of up to eight different combinations of HA and NA subtypes among isolates. These observations are discussed in relation to new vaccine developments for ISA. An ELISA that detects fish antibodies to ISAV was also developed and can be used to assess vaccine efficacy.

New peptide binding domain lineages found in MHC class I of rainbow trout (*Oncorhynchus mykiss*) shared with cyprinid species; conservation of variation

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Two new lineages were found for the α1 domains of the classical MHC class I in rainbow trout with higher homology to sequences found in cypriniformes than to previously described sequences in salmoniformes. Similar sequences have not been found in more primitive or more modern fish. These findings contribute to previous discoveries that some of the variation in the α1 domain is shared between cypriniformes and salmoniformes. The conservation of the α1 variation in these phylogenetic orders suggests that this variation, including more residues than only those lining the peptide-binding groove, is very important. The present study compares the α1 and α2 lineages found in different fish species, and discusses possible models for their evolution.

Pathogenicity of the oomycete, *Aphanomyces invadans*, in Atlantic menhaden, *Brevoortia tyrannus*

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Atlantic menhaden, *Brevoortia tyrannus*, develop characteristic skin ulcers in response to infection by the oomycete, *Aphanomyces invadans*. To investigate pathogenicity, we conducted a dose response study. Juvenile menhaden were inoculated subcutaneously with 0, 1, 5, 10, 100, and 500 secondary zoospores per fish and monitored for 37 d post-injection. Survival rates declined with increasing zoospore dose with significantly different survivorship curves observed at the different doses. Moribund and dead fish exhibited characteristic ulcerous lesions at the injection site starting at 13 d post-injection. None of the sham-injected control fish died. The LD_{50} for inoculated fish was estimated at 9.7 zoospores; however, some fish receiving a single zoospore developed infections that resulted in death. We also challenged menhaden by aqueous exposure and confirmed that *A. invadans* derived from a lesioned menhaden from the Wicomico River, Chesapeake Bay, was highly pathogenic by this more environmentally realistic route of exposure. Fish acclimated to culture conditions for 30 d and presumably free of skin damage were exposed to 100 zoospores mL^{-1}. These fish exhibited a 13.9% lesion prevalence and 11.1% mortality. Scanning electron microscopy of fish skin indicated that zoospores adhered avidly to intact epidermis, germinated and actively penetrated with a germ tube to infect the fish. Net-handled fish had a significantly higher lesion prevalence (64.3%) and mortality (64.3%). Control fish developed no lesions and did not die. Our results indicated that *A. invadans* is a primary pathogen of menhaden with an apparent very low minimum infective dose.

Undertaking an import risk analysis—the influence of knowns and unknowns in science

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Concerns over the spread of pests and diseases with the movement of live aquatic animals have led many countries to adopt both domestic and international controls. However, the risks associated with the movement of dead aquatic animal products is largely unknown, difficult to determine and often controversial. Import risk analysis (IRA) is a science-based process that can be used to evaluate the risk of foreign diseases being introduced with imported commodities. The process involves three stages: 1) identification of diseases that are potential hazards; 2) evaluation of the likelihood and consequences of their spread; and 3) evaluation of control measures to manage unacceptably high risks. The quality and confidence in the outcome of a risk analysis is reliant on the scientific information on which it is based. While considerable research has furthered our understanding of diseases of aquatic animals in recent years, much remains unknown regarding the taxonomy of disease agents, transmission of diseases, life-cycles, survival parameters and the validity of diagnostic techniques. This presentation will discuss how these matters are dealt with in the practical conduct of an IRA, where policies must be developed and justified (consistent with international obligations) in spite of this lack of information. The current Australian IRA on dead bivalve molluscs is used as a practical example.

Effects of *Ichthyophonus* on survival of Yukon River chinook salmon

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Recent observations have identified *Ichthyophonus* an emerging pathogen of adult Yukon River chinook salmon and have implicated the organism as a potential cause of prespawning mortality. Field and laboratory studies were conducted to: 1) sample fish at multiple sites, 2) relate annual changes in disease severity to changes in river conditions (e.g. temperature), 3) determine if infected adults (esp. females) die prior to reaching their natal streams, 4) identify the source of *Ichthyophonus* infections, 5) determine the effect of water temperature on the growth rate and pathogenicity of *Ichthyophonus*. We found approximately 30% of adult chinook enter the Yukon with subclinical infections, which become increasingly clinical as fish travel upriver. There was a relationship between elevated temperature and severity of disease. Historical temperature data showed that prior to the 1980s, river temperatures were considerably lower than at present. Spawn-outs collected in one tributary confirmed that no infected females were present, supporting our hypothesis that significant numbers of infected females died prior to reaching their spawning streams. Herring sampled from the Bering Sea were negative for *Ichthyophonus*. Marine fish experimentally exposed to *Ichthyophonus* and held at different temperatures showed a clear relationship between elevated temperature and increased severity of disease with dissemination of the pathogen within the infected fish.

Immuno-related genes expressed in fish stimulated by immunostimulant

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Immunostimulants are valuable for the control of fish diseases and have wide application in fish culture. The effects of immunostimulants in fish have been reported, but there are few studies done on genes expressed by immuno-stimulation. In this study, we analyzed genes expressed in head kidney cells of fish stimulated by peptidoglycan as an immunostimulant. Sequenced clones were compared with sequences in databases of GenBank, EMBL and DDBJ. The results contain the genes such as globins, several ribosomal proteins, cytochrome oxidase subunits and heat shock protein. Putatively identified immuno-related genes were immunoglobulin heavy and light chain, beta$_2$-microglobulin, CC chemokine, thymosin beta, lysozyme, Interleukin-1 beta and MHC class II.

Characterization of serum and mucosal antibody responses of rainbow trout (*Oncorhynchus mykiss*) to *Flavobacterium psychrophilum*

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Serum and mucosal antibody responses of juvenile rainbow trout (*Oncorhynchus mykiss*) were characterized by ELISA following immunization with various preparations of formalin-killed *Flavobacterium psychrophilum* cells. The protective nature of these preparations was then determined by immunizing rainbow trout fry and challenging with the bacterium. Juvenile rainbow trout immunized intraperitoneally (i.p.) with formalin-killed *F. psychrophilum* emulsified with Freund's complete adjuvant (FCA), and i.p. with formalin-killed *F. psychrophilum* either with or without culture supernatant generated significant serum antibody responses by 6 and 9 weeks, respectively. Significant mucosal antibody responses were detected by 9 weeks only in fish immunized i.p. with killed *F. psychrophilum*/FCA. Following immunization and bacterial challenge of rainbow trout fry, protective immunity was conferred in *F. psychrophilum*/FCA and saline/FCA groups with relative percent survival (RPS) values of up to 83 and 51%, respectively. Significant protection was not observed in treatment groups immunized by immersion or i.p. without adjuvant at the challenge doses tested. Results suggest that stimulation of non-specific immune factors enhance the ability of fish to mount a protective immune response, but specific antibody appears necessary to provide near complete protection. In this study, an ELISA was developed to monitor anti-*F. psychrophilum* antibody production in trout. The relationship of such responses to protective immunity suggests that future vaccination strategies against coldwater disease may require stimulation of both the innate and adaptive arms of the immune response.

***Paramoeba* sp. as a potential pathogen in seed clam (*Mercenaria* sp.) hatcheries in the Indian River Lagoon, Florida**

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In the last few years, several hard clam (*Mercenaria* sp.) hatcheries along the Indian River Lagoon, Florida have been experiencing a series of heavy seed clam mortalities. The mortalities appear to begin when batches of larvae settle out of the water column and start to set. Mortalities are acute and batches of larvae can be dead within a few days. Examination of environmental parameters within and external to the hatcheries, water quality, feed, and different management practices within individual hatcheries does not reveal any obvious common denominator. Batches of both larvae and setting clams from several hatcheries were examined grossly and by histopathology. A high incidence of *Paramoeba* sp. was found lining the mantle cavity in setting clams only. Free-swimming larvae did not appear to be infected. Likely influential factors are unknown chemical contaminants or biological phycotoxins that directly kill or stress larvae during the setting period and that may allow for secondary infection by opportunistic *Paramoeba* sp. Alternatively, *Paramoeba* sp. are primary pathogens that for as-yet-unknown reasons have become a more recent problem in this area. It is of interest to determine if these pathogens have played a role in the lowered recruitment of hard clams in the northern Indian River Lagoon during the last few years.

Diagnostic hematological changes in rainbow trout infected with INHV

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Hematological parameters are used routinely in aiding in the diagnosis of diseases. Information describing the morphological changes in blood and hematopoietic tissue of fish infected with virus diseases is limited. Stained smears of peripheral blood and anterior kidney imprints were examined from fish naturally and experimentally infected with IHNV. The most distinct and consistent changes included macrophages with cytoplasmic vacuolation and ingested cellular debris, nuclear pyknosis of hematopoietic cells, and the presence of degenerate nuclear and cytoplasmic debris. In addition to the above changes, the occurrence of bilobed erythrocytes appears to be pathognomonic for IHNV. IHNV concentrations of 10^4 to 10^5 plaque forming units/g were required before cellular changes could be observed. A presumptive diagnosis of IHN can be made rapidly using this methodology.

DNA vaccines against rhabdoviral disease of finfish: Tools for understanding virus defense mechanisms

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The results to date have shown that DNA vaccines against infectious hematopoietic necrosis virus (IHNV) and viral hemorrhagic septicemia virus (VHSV) can induce transient non-specific protection, elicit virus specific antibodies and reproducibly provide significant protection in rainbow trout at early (7 d) and late (28 d) time points after lethal virus infections, including waterborne and injection challenges. A variety of hypotheses are being investigated in an attempt to understand the mechanism(s) responsible for the potent effect of DNA vaccination including the priming of a specific humoral response, induction of non-specific anti-viral cytokine(s), the acceleration of virus clearance, and the induction of non-specific defenses that are effective against viral and bacterial pathogens. An additional study investigated the effect of the IHNV and VHSV DNA vaccines if they were delivered after the fish had been exposed to virulent IHNV. The results to date suggest that DNA vaccines against rhabdoviral diseases of finfish have great potential both as a practical biologic and as a significant tool for investigation of the teleost immune system.

Susceptibility of passively immunized rainbow trout and challenge survivors to *Flavobacterium psychrophilum*

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To further investigate the role that antibody plays in conferring protection to juvenile rainbow trout, adult rainbow trout were immunized with live *Flavobacterium psychrophilum*, the causative agent of bacterial cold water disease (CWD). The rainbow trout convalescent anti-*F. psychrophilum* sera were produced by injecting 1 kg fish with 1 mL of a 0.4 OD suspension of live bacteria three times at 4–6 week intervals. The sera were obtained from individual fish approximately 6 weeks later. Juvenile rainbow trout (mean weight, 1.3 g) were passively immunized with immune sera with different titers or saline and challenged by subcutaneous injection with a virulent strain (CSF 259-93) of *F. psychrophilum* 24 h after passive transfer. Relative percent survival ranged from 9–42 % and correlated with ELISA antibody titers that ranged from 1,600 to 102,400.The next objective was to determine if fish that survive CWD are susceptible to reinfection. Surviving fish from each treatment in the previous experiment were rechallenged using our standard methodology 6 weeks after the first challenge. Additional animals from each treatment that were not challenged previously were also infected. The relative protection of CWD survivors compared to their naive counterparts was 74–100% indicating a significant level of protection had been induced after the fish recovered from the initial challenge. These studies demonstrate that antibody to *F. psychrophilum* can be elicited following immunization and that antibody plays a role in conferring protection. Additionally, CWD survivors appeared solidly protected against reinfection providing a model to further understand a protective immune response.

Pathogenicity of white spot syndrome virus to the ornamental shrimp, *Lysmata wurdemanni*

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White spot syndrome virus (WSSV) is a serious viral disease affecting the penaeid shrimp culture industry, but has not been determined for any ornamental shrimp. Therefore, pathogenicity of WSSV to the peppermint shrimp *Lysmata wurdemanni* was examined. Three trials (25 days each) were conducted in which five adult *Lysmata* and ten juvenile *Litopenaeus vannamei* were individually fed WSSV infected white shrimp tissue at 10% of their body weight on day 0. Pacific white shrimp, *L. vannamei*, are susceptible to WSSV infection and were used for comparison. Presence of WSSV in all shrimp was determined by PCR and histology. Mortality (avg$\pm$s.d.) was not statistically different ($P<0.05$) between *Lysmata* and *L. vannamei* (67$\pm$30% and 60$\pm$10%, respectively). However, there were differences in length of time for mortalities to occur and presence of WSSV in shrimp. Time to 40% mortality was significantly ($P<0.001$) later for *Lysmata* (20.7$\pm$3.2 days) than for *L. vannamei* (3.3$\pm$0.58 days). All *L. vannamei* (dead and survivors) were WSSV positive, whereas in *Lysmata* there was much variation (Trial 1–100%, Trials 2 and 3–40%). It would appear from these trials that *Lysmata* can be infected with WSSV, but appears more resistant initially to WSSV infection than *L. vannamei*.

***Edwardsiella ictaluri* O polysaccharide biosynthesis gene cluster and O polysaccharide composition**

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We have previously reported the construction and phenotypic characterization of an isogenic *Edwardsiella ictaluri* O polysaccharide (OPS) negative mutant strain, 93-146 R6. This strain was highly attenuated in channel catfish compared to wild-type strain 93-146. We now report cloning the mutation responsible for the OPS negative phenotype of 93-146 R6 along with flanking sequence from the *E. ictaluri* chromosome. The mutation was located in a gene encoding a sugar epimerase (designated *wbeiT*), and the clone contained eight additional complete open reading frames and one partial open reading frame from the *E. ictaluri* OPS biosynthesis region. At least eight of these genes appeared to be organized in a single operon. An intact copy of the epimerase was amplified from wild-type *E. ictaluri* by PCR and cloned. Sequencing of this gene and the flanking genes provided a total of approximately 12 kb from the *E. ictaluri* OPS biosynthesis region, which has been deposited in GenBank (accession #AY057452). When the epimerase gene was expressed from a plasmid in the OPS mutant, OPS production was restored, indicating that the mutant strain's failure to produce OPS was not due to a polar effect on downstream genes in the OPS biosynthesis operon. In addition, we have conducted carbohydrate compositional analyses on purified OPS from wild type strain 93-146 and 93-146 R6. These data suggest that *E. ictaluri* OPS is composed of galactose, N-acetylgalactosamine, and galacturonic acid. This sugar composition correlated with the predicted functions of the enzymes encoded in the OPS biosynthesis cluster.

Differentiation of serologically related cyprinid rhabdoviruses by molecular genetic methods

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Spring viremia of carp virus (SVCV) is a fish rhabdovirus that causes severe disease in common carp and other fish. The disease causes high economic losses in carp farms and is notifiable to the OIE, and under national legislation in many countries. Fast and sensitive methods for detection of SVCV are essential for applying control measures (movement restrictions) for the disease. Currently, diagnosis is usually based on virus isolation in cell culture with subsequent identification by a serological method such as virus neutralisation, or the enzyme linked immunosorbent assay. The identification method must distinguish SVCV from the serologically related, but non-notifiable, pike fry rhabdovirus (PFR). However, new virus isolates have emerged that cause a SVC-like disease, but cannot be clearly identified as either SVCV or PFR by serological methods, and which consequently create a problem with regard to imposing legislative control measures. Reverse transcription-polymerase chain reaction (RT-PCR) followed by sequencing of the amplicons has led to the grouping of SVCV, PFR and serologically related isolates into four genogroups. We have produced four digoxigenin-labelled DNA probes, and have used them to distinguish between the isolates by Southern blotting of amplicons from the RT-PCR and by *in situ* hybridisation (ISH) using infected cell cultures. Comparisons of the two methods are presented. These studies may help to determine the real incidence of viruses from each genogroup and provide data to support a re-classification of SVCV, PFR and similar rhabdoviruses.

Mode of transmission of *Glugea plecoglossi* (Microspora) in the experimental infection model using rainbow trout

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Glugea plecoglossi (Microspora) forms numerous xenomas in the body cavity of cultured ayu, *Plecoglossus altivelis*. We studied the modes of transmission of *G. plecoglossi* in the experimental infection model using rainbow trout. To observe the attachment sites of *G. plecoglossi* spores, rainbow trout were immersed for 5min in the Uvitex 2B-labeled spore suspension and then fish were stained with 0.05 % trypan blue. The spores were observed mainly on the microscopic injuries positive for trypan blue. To examine the site of development of *G. plecoglossi*, spores were applied onto the limited area of the body surface using a cotton swab, then fish were maintained at 20°C for 30 days. *G. plecoglossi* xenomas developed only in the subdermal tissue under the applied area. On the other hand, xenomas were formed in the body cavity by the oral intubation of spores. To investigate the modes of transmission of *G. plecoglossi* in rainbow trout which were orally intubated or immersed in spore suspension, histological sections were prepared from the intestine and the skin of the fish just after inoculation, and *in situ* hybridization was performed to detect the early stages of *G. plecoglossi*. The sporoplasms were observed in the epithelium of the intestine and the skin 5 min post-inoculation. During the 24 hours, sporoplasms moved from the mucosal epithelium to submucosa in the intestine, while they migrated from the mucus cell layer to the muscle in the skin. It is suggested that transmission of *G. plecoglossi* in fish culture environment occurs through the oral route rather than the dermal route, considering the xenomas-forming site in the fish.

***Pfiesteria piscicida*: Molecular analysis of the life cycle does not support the presence of toxic amoeboid stages**

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Pfiesteria piscicida, recognized as a cause of morbidity and mortality in estuarine fish populations in the U.S., has previously been identified based upon the presumptive morphological identification of the algae's specific life stages via light microscopy, combined with confirming the presence or absence of *P. piscicida* in a small subsample via electron microscopy. However, it is now known that *P. piscicida* actually consists of a number of species (*Pfiesteria*-like organisms, PLOs) that are virtually impossible to distinguish via light microscopy. Since toxicity may vary between PLOs, and is dependent on the number of algae in the water and the life stage of those present, microscopic methods are not reliable for assessing the biological impact of PLOs. However, we have recently developed specific molecular probes that strongly indicate that the life cycle of *P. piscicida* is actually much simpler and typical of other marine dinoflagellates. Using a combination of peptide nucleic acid probes, nuclear staining techniques, high resolution differential interference contrast (DIC), and video microscopy, samples were either examined live using direct observation and time lapse photography, or were preserved and analyzed using 100X DIC, with either a *P. piscicida*-specific *in situ* hybridization probe, or nuclear staining with DAPI. Our results showed that *P. piscicida* has a seven-stage life cycle typical of marine dinoflagellates. Although true amoebae (Phylum Sarcomastigophora) were often observed as contaminants in some cultures, no amoeba-like stages of *P. piscicida* were ever detected despite exhaustive searching using conditions reported to produce these stages. Our results question the existence of these amoeboid life cycle stages, which were originally reported to comprise a majority of the toxic stages of this organism. This means that the putative toxicity of *P piscicida* should be re-examined given that life cycle stages, which do not exist, cannot be toxic. This research points out the critical importance of using well-characterized species-specific probes to definitively identify *Pfiesteria* and related organisms.

Development of a PCR-based procedure for the rapid detection of *Pseudomonas anguilliseptica*

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Pseudomonas anguilliseptica is considered today as an important emerging pathogen in marine aquaculture. Recently, this pathogen became one of the most important threats in the seabream and turbot culture in Spain. Due to the lack of clear sympthoms associated with this disease and the fastidious growth characteristics of its etiological agent, the diagnosis of fish pseudomonadiasis is usually difficult. In the present work, a rapid PCR-based protocol for the diagnosis of fish pseudomonadiasis caused by *Ps. anguilliseptica* was developed. Specific primers were designed on the basis of the 16S rRNA gene sequences obtained from the Gene Bank (accession N° X99541) and tested against a large collection of environmental isolated related to the *Pseudomonas* group, as well as a variety of bacterial fish pathogens. After the optimization of the amplification conditions, the developed procedure yielded a great sensitivity and specificity, the expected amplification product of 418 bp being observed only in the *Ps. anguilliseptica* isolates. The detection procedure was subsequentely tested in seeded tissues and in organs from experimentally infected fish showing promising results for its application in field samples.

Non-lethal detection of VHSV in fish blood by RT-PCR

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The viral hemorrhagic septicemia (VHS) virus is the causative agent of one of the most important fish diseases causing severe losses in salmonid farms in Europe. The EU made mandatory (directive 92/532/EEC) the control of fish stocks for detection of the disease. However, the official methods for detection of these viruses are destructive. Therefore, the fish farm companies requested a non-destructive method for screening the virus in high valuable breeders. In the present study we developed a non-lethal method for detection of VHS virus in fish blood by RT-PCR. Juvenile (12-15 cm) brown trout, obtained from a commercial trout farm, and cultured at 10 ± 2°C in the aquaculture facilities of the Instituto de Acuicultura, were inoculated i.p. with VHSV at a concentration of 10^5 TCID/ml. No external signs of disease were observed in the fish 75 d p.i. After being anesthetized, heparinized blood was collected from each fish, and afterwards the individuals were sacrificed and pools of kidney and spleen aseptically collected. Extraction of total RNA was performed from organs by means of Trizol LS Reagent (BRL, Invitrogen), and from blood by QIAamp RNA Blood (QIAGEN). The extracted RNA was subjected to RT-PCR with specific VHSV primers, using the kit SuperScript™ One-Step RT-PCR with Platinum® Taq (BRL, Invitrogen). Our results showed that the capacity of detection of the virus in blood samples is completely equivalent to detection in the fish tissues. This study provides a non-lethal method of diagnosis, which constitutes an important tool for selection of breeders free of VHSV in commercial fish farms.

Neutralization of a salmonid rhabdovirus by single-chain antibodies

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Viral hemorrhagic septicemia virus (VHSV) is one of the most important viral pathogens in European rainbow trout farms. Neutralizing antibodies are part of the immune defense components that can mediate protection against this disease in fish. In order to improve our understanding of how antibodies can neutralize the virus and possibly develop strategies for prevention of disease outbreaks, we studied the ability of recombinant monoclonal antibodies to neutralize virus at the molecular level. Based on variable domain genes from hybridoma cell lines producing neutralizing antibodies to VHSV, gene constructs encoding recombinant single chain antibodies were prepared. The resulting antibodies were expressed in *E. coli* or in fish cell cultures. The ability of the recombinant antibodies to neutralize various isolates of VHSV was analyzed in cell culture assays. The importance of selected amino acid residues in the variable immunoglobulin domains was examined using a panel of antibody variants prepared by site directed mutagenesis. Two variant antibodies appeared superior to the parent antibody in their neutralizing ability. One of these variants was used in passive immunization experiments. By employing a genetic administration strategy, it was possible to establish protective immunity to VHSV in rainbow trout fingerlings.

Modeling shrimp viral epidemics

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Epidemics of white spot syndrome virus (WSSV) and Taura syndrome virus (TSV) are devastating shrimp aquaculture throughout the world. We have developed compartment models using the Reed-Frost approach to transmission for epidemics of WSSV and TSV in *Litopenaeus vannamei*. The models include the following compartments, uninfected susceptible, latently infected, acutely infected, chronically infected, and dead infected shrimp. Transmission, patency, virulence, recovery, and decay (disappearance of dead infected shrimp) control the dynamics of the model. The models predict that a threshold density of susceptible shrimp exists below which outbreaks of WSSV and TSV will not occur. Increases in the transmission reduce the threshold density, whereas increases in mortality and removal increase the threshold density. The basic reproduction number (R_0) is another important measure of the potential for a pathogen to spread. For infectious disease the reproduction number is calculated as the mean life span of an infection times the number of transmissions that would occur over that infectious period. In the laboratory we have estimated some of the important parameters for each of the models and have estimated that the threshold density is lower for TSV than for WSSV and that R_0 is higher for TSV than for WSSV. This suggests that under the conditions of the experiments TSV is likely to spread more rapidly than WSSV in a population of *Litopenaeus vannamei*.

A glomerulopathy of chinook salmon (*Oncorhynchus tshawytscha*) in New Zealand

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The purpose of the project was to evaluate the extent and potential significance of a glomerulopathy seen in Chinook salmon with gastric dilation and air sacculitis (GDAS). Fish from several different age groups were caught by hook and line from net pens, bled from the caudal vein, and assessed for the severity of GDAS. Tissues from each fish were collected for histology and an objective score was developed for the glomerular lesion. Serum from each fish was analysed for creatinine, phosphorus, magnesium and calcium as well as, osmolality, sodium, potassium, total protein, albumin, urea, and bilirubin. In younger fish the glomerular lesion was often segmental. Affected glomerular membranes were typically bilaminar and markedly thickened with loss of epithelial and mesangial cells. The membrane did not stain uniformly with periodic-acid Schiff, however. While apparently clinically normal fish can have widespread glomerular lesions, the severity in some groups is marginally associated ($p<0.05$) with the degree of gastric dilation. The renal lesion is also significantly ($p<0.001$) associated with increased serum magnesium and calcium suggesting that it may have an impact on renal function. A rabbit antiserum raised against partially purified Chinook salmon immunoglobulin failed to react to thickened (or normal) glomerular membranes.

Experimental production of gastric dilation and its association with serum osmolality and biogenic amines in chinook salmon (*Oncorhynchus tshawytscha*)

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Chinook salmon smolt in fresh water fed a commercial diet known to produce minimal gastric dilation and air sacculitis (GDAS) were randomly assigned to four experimental tanks with flow-through seawater. All four groups were acclimatized to seawater for three weeks and fed a diet of minced fresh seafood. After three weeks the groups were fed either; seafood as before, a different commercial pelleted diet associated with the development of GDAS on the farm, or either diet supplemented with 500ppm putrescine, 300ppm cadaverine and 250ppm tyramine. Gastric dilation was produced in fish fed the commercial diet for one month but not by feeding a diet of minced seafood. The addition of putrescine, cadaverine and tyramine to either diet had no significant effect on the development of gastric dilation. Fish fed the commercial diet had significantly ($p<0.0001$) wider weight-adjusted stomach widths, less prominent longitudinal stomach folds ($p<0.0001$) and lower ($p<0.0001$) stomach-width ratios than fish fed the fresh seafood diet. There was no significant difference in serum osmolality or sodium concentration between fish from groups with or without gastric dilation or fed biogenic amines.

Evaluation of different oral vaccines against rainbow trout lactococcosis

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Lactococcosis, caused by *Lactococcus garvieae*, is an emerging disease in several fish species with economical importance in trout farm industry in Europe. Therapeutic measures are generally ineffective and, therefore, development of vaccines is essential to control the disease. Until now, the different bacterins tested by intraperitoneal injection (i.p.), although showing good protective rates, yielded very short periods of immunization which did not protect the fish until reach the marketable size. In this work, oral vaccination with encapsulated and non-encapsulated antigens was evaluated as alternative immunization procedures against trout lacotococcosis. The experimental design included four fish groups. Fish in Group A were oral immunized with a *L. garvieae* bacterin enriched with extracellular products that was encapsulated in alginate microgel capsules obtained by air-atomizing device. Microscopical and histological techniques were employed to determine the shape, size range, antigen release, and *in vivo* degradation dinamic of these microcapsules. Group B was oral vaccinated with a mixture of the same bacterin and dried food. Group C (positive control) was i.p. immunized, and Group D (negative control) included nonvaccinated fish. Four weeks after vaccination, fish were i.p. challenged with the homologous strain, and the protection was evaluated by determining the RPS in each group. The protective rates obtained were 8% and 43% for the oral non-encapsulated and the oral capsulated vaccines respectively. A RPS of 84% was achieved with the i.p. administered bacterin. These preliminary results suggest that the oral encapsulated bacterin tested can be a promising delivery system for the *L. garvieae* antigens, at least for booster vaccination. Further studies are currently in progress to confirm this hypothesis.

Infectious salmon anemia (ISA)—An industrial approach

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From being a spot-vice disease during most of the nineties, ISA re-emerged as a severe threat to industry in 2001 in Norway. Simultaneously, the disease developed with increasing severity in The Faeroes. Records comparing the number of outbreaks today with historic data will be shown both for Norway and The Faeroes. Although ISA always causes great losses at affected sites, the economical importance of this disease is changing dramatically. Although insurance programs effectively reduced economic losses for the scarce number of outbreaks that occurred during the nineties, the present situation is very different. As the disease emerges and number of affected sites increases, indemnification for ISA becomes less attractive to insurance companies. Consequently, the economic consequences are tremendous and forces both industry and governmental bodies to act jointly in order to establish effective measures against the disease. Figures demonstrating the economical impact of ISA to the industry are presented. The presentation will also discuss how structural changes in the industry during the last decade facilitated the emergence of a low virulent agent, like ISAV. Modern fish farming has become intensive food production and salmon are cultured more and more effectively. Feed conversion rates have dropped from 1.4 to 1.05 in the last ten years, while the number of fish per site has increased by a factor of 10. Furthermore, vaccines in use may also provide non-specific protection to the disease. Because the salmon industry will inevitably meet biological bottlenecks, it is imperative to deal with challenges seriously and interpret the presence of disease as biological lighthouses. Otherwise animal welfare, economy and the ultimate sustainability of the whole industry may suffer.

PCR development and screening of wild and captive-reared pallid (*Scaphirhynchus albus*) and shovelnose sturgeon (*S. platorynchus*) for the presence of iridovirus

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A new viral pathogen, shovelnose sturgeon iridovirus (SSIV), similar in appearance to the white sturgeon iridovirus (WSIV), has been identified in Missouri River sturgeon. Since the development of sturgeon aquaculture, iridovirus infections have been reported with increased frequency. The restoration and recovery plan for endangered pallid sturgeon, *Scaphirhynchus albus*, calls for the stocking of juvenile fish in several Missouri River sites. Wild adult pallid sturgeon and shovelnose sturgeon, *S. platorynchus*, are collected in the fall or spring, transported to hatcheries, spawned and eventually returned to the Missouri River Basin. Disease outbreaks attributed to SSIV in hatchery-reared progeny from wild adults have caused significant losses to the pallid sturgeon recovery program. A new detection method, the polymerase chain reaction (PCR), was developed to detect the iridovirus occurring in pallid and shovelnose sturgeon. This new test was essential since classic approaches to isolate the virus were unsuccessful even after developing new cell lines from these sturgeon species. Also, histological analyses and electron microscopy are costly and impractical methods to detect the virus, particularly when fish are not suffering mortality episodes. Investigations to validate the PCR test, establish the presence or absence of the virus in wild fish, determine if the virus can be transmitted from infected adults to their progeny, and confirm virus during captive rearing will be presented.

Specificity of developing channel catfish immune response to heterotypic bacterial challenge

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Previously we observed reduced immune responses in channel catfish fry vaccinated with *Edwardsiella ictaluri*. To determine if this effect was due to specific tolerance induction or general immunosupression, fry were exposed to either *E. ictaluri* or *Yersinia ruckeri* at hatch, 1 week post-hatch (wph), 2 wph, 3 wph and 4 wph. They were then divided into two groups: group 1 was exposed to *E. ictaluri* 4 weeks post the primary and evaluated for protection while group 2 received secondary exposures of either *E. ictaluri* or *Y. ruckeri* and six months later, their ability to clear *E. ictaluri* and *Y. ruckeri* was evaluated. Samples were taken before and 3 weeks after the last challenge and evaluated by ELIZA using whole bacteria as antigen. Exposure to either bacterium at hatch resulted in reduced antibody response when given *E. ictaluri* 4 weeks later, suggesting induction of a non-specific immune suppression or a shared antigen induced tolerance. In fish given later primary vaccinations, reduced responsiveness was not seen and induction of cross-reacting antibodies was seen on primary responses. After a secondary exposure to the same bacterium as the primary the cross-reacting antibody response was no longer evident. In the protection trials fish vaccinated with either bacterium at hatch had higher losses than unvaccinated controls. At later vaccination ages, *E. ictaluri* vaccination provided protection whereas *Y. ruckeri* vaccination generally resulted in higher losses when challenged with *E. ictaluri*. Induction of cross-reacting antibody was not associated with protection or increased bacterial clearance.

Survey of non-salmonid marine fishes for detection of infectious salmon anemia virus and other salmonid pathogens

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In an effort to identify potential reservoirs of salmonid pathogens, over 2300 fish, including alewife, American eel, herring, mackerel, cod, pollock, and winter flounder have been sampled from the natural environment, and pollock, cod and lumpfish have been sampled from within ISA-diseased cages. Assays included cell culture for listed salmonid viruses, direct fluorescent antibody test for *R. salmoninarum*, and/or RTPCR and indirect fluorescent antibody test for infectious salmon anemia virus. All of the fish collected from the natural environment tested negative by all assay methods. Two of 12 pollack taken from inside an ISA-diseased salmon cage were weakly RTPCR positive and cell culture negative, whereas 90 pollock collected outside a diseased cage tested negative for viruses and *R. salmoninarum*. One of 24 pools (5 fish/pool) of tissues from cod taken from the wellboat of a harvested clinically diseased cage produced CPE characteristic of ISAv on SHK cells and was confirmed by RTPCR of cell culture supernatant. Viral pathogens and *R. salmoninarum* were not detected in 26 lumpfish collected from inside diseased cages. This information points to industry attention to biosecurity practices concerning non-salmonids retained in and harvested from salmon cages. These results indicate that pollock and cod can harbor the ISA virus, however it is unknown if the virus can replicate within these hosts. The significance of these potential carriers to the epizootiology of ISA remains to be investigated.

Characterization of *Photobacterium damselae* subsp. *piscicida* strains isolated from cultured sole in Spain

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Characterization of several Spanish strains of *Photobacterium damselae* subsp. *piscicida* isolated from cultured sole (*Solea senegalensis*) was performed to investigate their biochemical, antigenic and genetic relatedness with the *Ph. damselae* subsp. *piscicida* strains isolated from other fish species throughout the world. In addition, the virulence capability of the sole isolates was also examinated. The taxonomical study revealed that all the strains exhibited the same biochemical and physiological characteristics which fit with the previous description of the pathogen. Antigenic analysis using agglutination tests and the dot blot assay indicated a serological homogeneity among the sole isolates corresponding to the unique serotype decribed until now for this bacterial pathogen regardless of their source. Molecular analysis of envelope LPS and proteins confirmed this antigenic homogeneity. The genetic studies revealed that the sole isolates belong to the previously described European genogroup represented by most of the gilthead seabream and seabass isolates. The sole isolates proved to be highly virulent for its host with LD_{50} values lower than 10^3 cells/ g fish.

Inhibition of rhabdoviral glycoprotein and nucleoprotein gene transcription by Japanese flounder Mx

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A homologous cell line, stably expressing recombinant Japanese flounder Mx (JFMx) was infected with hirame rhabdovirus (HIRRV) and viral hemorrhagic septicemia virus (VHSV). Both pathogens are negative single-stranded RNA viruses belonging to the *Rhabdoviridae* family. Following viral infection (10 $TCID_{50}$/ml units), RT-PCR analysis of JFMx-transfected cells showed possible low levels of rhabdoviral glycoprotein and nucleoprotein expression, in contrast with possible increasing levels of expression in non-transfected cells. This result was substantiated by the analysis using quantitative real-time PCR, which indicated a significant increase in the copy number in non-transfected cells either at the 2nd or 4th day post-infection. This supports the hypothesis that JFMx blocks transcription of the viral capsid and membrane protein genes, and may play crucial roles in inhibiting the virus during the early stages of infection. In addition, *in vitro* transcription-translation of the JFMx cDNA clone indicated that it translated a protein with a molecular mass of approximately 68 kDa.

Evaluation of infectious salmon anemia diagnostic tests

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Infectious Salmon Anemia is a viral disease characterized by lethargy, anorexia, anemia, internal organ damage, and death. Costly control methods used on New Brunswick Atlantic salmon farms include a surveillance testing program, early harvest of undersized fish, and indemnity packages. Although the surveillance diagnostic tests have not been evaluated, results are used to make sizeable monetary decisions. The objective of this study was to evaluate ISA diagnostic tests using data collected by the New Brunswick Department of Agriculture, Fisheries, and Aquaculture. Because there is no gold standard reference test, a pool of negative fish from farms that had never had the disease and a pool of positive fish from cages that were experiencing an outbreak defined by greater than 0.05% mortalities per day were obtained and assumed to be negative and positive respectively. There were 1135 (871 negative, 264 positive) fish in all. Depending on the cut-off value, the sensitivity and specificity for the histology test ranged from 30% to 73% and 73% to 99%, respectively. Depending on the cut-off value, IFAT had sensitivities and specificities in the range of 64% to 83% and 96% to 100%, respectively. For the RT-PCR, sensitivity and specificity were 93% and 98% respectively. Test performances were also evaluated factoring in possible clustering of test results of fish from the same farm that may be attributed to differences in disease severity or environmental factors. Slight changes in sensitivities and specificities were coupled with widening of confidence intervals.

The prevalence of infectious salmon anemia virus in farmed Atlantic salmon in New Brunswick

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Infectious Salmon Anemia (ISA) virus has been causing disease in New Brunswick since 1996. Viral control programs dictate slaughter of all fish in an outbreak cage. Evaluation of control success requires knowledge of the viral prevalence within outbreak cages and surrounding cages. The objective was to compare prevalence in cages experiencing an outbreak with healthy cages from the same farm, neighboring farms, and distant farms. Salmon from five different groups were tested using an RT-PCR test. Groups included moribund fish from a cage experiencing an outbreak (A), healthy fish from an outbreak cage (B), healthy fish from a negative cage from a farm experiencing an outbreak in a different cage(C), healthy fish from a negative farm near an outbreak farm (D), and healthy fish sampled at a negative farm located in an area with only negative farms (E). Survey data analysis techniques were used to evaluate the prevalence of ISA virus. Prevalences (std. err.) for the different groups (A-E) were 0.94 (0.040), 0.34 (0.064), 0.29 (0.040), 0.08 (0.048), and 0.08 (0.037) respectively. All groups were significantly different ($p<0.005$) from each other except between groups B and C and between groups D and E. Because the prevalence of the virus is significantly higher in the outbreak cage, early harvest of outbreak cages will remove one source of virus. ISA negative cages (C) that remain on the positive farm may act as a reservoir of virus, potentially spreading virus to other sites.

Regulatory aspects of ISA management in Scotland based on risk assessment principles

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Infectious Salmon Anaemia (ISA) was first detected in a Scottish salmon farm in May 1998. As a List I disease in EU regulations, the outbreak was subject to immediate control measures by the Competent Authority responsible for aquatic animal health measures. This included the removal of all stocks from the affected farm, the tracing of all contacts, the designation of infected and surveillance zones surrounding all foci of infection and an epizootiological investigation into the spread and possible sources of the infection. Although ISA had spread to 10 additional farms, the control measures implemented successfully removed the disease from Scottish salmon farms within a year of the first outbreak, without any subsequent recurrence. Decision making on all aspects of the management measures implemented was firmly based on risk assessment principles.

Comparisons of various ISA viral detection assays

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This talk examines how several Infectious Salmon Anemia virus (ISAv) detection assays were developed and implemented in the United States for pathogen detection and disease management. Current ISAv assays utilized by both private industry and USDA-APHIS include cell culture, molecular analysis (PCR), fluorescent antibody staining, and histology. Prototype serological tests were also developed for ISAv antibody detection. None of the aforementioned techniques have been validated. Although cell culture is regarded as the standard against which other assays are judged, experience demonstrated that problems relating to sensitivity and specificity exist for all tests. The element of timeliness of results plays a further role in the determination of optimal tests. Sample selection, collection and preservation procedures are reviewed with commentary on how the results of each diagnostic test are affected. Comparative cell culture techniques using different cell lines are also discussed, as well as, PCR optimization protocols using different extraction techniques and ISAv primer sets. Using the results from several blinded quality control studies, correlation of molecular assays, IFAT testing and histology with cell culture are reviewed. For amplification techniques such as cell culture and PCR, it is noted that because regulatory policy and actions may be based on essentially unquantified results, it is especially important to minimize false positives. This also applies to fluorescent antibody assays, which may be highly subjective. The discussion concludes with a presentation of recent information concerning development of serological and quantitative PCR analyses.

Histological characteristics of abnormalities and diseases in crustacean zooplankton from the Great Lakes region

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Crustacean zooplankton in the Great Lakes region have been reported with gross abnormalities (copepods) or reduced numbers (amphipods). Protruding lesions or tumor-like abnormalities (TLAs) have been observed on copepod species since 1995 and *Diporeia* amphipod populations have been declining since 1992. Two separate investigations assayed histologic characteristics of protrusions in copepods and disease in amphipods. Protrusions on copepods had diverse histological characteristics; over 50% of TLAs contained necrotic tissue; some were composed of acellular, granular, or non-staining material. Some TLAs had hyaline crystal-like structures either on the surface or embedded within the TLA. Nearly 40% of TLAs appeared to be herniated host tissue, usually muscle, hemocytes, or lipid, but occasionally gonad or gut. In histological sections, elongate transparent TLAs resembled ellobiopsid parasites containing granular material and eosinophilic round bodies. Initially the population decline in *Diporeia* spp. was thought to be due to zebra mussels *Dreissena polymorpha* intercepting food material before it settled to the bottom, but sampling efforts have shown there is sufficient food available to the amphipods. An alternative explanation for the amphipod population decline may be pathogens. Surveys revealed numerous parasites in amphipods including rickettsia-like microorganisms, yeast-like organisms, a haplosporidian-like organism, a microsporidian-like organism, external ciliates, gregarines, and worms. No one etiologic agent has been identified as causing the amphipod population decline but several parasites identified during this investigation likely result in amphipod mortalities.

The antibody response of wild striped snakehead *Channa striata* to the epizootic ulcerative syndrome pathogen, *Aphanomyces invadans*

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Epizootic Ulcerative Syndrome (EUS) is a devastating disease affecting both wild and farmed freshwater and brackish water fish in Southeast Asia. This disease has had serious implications for food sources and local economies of the region. The oomycete *Aphanomyces invadans* is the causative agent of EUS. In the present sudy, the antibody response of striped snakehead *Channa striata* to *A. invadans* was compared between fish collected from EUS-endemic and non-endemic regions in the Philippines. There was a considerable non-specific response to both *A. invadans* and other non-pathogenic oomycetes by the antisera. The highest antibody responses were detected in fish, which had recently been exposed to *A. invadans* for the first time. Sera from fish exposed for longer periods of time, on the other hand, showed the strongest inhibition of germination of *A. invadans in vitro*, in spite of relatively low concentrations of antibodies responding against the fungus. EUS-naïve snakeheads were passively immunised with sera from snakehead populations with various histories of exposure to EUS, then experimentally exposed to *A. invadans*. There was no difference in the prevalence of EUS between the treatment groups. However, fish immunised with sera from a population that had been exposed to EUS for a long period, which strongly inhibited the germination of *A. invadans*, had a significantly lower mortality than fish immunised with naïve sera. Immunisation with sera from fish that had recovered from their first exposure to EUS also resulted in reduced mortality levels.

APHIS Veterinary Services implements an infectious salmon anemia (ISA) program

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The Office of the Secretary of Agriculture announced that USDA funds are available to establish an ISA program that addresses the threat to the U.S. salmon industry. Approximately $8.3 million was authorized for APHIS VS to implement an ISA control and indemnity program for farm-raised salmon in the United States, effective as of December 13, 2001. In addition to the payment of indemnity, these funds are used to assist the State of Maine with program activities such as: depopulation and disposal, clean-up and disinfection, establishment of surveillance programs, epidemiology and diagnostic support, and training for producers and veterinarians. Maine has taken steps to prevent the spread of ISA; however, Federal assistance is deemed necessary to effectively control this disease, which poses a threat to animal health and the U.S. economy. The first case of ISA in the United States was confirmed in Maine on February 15, 2001; in December 2001, 14 marine net pen sites were confirmed as infected. Our goal is to control and contain the ISA virus through rapid detection and depopulation of salmon that have been infected with or exposed to ISA. It is believed that the virus can be controlled within high-risk zones through surveillance, vaccination, and best management practices. ISA control requires depopulation of all pens holding infected fish. Indemnification is necessary to provide an incentive for salmon farmers to report diseased fish and to continue testing.

The preference of mollusk-eating fish for three aquatic snails that vector fish trematodes

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Melanoides tuberculata, *Planorbella trivolvis* and *Physella heterostropha* are three aquatic snails which host trematodes that can infect both cultured and wild populations of fish causing serious problems. These three snail species were offered to black carp *Mylopharngodon piceus*, redear sunfish *Lepomis microlophus*, blue catfish *Ictalurus furcatus* and freshwater drum *Aplodinotus grunniens*, all known molluscivorous fish that could serve as biological snail controls in production systems. Tests were carried out in 70-L aquaria that were connected to a closed recirculating fresh water system with biofiltration. Individual fish were placed into the aquaria (4 fish per trial) and acclimated for 24 h. Twenty individuals of each snail species (60 total) were placed into each aquarium with fish and into 4 control aquaria without fish. Observations were taken hourly for 10 hours and then daily for up to 14 d. All snails used in these studies were of a size appropriate for the mouth gape of the fish tested. All trials were repeated. Initial test results indicated that black carp was the only fish to eat the *M. tuberculata*. However, they preferred the other two species consuming them at equal rates. *Physella heterostropha* were preferred over the other snails by both the redear sunfish and freshwater drum. The snail preference of blue catfish is yet to be determined.

Distribution of *Myxobolus cerebralis* in free-ranging fish populations in Idaho: associations with landscape variables

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Relatively few studies have been conducted on diseases and parasites of free ranging fish populations, and interpretations made from these studies are complicated by problems associated with sampling and sample analysis techniques. Understanding the role of parasites and diseases in natural populations is increasingly important to resource managers as global climate changes, habitat alterations, and species introductions provide altered environments for existing free ranging and native fish populations. Increased availability of data at landscape levels and new approaches to modeling, including use of Bayesian statistics, precipitates possibilities for innovative approaches. We are conducting studies to model the prevalence of *Myxobolus cerebralis* introduced into fish populations in Idaho and other interior Rocky Mountain states with variables that can be obtained from digital GIS-based data sets. Correlations are examined at several spatial scales. Basin size, gradient, and mean elevation can be correlated with water temperatures, and with sediment distributions. Our approach has been to use empirical data from fish cage studies in one Idaho watershed, and then to move to other watersheds to test these models. Our models can assist in restoration efforts so that the risks to fish populations from fish health challenges can be estimated and included into species recovery plans.

Withering syndrome, an epidemic rickettsial disease of wild and cultured abalone in California

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Withering syndrome (WS) was first identified when large numbers of dead and dying black abalone *(Haliotis cracherodii)* were observed at the Channel Islands off southern California in the mid 1980s. Affected animals exhibit lethargy and progressive shrinkage of the body mass followed by death. The disease spread epidemically to wild and farmed abalone populations throughout southern and central portions of the state. Susceptibility varies between species with black abalone most severely affected. We recently demonstrated the causative agent to be an intracellular Rickettsiales-like prokaryote (WS-RLP) that infects gastrointestinal epithelia. The bacterium replicates within vacuoles in host cells, forming large inclusions that burst into the gut lumen. Designated '*Candidatus* Xenohaliotis californiensis', it is Gram negative, pleomorphic, and its 16sRNA sequence indicates placement in the α-subclass of the Proteobacteria with affinities to mammalian pathogens *Anaplasma* spp and *Ehrlichia* spp. WS in red abalone *(H. rufescens)* exhibits a high degree of thermal modulation. Red abalone farms suffered severe losses of animals with WS clinical signs during the 1997-1998 El Nino that subsequently diminished despite continued presence of the pathogen. Feed-based oxytetracycline is highly effective at controlling infections. Using PCR-based diagnostics, we have developed non-lethal methods to detect the WS-RLP in feces, filter-feeding cohabitants and particles concentrated from water samples by tangential flow filtration. Black abalone have recently been proposed for listing under the federal Endangered Species Act; recovery plans for this and other abalone species in southern California need to consider the implications of the WS-RLP now endemic to this region. The few survivors of black abalone epidemics appear healthy, suggesting genetic variation in susceptibility to the WS-RLP, and therefore the potential for development of resistant stocks. Supported in part by California Sea Grant NA06RG0142 Project R/A-115 and the Marine Region, California Department of Fish and Game.

Management and control of ISA in New Brunswick, Canada

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While Infectious Salmon Anaemia (ISA) has been present in New Brunswick since 1996, it was not until 1997 that the causative agent was identified. As a result, ISA spread from just a few farms to several farms in three bay management areas. A number of management and control procedures have resulted in reducing the viral load and infection of ISA, they include: single year class farms, early detection and removal of infected fish, fallowing, control and containment of bloodwater and processing waste. However, ISA continues to be a serious disease of concern in New Brunswick. Discussions on these management and control strategies and what future measures may be required to further control ISA in New Brunswick will be presented and discussed.

Parasitic diseases of North Pacific wild fish and shellfish: do life history patterns of host mortalities exist?

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In recent years, several parasitic diseases have been diagnosed that may play roles in population abundance and distribution patterns of commercially important fish and shellfish. In general, overall prevalences of several parasitic diseases may range from 1 to 5% that, on the surface, would not appear significant. However, upon determining prevalences for various life history stages of affected fish and shellfish, different outcomes are suggested. For juvenile walleye pollock (*Theragra chalcogramma*), overall prevalences of a microsporidan (*Pleistophora* sp.) infection are less that 3%. However, *Pleistophora* infections of fish less than 50 mm range between 10 and 35%, and prevalences steadily drop as fish increase in size. A similar pattern exists in parasitic dinoflagellate (*Hematodinium* sp.) infections of snow (*Chionoecetes opilio*) and Tanner (*C. bairdi*) crabs. *Hematodinium* infections in small (< 55 mm) crab of both species range between 15 and 40%. With increase in size, disease prevalences in both host species drop significantly. In addition, a prevalence/temperature/depth correlation was discovered in *C. opilio*. For Dungeness crab (*Cancer magister*) infected by a parasitic ciliate, a different relation between disease prevalence and life history is suggested. Overall prevalence of a parasitic ciliate in Dungeness crabs over a three-year period was 15%. However, infections were associated with molting condition and size. In particular, infections were more likely to be encountered in larger rather than smaller crabs. These examples will be discussed in relation to ongoing disease studies of fish and shellfish in the North Pacific and their potential impact on population abundance and distribution patterns.

The effect of seasonality on the immune responses of rainbow trout (*Oncorhynchus mykiss*)

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Immune responses of ectotherms are known to vary seasonally, although little work has been carried out on the effect of seasonality on immune responses in fish. Environmental factors, mainly temperature and photoperiod, are known to undergo circadian and circumannual rhythms and have been proposed to be direct causative agents of these variations. The effects of variation and/or constant release melatonin implantation on the seasonal variation in the immune response of rainbow trout maintained under a simulated natural photoperiod were examined. Preliminary studies at the Institute have demonstrated that long-term administration (9 weeks) of melatonin via intramuscular implants significantly enhances a number of immune parameters and improves disease resistance. In conjunction with this trial, half the fish were maintained on EWOS "Boost" diet, which is rich in nucleotides, to see if it could alleviate seasonal immunosuppression when compared to a standard commercial diet. Immune parameters of both the specific and the non-specific immune system were assessed over the course of the trial. Seasonality was shown effect fish haematology and lysozyme activity. An improvement in survival following challenge with *Vibrio anguillarum* as a result of using the EWOS "Boost" diet and melatonin implants was also observed. It is anticipated that this examination of seasonality on basic immune function will be of benefit to the aquaculture industry. It will provide information that will allow administration of commercial diets containing functional supplements to be timed effectively and will facilitate our understanding of the epidemiology of specific fish pathogens.

The epidemiology of ISA in Scotland

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Infectious Salmon Anaemia (ISA) was conformed in May 1998 at Loch Nevis on the west-coast of Scotland. ISA rapidly spread from Argyll to Shetland, almost the entire region over which marine salmon farming occurs in Scotland. However, outbreaks were scattered over this area, 36 farms were suspected, of which 11 were confirmed out of a total of 343 active sites. Isolates were genetically identical and therefore outbreaks were linked by spread and were not due to some wide-spread environmental factor. The pattern of widely but, relatively, lightly scattered outbreaks is not that expected from spread through the environment. Instead it reflected patterns of movements within the aquaculture industry, in particular movements of well boats servicing the industry. Movements of live fish in well-boats spread ISA over a large area and well-boats collecting harvest explain most of the remaining spread. The relationships between the number of vessel visits for these purposes and suspicion or confirmation of ISA are statistically significant. There was no evidence of spread through the environment, but such processes may have been significant at scales of a few km, which the analysis of well boat-movements could not resolve. Restriction of movements around suspected sites, and slaughter at confirmed sites, together with fallowing, brought ISA under control. The last confirmed case was in May 1999 (the only confirmed case in 1999) and the last suspected cases were in November of that year. While isolated PCR positive results have since been found in salmon farms, and in wild salmonids, there has been no evidence of disease for over 2 years.

Persistence of vaccine components at the injection site of Atlantic salmon (*Salmon salar* L.) following intraperitoneal injection with oil-based vaccines against furunculosis

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The presence of vaccine components at the injection site of Atlantic salmon was monitored at 3, 6 and 12 months following intraperitoneal injection with oil-adjuvanted furunculosis vaccines. A monoclonal antibody that recognizes *Aeromonas salmonicida* lipopolysaccharide (LPS) was used in combination with a standard Steptavidin alkaline phosphatase immunohistochemical method for *in situ* identification of bacterium/ bacterial fragments that form part of these vaccines. The side effects and inflammatory reaction of Atlantic salmon were also evaluated using standard histological techniques. The vaccine components were demonstrated as red immuno-labeling in the periphery of oil droplets and in the cytoplasm of surrounding infiltrating macrophages. The vaccine components were consistently found in inflamed tissue located in the pancreatic region, between the pancreas and the blind sacs of the pyloric caeca and in the loose connective tissue and visceral peritoneum surrounding visceral fat and organs like the spleen. Persistent vaccine components at the injection site of Atlantic salmon following vaccination with oil-based vaccines seem to act as inflammatory stimulants that induce, maintain or perpetuate inflammatory reaction leading to the side effects seen at harvest. These findings are useful in the formulation of oil-based vaccines with no or reduced side effects of farmed Atlantic salmon.

Histopathologic and biochemical biomarker responses demonstrate improvement in flatfish health following remediation of a PAH-contaminated site in Eagle Harbor, in Puget Sound, WA

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Eagle Harbor became a Superfund site in 1987 due to high sediment concentrations of polycyclic aromatic hydrocarbons (PAHs) released chronically from a nearby creosoting facility. Studies there (1983-86) demonstrated high prevalences (~75%) of toxicopathic liver lesions, including neoplasms, in resident English sole. These lesions are consistently associated with PAH exposure in wild English sole, and have been induced experimentally in sole by injections of a PAH-rich fraction extracted from Eagle Harbor sediment. Further studies (1986-88) showed that values for biochemical biomarkers of PAH exposure and effect, including hepatic CYP1A expression, biliary fluorescent aromatic compounds (FACs), and hepatic DNA adducts in several flatfish species from Eagle Harbor were among the highest found in Puget Sound. Between 9/93 and 3/94, a one meter-thick cap of clean sediment was placed over 54 acres of the most PAH-contaminated area of Eagle Harbor. Lesion prevalences and biomarker values just before capping were reduced compared to historical data, consistent with facility closure and shore-based source controls. Data on liver lesion risk, hepatic CYP1A, and biliary FACs from fish collected just before capping and at intervals up to ~3 years after showed variable responses relative to pre-capping values. Over the entire monitoring period since cap initiation (up to 104 months, through 5/02), but particularly after ~3 years, there has been a significantly decreasing trend in hepatic lesion risk in English sole, as well as for biliary FACs and hepatic DNA adducts in English and rock soles, and starry flounder. CYP1A levels showed no trend relative to time of cap placement. These results show that the sediment capping process has been effective in reducing PAH exposure and associated effects in resident flatfish species, and that long term monitoring of biomarker responses in biota, such as resident flatfish, is useful to demonstrate the efficacy of this type of sediment remediation.

Antimicrobial activity of oyster lysozyme against *Vibrio species* and *Perkinsus marinus*

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Lysozymes are antimicrobial proteins which cleave glycosidic bounds between N-acetylmuramic acid and N-acetylglucosamine of peptidoglycan, a major component of bacterial cell walls. While lysozymes are generally considered more active against gram positive bacteria because their cell walls are largely made of peptidoglycan there is increasing evidence that lysozymes are also active against gram negative bacteria and other microorganisms through mechanisms not related to their enzymatic activity. The objective of this study was to determine the antimicrobial activity of lysozyme purified from plasma of eastern oysters against several clinical and environmental strains *of Vibrio vulnificus*, various *Vibrio* species pathogenic to human, fish and shellfish (*V. cholerae, V. parahaemolyticus, V. anguillarum, V. mimicus and V. tubiashii*) and against the oyster parasite *Perkinsus marinus*. No colony forming units (cfu) of the translucent *V. vulnificus* strain 1003 and the opaque *V. vulnificus* strain 1003 were observed following incubation with 100 and 200 µg/ml of lysozyme respectively. Lysozyme mimimum inhibitory concentration (MIC) was in the range of 25–100 µg/ml, depending on *V. vulnificus* strains and *Vibrio* species. *Perkinsus marinus* viability was significantly reduced (16-88%) after incubation with 75–600 µg/ml of lysozyme. *Perkinsus marinus* growth inhibition was dose-dependent in the range of 5–75 µg/ml lysozyme. Heat denaturation (15 min at 80°C) of oyster lysozyme significantly decreased its activity against *P. marinus* but had no effect on its antibacterial activity. The mechanisms by which oyster lysozyme kills *Vibrio* species and *P. marinus* will be investigated in future studies.

Innate immunity of rainbow trout (*Oncorhynchus mykiss*) is extensively affected by the environmental temperature

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The compounds of innate immunity in fish are supposed to be relatively temperature independent while specific immune defences are very temperature dependent. Therefore, innate immunity is assumed to be more important for fish than other endothermic vertebrates (Ellis, Dev Comp Immunol 2001, 25: 827-39). Even though the effect of seasonal and *in vitro* assay temperatures on fish adaptive immune functions are well established, there is still controversy over the effects of temperature on innate immune responses. In this report the effect of environmental temperature on the innate immunity of fish i.e. respiratory burst (RB) activity of phagocytes and bacteriolytic activity of complement were evaluated. The level of natural antibodies and the opsonization capacity of serum were determined as well. The non-vaccinated fish were acclimated for six weeks at different temperatures varying from 5°C to 20°C. Anticoagulated blood and serum were collected and the RB activity and the opsonization capacity were assessed as a chemiluminescence (CL) emission from highly diluted blood at various measuring temperatures ranging from 5 to 25°C while the level of natural antibodies and bacteriolytic activity of complement were assessed from serum at 20°C. The acclimation to cold temperatures inhibited all the measured activities. In particular, the the activities of phagocytes were affected extensively. The RB signal of fishes acclimated at 5°C was only 1/10 of that of fishes acclimated at 20°C and the peak time of RB increased simultaneously 2-fold. Contrary to previous opinions we suppose that innate immunity of fish is especially sensitive to environmental temperatures.

Respiratory burst activity (= phagocytosis) of rainbow trout (*Oncorhynchus mykiss*) phagocytes induced by *Aeromonas salmonicida* and various opsonins can be measured from highly diluted blood

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The respiratory burst (RB) activity of rainbow trout phagocytes was measured kinetically over a time period of 210 min as luminol-amplified chemiluminescence (CL) in a microplate by stimulating the phagocytes with variously opsonized avirulent, *Aeromonas salmonicida* sub. *salmonicida* MT004 (1.6 x 10^7CFU/reaction mixture). The activity of blood phagocytes was measured in highly diluted whole blood (0.5 µl of EDTA-anticoagulated whole blood in a reaction mixture of 300 µl) containing ~2000 phagocytes. Head kidney (HK) phagocytes were isolated with Percoll gradient centrifugation and RB activity was measured with cell numbers varying from 2000 to 250 000. *A. salmonicida* was opsonized with normal serum, immune serum, heat-inactivated normal serum, heat-inactivated immune serum and purified immune IgM preparations. When the RB activity of phagocytes of HK was compared to that of blood from the same individual it was noticed that the number of HK cells resembling the number of phagocytic cells in the diluted blood sample produced equal CL responses. The exception was that the response for bacteria opsonized with purified IgM needed 100-fold more HK cells than blood cells and the HK phagocyte response required much higher concentration of purified IgM in the opsonization. Our results suggest that in the presence of opsonins RB of fish phagocytes can be measured in a very diluted blood sample containing only few thousands of phagocytes. The relative role of various opsonins is easy to assess by kinetic measurements. The results suggest that RB responses closely resemble phagocytosis. The role of IgM in phagocytosis should be reconsidered.

Amoebic gill disease in cultured salmonids in Australia

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Amoebic Gill Disease (AGD) is the most serious health problem in sea-caged salmonid culture in Australia. While it increases production costs, AGD does not have any effect on the quality of the market product and it is not transmittable to humans. Current research aiming at reduction of AGD impact on salmon industry is coordinated by Cooperative Research Centre for Sustainable Aquaculture of Finfish (Aquafin CRC). The disease can be diagnosed using histopathology, immunodiagnostic techniques (IFAT, immunodotblot) and culture. AGD is characterised by hyperplastic gill lesions leading to lamellar fusion and formation of crypts. Despite gill lesions, respiration is not affected in fish with mild to moderate AGD; however heart pathology has been associated with AGD history. Freshwater bathing is the current treatment method used by the salmon industry. This treatment is not fully effective and fish become reinfected within a short period of time. Water quality can dramatically affect effectiveness of the treatment. New treatments, including chloramine-T are currently evaluated and show promising results. AGD risk factors and environmental distribution of *Neoparamoeba pemaquidensis*, the organism causing AGD, have been investigated. The pathogen has wide distribution and is present in sediment, water column and in net biofouling. As shown in surveys and infectivity trials wild marine fish are not affected by AGD.

Evaluation of health risks to the farmed southern bluefin tuna

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The aquaculture of southern bluefin tuna, *Thunnus maccoyii*, has been a major success story in the expansion of Australian aquaculture industry. The industry is based on grow-out of captive wild tuna in sea-pens for up to six months. S far, the industry has not been affected by major health problems. Sporadic problems in the past have included swimmer syndrome, blood fluke, and environmental factors. This lack of health problems is at least partly due to the fish age and relatively short duration of captivity ("all in all out"), sound husbandry techniques as well as advanced technology and good environmental conditions. However, in the face of possible future intensification of tuna farming and commencement of propagation program as well as culture of other species in the same area, there is a need to identify health risks, which the industry may have in the future. In particular, marine hatcheries often contribute more to health problems than grow-out phase. Qualitative fish health risk assessment, identification of areas of highest risk and management control measures for industry and research priorities for tuna health are the major objectives of this project. This project is part of Cooperative Research Centre for Sustainable Aquaculture of Finfish, Aquafin CRC, Health Program.

Molecular detection methods developed for a systemic rickettsia-like organism in *Penaeus monodon* (Decopoda: Crustacea)

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Molecular methods for detection of a rickettsial organism were developed to aid in the diagnosis of this pathogen which caused severe mortalities in farm raised *Penaeus monodon* in Madagascar. Using primers derived from the 16S rRNA gene of bacteria, a PCR assay was optimized to amplify this region of the genome of the bacterium, using extracted DNA from infected *P. monodon* tissue as the template. The resulting amplified PCR product (1538 bp) was sequenced. From the sequence data, two novel primers were selected from the variable region of the gene. These primers amplified a 532 bp fragment from DNA originating from rickettsia infected samples. The PCR assay was optimized and tested on DNA extracted from specific pathogen free (SPF) *Penaeus vannamei* tissue and several stains of bacteria. The PCR assay with the rickettsia-specific primers was found to be specific for this rickettsia-like organism and did not amplify the other DNA samples that were tested. The 532 bp PCR amplified fragment was labeled with digoxigenin (DIG) for *in situ* hybridization assays. This probe was tested on SPF, rickettsial and bacterial infected Davidson's fixed shrimp specimens. The probe was specific for both natural and experimentally generated rickettsial infections. Hybridization with this probe required a stringent temperature of 65°C, or cross-reactivity was observed with other types of bacteria.

ISA in the Faroe Islands

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Data for the introduction and spread of ISAV in Atlantic salmon farming in the Faroe Islands, production data and infrastructure of Faroish fishfarming that highlights structural advantages and disadvantages in fighting ISA, will be presented. The initial lack of routines and capacity for the handling of large amounts of high risk materials—dead fish and slaughter offals—revealed the need of detailed rules and regulations for production routines and disease prevention. The work with the Faroish Contingency plan including zoosanitary measurements and the possible use of a vaccine against ISAV will be presented and discussed.

Do fish have prions? Results of a one year study

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In recent years the range of species in which the existence of the normal cellular prion protein (PrP^c) has been discovered has greatly increased. The existence of PrP^c is the precondition to contract TSE (transmissible spongiform encephalopathy), a disease based on a conformational shift change of the physiological protein PrP^c to a pathological protein isoform. The question whether fish could contract a prion related disease and therefore pose a potential food safety risk to humans is an important issue because besides mammals and birds, fish are the most relevant vertebrate species among eatable vertebrates. The aim of this study was to investigate, whether the most important fish species in aquaculture in central Europe, rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*), express a homologue of PrP^c. Western Blots were performed using prion specific monoclonal antibodies as well as polyclonal anti PrP antisera. In two of the tested antibodies / antisera, binding to fish brain protein (rainbow trout and carp) was observed in a size range which would suit for a potential fish prion protein (ca. 40 kDa). Based on this finding, investigations were initiated to search for the hypothetical fish prion gene. This work is performed using degenerate primers. The results of those molecular studies will be presented.

Modulation of white perch immune function: investigations on fish health in selected tributaries of the Chesapeake Bay

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Functional assays were performed using leukocytes from white perch (*Morone americana*) taken from Chesapeake Bay tributaries. Data on macrophage function and on transforming growth factor-beta expression obtained from the perch collected in the Choptank, Severn, Back, Wicomico, and Pocomoke Rivers in June, August, and October of 1998 suggested that significant immunomodulation was occurring and that the degree of modulation varied by site and month of collection. This observation was supported by data on perch macrophage and lymphocyte functions, and on macrophage aggregates obtained during subsequent perch harvests from the Pocomoke, Chester and Patuxent Rivers (June, August, October 1999, bimonthly 2000, 2001, and 2002). Spatial and temporal differences in immune function are discussed in terms of seasonal, water quality and land-use influences.

Effects of temperature on WSSV and TSV in *Litopenaeus vannamei* in controlled experiments

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Water temperature serves as a major influence on infectivity of both TSV and WSSV to *Litopenaeus vannamei*. A series of experiments showed that low temperature influences infections of both viruses in TSV-sensitive specific-pathogen-free juveniles. Each experiment included a high temperature group, usually at 28□C, and one or more lower temperature groups ranging from 15 to 22°C. Some included an additional group at fluctuating moderate room temperatures. Test groups consisted of shrimp maintained in duplicate aquaria in waterbaths. In addition to positive and negative control groups held at constant temperatures, there were groups transferred from one to the other temperature at one or two periods after being administered the specific virus. In experiments with TSV, 15°C caused stress and some mortalities of shrimp, even in negative controls. At 16°C, shrimp were stressed less, and there was some inhibition or inactivation of the virus. At 18°C, there was inhibition when shrimp were moved from high to low, but not to room temperature and not when transferred from low to high temperature. In experiments with WSSV, roughly half the exposed shrimp in groups maintained at a constant 18 or 22°C survived. All shrimp transferred from high to low temperature and usually vice versa died, even though the period was less than in the exposed controls at constant 28°C. All shrimp, including an additional stock, died when exposed to constant 32°C, a temperature reported to inhibit infections. The study was funded by USDA, CSREES No. 98-38808-6019.

Adenovirus as a method for the delivery and expression of foreign genes in rainbow trout (*Oncorhynchus mykiss*)

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Various fish cell lines were used to assess the feasibility and potential limitations to using adenoviral vectors as gene delivery vehicles in fish. Several fish cell lines were tested for their infectivity by adenovirus and level of gene expression following infection. An adenoviral vector engineered from adenovirus type 2 was used along with a binding modified adenovirus. Both the tropism modified and the native tropism viruses contain the gene for firefly luciferase under the control of the cytomegalovirus promoter and were effective in infecting several cell lines. After demonstrating the effectiveness of Ad infectivity *in vitro* the ability of Ad to infect and express genes in fish *in vivo* was determined. Expression of luciferase was undetectable in 5 gram fry when they were immersed for one hour in PBS or water containing Ad5LucRGD or AdCMVLuc. Next, larger fish were individually injected by intraperitoneal injection with either of the Ad vectors. After dissecting and examining the individual organs of the injected fish for luciferase expression, low levels of expression were found in the muscle of injected fish. Direct intramuscular injection of fish verified the ability of these vectors to infect muscle cells and then express luciferase. In conclusion it was found that several teleost cell lines are capable of being infected and it appears that some cell lines express a human serotype adenoviral receptor homologue that aids in Ad infection, and that fish muscle is infected *in vivo* with adenovirus upon direct injection.

Expression level of immunological factors from rainbow trout (*Oncorhynchus mykiss*) after infection with either bacterial or viral pathogens

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In order to improve disease resistance in salmonid stocks it is necessary to better understand the genes necessary for certain types of immunological response to disease. To further enhance an understanding of immunological gene expression during infection with natural pathogens, a series of probes and primers were developed for the quantification of specific natural and adaptive immune factors using real-time PCR. Individual groups of fish were infected with either of three different pathogenic microorganisms, *Flavobacterium psychrofilum*, *Aeromonas salmonicida*, or infectious haematopoetic necrosis virus (IHNV). The pathogenic agents were dosed at three different levels and samples from infected and mock infected fish were taken at two different time points after infection. Sample points were 1 day and 5 days post infection. Ten fish were harvested for each experimental point and the liver, spleen, and head kidney were isolated for study. The organs from 5 of these animals were plated in tissue culture and examined for presence and level of pathogenic agent. These same organs were isolated from the remaining five animals and total RNA was extracted. The RNA from these organs was then examined for the level of expression of the following immunological related factors, interferon (MX-1), interleukin 8 (IL-8), CD-8, complement factor 3 (C-3), lysozyme, and tumor necrosis factor and they were also examined for the level of B-actin which was used as a standardization control for cellular RNA expression. Using the real-time PCR with fluorescent labeled probes it was found that infection with *F. psychrophylum* did not significantly change the expression level of most of the immune factors in the study. However, infection with *A. salmonis* and IHNV showed significant increases in response levels, some of which correlated highly with pathogen dose.

Investigations in the life cycle of *Enteromyxum* spp. (Myxozoa). A two-host cycle?

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A new genus of myxozoans, *Enteromyxum*, has recently been described. The parasites cause severe chronic enteritis and infections often result in emaciation and death of the affected fish host. Enteromyxosis by *E. scophthalmi* has been reported in cultured turbot, and infections by *E. leei* are common in Sparidae fish cultured in the Mediterranean. To date, known life cycles of myxozoans have been found to be heteroxenous, involving invertebrate intermediate hosts. Nevertheless, *Enteromyxum* spp. infections can be spontaneously transmitted fish-to-fish. Furthermore, recent findings demonstrate that the parasites can cross the host species barrier easily, infecting in some cases rather ubiquitous fish species. Epidemiological surveys screening invertebrates and wild fish are being carried out, in the search of natural reservoirs of these parasites in the wild. The putative existence of invertebrate hosts for *Enteromyxum* remains an open question, but the findings on the direct inter- and intra-specific transmission have obvious implications for the management of cultured stocks in sites where susceptible wild fish can acquire or spread these infections.

Abnormal hermaphroditism in shovelnose sturgeon (*Scaphirhynchus platorynchus*) from the Missouri River

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Sturgeon are gonochoristic and hermaphroditism is not a normal mode of reproduction in these fishes. Nevertheless, there have been occasional reports of individual sturgeon with both male and female gametes. We collected adult shovelnose sturgeon (*Scaphirhynchus platorynchus*) monthly from May 2001 through June 2002 from the lower Missouri River. Abnormal hermaphroditism was observed in some of the collected fish during September, December, January, February, and April. Gross observation indicated that gonadal tissues were mostly testicular. Ova at various developmental stages were embedded in the testicular tissue and not uniformly distributed. Ovigerous folds were observed in some areas of some testes. The proportion of hermaphroditic males ranged from 5% to 35% with the greatest percentage occurring in February. Although the conditions and factors causing abnormal hermaphroditism in sturgeons and other gonochoristic species are not known, there is some experimental evidence that altered or degraded environmental conditions may be responsible. Furthermore, it is not known if the reproductive biology of the shovelnose or the co-occurring and related endangered pallid sturgeon (*Scaphirhynchus albus*) may be impaired by the intersex condition. Further investigation must be completed before these observations of abnormal hermaphroditism in shovelnose sturgeon can be applied to our understanding of pallid sturgeon biology.

Immunomodulatory effects and efficacy of a DNA vaccine for aquatic mycobacteriosis

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Mycobacteriosis is a chronic progressive disease that leads to systemic infections and ultimately death. It is an economically important bacterial disease of virtually all species of freshwater and marine fish, and is usually caused by the intracellular pathogens, *Mycobacterium marinum*, *M. fortuitum*, or *M. chelonae*. While the immune response against aquatic *Mycobacterium* spp. has been characterized, an effective vaccine against mycobacteriosis has not been developed. Because of certain advantages and potential to induce protective immune responses against intracellular pathogens, a DNA vaccine was chosen as an appropriate construct for aquatic mycobacteriosis. A vaccine was assembled by cloning the *M. marinum* 85A gene in the pcDNA 3.1 eukaryotic expression vector (Invitrogen, San Diego, CA). In the resulting pCMV-85A construct, the 85A gene is expressed under a cytomegalovirus (CMV) promoter after injection into fish tissue. Hybrid striped bass (*Morone saxatilis* x *M. chrysops*), a proven susceptible species, were used as a model to test the vaccine. Intramuscular or intraperitoneal vaccinations of the pCMV-85A construct were administered on days 0 and 14, along with positive and negative control vaccinations. Every two weeks, samples were obtained from the fish to evaluate the humoral and cell-mediated responses by ELISA, lymphoproliferative assay, and cytokine assay. On day 70, the fish were challenged with *M. marinum*. Routine histological examination was performed and splenic bacterial counts evaluated to determine the relative degree of susceptibility among the different vaccination groups.

Ontogeny of the lymphoid tissues of the paddlefish, *Polyodon spathula*

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Polyodon spathula leukocytes and developing lymphoid tissues were characterized by enzyme cytochemistry and immunohistochemistry. Monocytic cells, T and B lymphocytes and granulocytes were positive for acid phosphatase. Monocytic cells and a population of T lymphocytes were positive for alpha-naphthyl butyrate esterase. Mature T and B lymphocytes were positive for beta-glucuronidase. Mature B lymphocytes were positive for the anti-light chain of white sturgeon (*Acipenser transmontanus*) immunoglobulin monoclonal antibody. All leukocytes were negative for Sudan Black B, and it is assumed that very few, if any neutrophils are present in *Polyodon*. Monocytic cells were present in the anterior and posterior renal hematopoietic tissue (ARHT, PRHT), spleen, cranial meningeal tissue (CMT), heart and spiral valve at 7 days post-hatch (dph). T lymphocytes were present in the ARHT, spleen, CMT and spiral valve at 7 dph. T lymphocytes were present in the heart by 21 dph and in the thymus, spleen and CMT at 28 dph. B lymphocytes were present in the ARHT, PRHT, thymus, CMT, heart and spiral valve at 7 dph and in the spleen at 56 dph. Granulocytes were present in the ARHT, PRHT, thymus, spleen, CMT, heart and spiral valve at 7 dph. Heart lymphoid tissue was not apparent until 56 dph. Peyer's patches were present in the gut lamina propria by 56 dph. The results of this study indicate that *Polyodon* lymphoid tissues are slow to develop and may not function similarly to juvenile or adult tissues until 28 to 56 dph.

Cloning and characterisation of a channel catfish (*Ictalurus punctatus*) Mx gene

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A 2.5 kb full-length cDNA clone of a channel catfish Mx gene was obtained using RACE (rapid amplification of cDNA ends) polymerase chain reaction (PCR) from RNA extracted from the liver of poly I:C stimulated channel catfish. The gene consists of an open reading frame (ORF) of 1905 nucleotides encoding a 635 amino acid protein. The predicted protein is 72.5 kDa and contains the dynamin family signature, a tripartite GTP binding motif and a leucine zipper, all characteristic of known Mx proteins. The catfish Mx protein exhibited 79 % identity with perch Mx and between 71 % and 74 % identity with the three Atlantic salmon and the three rainbow trout Mx proteins. Mx was constituitively expressed in channel catfish ovary (CCO) cells but in higher quantities in response to poly I:C treatment. Mx was found to be induced to a greater level and for a more prolonged period in channel catfish following infection with channel catfish virus (CCV) than injection with poly I:C.

Innate and acquired disease resistance of chinook salmon to *L. anguillarum* following dietary exposure to PCBS or PAHS

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Controlled laboratory challenges with *Listonella* (*Vibrio*) *anguillarum* were used to determine the effects of dietary exposure to Aroclor® 1254 (PCBs), or to a mixture of 14 polyaromatic hydrocarbon compounds (PAHs), on the immunocompetence of juvenile chinook salmon. Independent groups of salmon were fed different concentrations of PCBs or PAHs for a period of 4 weeks. These doses were designed to bracket the reported levels of these environmental contaminants in the stomach contents of Puget Sound salmon. To examine the effects on innate immunity, half of the fish were randomly assigned to replicate tanks, then challenged with pathogenic *Listonella anguillarum* and monitored for 14 days. Subsequently, the other half of the contaminant-dosed fish were vaccinated (excluding controls) and transferred to replicate challenge tanks. Specific immunity was allowed to develop for 3 weeks prior to bacterial challenge. All mortalities were individually necropsied and sampled for bacteria to identify the cause death. In one test, significant non-*Listonella* bacterial infections occurred as the fish transitioned into smolts toward the end of the study following vaccination. This test was repeated the following year and resulted in a much lower incidence of non-*Listonella* mortalities. A separate oral LD_{50} test that was run to measure acute toxicity resulted in no mortality over 96 hours, despite doses as high as 800 mg Aroclor 1254 per kg fish. The challenge data indicate that dietary PCB or PAH exposures, even at relatively high levels, did not have a significant effect on growth, disease resistance, or acquired immunity to *Listonella anguillarum*.

Insights into the pathophysiology and treatment of amoebic gill disease in Atlantic salmon

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Amoebic gill disease (AGD) is a major cost to aquaculture production of Atlantic salmon in Australia. Characterised by a multifocal hyperplasia of the secondary lamellae of the gills caused by the amphizoic parasite *Neoparamoeba pemaquidensis*, AGD pathophysiology involves mild respiratory and acid-base disturbances. In addition, an apparent cardiovascular hypertension and a concomitant change in ventricular shape with an increase in compact cardiac muscle and hyperplasia of the spongiosa have also been reported. Interestingly increases in guanyl cyclase activity stimulated by natriuretic peptides occur. Whether this is as a result of the hyperplastic response in the gill tissue or vasculature in response to the hypertension is unknown and under investigation. Treatment of AGD has relied upon the use of a 2-4 h freshwater bath. However, recent success using chemotherapeutic agents in freshwater and seawater at 10 ppm has shown that gill amoebae are readily removed from the gills. In seawater the removal of amoebae from the gills of AGD affected salmon can be achieved within 1 h of exposure. The use of chemotherapeutics and alternative treatments targeting the pathophysiology of AGD will be discussed.

Pathogenic diplomonad flagellates from fish: recent advances in species recognition, epizootiology, and pathology

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Diplomonad flagellates are common commensals and pathogenic parasites in hosts as diverse as fish and humans. They are widespread in wild and farmed fish, and have been reported as *Hexamita*, *Octomitus*, and *Spironucleus*. Diplomonads have a bewildering variety of relationships with fish, ranging from benign inhabitants of the digestive tract of wild fish, to devastating systemic invaders of farmed salmonids. We are undertaking a comprehensive study of diplomonads from fish, focusing on development of new techniques for recognition of species, via special stains for light microscopy, scanning and transmission electron microscopy (SEM, TEM), and molecular characterisation. These approaches allow us to accurately determine host and geographic ranges, and explain some of the factors associated with pathogenic infections in aquaculture. Transmission EM has shown that diplomonads from fish are *Spironucleus* species. Individual species can be reliably distinguished by a suite of external and internal ultrastructural features including surface ornamentation, patterns of bands of microtubules accompanying the recurrent flagella, auxiliary cytoskeleton, and cytoplasmic organelles: some of these features can also be recognised by special light microscopy stains. To date three species have been rigorously described from fish, and we are now able to determine host and geographic ranges: *S. barkhanus* and *S. torosa* have only been reported from salmonids and gadids respectively, *S. vortens* infects both cichlids and cyprinids; geographic ranges can be very wide, for example *S. vortens* occurs in Florida and Norway. Some devastating systemic infections in aquaculture are caused by species existing as commensals in wild fish.

Protocol of use of a marine probiotic strain in aquaculture

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In previous work, we have established the effectiveness of a probiotic marine bacterium, PP-154 (identified as a member of genus *Roseobacter*), that enhances survival of bivalve larvae in micro and meso-scale cultures, because of its ability for inhibiting bacterial pathogens. Posterior studies were focused to establish a protocol of application of its use in aquaculture industries. With this purpose, a screening of antibacterial activity was performed, using the probiotic strain against molluscs, fishes and crustaceans pathogens. In order to know the response of strain PP-154 to variations of environmental conditions of distinct aquaculture systems, we studied the growth of this strain in a range of temperatures and salinities. We established the relation between growth curve and the antibacterial activity. Finally, the behaviour of the probiotic in the seawater and phytoplankton environment (used as food in larval cultures) was analysed. We have also determined the molecular size of the bioactive substance.

Comparison of culture conditions on development, survival and growth of *Ichthyophthirius multifiliis in vitro*

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Ichthyophthiriasis is an infectious disease of major importance in fish culture. The availability of methods for the *in vitro* culture of *Ichthyophthirius multifiliis* would greatly facilitate the study of the pathogenic mechanisms associated with the disease, and would beneficial for the development of therapeutic treatments or vaccination. To date, however, the parasite can be cultured *in vitro* for short periods only, and it is not possible to maintain the complete life cycle of *I. multifiliis* under *in vitro* conditions. The aim of the present study is to search for culture media that positively influence the *in vitro* performance of *I. multifiliis*. Temporary isolates of the parasite were prepared from infected rainbow trout, *Oncorhynchus mykiss*, and the theronts escaping from tomontocysts were kept in the following media: (1) in water, (2) in Minimum Essential Medium (MEM) for vertebrate cell culture, (3) in co-culture with the fish cell line, EPC (Epithelioma Papulosum Cyprinids), and (4) in co-culture with freshly isolated peripheral blood leucocytes (PBL) of rainbow trout. Both EPC cells and PBL were maintained in MEM. The effects of the four media on *I. multifiliis* were assessed by measuring *in vitro* development, growth and survival of the parasite. The major finding from this study is that the presence of blood leucocytes form the host appears to support *in vitro* development and survival of the parasite.

Biochemical and molecular characterization of a novel ranavirus isolated from diseased grouper, *Epinephelus* spp.

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A pathogenic virus was isolated from grouper, *Epinephelus* spp. by cell culture. The virus was sensitive to acid, ether and heat treatments. Virus replication was inhibited by 5-iodo-2-deoxyuridine (IUDR). Transmission electron microscopy revealed abundant cytoplasmic icosahedral virions in virus-infected grouper cells with a size of about 165 nm. SDS-PAGE revealed more than 20 structural protein bands and a major capsid protein (MCP) of 49 kDa. Antigenic properties of the virus were investigated using rabbit IgG against the virus particles. The surface viral antigens and antigenic-related proteins were visualized and discriminated by immunogold electron microscopy, immunofluorescence microscopy and Western blot. A partial MCP gene of ~500 nucleotides was amplified and sequenced from the viral genome by polymerase chain reaction (PCR) using the primers from the conserved regions of MCP gene of frog virus 3 (FV3), the type species of *Ranavirus*. Sequence multiple alignment and phylogenetic analysis showed that the newly isolated virus was more closely related to large mouth bass virus (LMBV) and FV3. The present data suggest that the grouper virus isolate is a novel member of genus *Ranavirus*, the family *Iridoviridae*.

Genetic analysis of fish isolates of *Lactococcus garvieae* by RAPD analysis

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Lactococcus garvieae is considered one of the major fish pathogenic Gram positive cocci, producing fatal septicaemia among fish species living in very diverse environments. Infections caused by this microorganism have been reported in cultured salt and freshwater fish species like yellowtail in Japan and rainbow trout in Europe and Australia. In Spain, the disease is one of the most important risk factors in the trout industry during the summer months. In this work, we present the results obtained in the genetic characterization of 52 fish isolates of *L. garvieae* with different host and geographical origin. A great genetic variability was observed among the isolates, being detected 11 different RAPD profiles. The majority of the Spanish isolates joined together in a unique profile (RAPD I), showing a close relationship with the isolates from Turkey (RAPD II) and the Italian catfish strain (RAPD III). On the other hand, the French strains were distributed into three different profiles (RAPD V, VI and IX) while the Italian isolates from trout were clustered together in profile VII. Moreover, all the yellowtail isolates from Japan belonged to RAPD type X. Three profiles contained a unique strain, profile IV which included the Spanish isolate from broodstock 332, profile VII constituted by the Spanish strain 344 isolated from carrier fish, and profile XI including the reference strain NCDO 2155 with bovine origin. All these RAPD types could be grouped in two clonal lineages showing a 34% similarity. One lineage clustered the RAPD I to IV and the other lineage comprised the other RAPD types. All these results indicate that RAPD technique can be useful for epidemiological studies of *Lactococcus garvieae*.

Molecular genetics of major soluble antigen (MSA) in the salmonid pathogen, *Renibacterium salmoninarum*

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Bacterial kidney disease (BKD), a debilitating systemic disease of both wild and cultured salmonids, is caused by the Gram-positive bacterium *Renibacterium salmoninarum*. Fish infected by *R. salmoninarum* generate an antibody response directed against an abundant surface protein called major soluble antigen (MSA). There are at least two copies of the gene encoding MSA (*msa1* and *msa2*), and their promoter regions differ significantly beyond 40 bp upstream of the translation start site. The high abundance of the MSA protein and the 100% nucleotide identity between the *msa1* and *msa2* open reading frames led us to hypothesize that both gene copies are expressed. We constructed *msa*-green fluorescent protein (*gfp*) gene fusions containing 500-600 bp of 5' flanking sequence from *msa1* or *msa2*, and transformed *R. salmoninarum* ATCC type strain 33209 with these constructs. The reporter plasmids precisely integrated into their cognate chromosomal locations by single cross-over homologous recombination, providing the first demonstration of genetic manipulation of *R. salmoninarum*. GFP mRNA and protein were expressed by *R. salmoninarum* clones bearing integrated *msa-gfp* reporter fusions, indicating that both *msa1* and *msa2* are expressed under *in vitro* culture conditions. Although *R. salmoninarum* possesses two functional *msa* genes, further analysis indicates that additional copies may be present. These results suggest MSA is a significant, possibly essential, gene in *R. salmoninarum*.

Experimental mycobacteriosis in striped bass *(Morone saxatilis)*: microbiological results

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Mycobacterium marinum is one of three species considered the primary agents of mycobacteriosis in fish, including striped bass (*Morone saxatilis*). During a recent epizootic of striped bass in the Chesapeake Bay, USA mycobacteria were cultured from the spleens of approximately 76 % of aseptically examined fish (N=196). A variety of *Mycobacterium* species were recovered including a newly proposed species, *M. shottsii*, which was the most frequently cultured mycobacterium (77%), typically at densities of 10^{4-6} colony forming units per gram (CFU/g) splenic tissue. In laboratory challenge studies, striped bass were injected intracoelomically with ~10^5 CFU of either *M. marinum*, *M. gordonae* or *M. shottsii* isolated from infected Cheapeake Bay striped bass. Fish were maintained in separate flow through systems at 18-22°C, and sampled bimonthly for10 months. Mean densities of *M. marinum* at 2 months were ~ 10^7 CFU/g splenic tissue and increased to maximum values of ~ 10^9 CFU/g at 8 months. In contrast, densities of *M. gordonae* and *M. shottsii* at 2 months were ~ 10^{3-4}/g and maximum mean densities of ~ 10^7 and ~ 10^4/g were observed respectively at 8 months. In vitro growth studies demonstrated that *M. marinum* is a relatively rapid grower having a generation time of 2-3 days at 23°C compared to *M. shottsii* which has a generation of time of ~10 days. Only at one sampling time (8 months) did the majority of fish challenged with *M. shottsii* exhibit densities as elevated as those frequently observed in wild fish with mycobacteriosis.

Current eradication status of the exotic sabellid worm in the state of California

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In California during the late 1980's the introduction of an exotic parasite from South Africa was observed in red abalone (*Haliotis rufescens*) culture facilities. This previously unidentified sabellid polychaete, *Terebrasabella heterouncinata* continued to plague commercially-grown abalone until its profusion escalated in the mid 1990's, infesting every California commercial abalone farm and many research facilities. *T. heterouncinata* infests the shells of abalones and other gastropods causing a deformed appearance. Shell nacre deposition is altered due to the presence of the worm creating vertical versus lateral growth. Reduced growth results in a sub-optimal terminal market product and increased mortality. The aquaculture industry, University of California Santa Barbara researchers and the Department of Fish and Game initiated an aggressive pest eradication program in 1996, and Departmental policy was established in 1997 to prevent further spread of the sabellid. Culling of infested stocks and freshwater treatment of tanks in combination with strict hygiene protocols proved effective methods for curbing new infestations. The sabellid worm however, was determined to be capable of persisting at low-levels with the ability to become re-infestive, therefore extensive sampling of abalone populations became necessary to thoroughly determine its prevalence. Facilities have undergone both "spot" (random checks) and/or "certification" (facility-wide) inspections. In 2001/02 approximately 24,000 shells or live abalone were inspected and all but one facility passed spot inspections for the first time since program inception. Additionally, three abalone culturists have requested and passed sabellid certification inspections successfully. Over 28 wild populations of red abalone (14 of which had been exposed to potentially infested outplanted abalone) have been examined since 1998, with no sabellids found. These investigations suggest that control efforts have led to near eradication of the sabellid pest, but that continued monitoring is necessary.

The ASK cell line: an improved diagnostic tool for isolation, propagation and titration of the infectious salmon anemia virus (ISAV)

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Infectious salmon anemia (ISA) is an important viral disease of cultured Atlantic salmon (*Salmo salar*) in Norway, Scotland, Canada, Denmark, Chile and more recently, the United States. Current diagnostic methods include viral isolation in cell culture and RT-PCR. Due to cost and availability, cell culture is often the preferred diagnostic tool. The most commonly used cell line for isolation, propagation and titration of ISAV is the SHK-1 (salmon head kidney) cell line developed from Atlantic salmon pronephros. The SHK-1 cell line appears to be of mixed morphology consisting mostly of fibroblast-like cells. This cell line is delicate, has a split ratio of 1:2 approximately every 10-14 days and requires Liebowitz's L-15 medium supplemented with fetal bovine serum (FBS) and beta-mercaptoethanol. The cell line appears to have an unstable morphology requiring periodic replacement of high passage cells with low passage frozen stocks. We compared the SHK-1 cell line to another cell line also developed from Atlantic salmon pronephros, the ASK-2 (Atlantic salmon kidney) cell line. The ASK-2 cell line has split ratios and medium requirements similar to the SHK-1 cells, although ASK-2s do not require beta-mercaptoethanol. ASK-2 cells also produce ISAV titers similar to the SHK-1 cell line; however, inoculation of the ASK-2 cell line with ISAV results in distinct and complete CPE facilitating titer determination. In addition, the morphology of the cell line appears to be stable and the cell line is generally less delicate than SHK-1 cells.

Relative resistance of Pacific salmon to infectious salmon anemia virus (ISAV)

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The aim of this study was to determine the susceptibilities of Pacific salmonid species to Infectious salmon anemia virus (ISAV) compared to Atlantic salmon (*Salmo salar*). Chum (*Oncorhynchus keta*), steelhead (*O. mykiss*), chinook (*O. tsawytscha*), coho (*O. kisutch*) and Atlantic salmon were distributed into duplicate tanks of 25 fish per species per ISAV dosage. In Experiment 1, groups were injected intraperitoneally (i.p.) with a high (10^8 $TCID_{50}$ ml^{-1}), medium (10^6 $TCID_{50}$ ml^{-1}) or low (10^4 $TCID_{50}$ ml^{-1}) dosage of ISAV (Bremnes, Norway). Experiment 2 was a high dosage (10^8 $TCID_{50}$ ml^{-1}) i.p. challenge of all species, except chum salmon, with ISAV of either the New Brunswick, Canada or the Bremnes, Norway strain. The cumulative mortality of Atlantic salmon in Experiment 1 was 12% in the high dosage group, 20% in the medium dosage group and 16% in the low dosage group. The average cumulative mortality of Atlantic salmon in Experiment 2 was 98%. No ISAV-related mortality occurred among any of the *Oncorhynchus* spp. in either experiment although ISAV was detected in random samplings of all species except Chinook salmon, suggesting that infection is possible. The results indicate that *Oncorhynchus* spp. are relatively resistant to ISAV infection. However, the potential for ISAV to affect *Oncorhynchus* spp. should not be ignored as this virus belongs to the Orthomyxoviridae, a highly adaptive family of viruses including the influenza virus.

Efficacy of an ISAV vaccine in Atlantic salmon in freshwater and seawater

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Infectious salmon anemia virus (ISAV) is a severe viral pathogen in the orthomyxovirus family, which affects Atlantic salmon in Norway, Scotland, Faeroe Islands, New Brunswick, Canada and Maine, USA. Although bio-regulatory control measures have been implemented in all effected areas, vaccination against the disease has been mostly limited to Canada. An inactivated multivalent vaccine (Forte V1) has induced a high level of protection against cohabitation challenge with ISAV in freshwater (relative percent survival: 84%) with 74% mortality in the controls. Bio-containment measures have made it difficult to determine field efficacy in clinical trials. In the fall of 2000, S_0 Atlantic salmon (80 g) were vaccinated at a freshwater hatchery and a sub-population transferred to a land based saltwater holding facility. At 1200, 1500 and 2200 degree days (dd) post vaccination, fish vaccinated with either Forte V1 or a negative reference vaccine (a multivalent bacterin, Lipogen Forte) were challenged by co-habitation with naïve fish injected with $2 \times 10^{5.4}$ $TCID_{50}$ of ISAV (Bay of Fundy isolate). Protection was evident for at least 10 months (2200 dd), the extent of the study. Molecular differences between European and North American ISAV isolates have been reported. To show the potential use of this vaccine in other geographical regions results of cross-protection studies with European isolates will be presented. To address the potential that virus recombination influences vaccine efficacy, results of vaccination-challenge studies with ISAV from the Bay of Fundy in consecutive years will also be presented.

Infectious disease model development using zebrafish (*Danio rerio*) and a viral pathogen

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The laboratory cyprinid zebrafish (*Danio rerio*) is currently utilized for biomedical developmental and genetic studies. We hypothesized that zebrafish might be a useful model for improving our understanding of the genetic basis of disease as it relates to cyprinids. Therefore we developed an infectious disease model for zebrafish using spring viremia of carp virus (SVCV), *Rhabdovirus carpio*. After spawning a wild type strain of zebrafish, 200 fish were reared to adults and were transferred into an aquatic biohazard level-3 facility. Replicate groups of 10 fish were exposed, via immersion, to various concentrations [10^3, 10^4, 10^5 plaque forming units per ml (PFU/ml)] of SVCV at different temperature regimes. Clinical signs became evident approximately seven days after viral exposure and were observed most consistently in the 1×10^5 PFU/ml exposure group. In zebrafish, SVCV produced anorexia, listlessness, multifocal epidermal petechial hemorrhages and death, similar to clinical signs observed in naïve cyprinid species in Europe. Evaluation of tissue from infected fish by viral plaque assays and polymerase chain reaction revealed the presence of SVCV and titers $\geq 1 \times 10^4$ PFU/ml. Histopathologic lesions include multifocal hepatic necrosis, and melanomacrophage proliferation in the gills, liver, and kidneys. The future use of this disease model with various stocks of mutant zebrafish will be of assistance in the evaluation of the roles that genes play in piscine viral disease pathogenesis and elucidation of potential genetic differences.

Isolation and characterization of a novel CXC chemokine in common carp (*Cyprinus carpio* L.)

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Chemokines are essential mediators of normal leukocyte trafficking as well as leukocyte recruitment during inflammation. A novel CXC chemokine was identified for the first time in a teleost, the common carp, *Cyprinus carpio* L. The cDNA is composed of 619 bp with a 37 bp 5 untranslated region (UTR) and a 287 bp 3 UTR. An open reading frame of 368 bp encodes a 97 amino acid peptide, with a putative signal peptide of 20 aminoacids. The gene has four cysteines residues, which are conserved, with the first two cysteines separated by phenylalanine. Homology and phylogenetic analysis revealed that carp was found to be closer to human IP-10. Identities were significantly low to the CXC chemokines cloned from fish. The carp CXC chemokine contains four exons interrupted by three introns. The gene was transcribed from an early time point by stimulation with lipopolysaccharide and concanavalin A. Organs in resting as well as stimulated phase expressed the gene in various tissues.

Role of the veterinary profession and the American Veterinary Medical Association in aquatic animal health

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The mission of the AVMA is to advance the science and art of veterinary medicine including its relationship to public health, biological science, and agriculture, through the support of, and service to, its ~67,000 veterinarian members. Since the 1860s, the health care, management and medical services provided to all animal species by veterinarians has evolved with societal needs, medical knowledge, technology, and the evolution of animal health and disease. The AVMA and profession have responded strongly to assist and enhance the practice of aquatic veterinary medicine covering aquaculture, ornamental (pet), wild fisheries, and seafood safety through developing programs, services, and policies that affect its members, their clients, and aquatic animal industries they serve. With increasing focus on aquatic veterinary issues since the early 1990s, the veterinary profession has increased emphasis on emerging aquatic diseases, diagnostics, drug availability, education, legislation and regulations, animal welfare, seafood safety. Aquatic issues, solutions, and program initiation originated from, or filter through, the Aquaculture and Seafood Advisory Committee (ASAC). ASAC presently consists of specialists representing all major areas of veterinary aquatics and including non-veterinary aquaculture production, governmental consultants from USDA-APHIS-VS, FDA-CVM, FDA-CFSAN, and others. Working through its ~30 Councils and Committees, and in dialogue with ~450 regional, state and local and ~ 40 veterinary specialty organizations, allied to the AVMA, the Association is the authorized voice of the profession in presenting its views to government, academia, agriculture, animal owners, the media, and other concerned public entities. Through formal ASAC liaisons to several subgroups of the federal interagency Joint Subcommittee on Aquaculture (Quality Assurance, Aquatic Animal Health, Effluents) and other organizations, the AVMA provides veterinary input to developing national and international programs, legislation, and regulations dealing with drug availability and use, national and local responses to endemic, epizootic and foreign animal diseases, aquatic pollution, and seafood safety.

Comparative pathogenicity of dermo (*Perkinsus marinus*) between the Caribbean and American oyster

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The Caribbean oyster *Crassostrea rhizophorae* is a closely related species of *C. virginica*, but little is known of its susceptibility to Dermo. Therefore, pathogenicity of Dermo was compared between oyster species. Dermo-free oysters were challenged once via shell-cavity inoculation ($1x10^5$ *P. marinus* per gram of estimated wet tissue weight) with either saline or one of two genetically distinct North American isolates of *P. marinus* and observed for 16 weeks. A natural infection study was also performed by examining oysters placed in land-based tanks, which received 7-10 L/min unfiltered seawater, for 214 days. Infections were determined using the whole-body burden method. Fifteen American oysters and one Caribbean oyster died during the challenge experiment. Log10 transformed *P. marinus* burdens in surviving oysters were significantly heavier for Caribbean oysters (1.25±0.08) compared to American oysters (0.72±0.06). Control oysters did not develop infections (0.09±0.01). In the natural infection study, parasites were detected in all groups after 103 days: intensities were negligible and there was no difference in burdens between the two species. At the end of the study most oysters had died (89-98%). *Perkinsus* prevalence was 100% in all surviving *C. virginica*, but 10% of the *C. rhizophorae* had escaped infection. There was no significant difference between oyster species in body burdens of survivors. Results from the two studies indicate that Caribbean oysters are as susceptible to North American isolates of *Perkinsus*, but may be somewhat more tolerant of heavier parasite loads.

Gene expression and proteomics: the application of microarray techniques and SELDI mass spectroscopy for the identification of biomarkers/bioindicators

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Eukaryotes have numerous mechanisms for dealing with environmental stresses and pathogens. While animals are exposed to pathogens regularly, disease is manifested only when the immune system is not able to counter the invasion and multiplication strategies of the infectious agent. Often, the immune system succumbs as a result of pressure from perturbed environmental situations and the resulting stress. Episodes of animal and human disease have recently increased dramatically in association with freshwater, aquatic, and marine coastal systems. In the Chesapeake Bay, for example, blooms of toxic algae, dinoflagellates, and fungus have been reported to have caused fish kills and lesions and perhaps human illness. New technologies for gene identification and measurement of gene expression have exploded in recent years. One of these methods, the microarray, allows for the measurement of the activity of thousands of genes simultaneously. Most of the information from these types of experiments, however, lies in teasing out patterns of gene expression rather than simply quantification of expression on a gene-by-gene basis. That is, the discoveries lie in defining networks of genes that show coordinate expression under various conditions. Powerful as they may be, these global measurements of simple gene expression do not totally describe cellular activities. Posttranslational modification and control mechanisms also play significant roles as do nuclear trafficking systems. Proteomic and cellular studies are required to complete the story. We describe applications of two new methodologies, microarray analysis and SELDI (Surface-Enhanced Laser Desorption/Ionization) mass spectroscopy in the rapidly evolving field of toxicogenomics.

An unusual DNA virus responsible for tumor development in damselfish

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Damselfish neurofibromatosis (DNF) is a transmissible, neoplastic disease affecting bicolor damselfish (*Stegastes partitus*) on South Florida reefs. This disease is characterized by multiple neurofibromas and chromatophoromas. The agent responsible for DNF appears to be an unusual DNA virus. Extrachromosomal viral DNAs of discrete sizes, ranging from 1.2 to approximately 7 kb, accumulate in infected cells. The genome is most likely 2.5 kb dsDNA. This DNA does not show homology to any sequences in GenBank. Viral DNAs isolated from damselfish with naturally occurring and induced tumors and from long-term cell lines have shown an almost perfect sequence conservation suggesting that strong selection pressure must be acting to stabilize this sequence. Associated RNAs are approximately 300 bp, 600 bp, 1.2 kb and 2.4 kb in size. Predicted particle size for a 2.5 kb genome would be in the range of 25 nm and appropriate sized particles have been observed in tumor tissues. However, particles have been difficult to isolate from either tissues or cultured cells and appear to have a very labile viral coat. The presumed coating of the particles, the presence of which is defined by resistance of particles to DNase, can be disrupted by even mild organic solvents, such as chloroform and Freon, treatments to which most known viral capsids are resistant (even in enveloped viruses). The nature of the viral coat and presumptive viral proteins are unclear. This agent is unique in that DNF is the only naturally occurring, transmissible cancer affecting a neuroectodermal cell type (Schwann cells and chromatophores in the case of DNF).

Environmental contaminants and developmental mortality in fish and alligators

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Over the past years, our laboratory has been evaluating the incidence of developmental mortality in largemouth bass (*Micropterus salmoides*) and American alligator (*Alligator mississippiensis*) as a function of environmental exposure to chlorinated pesticides (OCP). Sampling areas have included several reference and contaminated lakes, as well as reclaimed agricultural properties ("muck farms") in Central Florida. The predominant contaminants from these sites are DDT and derivatives, toxaphene, dieldrin, and chlordane. In largemouth bass, exposure to OCP causes endocrine changes (reduction in sex steroids and vitellogenin) that are not translated in reduced spawning or hatch rates. However, exposure to OCP results in an increase in fry mortality at the swim-up stage. In alligators, hatch rates are reduced in contaminated sites when compared to the reference. Low hatch rates are due to increased neonatal mortality, mainly during the first 35 days of development. Detailed analyses of alligator eggs and embryos have not indicated developmental anomalies or disease as the cause for this mortality, but rather an overall retardation of growth in clutches from high-OCP sites. Potential mechanisms for the fish fry and alligator embryo mortalities observed at these sites are discussed.

Aspects of the pathology of *Hematodinium* sp. infections in snow crabs (*Chionoecetes opilio*) from Newfoundland, Canada

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Bitter crab disease (BCD) of snow crabs, *Chionoecetes opilio*, is caused by a parasitic dinoflagellate, *Hematodinium* sp. The disease has shown an alarming increase in prevalence in Newfoundland's commercial fishery since it was first recorded there in the early 1990s. We examined the pathology of several infected snow crabs and documented the alterations to the gills during sporulation of the parasite. Sporulation events (sporogony) can lead to high densities of dinospores in the hemolymph (10^8 dinospores ml^{-1}) and may cause significant alterations to the crab leading to host death. The damage to the gills varied but in some cases it was severe involving an apparent thinning of the cuticular layers, necrosis and loss of host epithelial cells, and fusion of the membranous layers of adjacent gill lamellae. Affected lamellae exhibited varying degrees of hypertrophy with the loss of trabecular cells, hemocyte infiltrations, and the appearance of clubbing along the distal margins. Necrotic host tissues, when present, were clumped along bands or zones of individual and fused lamellae. Large numbers of zoospores were located distally along the distal margins of affected lamellae suggesting that sporulation may result in the lysis or bursting of the lamellar cuticle to release spores. It is unclear how dinospores transmit the disease to the next host. Transmission and mortality studies are warranted to better understand the effect of the disease on its commercially important host.

Characterization of fish mortalities caused by *Pfiesteria shumwayae* (Strain CCMP 2089)

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Long-term, large-scale fish bioassays are currently used as the standard for identifying toxic *Pfiesteria* spp. We investigated the nature of mortalities in fish exposed to short- and long-term challenges with *Pfiesteria shumwayae* in large-scale assays (38 L) and in small-scale assays (10 mL). Exposures consisted of 25–30 tilapia or 10–25 menhaden in 38-L aquaria inoculated with clonal isolates of CCMP2089 (VIMS 1049). In short-term assays, fish died rapidly in relation to high cell densities with pathological changes to the epidermis and gills. In long-term assays, mortalities began from 21–27 d in aquaria seeded with clonal cultures that previously had been fed algae for 9–14 months. Fish mortalities exhibited temporal peaks separated by quiescent periods of 7–30 d. Long-term assays and subcultures from long-term assays continued to kill fish for over 1 yr. Differential centrifugation and filtration of these fish-killing cultures was used to produce fractions enriched in dinoflagellates and possible "exotoxins" (supernate) which were then assayed with larval fish. In a series of fractionation assays, mortalities occurred only in treatments containing live dinoflagellates, with no mortalities in supernate fractions or controls. The use of fractionation studies in the long-term bioassays indicated that the nature of the mortalities did not change. Strain CCMP2089 readily killed juvenile tilapia, menhaden and larval minnows, but it did not produce a fish-killing toxin. Our findings bring into question the toxigenicity of *Pfiesteria* spp.

A subunit vaccine for infectious pancreatic necrosis virus (IPNV) using a baculovirus/insect larvae system

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Infectious pancreatic necrosis virus (IPNV) can cause a highly contagious disease of juvenile rainbow and brook trout and Atlantic salmon and can infect a variety of salmonid and non-salmonid fishes. Various attempts to develop a vaccine against IPNV have not yielded consistent results. Thus, at present, no commercial vaccine is available that can be used with confidence to immunize fry of salmon and trout. We are attempting to develop an effective sub-unit vaccine against IPNV. We generated a full-length cDNA clone of the large genome segment A of Sp strain of IPNV and expressed structural protein genes VP2, VP3 and VP4 in insect cell line and cabbage looper (*Trichoplusia ni)* larvae using a baculovirus expression system. Green fluorescent protein (GFP) was also co-expressed as a reporter molecule. High yields of IPNV protein were obtained and the structural proteins self assembled to form virus like particles (VLPs). We are currently testing the efficacy of a putative VLP vaccine. Young-of-the-year rainbow trout were vaccinated at two VLP concentrations and are being challenged using Sp and VR-299 strains of IPNV.

Endogenous antibiotic defenses in hybrid striped bass and other fish

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Antimicrobial polypeptides are increasingly recognized as a critical component of innate immunity. Recently, antimicrobial polypeptides have been isolated from some species of fish by several laboratories. Our laboratory has isolated several types of polypeptide antibiotics from hybrid striped bass (*Morone saxatilis* x *M. chrysops*). Histone-like proteins (HLPs), small proteins closely related to histones H2B and H1, are present in high concentrations in the gill, skin and spleen. HLPs are lethal to bacteria and water molds, as well as to *Amyloodinium ocellatum*, one of the most common and pathogenic parasites of marine fish. Piscidins, a novel family of linear, amphipathic polypeptides, also have potent activity against important bacterial pathogens (e.g., *Aeromonas hydrophila, Aeromonas salmonicida, Lactococcus garvieae, Streptococcus iniae*) including isolates which are resistant to traditional antibiotics. Piscidins reside in many tissues, including skin, gill, and gastrointestinal tract, and are expressed in mast cells (eosinophilic granular cells). This is the first time that polypeptide antibiotics have been identified from the mast cells of any vertebrate and suggests a new role for this ubiquitous immune cell. We have evidence that mast cells of a number of other unrelated fish also have piscidin-like antibiotics. Hybrid striped bass also express other polypeptide antibiotics which have broad-spectrum activity. The discovery of such diverse and potent antimicrobial polypeptides supports the prominent role of this class of molecules in host defense against infectious diseases. These findings could lead to ways to protect hybrid striped bass and other fish against diseases without using traditional drugs.

Assessing cortisol responsiveness between strains and *Edwardsiella ictaluri* susceptible and resistant families of channel catfish, *Ictalurus punctatus*

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Edwardsiella ictaluri, the bacterial pathogen that causes enteric septicemia of catfish (ESC), is a prevalent source of substantial economic loss to catfish farmers. Stress has been shown to increase *E. ictaluri* susceptibility in catfish. The secretion of cortisol is one of many physiological changes that occur during stress in fish. Disease outbreaks following a period of stress are often considered to be the result of cortisol induced immunosupression. These experiments were done to determine whether plasma cortisol levels following a stressor (low-water confinement) and during an *E. ictaluri* challenge correlate to the genetic predisposition of a catfish family for *E. ictaluri* resistance or susceptibility. No differences ($P > 0.05$) in the cortisol stress response following a 1-h stressor and 1-h recovery were determined between catfish strains (Norris and USDA-103) or families within a strain. Stress induced and recovery plasma cortisol levels were not significantly ($P > 0.05$) correlated to mortality resulting from *E. ictaluri* challenges ($r^2 = 0.02$ and 0.49, respectively). Plasma cortisol response during an *E. ictaluri* challenge, however, differed significantly ($P < 0.05$) 48-h post-challenge between highly susceptible and resistant families. While the present data indicate that the magnitude of cortisol response to a single low-water confinement stress does not correlate to *E. ictaluri* susceptibility, the observed familial differences in cortisol response during a *E. ictaluri* challenge suggest cortisol may still play a role in pathogen resistance.

Diagnosis of *Hematodinium* infection in the Norway lobster *Nephrops norvegicus* by ELISA, PCR, dot blot, and *in situ* hybridization

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Norway lobsters (*Nephrops norvegicus*) from the coastal waters of Scotland are seasonally infected by a parasitic dinoflagellate of the genus *Hematodinium*. An enzyme-linked immunosorbent assay (ELISA), a polymerase chain reaction (PCR) assay and a DNA probe for use in dot blot and in situ hybridization have been developed for the detection of *Hematodinium* species infection in the haemolymph and tissues *of N. norvegicus* and other crustaceans. The ELISA method is simple and quick to perform with a detection limit of 5×10^4 parasites ml^{-1} haemolymph, which facilitates the screening of large numbers of samples. The nucleotide sequence encoding the partial ribosomal small subunit and first internal transcribed spacer region of the parasite has allowed the synthesis of specific oligonucleotide primers and a DNA probe. All techniques are capable of detecting low-level infections, and are currently being evaluated as tools to investigate the prevalence and seasonality of *Hematodinium* infection in *N. norvegicus* and other crustacean hosts.

(Infectious?) Hemolytic anemia of salmon: an emerging disease occurring in seawater cultured coho salmon (*Oncorhynchus kisutch*) in Chile

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In the last six years a new disease, colloquially referred to as "*guata amarilla*" ("yellow belly"), affecting coho salmon reared in sea water cages has been emerging in southern regions of Chile. An orthomyxovirus (ISAV) has been isolated and reported to be its etiological agent. An extensive field sampling, including whole fish, blood, sera, fish tissues, food and water, was carried out from six different sea sites where outbreaks of this disease were occurring. Gross lesions were yellow color of the skin of the abdominal region, bases of the paired fins and periorbital cartilages. Internally, fish had pale gills, ascites, abundant visceral fat with yellow color, brown liver with plethoric gall bladder, dark spleen, hydropericardium, pale kidney and no food in the stomach and gut. Histopathology showed erythrocyte depletion in blood vessels in most organs, hemosiderosis in spleen and liver esteatosis. A degenerative process resembling the Zenker disease was found in skeletic muscle and myocardium. Hematological analysis showed hemolytic anemia. Bacteriology only showed low levels of endemic microorganisms. After 30 days postinoculation with filtrates of homogenates of organs of affected fish, the disease was not reproduced in rainbow trout and coho salmon. These results suggest that a virus would not be the single etiological agent of this condition, or if so, it would have a low virulence. The striking differences in the pathology of this disease compared with that of ISA and the fact that it only affects coho salmon but not other species cultured in the same sites, particularly Atlantic salmon (*Salmo salar*), which is described as the most susceptible species to ISA, strongly suggest that this is not the same disease condition. Although this disease might be associated with an orthomyxovirus its etiology is more likely to have a multifactorial origin and probably linked to some metabolic disturbances. Financed by INTESAL and Fondecyt 1010544.

Pathology associated with a *Tetrahymena*-like organism in southern flounder, *Paralichthys lethostigma*

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A small population of commercially-reared juvenile southern flounder, *Paralichthys lethostigma*, was experiencing a sudden onset of morbidity and mortality. Clinical signs included distended abdomens, petechial hemorrhage and focal ulcerations of the skin. Skin biopsies revealed a heavy infestation of *Trichodina* sp. and an excessive amount of mucus. Scrapes directly from the ulcerated skin lesions revealed a heavy infestation of an unidentified ciliated protozoan. Bacterial cultures from the posterior kidney were negative for any bacterial growth. Bacterial cultures from the ulcerated skin lesions grew *Vibrio furnissii*, *Chryseobacterium meningosepticum* and *Pseudomonas alcaligenes*. Histopathological evaluation revealed epithelial hyperplasia and localized cellular necrosis of the skin. Microscopic examination documented that the ciliated protozoan had penetrated the deeper tissues of the fish. These *Tetrahymena*-like organisms were localized to areas of epithelial compromise but were also found in the deeper dermal and muscular layers. Accompanying these burrowing organisms was a moderate to severe infiltration of inflammatory cells. In addition, organisms were observed in the peritoneal cavity accompanied by a significant peritonitis. It was speculated that the heavy *Trichodina* sp. infestation in this cultured fish population lead to epithelial compromise and the secondary invasion by the *Tetrahymena*-like organism and bacteria. Significant improvement of affected fish was observed in this population after a 20 ppm formalin treatment.

Potential impacts of selected contaminants on endocrine biomarkers in carp and lake trout from sites in the Great Lakes

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A reconnaissance investigation of Great Lakes fish in connecting channels, tributaries and Lake Michigan open water was conducted in 1999 to assess the occurrence and distribution of the chemical class alkylphenol ethoxylates. Common carp (*Cyprinus carpio*) were collected at several riverine sites near Chicago, Illinois; Detroit, Michigan; and Alpena, Michigan. Lake trout (*Salvelinus namaycush*) were collected in the open water of Lake Michigan near Sturgeon Bay, Wisconsin; and Saugatuck, Michigan. All fish were analyzed to determine chemical residue in whole fish for organochlorine pesticides, polychlorinated biphenyls (PCBs), polybrominated diphenylethers (PBDEs) and selected alkylphenol ethoxylates (including their metabolites, nonyl- and octylphenols and shorter chain carboxylates). Additionally, a comprehensive analysis of fish health was made for each fish, which included the collection of endocrine biomarkers for histopathological analysis of specific organs and of blood samples for analysis of hormones and vitellogenin. Fish tissue residue, endocrine biomarkers, bed sediments and water chemical residue will be compared for each species

Transferrin and the innate immune response of fish: identification of a novel and highly conserved mechanism of macrophage activation

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Macrophages are immune cells that play a central role in the elimination of foreign invaders. In response to an infection, these cells become activated and inhibit microbial replication by producing an array of antimicrobial products including nitric oxide (NO). The primary macrophage activating factors in mammals are a class of soluble signaling proteins called cytokines. Recently, by studying goldfish (*Carassius auratus*) macrophages, we have discovered that a non-cytokine serum protein called transferrin is a primary activating molecule of the goldfish macrophage antimicrobial responses. We report that: 1) transferrin must be proteolytically cleaved before it is biologically active; 2) proteolytic cleavage was mediated by mitogen-activated leukocyte supernatants and by enzymes released by damaged cells; 3) addition of transferrin enhanced the killing response of goldfish macrophages exposed to different pathogens or bacterial products (e.g. lipopolysaccharide, *Mycobacterium chelonae*, *Trypanosoma danilewskyi*, and *Aermonas salmonicida*); and 4) products in fish serum neutralized transferrin activity and inhibited induction of NO response of macrophages. While transferrin is not a cytokine and "normally" functions as an iron-binding protein, the ability of the enzymatically-cleaved forms of this protein to modulate fish macrophage function is novel and may represent a primitive and evolutionary conserved mechanism for the induction of NO response of macrophages.

The eradication of an epidemic of ISA in Scotland

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In 1998, an epidemic of Infectious Salmon Anaemia (ISA) occurred in marine salmon farms in Scotland. In all 11 farms were confirmed and a further 25 farms were suspected of being infected. The last suspect case was in November 1999 and the epidemic was officially over on 16 January when the last farm in the same coastal waters as a suspect case had completed the requisite fallow period. Active surveillance will continue for 2 years. In the European Union ISA is a List I disease and a strong legislative framework (Directive 93/53/EC) had already been established in 1993 to enable eradication of the disease. This framework made the disease notifiable and provided measures to contain and limit spread and to eliminate the source of infection. On suspect farms, and farms in the same coastal waters, it provided for restrictions on the movements of live fish and fish to harvest and mandatory biosecurity provisions under the supervision of the Official Service. On confirmed farms immediate depopulation followed by fallowing and disinfection was required. The average time for withdrawal of all fish in the Scottish epidemic was 21 days depending on logistics and the size of the farm. This, together with the established legal framework to limit spread, is thought to have been an important factor in the success of the eradication programme compared with other countries. Despite the evidence for a background level of infection, it is important to understand the benefits of eradicating an epidemic, which was transmittable easily from farm to farm and had potential to cause significant and sustained losses if it had become established in Scotland. Diseases of this nature provide a considerable challenge for both scientific and legislative structures if we are to maintain the health status of fish populations in an era of free trade.

A real-time nucleic acid sequence based amplification (NASBA) procedure for detection of fish nodaviruses

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Nucleic acid based sequence amplification (NASBA) is an isothermal nucleic acid amplification procedure based on target-specific primers and probes, and the co-ordinate activity of three enzymes: AMV reverse transcriptase, RNase H, and T7 RNA polymerase. We have developed a real-time NASBA detection procedure for piscine nodaviruses, which have emerged as major pathogens of marine fish. Viral RNA was isolated from clinical samples by guanidine thiocyanate lysis followed by purification on silica particles. Oligonucleotide primers were designed to target sequences in the nodavirus capsid protein gene yielding an amplification product of 161 nucleotides. The downstream primer also contained a T7 RNA polymerase promoter sequence. Amplification products were detected with a molecular beacon (FAM labelled/methyl-red quenched) which recognised an internal region of the amplification product. Amplification and detection were performed at 41°C for 90 minutes in a Corbett Research Rotorgene. In preliminary experiments, the real-time NASBA procedure exhibited a dynamic range of over 7 log10, and was found to be approximately 100 fold more sensitive than single tube RT-PCR for the detection of a nodavirus isolated from sea bass. The real-time NASBA procedure was then used to study a panel of 41 clinical samples submitted to our virology laboratory for nodavirus diagnosis based on histopathology and cell culture isolation. Real-time NASBA correctly identified 41 samples (100%) whereas RT-PCR identified 38 (92.7%). These results suggest that real-time NASBA may represent a sensitive and specific diagnostic procedure for piscine nodaviruses.

Morphometric evaluation of hepatic lesions in killifish, *Fundulus heteroclitus*, exposed to polyaromatic hydrocarbons

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Stereology of biological tissues involves the derivation of 2-dimensional data into 3-dimensional constructs. We applied a stereology technique to discern morphometric attributes of hepatic lesions from a population of killifish, *Fundulus heteroclitus*, sampled from the Elizabeth River of the Chesapeake Bay. The lesions in these fish were associated with exposure to Elizabeth River sediments laden with PAHs. Histological step sections were taken throughout each of several livers to evaluate lesion types, volumes, shapes, and distributions. Images of the liver sections were digitized, lesions were highlighted, 2-dimensional areas were determined, and 3-dimensional volumetric data for the whole liver and the lesions were established. The total number of hepatic alterations (eosinophilic, basophilic and clear cell foci, hepatocellular carcinomas, hemangiopericytomas and cholangiomas) ranged from 10-125 per fish. Lesion volumes ranged from 0.00012-63.87mm^3 and represented 0.21% to 67.36% of total liver volume. There was a tendency for the lesions to be more pancake-like than spherical or rope-like when observed from longitudinal sections, and the lesions had notable inter-individual variation in their hepatic distributions. Our data indicate that, on average, six evenly spaced histological sections are required to accurately estimate lesion volume and extent in our sample population of fish. This study provides a foundation for studying piscine carcinogenesis, and developing histological sampling techniques for observing lesion volume changes over time.

Re-evaluating SSO disease: *Haplosporidium costale* infections of the Eastern oyster in Virginia, Connecticut, and Massachusetts

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Haplosporidium costale, the causative agent of SSO disease, is a haplosporidian parasite of the eastern oyster, *Crassostrea virginica*. This parasite has been reported in US Atlantic coast oysters from Virginia to Maine. It is closely related to the more serious disease agent, *Haplosporidium nelsoni* (MSX), and plasmodia of these two parasites can be very difficult to distinguish by light microscopy. Oysters from Virginia (VA), Connecticut (CT), and Massachusetts (MA) have been sampled monthly for two years and diagnosed for *H. costale* and *H. nelsoni* by histological examination and PCR. By both histological and PCR diagnoses, infections were mostly *H. nelsoni* in VA, both *H. costale* and *H. nelsoni* in CT, and only *H. costale* in MA. The histological and PCR diagnoses for *H. costale* had only about 50% agreement for infections from CT and MA using PCR SSO primer set I. When the histology +/PCR – samples for *H. costale* were tested with SSO primer set II, most of the MA infections were then positive by PCR, but the CT infections were still negative. The MA *H. costale* infections followed the reported disease cycle of infections in April-June; however, CT *H. costale* infections were in October-December. It is possible that there are two strains of *H. costale* in MA and either a different strain of *H. costale* or another species morphologically similar to *H. costale* in CT that is not detectable by the PCR primer sets tested. Sequencing studies are underway to address these possibilities.

Role of complement C3b in adaptive immunity in teleost fish

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As result of complement activation, the C3 protein is cleaved into C3b and C3a fragments. Through the C3b molecule, the complement system plays an important role in antigen uptake and internalization which leads to an amplification of the adaptive immune response. In this study we investigated the presence of C3b receptors in fish and the role of C3b in enhancing the immune response of fish. Purified trout C3b-1 (generated from isoform C3-1) was bound to trout leukocytes in a dose responsive and saturable manner by flow cytometric analysis. Thereafter, we determined whether the complex formed between trout C3b-1 and an antigen (Hen Egg Lysozyme [HEL]) was able to enhance the proliferation of leukocytes from HEL-immunized trout leukocytes. For this purpose, leukocytes from peripheral blood (PBL) and head kidney (HKL) of immunized and nonimmunized trout were tested for proliferation responses against HEL alone, HEL complexed to trout C3b-1, and C3b-1 alone. Leukocytes from immunized fish had a markedly increased antigen-dependent proliferation response when stimulated with 1000 μg/ml of HEL. Leukocytes stimulated only with 1 μg/ml of HEL coupled to C3b-1 induced an elevated proliferative response that was comparable to the response obtained with 1000 μg/ml of HEL alone. These results indicate that when HEL is coupled to C3b-1, the amount of HEL required to elicit an antigen-dependent proliferative response is significantly reduced. These results provide the first indication that in fish, complement may have already evolved into a shared effector system between innate and adaptive immunity.

Geographic variations among infectious hypodermal and hematopoetic necrosis virus (IHHNV) isolates and characteristics of their infection

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Nucleotide sequence variations of a 2.9 kb fragment of infectious hypodermal and hematopoietic necrosis virus (IHHNV) isolated from samples of *Penaeus monodon* were determined and compared with an isolate from Hawaii. The infection characteristics of these isolates were examined by histology, in situ hybridization, and laboratory challenge studies with *Penaeus vannamei*. Isolates of IHHNV were obtained from samples collected from the Southeast Asia region (the Philippines, Thailand, and Taiwan). Isolates of putative IHHNV were obtained from African samples (Tanzania, Madagascar, and Mauritius). The Philippine isolate was found to have a very high similarity, 99.8%, to Hawaii IHHNV. The Thailand isolate showed a slightly lower similarity, 96.2%. The putative IHHNV sequences collected from Tanzania and Madagascar showed greater divergence from Hawaii IHHNV, 8.2% difference for Tanzania and 14.1% difference for Madagascar. A phylogenetic analysis showed that the Philippine IHHNV clustered with IHHNV found in the western hemisphere. This suggests that the Philippines was the origin of IHHNV that was first detected in Hawaii and subsequently in most shrimp farming areas of the Americas. In the laboratory infection study, both the Philippine and Thailand IHHNV were passed into *P. vannamei*, and the infected shrimp did not suffer any mortalities. In another laboratory infection, *P. vannamei* injected with a tissue homogenate of *P. monodon* collected in Madagascar did not demonstrate IHHNV infection, suggesting that this putative IHHNV is not infectious to *P. vannamei*.

Induced resistance to white spot syndrome virus (WSSV) infection in *Penaeus stylirostris* through pre-infection with infectious hypodermal and hematopietic necrosis virus (IHHNV)

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White spot syndrome virus (WSSV) is highly virulent and responsible for serious economic losses on shrimp farms throughout the world. Infectious hypodermal and hematopoietic necrosis virus (IHHNV) also infects penaeid shrimp; although once virulent to cultured stocks of *Penaeus stylirostris*, it has not been associated with mass mortalities in recent years. Through laboratory challenge studies, we discovered an interaction between these two viruses in *P. stylirostris*. Juvenile *P. stylirostris* were infected with IHHNV by feeding them IHHNV-infected tissue. These shrimp, along with a non-infected control group, were then fed WSSV-infected tissue. Two days after the WSSV challenge, mortalities began to occur. All of the control shrimp, not previously exposed to IHHNV, died within 5 days, while 31-44% of the IHHNV-infected shrimp were alive ten days later. Quantitation by real-time PCR determined that surviving shrimp had high levels (10^{9} copies per µg DNA) of IHHNV and low levels (50-1,000 copies per µg DNA) of WSSV DNA. Moribund shrimp had high levels (10^{6}-10^{7} copies per µg DNA) of WSSV and low levels (10^{4} -10^{6} copies per µg DNA) of IHHNV DNA. A confirmatory challenge study with *P. stylirostris* that were heavily infected with IHHNV showed 21 of 23 shrimp survived 20 days after exposure to WSSV. Ten of these surviving shrimp were re-exposed to WSSV, and 6 of these were still alive after 3 weeks. In situ hybridization analysis confirmed that surviving shrimp were strongly infected with IHHNV and had low levels, if any, of WSSV. In conclusion, juvenile *P. stylirostris* highly infected with IHHNV become resistant to infection with WSSV.

The effect of CpG oligodeoxynucleotides on the innate immune response of common carp, *Cyprinus carpio* L.

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Unmethylated CpG dinucleotides within bacterial DNA or synthetic oligodeoxynucleotides (ODNs) can activate immune cells. A panel of synthetic oligodeoxynucleotides was evaluated *in vitro* and *in vivo* for its ability to enhance the innate immune response of common carp, (*Cyprinus carpio* L). *In vitro* addition of CpG-ODNs to fish, resulted in enhanced responses of phagocytic and nitroblue tetrazolium (NBT) activities in kidney phagocytic cells. The CpG-ODNs also induced lymphocyte proliferation in the fish kidney cells. Intraperitoneal injection of CpG-ODNs into fish, resulted in increased responses of phagocytic and NBT activities in kidney phagocytic cells. The serum lysozyme activity also increased in fish treated with CpG-ODNs. The ODNs containing multiple CpGs generally resulted in greater stimulatory capacity, although CpGs located at the terminus of an ODN were ineffective. These results indicated that CpG-ODNs enhance the innate immune response of common carp.

Pathogenicity of morphologically and genetically characterized *Flavobacterium columnare* strains in Channel catfish

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A major disease of warm water fish is columnaris caused by the bacterium *Flavobacterium columnare*. The disease causes significant losses in the catfish and baitfish industries, but little is known about the different strains of this bacterium and their pathogenicity. Eleven different isolates that formed characteristic flat, rhizoid, yellow pigmented colonies on Sheih Agar and have the typical biochemical characteristics of *F. columnare* were collected from several species of moribund fish including *Ictalurus punctatus, Notemigonus crysoleucas, Pimephales promelas, Xiphophorus maculatus* and *Cyprinus carpio*. The bacteria were long, slender, Gram-negative rods and exhibited length differences among isolates. The short isolates varied from 1.5–3.0μm and the long isolates varied from 4.0–8.0μm in length. The isolates varied in width from 0.45–6.0μm. The DNA sequence similarity of the isolates was analyzed by Random Amplification of Polymorphic DNA (RAPD) with five different 10-mers. The RAPD profile and morphology data revealed the presence of seven different strains among our isolates. Channel catfish were challenged with the isolates by immersion in a bath of 5 x 10^6 *F. columnare*/ml. Mortality varied from 0–90% among the isolates used in our challenge experiments. There were no correlations between pathogenicity and the fish species from which the isolates were obtained.

Comparison of fish-to-fish and waterborne transmission of channel catfish virus in a pond environment

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Fish-to-fish vs. waterborne transmission of channel catfish virus (CCV) was directly tested in a pond environment. Susceptible channel catfish fingerlings were stocked into 16 pond-side tanks that received pond water (28-32°C) from a 0.1-acre reservoir pond. Fish in 8 tanks were challenged by cohabitation (fish-to-fish transmission) and fish in the remaining tanks were challenged by strict waterborne exposure from water received from the reservoir pond. Posterior kidneys of two fish from each tank were collected at 10 sampling intervals over a 2-week period and screened for the presence of CCV on channel catfish ovary cells. Infection was confirmed by isolating virus from cohabitation fish following experimental infection. At study termination, 15 fish from each tank were screened for the presence of CCV by diagnostic PCR. Results indicate a difference in the proportion of fish infected by cohabitation vs. strict waterborne exposure. Bioassays evaluating free virus in pond water agree with work done by Brady and Ellender (1982) indicating that CCV virions rapidly complex with colloids. We found that free virus was not detectable from pond water. However, when colloid-virus mixtures were injected into catfish infectious virus could be reisolated. These results may account for poor waterborne transmission.

Experience with regulatory responses to infectious salmon anemia (ISA) in Norway

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Norway was the first country to experience the challenge of ISA. After the first outbreak in 1984, the number of cases of ISA "exploded" in 1989, and reached a peak of 80 new infected farms in 1990. The disease became notifiable in 1988, when it was realized that ISA was a contagious disease, probably caused by a virus. As the disease became a serious loss factor to fish farming, regulatory responses were developed in parallel with increased scientific knowledge about the virus and risk factors of the disease. To eradicate ISA from infected farms and infected areas, the main measure was to depopulate the farm and eventually the whole area. To prevent introduction of ISA into non-infected areas, the most effective measure has been control of transfer between areas of live and dead salmon exposed to seawater. In 1990, the virus had not yet been identified, and today's diagnostic tools were not available. Still, the strategy for combat of ISA was successful. The last 3-4 years, the number of outbreaks has increased again. There may be several reasons for this; none of them being scientifically reviewed. Structural changes in the industry leading to long transports of salmon for rearing and for slaughter may be one factor. Unspecific immunization due to vaccination procedures against other diseases may be another, and in particular the combination of such factors. Vaccination was not an alternative in 1990. Today, vaccination is considered a possible tool in the combat of ISA within infected areas.

Essential role of the NV protein for the pathogenicity of *Novirhabdovirus* in rainbow trout

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Novirhabdovirus, like the infectious hematopoietic necrosis virus (IHNV) and the viral hemorrhagic septicemia virus (VHSV), are fish rhabdoviruses which, in comparison to the other rhabdoviruses, contain in their genome an additional gene encoding for a small non structural NV protein of unassigned function. The growth in cell culture of a recombinant IHNV knockout for NV (rIHNV-ΔNV-GFP) has previously been shown to be severely impaired, suggesting a crucial role of NV for the optimal IHNV replication. In the present study, we show that a normal growth of rIHNV-ΔNV-GFP can be restored when NV is provided *in trans* by using cell lines constitutively expressing the NV protein (EPC-NV). Moreover, when EPC-NV cells were infected with the wild type IHNV (wtIHNV), a 10-fold increase of the viral titer was observed compared to wtIHNV-infected EPC cells. These observations tend to hypothesize a replication-regulatory role for NV. Although IHNV and VHSV NV proteins do not share any significant identity, we show that both NV proteins play a similar role since a recombinant IHNV virus, rIHNV-NVvhsv, in which the NV gene has been replaced by that of VHSV, was produced and shown to replicate as well as the wtIHNV into EPC cells. The data provided by the experimental fish infections using the various recombinant viruses demonstrate an essential role of the NV protein for the pathogenicity of IHNV. Thus the knowledge that IHNV is no more pathogenic for trouts when NV gene is deleted and also that heterologous glycoproteins like that of VHSV can be efficiently inserted in the IHNV virions, allowed us to generate a potential live bivalent vaccine against IHNV and VHSV by deleting the NV gene and inserting the VHSV glycoprotein gene. That recombinant virus (rIHNV-Gvhsv-Gihnv) was shown to express both glycoproteins from VHSV and IHNV and to be totally non-pathogenic for trout by bath immersion and injection as well.

The use of signature tagged mutagenesis to identify factors involved in the pathogenesis of *Edwardsiella ictaluri*

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Enteric septicemia of catfish (ESC) is caused by *Edwardsiella ictaluri* and is recognized as the most serious disease facing the commercial channel catfish industry, yet very little is known about the virulence factors associated with infection. Signature tagged mutagenesis (STM) is a transpositional mutagenesis system that uses transposons carrying unique DNA tags. Using the unique tags and a common, complimentary oligonucleotide from the transposon as primers in the polymerase chain reaction, mutants carrying insertions in genes required for invasion and survival in the host were identified by comparing the amplification results for mutants used to challenge catfish (in pool) to the results for mutants isolated from moribund or dead fish (out pool). DNA containing the insertion sites from the individual identified mutants was cloned into pBluescript and the sequence of the bacterial DNA flanking the transposon was determined using the unique tag as the primer. Genetic database searches were used to identify similar genes and to provide insight into the function of the mutated genes. To date, 179 pools containing a total of 1611 individual mutants have been screened in channel catfish. Fifty-three mutants that did not survive in the host have been cloned into pBluescript, and sequencing data is available for 33. Database searching to date has identified 2 genes with no data base matches and 3 with matches to putative membrane or exported proteins. Nineteen mutants carried insertions in nutritional or housekeeping genes. Nine mutants carried insertions in putative virulence factors that could be directly involved in *E. ictaluri* pathogenesis.

Starvation of *Flavobacterium psychrophilum* in stream water, broth and distilled water

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Flavobacterium psycrophilum is the causative agent of rainbow trout fry syndrome and bacterial cold water disease (BCWD) in salmonid fish. Little is known of the epidimiology of the disease or its survival outside its host. It is considered a very fastidious organism, and many authors have reported problems with its culture. Under adverse conditions it is possible that the bacterium enters a viable, but non-culturable state, as with many other bacterial species, such as *Salmonella* sp and *Yersinia ruckeri*. This characteristic results in an inability to detect the pathogen in environmental samples when culture is used as the only means of identification. Physical changes in *F. psychrophilum* were examined over a 19-week period of starvation. Bacteria were maintained in either filtered stream water, *Cytophaga* broth, filtered distilled water or maintained in broth after disinfection. Culturability and viability of the bacterium were then assessed using Colony Forming Units (CFUs) and a Live/Dead kit. Antigenic profiles and general morphology of the bacterium were also examined, using Western blot analysis and electron microscopy, respectively. In stream water, the bacterium stop multiplying, became small and rounded and its culturability declined until it was no longer possible to obtain colonies on agar plates after 19 weeks. Results from the Live/Dead kit did not correspond with the viability obtained as CFUs by culture. The ability of *F. psychrophilum* to survive under conditions of starvation and changes in the organism so as to adapt to its environment will be discussed.

Atlantic salmon major histocompatibility complex II α and β promoters—expression studies

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The Atlantic salmon Major Histocompatibility Complex class II (MHCII) α and β promoters were isolated, and found to have the same organisation of regulatory boxes as mammals. In order to test the transcriptional activity of the promoters, reporter plasmids were constructed with the Atlantic salmon promoters, using GFP and Lac Z as reporter genes. Transient transfection of Atlantic Salmon Head Kidney (SHK-1) cell line, Dog ADH lymphb cell line (DH 82) and Rabbit ADH lymphb cell line (HybL-L) was conducted by these promoter constructs.The reporter plasmids were also transiently microinjected into zeebrafish eggs. Both MHCII α and β promoters were found to express the reportergene in all the cell lines mentioned. The expression could also be observed in transgene zebrafish.

Experimental infection of necrotizing hepatopancreatitis (NHP) in *Litopenaeus vannamei* by injection and ingestion

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Necrotizing hepatopancreatitis (NHP) is a severe bacterial disease of cultured *Litopenaeus vannamei*, the Pacific white shrimp. In this experiment, individuals of *L. vannamei* were experimentally infected with NHP by injection and ingestion. Kona stock *L. vannamei* shrimps were individually placed in aerated 1-liter containers. In treatment 1, twenty shrimps were injected with 1% weight/volume of an aqueous extract of NHP-infected hepatopancreas filtered to 1 μm. Injectant was prepared from hepatopancreas, pooled from several infected shrimps. Ten control shrimp were exposed using the same method as treatment 1 but with NHP-negative hepatopancreases. In treatment 2, twenty shrimps were individually fed a quarter-piece of NHP-infected hepatopancreas. Ten control shrimps were exposed to inocula prepared from NHP-negative hepatopancreas. Shrimps were maintained at 30 ppt salinity and 30°C for 21 d. Each shrimp was fed a food pellet every third day. Freshwater was added to containers to replace evaporation but water was not exchanged. Survival analyses detected significantly lower mean survival time of shrimps exposed to NHP by injection than by feeding. Mean survival time of shrimps injected with NHP was significantly lower than in NHP-negative control injected shrimps. Shrimps exposed to NHP through feeding also showed lower mean survival times than NHP-negative control fed shrimps.

Chitinolytic activities of bacteria associated with shell disease syndrome in the edible crab, *Cancer pagurus*

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Chitinolytic bacteria are the primary aetiological agents of shell disease, a degradative condition of the crustacean exoskeleton that results in the formation of black-spot lesions. Variations in the levels of shell disease lesions from natural populations of the edible crab (*Cancer pagurus*) were surveyed in Gower, South Wales. The greatest severities of the disease were attributed to sand abrasion injuries during back-burrowing behaviour and prolonged exposure to high numbers of chitinolytic bacteria. Lower severities of shell disease were found in sites where sediment structure largely prevented back-burrowing and hence animals were only exposed to low levels of chitinolytic bacteria in the water column. However in areas were back-burrowing was permitted, sediment chitinase activities from cultured bacteria were found to be significantly lower (0.74 ± 0.14 µmol GlcNac production.$ml^{-1}.hr^{-1}$) than sediments cultured from the high shell disease severity site (1.57 ± 0.19 µmol GlcNac production.$ml^{-1}.hr^{-1}$). A number of chitinolytic bacteria were isolated from lesion and non-lesion areas of *Cancer pagurus*. All were capable of growth in a minimal media consisting of chitin powder from crab shells, but differed in their speed of growth and nature of chitinolytic activity suggesting that they have different roles within the lesion community. Preliminary studies on these isolates and sediment samples have revealed the potential of metal ions to enhance (Fe^{2+}, Mg^{2+}, Cu^{2+}) or inhibit (Hg^{2+}, Zn^{2+}) microbial chitinases perhaps suggesting that the putative link between shell disease and pollution may be due to regional enhancement of chitinase activity in the presence of specific metal ions.

***Pfiesteria shumwayae* kills fish by myzocytosis not exotoxin secretion**

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Dinoflagellates of the toxic *Pfiesteria* complex (TPC) reportedly secrete potent exotoxins believed responsible for fish lesion events, acute fish kills and human disease in mid-Atlantic USA estuaries. However, to date no *Pfiesteria* toxin has been isolated or characterized. Using a new larval fish bioassay, we investigated mechanisms of fish killing by *Pfiesteria shumwayae*. Water from actively fish-killing cultures was processed by differential centrifugation and filtration to produce separate aqueous fractions enriched in dinoflagellates, contaminating bacteria and possible "exotoxins" (supernate). Fish mortalities (60-100%: < 24 hrs) occurred only in treatments containing live dinoflagellates, with no mortalities in supernate, bacterial fractions or controls. Using the larval fish bioassay, we developed a new approach to assess for the presence of possible fish-killing "exotoxins" in *Pfiesteria* cultures using various membranes. Results from this new approach support findings from the fractionation studies. Videomicrography, histopathology and electron microscopy showed dinoflagellates attaching to skin, actively feeding, and rapidly denuding fish of epidermis. We show here that potent exotoxins are not produced by our actively fish-killing *P. shumwayae* cultures. Rather, the loss of epidermis and fish mortality is the direct result of a micro-predatory feeding process called myzocytosis. Our findings bring into question production of potent exotoxins by *P. shumwayae* and suggest that all strains and species of *Pfiesteria* be reevaluated using a bioassay able to distinguish between toxigenicity and fish killing by alternate mechanisms. Supported by ECOHAB Grants CR826791-01-0 and CR-828225-01-0.

Prevalence, histopathology, and ultrastructure of vascular neoplasms in mummichogs (*Fundulus heteroclitus*) from a creosote-contaminated site

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Mummichogs (*Fundulus heteroclitus*) inhabiting chemically contaminated estuarine environments in southeastern Virginia USA and elsewhere, exhibit greatly elevated prevalences of hepatic neoplasia and associated lesions. Recent exposure studies in our lab have demonstrated a direct causative relationship between development of these liver lesions and exposure of the fish to polycyclic aromatic hydrocarbons (PAH) in their local environments. High prevalences of exocrine pancreatic and vascular neoplasms also occur in these fish. Whereas the pancreatic neoplasms have been previously linked to contaminant exposure in wild fishes, to our knowledge vascular neoplasms have not. Vascular tumors occurring in mummichogs from a creosote-contaminated site in the Elizabeth River, VA belonged to two basic histologic types, the angiosarcomas and hemangiopericytomas. Most of these lesions arose within the liver tissue, a primary target organ for the carcinogenic effects of PAHs in fishes. The vascular lesions in this fish population therefore are also believed associated with contaminant exposure. Some of them resulted in the death of fish maintained long-term in the laboratory. We describe here the lesion prevalence, histomorphology and ultrastructural pathology of these interesting neoplasms. Supported in part by EPA Contracts CR818165-01 and CR827131-01.

Identification of the gene responsible for hemolysis in *Edwardsiella ictaluri*

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Edwardsiella ictaluri is the causative agent of enteric septicemia of catfish (ESC), an important disease for the channel catfish industry. The pathogenesis of ESC is not fully understood; however, several characteristics have been examined as potential virulence factors, including a cell-associated hemolysin. Previous research has determined that there was no clear relationship between hemolytic activity and virulence of *E. ictaluri*. We hypothesize that hemolysin is important for virulence in *E. ictaluri*; therefore, we developed an isogenic hemolysin negative mutant using transposon mutagenesis. Plasmid pLOFKm, which contains a composite mini-transposon with the Tn*903* kanamycin resistance gene, was used to generate a bank of random *E. ictaluri* mutants. Two hemolysin negative mutants from a pool of 6000 transconjugates were isolated. Next, we determined whether these two mutants contained insertions into the same gene by performing a Southern hybridization of selected restriction enzyme digests of genomic DNA from the two *E. ictaluri* mutants, using the Tn*903* kanamycin resistance gene as the probe. This resulted in identical banding patterns between the two mutants, indicating that the mutations reside in the same gene. Then, we determined the identity of the mutated gene by ligating *Kpn*I-digested chromosomal DNA from one of the mutants into pBlueScript cloning vector and selecting *E. coli* transformants for kanamycin resistance. These clones were sequenced and shown to have a 90% nucleotide identity to the hemolysin A gene of *Edwardsiella tarda* (GenBank number D89876).

Use of transgenic medaka for assessing mutagenicity of chemical contaminants in aquatic systems

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Chemical contamination of aquatic environments remains of significant concern because, while it is understood that aquatic systems serve as major conduits for distribution and deposition of many toxic agents, relatively few methods provide sufficient sensitivity, accuracy, and practicality necessary for routine assessment of chemical toxicity. Fish are well recognized for their value as sensitive indicators of environmental contamination. Most recently, genetically modified, or transgenic fish have been generated to enhance the role of fish in assessing potential mutagenicity of chemical contaminants. Lineages of the small aquaria fish, medaka (*Oryzias latipes*), carry a bacteriophage (lambda) vector that harbors bacterial genes (*cII* and *lacI*) that serve as targets for mutations. The generalized approach entails exposing the fish to the chemical using a variety of laboratory exposure regimens, allowing sufficient time for the mutations to manifest. DNA is then isolated from various tissues, the vector is separated and transferred into specialized bacteria where the numbers of mutant target genes are quantified. Results of several studies using different study designs, exposure regimens, chemical mutagens, and tissues are highlighted to illustrate the benefits of the transgenic fish mutation assay supporting its continued use. Among the advantages include the ability to compare mutations in a variety of tissues, to correlate mutations with other endpoints such as neoplasia in the same animal, to use few animals/treatment to detect statistically significant differences, and to detect mutations at the level of single nucleotides.

Toxicity of aerially applied pesticides to fish and shrimp: identification of compounds likely to cause mortality in aquaculture

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Many warm water fish farms are located near crop production areas where pesticides are applied by aerial application. There is a widespread perception by farmers that pesticide drift is a major source of fish losses, however, much of the information needed to evaluate the potential of pesticides to kill fish is not available. Label LC_{50} data is not sufficient because it does not include the most common aquacultural species or life stages, and does not provide information about safe levels. In our work, we are testing 47 pesticides that were selected based on their frequency of use and on their apparent risk as determined by comparing the LC_{50} to the pesticide concentration that would result if the pond received the full field dose of chemical. Species studied include channel catfish *Ictalurus punctatus*, largemouth bass *Micropterus salmoides*, golden shiner *Notemigonus crysoleucas*, fathead minnow *Pimephales promelas*, and freshwater shrimp *Macrobranchium rosenbergii*. The study includes fry, fingerling, adult fish, and post-larval shrimp. The experiments were conducted in static, clear water. Chemicals were tested at their full field dose equivalent (FDE). Concentrations that produced observable changes in fish behavior or survival were further diluted to determine their No Observable Effect Concentration (NOEC). Contrary to farmer perceptions, the NOEC of all herbicides and herbicide mixtures tested on fish and shrimp were greater than the FDE. Experiments with insecticides are not yet complete, but results to date clearly show insecticides pose a greater threat and many produced adverse effects at dosages less than one half the FDE.

Unusual findings in guppies (*Poecilia reticulata*) used in toxicological studies

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A variety of unusual histopathological alterations were observed in guppies utilized in several toxicological studies. Categories of changes include: degenerative (fibrovascular proliferation of the bulbus arteriosus, hyalinization of splenic arteries); hyperplastic/hypertrophic (chromaffin tissue hyperplasia, pancreatic duct hyperplasia, thyroid follicular hyperplasia, islet cell hypertrophy); neoplastic (retinoblastoma, cardiac ventricular sarcoma, renal hemangioma, invasive pancreatic carcinoma); and congenital (ectopic pituitary tissue, ectopic ova within the gallbladder, thymic cysts). All of these findings will be illustrated, and differential diagnoses and possible causes will be discussed.

Unusual findings in medaka used as test animals in toxicologic studies

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A number of interesting and unusual lesions have been diagnosed in medaka that have been evaluated from toxicologic tests over a twelve year period. These lesions include rare neoplasms such as lepidocytoma (a neoplasm arising from scales), an olfactory neuroblastoma arising in the nares, and Esophageal carcinomas. Non-neoplastic findings of interest include normal tissue that occurs in unusual locations. Thyroid tissue, for example, is commonly found distant from its normal location in medaka. However, less commonly, brainlike tissue and gonadal tissue have been observed in unusual locations. A lesion that occurs most often in medaka that are nine months old or older is a degenerative lesion of the bulbus arteriosus characterized by mineralization, granulomatous inflammation and, occasionally, dissecting aneurysm. A spectrum of lesions that involves thickening of the peritoneum also occurs sporadically in medaka. These lesions may or may not represent one entity, and they have been diagnosed variously as inflammatory or neoplastic. One other lesion that is questionable as to whether it is neoplastic or inflammatory is an enlargement of the tip of either the upper or lower jaw. All of these findings will be illustrated, and differential diagnoses and possible causes will be discussed.

Haemocyte-parasite interactions in the edible cockle, *Cerastoderma edule*

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Metazoan parasites, particularly digenean trematodes, are very common in marine bivalves and exert considerable stress on their hosts, often resulting in reduced resistance to environmental stressors, tissue damage and behavioural changes. In the most severe cases, mass mortalities can occur. To date, there is little information available on host resistance mechanisms to trematode parasites in bivalve molluscs. This is surprising considering the high prevalence of these parasitic infestations and the economic value of many marine shellfish. Our research aims to characterise host immune responses against digenean parasites in the edible cockle, *Cerastoderma edule*. *C. edule* is a common host for digenean trematodes, and is of national and European commercial importance. Current investigations are focused on the encapsulation response of *C.edule*. Haemocytic encapsulation is an important immune defence response against parasites in invertebrates. Using the cysts of *Himasthla elongata*, a digenean parasite found encysted in the foot of *C. edule*, as well as synthetic beads and thread, the dynamics of capsule formation have been studied both *in vivo* and *in vitro*. Histological studies were used to assess *in vivo* encapsulation of *H. elongata* cysts, whilst *in vitro* experiments have resulted in the isolation of several encapsulation-related proteins (ERPs) from injected synthetic targets. Work is continuing in all aspects of encapsulation, including cell adhesion, a key component of encapsulation, as well as the purification and amino acid sequencing of the ERPs. Subsequently, comparisons will be made with recognition factors found in higher vertebrates.

Mycobacteriosis in a collection of frogfish (*Antennarius striatus*): an atypical presentation

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In late 1999, six striated frogfish (*Antennarius striatus*) were imported from Brazil and purchased through a wholesaler by Mote Marine Laboratory Aquarium (Sarasota, Florida, USA). The fish were separated into two groups: 1) one male, two females and 2) two males, one female. Each group was placed in a separate quarantine tank. Approximately five months later, spawning behavior was observed in each tank. Spawns were fertile but fry survival was poor. Over the next nine months, each of the six individuals from both breeding groups of frogfish became clinically diseased and/or died. Ante-mortem clinical signs and lesions included: poor buoyancy control; anorexia; abnormal swimming; spawning difficulty (retained egg masses); lethargy; ascites; skin lesions including melanotic foci; ocular opacity; and reduced growth. Gross necropsy and wet mounts were, not very informative, although in some fish small brown foci were seen in gill filaments, and livers were grossly fatty. Aerobic cultures on TSA with 5% sheep's blood were inconsistent, but generally negative. Histopathologic lesions were summarized as widespread multifocal histiocytic inflammation and necrosis with myriad intralesional and intravascular acid-fast bacteria. Liver culture from one fish grew *Mycobacterium marinum*. Granulomatous disease, a typical finding in piscine mycobacteriosis, was not generally evident or present in these fish. Therefore, acid-fast staining and mycobacterial culture are recommended as a general procedure for evaluation of any group of fish with a history of chronic, low level mortalities, spawning difficulties, with or without buoyancy control problems, especially if H & E stained tissues indicate chronic inflammation.

Single-dose pharmacokinetics of florfenicol in koi (*Cyprinus carpio*) and gourami (*Trichogaster trichopterus*)

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Florfenicol has been shown to be very effective against many bacterial diseases in fish. Pilot studies were undertaken to determine single dose pharmacokinetics in two species of ornamental fish, koi (*Cyprinus carpio*) and gouramis (*Trichogaster trichopterus*) after oral, bath, and intramuscular administration. Minimal absorption occurred during bath administration. Oral and intramuscular administration results were interpreted using a one-compartment, population pharmacokinetic model of florfenicol, developed with KINETICA® software (Version 4.0.1). For koi: a) 50 mg/kg, oral administration: T1/2, Vd/F and Cl/F were 2.88 h, 0.5 L/Kg, and 0.12 L/kg.hr, respectively; Tmax was 12 h, C max was 11.74 µg/mL; MRT: 15.26 hr. b) 25 mg/kg intramuscular administration (polyethylene glycol carrier): T1/2, Vd/F, and Cl/F were 17.3 h, 0.64 L/Kg, and 0.0384 L/kg.hr, respectively; Tmax was 24 hr; Cmax was 17.91 µg/mL; MRT: 30.96 hr. For gouramis: a) 50 mg/kg oral administration: T1/2, Vd/F, and Cl/F were 3.85 h, 2.03 L/kg, 0.345 L/kg.hr, respectively; Tmax was 7 hr; Cmax was 2.21 µg/mL; MRT: 6.52 hr. b) 50 mg/kg intramuscular administration: T1/2, Vd/F, and Cl/F were 3.85 hr, 1.55 L/kg, 0.264 L/kg.hr, respectively; Tmax was 0.5 hour, Cmax was 23.33 µg/mL; MRT: 6.04 hr. Based on the pharmacokinetic parameter estimates multiple dose scenarios were simulated. The dose recommendations were based on predicted Cmin and Css values. For targeted MIC values of 1, 3 and 6 µg/ml the suggested dosage regimen for koi would be 10, 25 and 50 mg/kg respectively administered once daily. For gouramis, the same dose should be administered every 12h.

Development of *Myxidium* spp. (Myxozoa) in the intestine of tiger puffer experimentally fed with infected gut tissue

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Fish-to-fish transmission of marine myxosporeans belonging to the genus *Myxidium* has been recently demonstrated. In this study, experimental transmission of *Myxidium fugu* and *Myxidium* sp. TP from the intestine of tiger puffer *Takifugu rubripes* exhibiting the emaciation disease was attempted by feeding infected gut tissue. Experimentally infected fish were reared in temperature-controlled tanks (15, 20, 25°C), and the development of myxosporeans were periodically examined. *M. fugu*, attaching on the intestinal epithelium, spread rapidly in the whole intestine, and prevailed in 100% of the fish Day 19 post-exposure (PE), regardless of the rearing temperature. *Myxidium* sp. TP, proliferating inside the intestinal epithelium, developed at 20° and 25°C but did not grow at 15°C. It was also indicated that *Myxidium* sp. TP became "cryptic" infection at 15°C. Mortalities and morbidities of experimentally infected fish were observed from Day 37 PE at 20°C. The present study revealed experimentally the time-course development and the temperature dependency of *Myxidium* spp. in the intestine of tiger puffer.

Biological control of fish viral disease with anti-viral substances produced by bacteria

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Many bacteria producing anti-viral substances were isolated from the aquatic environment. Fish intestinal bacteria such as *Aeromonas* spp. and *Vibrio* spp. producing anti-viral substances were isolated from intestinal contents of masu salmon (*Oncorhynchus masou*), Japanese flounder (*Paralichthys olivaceus*) and barfin flounder (*Verasper moseri*). *Aeromonas* strains produced anti-infectious haematopoietic necrosis (IHNV) substances and *Vibrio* strains showed anti-IHNV, *Oncorhynchus masou* virus (OMV) and barfin flounder nervous necrosis virus (BF-NNV) activities. When *Aeromonas* spp. strains M-26 and M-38 were mixed with food pellets and fed to rainbow trout (*O. mykiss*) and masu salmon, both bacteria became dominant in the intestinal microflora and anti-IHNV activity was observed in homogenates of intestinal contents. These rainbow trout and masu salmon fed the *Aeromonas* spp. showed more resistance to the artificial IHNV challenge test. Barfin flounder fed *Vibrio* spp. strains 2IF6a and V-15 with *Altemia salina* showed anti-IHNV, OMV and BF-NNV activities in the intestinal contents. Larvae fed the *Vibrio* spp. showed a higher survival rate than the fish cultured using the virus free seawater and non-treated seawater.

Protective effect of cutaneous antibody against *Ichthyophthirius* on channel catfish

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Fish recovered from sublethal ichthyophthiriasis acquire protective immunity against *Ichthyophthirius* (Ich). The objective of this study was to evaluate the protective effect of cutaneous antibody produced by channel catfish immune to Ich on cohabited non-immune catfish after exposure to 15,000 theronts/fish. Non-immune and immune fish controls were separately maintained and infected with theronts. Ich infection was assessed by scoring 0, <50, 50-100 and >100 trophonts/fish at 5 days post-infection. The results of infection showed that cohabited fish at the ratio of 15 non-immune to 2 immune fish had <50 trophonts/fish. Eighty percent of the cohabited fish at the ratio of 10 non-immune to 2 immune fish showed 0 or <50 trophonts/fish. The control non-immune fish had 50-100 trophonts/fish or >100 trophonts/fish. The infection score of the control immune fish was 0 trophonts/fish. Anti-Ich antibody was detected in water samples taken from tanks containing immune fish by ELISA after the water samples were concentrated 40X. Immunoadsorption of these water samples with theronts reduced the level of anti-Ich antibodies. The results of the study suggest that immune fish cohabited with non-immune fish may protect non-immune fish against Ich infection.

Poster Sessions
Abstracts

1. **Infectious Disease Models for the Zebrafish *(Danio rerio)***

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We present a model for studying pathogenesis in the zebrafish *(Danio rerio)* using the viral pathogen snakehead rhabdovirus (SHRV) and the bacterial pathogen *Edwardsiella tarda*. These pathogens were chosen based on optimal growth temperature and host range. *Edwardsiella tarda* has been found to infect a variety of fish including flounder, tilapia, carp, channel catfish, and gourami. Infection with *E. tarda* leads to bacterial septicemia which can give rise to internal hyperemia, abscesses on internal organs and external ulcerative lesions. Snakehead rhabdovirus is a tentative member of the Novirhabdovirus family which has been found to infect snakehead fish, catfish, and the sand goby. Although SHRV is known to cause necrotic ulcerations, little else is known about SHRV pathogenesis. In our challenge experiments, AB inbred strain zebrafish were infected by static immersion and intraperitoneal (i.p.) injection at various ages ranging from 24 hours post fertilization (hpf) to adulthood (3 months and older). Fish infected at time points 24 hpf and 72 hpf were susceptible to infection by immersion using both *E. tarda* and SHRV. Adult fish were susceptible to challenge by i.p. injection of both pathogens. Adult fish infected with SHRV exhibited lethargic swimming behavior and severe petechial hemorrhages in the abdominal region. Adult fish injected with *E. tarda* exhibited distended abdomens and as well as erratic swimming behavior. The disease model developed in this study also will provide a tool for studying immune function, specifically innate immune pathways such as the Toll pathway in zebrafish.

2. **Development of novel multiplex PCR detection systems for economical important viral diseases of fish**

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The great development of aquaculture in past decades has led to an increase of viral diseases, producing important problems particularly in intensive fish production. Since no treatment is available and virus vaccines are not widely used in fish medicine, viral disease control is based on the enforcement of strict sanitary measures (facilities and fish movements) and rapid laboratory diagnosis. PCR methods have shown to be high sensitive and specific and are being widely used for diagnosis of viral diseases in mammals. Two novel Multiplex PCR systems have been developed for simultaneous diagnosis of economical important viruses of fish. The clusters included a) OIE notifiable diseases (Viral Hemorrhagic Septicemia virus (VHSV), Infectious Hematopoietic Necrosis virus (IHNV)), and b) significant/emerging diseases (Infectious Pancreatic Necrosis virus (IPNV) and Sleeping disease virus (SD)). By sequence analysis of 39 VHSV isolates and 31 IHNV isolates, specific primer sets for VHSV and IHNV were selected from the protein G coding region, compatible for their use in an individual and multiplex PCR. *One-step* RT-PCR assays using VHSV or IHNV infected culture samples showed each set of primer amplified specific products of 250bp and 200bp respectively. VHSV/IHNV Multiplex PCR allows specific detection of VHSV or IHNV, with a sensitivity of 0.032 $TCID_{50}$/PCR and 0.11 $TCID_{50}$/PCR respectively. Specific primer sets for IPNV and SDV were selected from VP2 and glicoprotein E2 genes respectively, after sequence analysis of 27 IPNV isolates and 1 SDV isolate. Primer sets were compatible for their use in an individual and multiplex PCR. *One-step* RT-PCR assays using IPNV infected culture samples and biological samples clinically suspected of SDV infections showed each set of primers amplified specific products of 104 bp and 250 bp respectively. IPNV/SDV Multiplex PCR allows specific detection of IPNV with a sensitivity of 0.24 $TCID_{50}$/PCR. Samples from SD suspected trout were confirmed as positives by individual or multiplex PCR assays.

3. **Tissue distribution kinetics of grouper iridovirus in Taiwan (TGIV) during infection: an *in situ* hybridization study**

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Grouper Iridovirus in Taiwan (TGIV) has caused heavy losses in grouper aquaculture in Taiwan. Phylogenetically the virus is more closely related to Infectious Spleen and Kidney Necrosis Virus (ISKNV) and Red Seabream Iridovirus (RSIV) than to Epizootic Hematopoietic Necrosis Virus (EHNV) or Largemouth Bass Virus (LMBV). Common characteristic pathology found in TGIV, ISKNV and RSIV is the widespread distribution of enlarged eosinophilic and basophilic cells in tissues. Tissue labelling using *in situ* hybridization of a DNA probe (CY15 fragment) specific to TGIV indicates that it is the basophilic enlarged cells, but not the eosinophilic enlarged cells, contain TGIV DNA in infected hybrid groupers (*Epinephelus malabaricus* x *E. akaara*). These basophilic enlarged cells appear mainly in kidney, head kidney and spleen. The number of these cells peaked at day 5 post injection, then declined with time. There was, however, an early peak in cell numbers in the spleen at approximately day 3, then they declined until a second peak at day 5. Basophilic enlarged cells could also be found in liver, muscle, heart, gill, intestine and eye but in lower numbers. TGIV may first infect and replicate inside the spleen before day 3, and then be transported through the blood to other organs.

4. **Evaluation of methods of extraction of viral RNA from infected cell monolayers**

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Since the last decade, an increasing number of companies and laboratories have developed molecular methods for detection of virus, especially to be used as a tool for diagnosis and control of infectious diseases in commercial fish farms. Those techniques provide considerable savings in terms of time and labour over the conventional procedures such as the serological ones. However, their sensitivity depends upon the quantity and quality of the nucleic acid extracted from tissues or infected cell cultures. In the present study, several methods for extraction of total RNA (TRIZOL LS-Reagent, GIBCO-BRL; RNeasy Mini Kit, QIAGEN; NucleoSpin RNA, MACHEREY-NAGEL; Perfect RNA^{TM} Eukaryotic Mini, EPPENDORF) were evaluated, using the traditional method of extraction by Proteinase K / Phenol-Chloroform as the reference assay. The amount and purity of the extracted RNA was measured by spectrophotometry and SDS-PAGE. In addition, we applied RT-PCR to evaluate the quality of the RNA extracted by the different methods. The results obtained were different depending on the technique employed for the evaluation of the extraction method. In this sense, when we used spectrophotometry, TRIZOL LS yielded the highest concentrations of total RNA, although its quality (in terms of ratio A_{260}/A_{280}) was relatively low. The resin-based methods provide low concentrations but higher ratios. When the extracted RNA samples were evaluated by acrylamide gels, the best method was Nucleospin. Finally, the Perfect-RNA^{TM} and Nucleospin methods showed the best results for amplification of the viral RNA by RT-PCR, even for small volumes of raw virus.

5. **The effect of stocking density and other parameters of the susceptibility of Atlantic salmon (*Salmo salar* L.) to infectious pancreatic necrosis virus**

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The study of the effect of infectious pancreatic necrosis virus on adult Atlantic salmon has been restricted by the lack of a reproducible challenge protocol. We have recently published a method for inducing mortality in post smolt salmon and have been looking at the effect of a range of parameters in order to understand the factors that promote the development of this disease. Preliminary studies had suggested that stocking density was an important factor in the progression of the disease and we have studied this in more detail. Results show that there are clear effects relating to stocking density with low stocking density producing low mortality. Other parameters were investigated including viral concentration and time of infection following seawater transfer. Viral concentration variation showed that low doses only extend the onset time. There seemed to be little variation in onset and mortality in relation to the time of challenge following seawater transfer. We have also conducted some preliminary investigations into the induction of antiviral proteins. These are discussed.

6. **Interference of the life cycle of fish nodavirus with fish retrovirus**

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The interference of the life cycle of grouper nervous necrosis virus (GNNV), a member of the fish nodaviruses, by snakehead fish retrovirus (SnRV) has been studied *in vitro*. A new SnRV-persistently infected fish cell line was induced by inoculating SnRV into a grouper fin cell line GF-1, and was designated as SGF-1. The culture supernatants and cell pellets from GNNV-infected SGF-1 cells and from GNNV-infected GF-1 cells were collected and employed for viral productivity analysis. The yield of GNNV RNA and capsid protein in GNNV-infected SGF-1 cells were similar to that in GNNV-infected GF-1 cells. However, when GF-1 cells were used as a titration system, the titer of the culture supernatant from GNNV-infected SGF-1 cells was much higher than the titer of culture supernatant from GNNV-infected GF-1 cells. The titration result suggested that SnRV enhanced the infection or cytopathic effect (CPE) of GNNV during GNNV/SnRV coinfection in GF-1 cell titration system, although SnRV can not induce any CPE in GF-1 cells alone, nor increase the yield of GNNV after GNNV superinfection of SnRV-persistently infected SGF-1 cells. Moreover, GNNV cDNA was detected both in the pellet and the supernatant of GNNV-infected SGF-1 cells. This result indicated that fish retrovirus reverse-transcribed GNNV single strand genomic RNA into cDNA during GNNV superinfection in SGF-1 cells, and created a new DNA stage in the life cycle of fish nodavirus.

7. **Molecular diagnosis of fish diseases: the way forward**

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The advent of molecular techniques to detect and analyse fish pathogens has given rise to a great number of publications describing development of new methods for disease diagnosis. Some of these have already proven invaluable in the detection, identification and epizootiology of pathogens such as infectious salmon anaemia virus (ISAV), *Gyrodactylus* parasites, and viral haemorrhagic septicaemia virus (VHSV), and examples of their use in Scotland will be presented. However, molecular techniques are not a panacea and care needs to be taken in their adoption and interpretation. A number of relevant concerns and areas where more work is required will be highlighted and the present condition of the field discussed. In deciding whether to adopt a certain molecular diagnostic method, a number of important components must be considered. These factors, together with the prospect for future application of molecular techniques will be described.

8. **Fingerprinting of *Flavobacterium psychrophilum* isolates by plasmid profile**

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Plasmid profiling was used to determine genomic variation in 169 strains of *Flavobacterium psychrophilum* isolated from ayu, rainbow trout, other salmonids and cyprinids in Japan or other countries. The nucleotide sequences of two plasmids, pFPC814 and pFPC840 obtained from *F. psychrophilum* FPC814 (an isolate from rainbow trout) and FPC840 (an isolate from ayu), respectively, were determined. Based on these sequences, plasmid-specific primer pairs were synthesized and plasmid profiles were determined by PCR. A total of 72 strains of *F. psychrophilum* were separated into 3 groups (I, II and III) based on the plasmid profiles. Ninety-seven strains did not harbor both plasmids (III). Of the 31 strains with pFPC814 (I), 29 strains were isolated from rainbow trout and two were from other fish species. The isolates from ayu were accounted for 85% of the strains with pFPC840 (II) (35/41). This may suggest that outbreaks of bacterial cold-water disease have been occurring among ayu independent of other fish species.

9. Genotyping of *Flavobacterium psychrophilum* by PCR-RFLP analysis

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Genetic variability among strains of *Flavobacterium psychrophilum* was characterized using restriction fragment length polymorphisim (RFLP) analysis of their polymerase chain reaction (PCR) products. In the first experiment, the universal primers of GYR-1 and GYR-1R were used to amplify gyrase subunit B gene (*gyrB*). The universal primer pair yielded approximately 3000 bp, 1200 bp and 300 bp PCR products from all *F. psychrophilum* strains. In the RFLP analysis of the products, the restriction enzyme *Hinf*I produced two cleavage patterns (genotypes A and B) of the 300 bp products, which was not considered to be the *gyrB* because of its size. Genotype A was found only in isolates from ayu (n=105). Genotype B was found in isolates from coho salmon (n=11), ayu (n=31), rainbow trout (n=42) and other fishes (n=32). In the second experiment, the primers of PSY-G1F and PSY-G1R specific for *F. psychrophilum* were used to amplify the *gyrB*. The specific primer pair amplified the expected size (1017 bp) PCR products from all *F. psychrophilum* strains. In the RFLP analysis of the *gyrB*, the restriction enzyme *Rsa*I produced two genotypes R and S. Genotype R was found in isolates from coho salmon (n=6), ayu (n=26), rainbow trout (n=38) and other fishes (n=2). Genotype S was found in isolates from coho salmon (n=5), ayu (n=110), rainbow trout (n=4) and other fishes (n=30). There were no geographical features of the genotypes.

10. Studying mycobacterial infection using small fish models and *M. marinum* expressing GFP

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The intracellular pathogen *Mycobacterium tuberculosis* is responsible for ~3 million deaths per year. One of the defining characteristics of the chronic state of tuberculosis is the formation of granulomas. *Mycobacterium marinum* is the species of mycobacterium most closely related to *M. tuberculosis*, with a sequence homology of 99.4%. Systemic infection and the formation of granulomas characterize *M. marinum* infection in fish. It has been shown to be possible to induce both an acute and chronic infection in goldfish, *Carassius auratus*, depending on the dose of *M. marinum* administered. This makes it an effective model for the study of mycobacterial pathogenesis. In this study, goldfish were injected intraperitoneally through the lateral abdominal musculature by suspensions of *M. marinum*, which carried a plasmid containing a strong promoter sequence fused to the mGFP reporter gene. Fluorescence microscopy was utilized to view the bacteria in fresh tissues. Infection by the bacteria was observed by microscopy and plating of homogenized tissues at various points post infection. These studies are being extended into larger-scale experiments involving other small fish models, in particular medaka (*Oryzias latipes*).

11. A microtiter plate assay method for mucosal adhesion of *Aeromonas hydrophila* using a water-soluble tetrazolium salt (WST-1)

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Aeromonas hydrophila is a pathogenic bacterium causing severe skin ulcers in a variety of fish species, and its adhesion to skin surface has been recognized as the initial stage of infection. In the case of fish, this skin surface is covered by a mucus layer, which is secreted by goblet cells located in the epidermal layer. However, the significance of fish mucus as the first layer involved in microbial adhesion or as a defense barrier against pathogenic invasion remains to be elucidated. This study was undertaken to develop a simple microtiter plate assay method for measuring mucosal adhesion of *A. hydrophila* using a water-soluble tetrazolium salt (WST-1). Skin mucus extract samples (SMES), which were prepared from eel skin, were placed in wells of a 96 well-microplate and incubated overnight at 4℃. Then, each well was washed three times with PBS to remove the excess SMES. After then, bacterial suspensions at different densities were added into wells and the plate was incubated for several hours at 25℃. The wells were washed three times with PBS, incubated with WST-1 for 3h at 25℃, and measured by a model 550 Bio-Rad microplate reader at 450nm. As a result, it was found that adhesion of *A. hydrophila* to mucus was dependent on bacterial density loaded and incubation time. Moreover, results of the new assay method were comparable to those of the direct count method using Giemsa staining. These results strongly suggest that the newly developed method is effective for the study of mucosal adhesion of bacteria.

12. First isolation of *Vibrio anguillarum* serotype O1 from sole (*Solea solea*) in Italy

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During the last ten years a new interest in the farming of sole, *Solea solea*, has been stimulated for the existing marine fish farming industry in order to diversify their production. In September 2001 a group of 3,000 young common sole was caught from the Venice lagoon and introduced into a culture facility in the North East of Italy. Six months later, when the temperature was increasing, an acute mortality occured. Fish showing clinical signs were sampled and analized in our laboratory. Diseased fish showed no apparent lesions and some depigmented and erosive areas of the skin have been detected. Virological and parasitological tests were negative, while *Vibrio anguillarum* serotype O1 has been isolated from the brain tissues. This strain was tested by agar diffusion method against some antibacterial agents and after a therapeutic treatment mortality stopped. This is the first report of Vibriosis in reared sole, *Solea solea*, in Italy.

13. Cold shock response of *Vibrio vulnificus*: with and without acclimation periods

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Vibrio vulnificus is a gram negative, halophyllic, mesophile that is a human health concern. It is primarily transmitted via ingestion of raw molluscan shellfish, especially oysters and can cause disease and even fatalities. The approach of this study was to examine the effects of cold shock on protein levels and expression using one dimensional glycine and tricine gel electrophoresis. Cells were cold shocked from 37°C to 15 and 4°C for up to 24 hours. Glycine gel electrophoresis indicates possible shifts in glycolytic enzyme presence. The tricine gel electrophoresis was performed to examine the presence of low molecular weight cold shock proteins (csp), for example the major *E. coli* cspA molecular weight 7.4 kDa. Preliminary trials indicate possible presence of this protein which will be further investigated using radio isotope labeling, and two-dimensional gel electrophoresis. The affects of a 2 hour, 4°C shock prior to sub-0°C incubation was also investigated. Plate count analysis indicates decreased die off rates of cultures shocked prior to sub-0°C incubation. This information is critical to the oyster industry because it will determine proper handling of the oysters immediately post harvest. If it holds true that *V. vulnificus* counts increase if given an acclimation period; chilling oysters immediately post harvest will prove contradictory to the ultimate goal of reducing *V. vulnificus* counts when they reach the consumer.

14. Discriminant analysis on the blood chemistry of *Streptococcus iniae* and *Vibrio vulnificus*-infected tilapia, with references to histopathology

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Blood chemistry of Nile tilapia (pure strain *Oreochromis niloticus*) kept in a laboratory setting was studied. Plasma samples were obtained from 30 healthy, 30 *Streptococcus iniae*-infected, and 30 *Vibrio vulnificus*-infected tilapia. Thirty parameters were then measured and twenty were used in discriminant analysis. The full model (20 parameters) yielded 93% correctness in predicting membership to the three groups (healthy and two infections). A reduced 8-parameter model containing sodium, potassium, sodium-to-potassium ratio, chloride, calcium, iron, anion gap, total protein yielded 92%, while a 3-parameter model containing iron, anion gap and total protein yielded 88%. This indicates that these parameters contain extraneous information and careful selection of parameters can still maintain enough predicting ability. Histopathology of fish from both infected groups were coded and their correlation to blood chemistry parameters were calculated. Hepatic pathology is correlated (correlation coefficient > 0.4) to bicarbonate, phosphate, total protein, aspartate aminotransferase and creatine kinase. Renal pathology is correlated to potassium, chloride, bicarbonate, anion gap, total protein and creatine kinase. Spleen pathology is correlated to potassium, chloride, bicarbonate, anion gap, total protein and cholesterol. Pathology in head kidney is correlated to potassium, chloride, anion gap, total protein, glucose, cholesterol and lipemia.

15. Antimicrobial profiles of members of genus *Serratia* considered typical fish pathogens

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Most of the members of Genus *Serratia* described so far have been isolated from fresh water and fish, suggesting they may be potential opportunistic pathogens for animals and humans. *Serratia* spp. grows well on ordinary media under anaerobic and aerobic conditions. Optimum growth of all strains has been observed at temperatures from 10-37ºC. In this work we have evaluated a total of 26 antimicrobial compounds including drugs commonly used in fisheries and other chemotherapeutics with efficacy against members of Genus *Serratia*, considered typical fish pathogens that not been determinated until present. Drug resistence patterns of the isolates were determined by the disc diffusion method on Müller-Hinton agar using the chemotherapeutics agents. The minimum inhibitory concentrations (MICs) were determined using the plate dilution technique.

16. Prevalence of Type E botulism in fish in the Lower Great Lakes

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Botulism has been documented in waterfowl during several mass mortality events on Lake Erie in 1999-2001. This project is focusing on the role of fish in these outbreaks. The specific objectives of the project are to determine the prevalence of *Clostridium botulinum* in apparently healthy, moribund, and dead fish in areas of confirmed outbreaks of avian botulism and unaffected areas within the Lower Great Lakes. We are also quantitating the amount of *C. botulinum* and toxin in carrier fish. In a cooperative effort with the NYSDEC, we are collecting fish from both Lake Erie and Lake Ontario. The focus is on species of fish that have died in recent fish kills such as freshwater drum, smallmouth bass, and round goby. Scheduled collections of these species of fish, as well as sampling during fish kills and active outbreaks of botulism in waterfowl have or will be taking place. Standard necropsies are being performed on all fish sampled including skin scrape, gill clip, and bacteriological culture of the kidney. Tissue samples that are being archived for PCR assay of the type E botulinum toxin gene include liver, blood, and intestinal contents. Those samples that test positive for *C. botulinum* also will be assayed for toxin by the traditional mouse bioassay and the number of bacteria present will be enumerated by quantitative PCR. We hope to better understand the circumstances under which fish-eating birds can become intoxicated with type E botulism from eating live or moribund fish.

17. Mucus adhesion of bacteria isolated from intestinal tract of carp, *Cyprinus carpio*

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Although adhesion to the intestinal mucosa is in general regarded as the prerequisite for colonization, growth and pathogenicity of microorganisms, little is known about the significance in fish. Therefore, in this study, intestinal bacteria isolated from carp (*Cyprinus carpio*) were examined for their abilities to adhere to fish mucus. A total of 400 bacteria were isolated from the content and wall surface of carp's intestines. Mucus was collected from carp and fixed on wells of a 96-well microplate. Bacterial suspension was added into each well and washed with PBS. After then, bacteria adhered to mucus was assessed by a colorimetric assay using WST-1. In addition, the partial fragments of amplified 16S rDNA from 62 bacterial isolates, which showed high abilities to adhere to fish mucus, were sequenced and their phylogenetic positions were analyzed. As a result, they were classified into six taxa, including *Aeromonas veronii, A. hydrophila, Citrobacter freundii, Acinetobacter junii, Plesiomonas shigelloides*, and *Pseudomonas gingeri*. Of these bacteria, *A. veronii* and *A. hydrophila* were major species.

18. Screening of tuna's microbiota captured in different fishing grounds (Pacific, Indian and Atlantic oceans)

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The sea fish has own microorganisms that are innocuous for human, however inadequate elaboration process can introduce exogenous microorganisms who became this product in a risk for the consumer health. The security of fishing products depends on the present pathogenic agents in the fish just captured, due to the presence of pathogenic microorganisms present in the aquatic ecosystem, based on coastal contamination or manipulation during the processing by the workers and means. The aquatic environment is then one of the important factors that influence in the type of microbiota that is developed in the fish. Parameters like water temperature, mineral concentration and own fish influence in the type of microorganisms present in the fish. In this study we tried to compare microbiota present in variety tuna fish captured in different zones. For it, the percentage of different microorganisms in tunas captured in fisheries has been determinated from the Pacific, Indian and Atlantic oceans. The transport of the samples was made at freezing temperature, and once in the laboratory they stayed to room temperature before analitical assay. After defrosting, the analysis was made following official methods of microbiological analyses.

19. Effect of lactic acid and the supernatants from different lactic-acid bacteria strains against *Vibrio* strain

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In the present work, the effect of lactic acid and the supernatants from different lactic-acid bacteria against an isolate of *Vibrio* sp. pathogenic for turbot larvae was studied. Different lactic acid concentrations were added to microcosms of sterile marine water containing 10^5 CFU/mL of *Vibrio* strain, maintained during 72 h at 22ºC. Aliquots were taken from different microcosms at regular intervals (0 h, 24 h, 48 h and 72 h) spread onto marine agar plates and incubated at 22ºC for 24 h. The supernatants of lactic-acid bacteria were obtained after centrifugation of 10^9 CFU/mL and 10^5 CFU/mL at 2500 rpm during 5 minutes. The filtered supernatants obtained were added to each microcosm, maintained during 72 h at 22ºC. Aliquots were taken from different microcosms at different intervals time (0 h, 24 h, 48 h and 72 h) spread onto marine agar plates and incubated at 22ºC for 24h.

20. Interaction between different populations of lactic-acid bacteria and typical virulent *Vibrio* strain

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In the present work the interaction between a pathogenic strain of *Vibrio* sp. isolated from turbot larvae and different stocks from lactic-acid bacteria were evaluated. The experiment was carried out using six different lactic-acid bacteria strains against *Vibrio* strain in microcosm (sterile marine water). A concentration of 10^9 CFU/mL lactic-acid bacteria and 10^9 CFU/mL *Vibrio* sp. were used. Another concentration of 10^8 CFU/mL lactic-acid bacteria and 10^2 CFU/mL of *Vibrio* sp. were used in order to compare the possible effect between populations. Aliquots from microcosms were taken at regular intervals (0 h, 24 h, 48 h and 72 h) and spread onto MRS plates for lactic-acid bacteria and marine agar for *Vibrio* strain. MRS plates were incubated at 28ºC during 48 h and marine agar plates were incubated at 22ºC during 24 h. Control microcosms were performed with only one strain in sterile marine water in the same incubation conditions. In the microcosm with a concentration 10^9 CFU/mL of lactic-acid bacteria and 10^6 CFU/mL of Vibrio sp., was observed an independent behaviour between microorganisms, similar to the control tubes. The *Vibrio* strain was not inhibited by the presence of lactic-acid bacteria. When the microcosm used with a concentration of 10^8 CFU/mL of lactic-acid bacteria and 10^2 CFU/mL of *Vibrio* sp., was observed an exponential growth of the pathogenic strain during the first 24 h, that does not agree with the behaviour of *Vibrio* sp of the control tube. The lactic-acid bacteria strains were not affected by the pathogenic strain, and its behaviour was similar to those control tubes obtained in.

21. Cloning and characterization of a phospholipase gene of *Pasteurella piscicida*: this enzyme shows hemolytic activity

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Pasteurellosis, caused by *Pasteurella piscicida* is a serious bacterial disease affecting yellowtail *Seriola quinqueradiata* aquaculture in Japan. It also results to high losses in economically important cultured marine fishes in the U.S.A. and Europe. *P. piscicida* virulence is presently little understood. It is implicated that *P. piscicida* extracelluar product (ECP) was strongly toxic to fish, and all the ECP samples examined have notable phosholipase activity. Phospholipase is reported as a virulence factor in several pathogenic bacteria. We cloned the *P. piscicida* phospholipase gene by screening a genomic cosmid library using egg-yolk agar. Sequence analysis of this clone showed that the open reading frame of *P. piscicida* phospholipase is composed of 1.218 bp and 405 amino acid residues. Comparison of the amino acid sequence of *P. piscicida* phospholipase gene with that of other bacteria revealed high homology with the phospholipase or hemolysin of *Vibrio* species. The molecular size of *P. piscicida* phospholipase is approximately 46 kDa, based on SDS-PAGE analysis. Blood agar assay of the concentrated supernatant of *E. coli* transformed with *P. piscicida* phospholipase showed that *P. piscicida* phospholipase could directly degrade fish erythrocyte phospholipid, and indirectly degrade mammalian erythrocyte phospholipid depending on the addition of lecithin. The single gene knock-out of this gene did not show phospholipase and hemolytic activity, and we suggest that this is the gene which shows phospholipase and hemolytic activity in *P. piscicida*.

22. PCR detection of *Pseudomonas anguilliseptica* from winter disease outbreaks in sea bream

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Pseudomonas anguilliseptica is an emerging fish pathogen that has gained clinical significance for being responsible of mortality outbreaks in different fish species. Recent microbiological studies have associated infection by *P. anguilliseptica* to the etiology of winter disease (WD) in sea bream. WD is a multifactorial syndrome associated with the nutritional and immune status of fish and stressful environmental conditions. During the last years, several WD outbreaks in sea bream have been reported in different marine fishfarms of the Mediterranean area. The severe mortality that can be observed during the WD outbreaks, together with the fact that sea bream is one of the main sea cultured non-salmonid fish species, make *P. anguilliseptica* a fish pathogen of emerging clinical significance. The diagnosis of *P. anguilliseptica* infections is made difficult by the slow growth rate and weak reactivity in most biochemical tests of this microorganism. The detection and identification of fastidious microorganisms, like *P. anguilliseptica*, can be significantly improved by using molecular techniques. A PCR-based detection system for *Pseudomonas anguilliseptica* was developed based on primers designed to amplify a fragment of one variable region of the 16S rRNA. The primer combination PAF-PAR gave a unique and specific amplification product of 439 bp at an annealing temperature of 46ºC with all the *P. anguilliseptica* isolates and strains (n=56) but no amplification products were observed with any other *Pseudomonas* species or phylogenetically related bacteria tested. The PCR assay had a detection limit of 170 to 200 cells per PCR tube, which was improved 8-fold when the PCR amplification product was used as a nonradioactive probe in blotting hybridization experiments. The PCR assay allowed the specific and reliable detection of *P. anguilliseptica* within 8 h, compared with up to 10 days required for its isolation and further characterization by conventional microbiological approaches.

23. Evaluation of API Staph for identification of different species of genus *Staphylococcus* isolated from diseased rainbow trout

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Staphylococcus spp. have been isolated from aquarium water and have been found in aquaculture pond water in Africa. *Staphylococcus aureus* and *Staphylococcus epidermidis* cause staphylococcosis in fish. In Spain the authors report the first isolation of *Staphylococcus warneri* from diseased rainbow trout. *Staphylococcus warneri* is usually found on human skin in very small populations. It may be associated with a variety of human infections, such as septicemia, endocarditis, conjunctivitis and urinary tract and wound infections. In the present study we have compared the accuracies of commercially available identification system (API Staph., Biomerieux, Spain) with those conventional reference biochemical tests for the identification of members of Genus *Staphylococcus* incubated at 22°C and 37°C.

24. Survival of virulent and avirulent *Hafnia alvei* strains in fresh water microcosms

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Hafnia alvei has been reported to cause epizootic haemorrhagic septicaemia in rainbow trout, brown trout and causative agent of kidney pathology in cherry salmon. *Hafnia alvei* is a normal habitant of surface water and soil. The influence of different environmental factors (temperature and presence of sediment) on the survival of three strains of *Hafnia alvei* was studied according to laboratory desings. Two *Hafnia alvei* virulent strains, isolated from diferent sources (X1 and OR-1), and one avirulent strain ATTCC 13337 were used. The ability of these fish pathogenic bacteria to survive in fresh water was studied in laboratory microcosms at 11°C and 22°C in presence and absence of two typical fresh water sediments (sewage and sandy ground). The microcosm were prepared in flask containing 100 mL of natural fresh water and inoculated to an initial concentration of approximately 10^5 bacteria/mL. This bacteria were enumerated using selective medium (Salmonella Shigella, agar) and general medium (TSA, agar) normal and ten fold diluted. The influence of temperature was clear being the viability greater at 11°C than at 22°C. The presence of sediment in the microcosm significantly enhanced the survival of the examined bacteria compared to the microcosms containing only water.

25. Hydrophobicity assays using virulent and avirulent *Hafnia alvei* strains

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Hafnia alvei isolates were evaluated for their relative cell surface hydrophobicity by the salt aggregation test (SAT). 25 virulent and avirulent *Hafnia alvei* strains were routinely cultured on tripticase soy agar at 37ºC and 22ºC for 72 h. The bacteria were washed and suspended in sodium phosphate buffer (pH=6.8) and centrifugated a 4000 r.p.m. during 20 min. The pellets obtained were resuspended in sodium phosphate buffer until optical density of the suspension was adjusted to 1.0 at 610 nm. An aliquot of 30µL of this suspension was mixed with an equal volume of differents concentrations (0.05- 4 M) of ammonium sulphate $(NH_4)_2SO_4$ on glass slides and rotated for 2 min at room temperature. The SAT value was defined as the lowest molarity of $(NH_4)_2SO_4$ that caused a visible agglutination of the test organism.

26. Siderochrome production by virulent and avirulent strains of *Hafnia alvei*

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The role of *Hafnia alvei* as an environmental bacterium has been reported many times. *H. alvei* is a normal inhabitant of surface water and soil. And this organism has generally been considered an opportunistic pathogen occurring with other predisposing factors and recently has been reported to cause haemorrhagic septicaemia in brown trout. The availability of iron has been shown to be an important factor in the pathogenicity of microorganisms associated with fish diseases. The aim of the present work was to evaluate the possible relationship between siderochromes production with virulent and avirulent *Hafnia alvei* strains. Twenty-five *Hafnia alvei* strains isolated from different sources and reference strains were examined. The production of siderochromes was determined by chemical method of Schwyn and Neilands (1987). The medium MM9 augmented with Chromeazurol S (Sigma) and hexadecyltrimetylammonium bromide (HDTMA, Sigma) was used.

27. Effect of mainteinance at refrigerated temperatures of *Hafnia alvei* strains on their biochemical profiles

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Hafnia alvei is an environmental bacterium that has showed to be an opportunistic pathogen in fish, causing kidney pathology in cherry salmon and septicemia in brown trout where typical fresh water temperature is arround 11ºC. The aim of the present study was to evaluate the influence of this low temperature (11ºC) in a typical mesophylic bacterium in terms of feasible changes on biochemical profiles of *Hafnia alvei*. Twenty-five typical and atypical *Hafnia alvei* strains isolated from different sources and reference strains were selected for testing. Strains were incubated at 11ºC using TSA with 5% fish blood during one month in order to simulate the natural conditions of these bacteria inside fish. The following biochemical test were performed: methyl red, Voges Proskauer reaction, indole production, nitrate reduction, Citrate Simmons utilization, hydrogen sulphide production, gelatinase production, acid production from glucose, sucrose, rhamnose, arabinose, melibiose, sorbitol, mannitol, inositol, arginine dihidrolase production, lysine and ornitine decarboxylase production.

28. *Hafnia alvei:* determination of time and temperature correlation when using API 20E

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Hafnia alvei as an opportunistic pathogen causing mortality in brown trout, septicaemia in rainbow trout and kidney pathology in cherry salmon and usually surviving for long in different environmental sources as water, soil or mud. With the expansion of aquaculture there has been an increasing interest in reducing time required for the identification of microorganisms associated with fish diseases. The API 20E is currently one of the most used miniaturized systems of rapid diagnosis of bacterial fish diseases. Different researchers used different times and temperatures with different results. The aim of this study was to determine the most suitable time and temperature for diagnosing *Hafnia alvei*. We have collected virulent and avirulent *Hafnia alvei* strains from different sources and reference strains from culture collections. All strains were subcultured on tryptone soy agar (TSA) at 11ºC and 22ºC. The API 20E system was run on 24- to 72-hour cultures depending upon rate of growth. Incubations were performed at 11ºC and 22ºC for 48 and 72 hours. We concluded that some strains of *Hafnia alvei* are misidentified.

29. Early events of the infection of *Piscirickettsia salmonis* of CHSE-214 cells by confocal microscopy

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The aim of the present work was to document the infection process of *P. salmonis* in CHSE-214 cells by confocal microscopy. The SLGO-95 strain of *P. salmonis* was used to infect monolayers of cells grown in round coverslips. Observation was performed at the following post inoculation times (*pi*): 5min, 10min, 15min, 30min, 1h, 3h, 6h, 12h and 24h. *P. salmonis* was visualized using two methods. One of them was fixing the microscopical preparations and labelling the bacteria by means of an IFAT using monoclonal antibodies. The other method consisted of labelling the bacteria with a vital fluorescent dye in order to observe *in vivo* the fate of *P. salmonis* in the CHSE-214 cell monolayers. The membranes of the eucaryotic cells were labelled with another fluorescent probe, with a different wavelength absorption and emission spectrum, in order to facilitate the bacteria location on the live fluorescent specimens. Results showed that *P. salmonis* was found attached to the plasma membrane as early as 5min *pi*. The microorganisms remained on the cell surface from 5 to 30min *pi*. At 1 and 3h *pi* the bacteria could be observed on the cell surface and/or embedded in the plasma membranes. Finally, from 6h and longer *pi* times (12 and 24h) *P. salmonis* was found inside the cytoplasm of the cells. According to the results it seems that the early infectivity events of *P. salmonis* in CHSE-214 cells involve a fast attachment step (≤ 5min *pi*) and a further cell penetration through the plasma membrane which occurs mainly between 3 and 6h *pi*. Financed by Fondecyt grants 1010544 and 1000788.

30. Vertical transmission of *Piscirickettsia salmonis* and a study of the mode of entrance into the ovum

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Piscirickettsia salmonis is a pathogenic rickettsial agent causing septicaemic disease in salmon that was first isolated in Chile in 1989, where it has since produced high mortality rates in salmon farms. Little information exists regarding the mechanisms of vertical transmission of this pathogen. Experimental vertical transmission was established in the present study by inoculation of male and female breeders with *Piscirickettsia salmonis*. *P. salmonis* was detected using indirect immunofluorescence in semen and celomic fluid of the majority of inoculated breeders (14/15). Fry infection occurred when either both or only one parent was inoculated, although none of the infected fry presented symptoms of the disease. Positive progeny were also obtained through fertilization of a group of ova proceeding from non-inoculated females in a medium containing a rickettsial suspension, demonstrating transmission during the process of fertilization. A group of ova infected *in vitro* were studied at different times by scanning electron microscopy, showing that the rickettsia attaches to the ova by means of membrane extensions similar to multiple podosomes, structures which we have called Piscirickettsial Attachment Complex (PAC) and which would allow later penetration into the ovum.

31. Infectivity study of *Piscirickettsia salmonis* in CHSE-214 cells by immunogold and standard transmission electron microscopy

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The purpose of this work was to observe the early events of the infection process of *P. salmonis* on CHSE-214 cells by standard electron microscopy (TEM) and immunogold. The SLGO-95 strain of *P. salmonis* was used. The bacteria were grown in monolayers of CHSE-214 cells as described by Fryer et al. (1990). Inocula were prepared from spent supernatants which were filtered (5μm) to remove clumps and the nondisrupted CHSE-214 cells. The bacteria were used to infect monolayers of CHSE-214 cells grown in 25 cm^2 flasks. Standard procedures were used for TEM. For the immunogold, a polyclonal antibody was used as the first antibody (Lannan and Fryer, 1994) and a commercial gold (10nm) labelled antibody as the second one. Observation was performed at the following post inoculation times (*pi*): 15min, 1h, 4h, 6h, 24 h, 45 h and 7 days. At 15 min, 1h and 4h *pi* no bacteria could be found inside the cytoplasm of the CHSE-214 cells. At 15 min *pi* a number of rounded empty structures, only made up of a membrane and similar in size to *P. salmonis*, were found outside but close to the plasma membrane of the cells. At 4 h *pi P. salmonis* was observed very close to the plasma membrane. Finally, from 6h and longer *pi* times (24 and 45 h and 7 days) *P. salmonis* was found inside the cytoplasm of the cells. These results are similar to those obtained in another study of the same kind but which used confocal microscopy. Financed by Fondecyt grants 1010544 and 1000788.

32. Experimental infection of coho salmon (*Oncorhynchus kisutch*) exposing the surface of skin, gills and intestine with *Piscirickettsia salmonis*

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In a previous work it was determined that skin and gills, and primarily the former, were the main routes of entry of *Piscirickettsia salmonis* in rainbow trout (*O. mykiss*) (Smith et al., 1999). In order to get further information on piscirickettsiosis pathogenesis, some tissues were tested as entry portals of *P. salmonis* in coho salmon. Juvenile fish (n = 150), weighting 10 g, were used in this trial. Inocula were prepared using the strain SLGO-95 of *P. salmonis*. The microorganism was cultured in the CHSE-214 cell line as it has been previously described by Fryer et al. (1990) and three doses containing 10^6, $10^5$and 10^4 $TCID_{50}$, were prepared. Each dose was used to infect the fish in the surface of the skin, gills and intestine. Skin and gills were exposed by a drop, and the intestine by an intubation through the anal opening. After exposure fish were held by 30 min immersed in minimal essential medium. In addition some fish were injected intreperitoneally with the same *P. salmonis* doses, as positive virulence controls. Appropriate negative controls were also included. Piscirickettsiosis was experimentally reproduced with all the inoculation methods employed herein. There was a dose-response pattern en every case. Cumulative mortalities and survival analyses showed that the most effective way of entrance was skin followed by intestinal intubation and finally by gill infection. In previous studies in rainbow trout, gill exposure resulted in higher mortalities than the ones obtained by intestinal intubation, but in both salmonid species the skin proved to be the most efficient way of entrance of *P. salmonis*. Financed by grants Fondecyt 1010544 and 1000788.

33. Characterization of a piscirickettsiosis-like disease in Hawaiian tilapia

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Early in the 1990s tilapia in Hawaii, USA, started showing signs of a disease syndrome similar to piscirickettsiosis, caused by *Piscirickettsia salmonis*, an obligate intracellular bacterial pathogen of salmonids. Infected tilapia often swim erratically, appear to have trouble staying at depth, have occasional cutaneous hemorrhages in the skin and regularly have exophthalmia. But frequently the first sign of disease is death. The gills show epithelial hyperplasia, with some severe consolidation of secondary lamellae. Multiple white granulomas are observed in the gills and tissues. The piscirickettsiaosis-like syndrome does not form ring shaped lesions in the liver as seen with *P. salmonis* infections and the agent is active at temperatures higher than *P. salmonis*. The organisms are inconsistently visualized with Giemsa and Warthen Starry stain , but stain well with Lillie Twort stain. In blood smears moderate to large numbers of intracellular bacteria are noted with rare circulating monocytes. Some predilection for nervous tissue including peripheral nerves, spinal cord and brain is observed in histological sections. In TEM pleomorphic bacterial organisms, generally coccoid in shape, 0.4 – 0.67 x 0.5 – 0.89 µm, are observed free in the cytoplasm and within phagolysosomes. The organisms have a double cell wall with no defined nucleus and variable electron dense and electron lucent areas.

34. Investigation of fish to fish variation in oxolinic acid concentrations in commercially farmed fish and laboratory held fish following commercial oral therapy

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In the context of antimicrobial therapy in commercial fish farms, the predictions of the probable outcomes of the antimicrobial therapies are made through comparisons of the susceptibility of pathogenic bacteria (MIC) with the concentration of antimicrobial agent concentrations (plasma Cmax) that can be achieved in the host. The majority of published pharmacokinetic studies have not studied multiple dose oral administrations by natural feeding. Even when this commercially relevant method of administration has been employed, the fish studied have frequently been small populations of healthy fish held under laboratory conditions. In commercial farms, however, variations in feeding rates between individual fish and between healthy and infected fish may be predicted to contribute to a significant heterogeneity of Cmax values in a treated population. In order to assess the extent of this heterogeneity and the commercial relevance of mean plasma Cmax values generated in laboratory based studies, paired laboratory and field studies were performed. Plasma oxolinic acid (OXA) concentrations were determined in large samples of fish collected during administration of this agent in two independent commercial fish farms. Two laboratory studies were designed to replicate these field studies with respect to species, temperature, salinity, dose and administration method. Data on mean plasma OXA concentrations and its variance were obtained under farming conditions from apparently healthy rainbow trout (n = 20) and Atlantic salmon (n = 18) in seawater farmed at approx. 12°C and 6°C, respectively. These are compared to similar data from healthy rainbow trout and Atlantic salmon held under laboratory conditions.

35. ***In vitro* metabolic profiles to characterize and predict drug residues in aquacultured finfish**

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As infectious and parasitic diseases are major obstacles for aquaculture growth, there is a vital need to expand the repertoire of therapeutic drugs for veterinary use in fish species. Compared with traditional farm species, very few drugs are currently approved by the US FDA for use in aquaculture. In order to facilitate the drug approval process for cultured fish species, it is desirable to establish species crop groupings based on similar drug metabolic profiles and residue patterns between species. These factors determine the appropriateness of therapeutic drugs used in aquacultured species destined for human consumption. Studies of mammalian drug metabolism *in vitro* are predictive of the fate of particular metabolic capacity. Our study investigates drug metabolism *in vitro*, in multiple fish species, and will develop a model to predict drug residue patterns in edible tissues. Fish species evaluated in this study include rainbow trout, Atlantic salmon, channel catfish, hybrid striped bass, tilapia, bluegill, yellow perch and largemouth bass. Both phase I (cytochrome P-450 dependent) and phase II (conjugation) pathways of drug metabolism are being studied to evaluate differences or similarities in drug metabolizing kinetics among species. The metabolic profiles determined *in vitro* will be compared with data obtained from *in vivo* drug residue experiments. The likelihood of deriving species groupings will be based on similarities or dissimilarities in biotransformation profiles between species.

36. **Resistance to oxytetracycline in bacteria from salmon farming in Chile**

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The use of oxytetracycline for preventing and controlling bacterial pathogens in salmon farming is a frequent practice in Chile. In this work the frequency of oxytetracycline-resistant bacteria from water, pelletized feed and fingerlings from four Chilean freshwater Atlantic salmon farms, as well as the level of resistance of selected strains was investigated. High proportions of low- and high-level oxytetracycline resistant bacteria (selected in agar plates containing 30 μg/ml and 100 μg/ml, respectively), mainly from pelletized feed and effluent samples of the fish farms were found. The highest proportions of resistant bacteria were found in the effluent samples, and were significantly higher ($P<0.05$, Tukey's test) than those from the other samples studied. On the contrary, influent samples exhibited the lowest proportions of resistant bacteria. One-hundred and three resistant Gram-negative isolates, which represented the oxytetracycline-resistant bacterial populations, were isolated. A large number of non-fermenting bacteria (77.7%) were identified. *Pseudomonas fluorescens* (28.2%), *Aeromonas hydrophila* (9.7%), *Stenotrophomonas maltophilia* (5.8%), *Sphingomonas paucimobilis* (5.8%), *Acinetobacter lwoffii* (4.8%), and *Pseudomonas putida* (4.8%) were the most frequent. *P. fluorescens* and *A. hydrophila* predominated in salmon fingerlings, whereas *A. lwoffii* and *S. maltophilia* were predominant in pellet samples. Selected strains exhibited high levels of oxytetracycline resistance, with minimum inhibitory concentrations (MICs) ranging from 64 to 2048 μg/ml, whereas MIC_{90} of oxytetracycline for each farm varied between 1024 μg/ml and 2048 μg/ml. The high incidence of oxytetracycline resistant bacteria in Chilean salmon farms e an important risk to public health for workers involved in fish culturing and processing.

37. Metomidate anaesthesia in turbot

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Metomidate is a hypnotic agent with a large therapeutic range used in marine fish species. We have studied pharmacology of metomidate in turbot following intravenous, oral and bath administration. Estimates of elimination half-life, volume of distribution, clearance, bioavailability, and induction- and recovery times are presented. Anaesthetizing/immobilizing fish is usually performed as a bath treatment. In free-swimming fish (large aquariums), the capturing/netting for handling procedures, including anaesthesia, often impose stress and damage to the fish. Further studies are needed to explore the possibility of using oral administration of metomidate to anaesthetize/immobilize/calm fish.

38. Effectiveness of commercial disinfectants against typical bacterial fish pathogens

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Sanitary conditions and biosecurity are essential in combating and preventing fish diseases. With the rapid increase in intensive aquaculture, the application of disinfectants has also increased. Entry and growth of pathogens into the fish must be minimized through use of disinfectants in water, tanks, equipment, or in the owner fish. The effects of typical aquaculture disinfectants are indicated at all types of infectious agents (bacteria, fungi, viruses and protozoa). It is quite difficult to find disinfectant treatment against a specific microorganism. The disinfectant must come into direct contact with the organism. It is important that surfaces be clean of organic matter before disinfection. This is best done by physically cleaning the surfaces with soap and water prior to using the disinfectant. The choice of disinfectant is affected by not only cost but also by efficacy, speed of action and other hazard related concerns. The aim of the present work was to evaluate the effectivity of different commercial aquaculture disinfectants against typical bacteria fish pathogens. *Vibrio* p., *Staphylococcus warneri*, *Hafnia alvei*, *Aeromonas hydrophila*, *Aeromonas salmonicida*, and *Yersinia ruckeri* were used. The evaluation was realized with a qualitative verification of the inhibiting effect by means of a method of diffusion in agar (DRAWERT, 1982), a quantitative determination based on the Minimum Inhibitory Concentrations (CMI) (BECK ET al., 1977; BORNEFF ET al., 1981), and the determination of the lethal effect by a method of suspension (BECK ET al., 1977; BORNEFF ET al., 1981; German Agricultural Association, 1983).

39. Lesions of estuarine fish in Florida: are they caused by the same pathogen?

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The occurrence of ulcerative mycosis (UM) in fish along the eastern seaboard of the United States has been well documented. In Florida, the appearance of lesions in estuarine fish has been reported since the 1970's. These skin lesions are generally bloody in appearance, have raised irregular margins, and usually have deeply penetrating fungal hyphae in the surrounding muscle tissue. The most commonly affected species of estuarine fish are striped mullet (*Mugil cephalus*) and silver mullet (*Mugil curema*). The majority of these lesions seem to occur in areas of lower salinity or in estuaries that commonly experience heavy freshwater influx. Lesioned fish were collected from three different areas in Florida and examined histopathologically and in fresh squash preparations for the presence of fungal hyphae. The fungal pathogen, *Aphanomyces invadans*, was isolated and identified by PCR analysis from lesioned American shad (*Alosa sapidissima*) collected in the St. Johns River. Other fungal isolates have been obtained from lesioned fish in the St. Lucie and Caloosahatchee Rivers. All isolations were performed using a glucose-peptone growth medium and all incubations were maintained at 22° C. Sporulation was achieved by transferring the isolates from freshwater to a salinity of 1 psu using previously described methods. Secondary zoospores were noted after 24 hours and continued to produce for four days at room temperature. PCR analysis is being used to determine if the fungal isolates obtained from the St. Lucie and Caloosahatchee Rivers are also *Aphanomyces invadans*.

40. Mixed infection of fungal disease in snakehead, *Channa striatus* (Bloch) in Thailand

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In January, 2000, a single diseased snakehead, *Channa striatus* (Bloch) was found in a fish culture pond in Thailand. Two species of fungus, *Aphanomyces piscicida* and *Achlya* sp. were isolated from an ulcer lesion on this fish. The lesions were mainly observed on the head, body surface, dorsal fin and caudal fin. The fungal pathogens were identified based on their morphological and biological characteristics. Mycotic granulomatosis was seen in histological sections of ulcerative lesions. The pathogenicities of the two isolates were determined by artificial infection tests. Goldfish (*Carasius auratus*) were injected with *A. piscicida*, and platies (*Xiphophorua maculatus* var.) were placed in a cooled bath containing *Achlya* sp. These tests revealed that *A. piscicida* was highly pathogenic to goldfish and *Achlya* sp. was moderately pathogenic to platies. These findings indicate that *Achlya* sp. is an opportunistic pathogen that can become established in the presence of some other stressor. In goldfish, but not in platies, granulomas with fungal hyphae formed in the muscle.

41. Ulcerative mycosis, salinity, and granuloma formation in Florida fish

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Ulcerative mycosis (UM) is a widespread disease problem in estuarine fish along the eastern seaboard of the United States. *Aphanomyces* is a fungus that has consistently been isolated from fish lesions and is thought to be the primary pathogen in UM. Over 600 histological slides of various tissues from fish collected during UM events in Florida since 1980 were examined. Environmental data was also correlated with UM presence. Skin, ulcer, and muscle tissue samples of *Mugil cephalus, Mugil curema, Brevoortia tyrannus, Alosa sapidissima, Cynoscion regalis,* and *Archosargus probatocephalus* were included. Analysis of these slides and the correlative environmental data suggests that salinity appears to play an important role in the tissue response of the fish to the fungal hyphae. Granuloma development appeared to correspond to increasing salinity. Tissues from fish found in areas of higher salinity (greater than 15 ppt) had more granulomas—walling off of the hyphae in an attempt to destroy them and to minimize hyphal penetration. However, fish from lower salinity areas (15 ppt or less) had fewer granulomas with more free hyphae in the tissues. Since *Aphanomyces* is more typically tolerant of lower salinities, it is likely that under these conditions the fungus penetrates the tissues quickly before the fish's immune system can mount a cellular granulomatous response. Water management activities may have a significant influence on fish health in estuarine systems, where sudden salinity decreases may stress fish and allow for infection.

42. Sanitary/epizootic state of sturgeon fish plants (SFP) from the Azov sea basin

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The main source and maintenance of sturgeon stocks in present conditions is the rearing of juveniles at fish plants and commercial fish farms. For sturgeons being reared several infectious diseases (fungal, bacterial, viral), invasive (trichodiniosis, polypodioses etc.) and non-infectious (gill necroses, gas vesicle disease, alimentarian) nature were described. In 2000-2001, infection of sturgeon by parasites did not rise to an epizootically significant level. Therewith in literature described cases of diseases and mortality of juvenile sturgeons caused by representatives of families Chilodonellidae, Trichodinidae, and Diplostomidae. At some plants losses of caviar and juveniles were caused by the fungus *Saprolegnia* (*Saprolegnia parasitica, S. ferax, S. mixta,* etc.). One hundred twenty-seven relatively pathogenic cultures (10 species) were identified: 72 strains from water and 55 strains from parenchymatous organs of sturgeons. Aeromonads were dominant in the spectrum of relatively pathogenic organisms. To define relatively pathogenic organisms we took some species of oxidase-positive bacteria, which had been mentioned by several authors as agents accompanying infectious fish diseases, and oxidase-negative bacteria from 3 genera: *Proteus, Citobacter,* and *Enterobacter.* Bacterial cell counts for sturgeon juveniles from fish plants were 0.9 x 102–1.98 x 105 CFU/g and oxidase-positive bacteria were 2.2–23.1% from defined microflora.

43. Parasitological and bacteriological findings in freshwater ornamental fish imported in Italy

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From 1997 to 2001 a parasitological survey of 747 imported freshwater ornamental fish was carried out. The fish were from Singapore (494 subjects), Malaysia (82), Sri Lanka (52), Thailand (45), China (30), Israel (15), Hong Kong (13), Colombia (10) and the Amazon (6). Parasites were detected in 406 (54.3%) out of 747 examined fish and the highest prevalence was found in fish from Malaysia (90.2%), Israel (60%) and Singapore (56.1%). The most frequent genera of parasites isolated were: *Myxobolus*, *Dactylogyrus*, *Goussia* and *Centrocestus*. The following genera were observed with a lower prevalence (<10%): *Gyrodactylus*, *Cryptobia*, *Trichodina*, *Chilodonella*, *Ichthyobodo* and *Ichthyophthirius*. Some Myxozoa, i.e. *Thelohanellus*, *Sphaerospora*, *Zschokkella*, *Myxidium* and *Kudoa* were occasionally found. Starting in 1999, bacteriological analysis on 334 imported ornamental fishes and 31 samples of shipping water were also carried out. The most frequent bacteria isolated were *Aeromonas hydrophila*, *Vibrio cholerae* NAG (non O1), *Vibrio* spp. and *Shewanella putrefaciens*. Three water samples were positive for *V. cholerae* NAG (non O1), whereas all the samples were negative for *Salmonella* spp. Furthermore, few samples of fish and water were positive for *Mycobacterium* spp. According to these preliminary results we could assume that the risk of introducing non-indigenous parasites or bacterial diseases by means of the imported exotic freshwater fish in Italy must be taken into consideration and the importance of carrying out further surveys in this field must be stressed. This research was partially supported by grant Ministry of Health - IZSVE 06/99.

44. Health management for offshore aquaculture of red drum

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The potential emergence of offshore aquaculture in the Gulf of Mexico presents new challenges for health management of finfish in a unique environment. In addition to the paradigm "know your fish, know your disease, know your treatment," consideration must be given to various stressors associated with the unique production scenario. Factors to be considered in a long term health management plan include: fish health education/training of fishery personnel, quality of brood stock and offspring, nutrition, health examination (and status)of the fish at various production stages, periodic assessment for stress indicators, resistance/immunity to well recognized pathogenic agents, and the impact of production design on fish health considerations.

45. SDS-PAGE and Western blot analysis of the Triactinomyxon spores, the cause of whirling disease in salmonid fish

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Considerable research is directed towards whirling disease because it is an economically devastating parasitic disease of salmonid fish in Europe and USA. In this presentation, we will provide the preliminary results of molecular studies on the triactinomyxon spores of *Myxobolus cerebralis*, the cause of whirling disease. The soluble whole-cell protein profile of the triactinomyxon spores was examined using sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE). The pattern was reproducible and polypeptides of various molecular weights were detected. Western blot analysis was carried out to detect the antigenic proteins associated with the soluble protein of the spores. A strong reaction was observed with specific antibodies against the parasite raised in rabbit. Some antigenic polypeptides which were detected following western blotting did not appear in the SDS-PAGE silver-stained gels indicating the superior sensitivity of the western blotting analysis.

46. First report of subsidiary protrusions on the caudal processes of two novel types of hexactinomyxon spores (Myxozoa)

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In the course of documenting actinosporeans from freshwater oligochaetes in Bavaria, Germany, we isolated two novel hexactinomyxons that differ from previously described forms both in morphometrics and in that they possess additional structures on their caudal processes. *Hexactinomyxon* type 1 nov. and *Hexactinomyxon* type 2 nov. spores are triradially symmetrical and are comprised of a spore body, style, and six caudal processes. The caudal processes arise from the division of each of the three valve cells into an equal pair of projections at the base of the style. One of each pair is fused conspicuously to its nearest neighbour for one-fifth to one-quarter of their total length. Distally, each process possesses subsidiary protrusions which are irregularly shaped and irregularly positioned extensions of the valve cell: a feature not observed previously in actinosporeans. Scanning electron microscopy of Hexactinomyxon type 2 nov. revealed that these protrusions are a seamless extension of the valve cell wall. The protrusions branch distally, and terminate in a distinct bulbous structure. The small subunit ribosomal (18S) DNA genes of both hexactinomyxon types were amplified through nested PCRs and partially sequenced. Each hexactinomyxon had a unique sequence. These are the first sequence data for this group of actinosporeans.

47. Coccidiosis in bluegill

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In February 2002, a large lake in eastern Virginia experienced moderate mortalities among young-of-the-year bluegill (*Lepomis macrochirus*). A sample of the affected population was submitted to the Aquatic Medicine Laboratory of the Virginia-Maryland Regional College of Veterinary Medicine for diagnostic evaluation. Gross external examination revealed small, anorexic, lethargic bluegill. Wet mount biopsies of gill samples showed moderate mucus production and mild telangectasia. Gross internal examination elicited the presence of numerous white nodules on the liver, gastrointestinal tract, spleen, and posterior kidney. Squash preparations of these nodules showed that they contained larval digenetic trematodes. Bacterial cultures taken from the posterior kidney did not yield significant growth. Histopathology confirmed a mild infestation of larval digenetic trematodes in the liver, spleen, and muscular layer of the gastrointestinal tract. The presence of a few nematodes and cestodes in the lumen of the intestines was associated with minimal pathology. The gastrointestinal tract of fish also exhibited a mild to moderate coccidian infestation, which was associated with sloughing of the intestinal mucosa. While coccidia infestations have been reported in wild populations of sunfish and bass, to the authors' knowledge, this is the first reported coccidia infection in bluegill. It is not known whether the coccidial infestation was sufficient to cause the observed mortalities in the population. However, this case suggests that such an infestation can contribute to mortalities among smaller fish. Additional samples from this population were obtained for further examination of the parasite.

48. Tricaine dramatically reduces the ability to diagnose protozoan ectoparasite (*Ichthyobodo necator*) infections

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Tricaine (MS-222) is a widely used anaesthetic and euthanasia agent for fish. While evaluating hybrid striped bass (*Morone saxatilis* male x *M. chrysops* female) for the presence of the kinetoplastid ectoparasite, *Ichthyobodo necator* ("costia"), we discovered that unbuffered tricaine caused the rapid detachment and mortality of this parasite. Solutions of unbuffered and buffered (with sodium bicarbonate) tricaine were prepared and serially diluted to final concentrations of 1000, 250, 100, 50, 25, and 0 mg tricaine/L. Blind trials were conducted by placing scales, heavily infected with costia, into different treatments following a split plot design. Parasite motility was then scored and statistically evaluated using ANOVA. The motility of costia decreased with increasing unbuffered tricaine concentrations. Motility in buffered tricaine remained unchanged. As the unbuffered tricaine concentration increased, the pH of the solution dramatically decreased to as low as pH 3.3. To rule out the effect of pH, water was adjusted to either pH 7.4 or 3.3 and parasite motility scored as before. Differences in motility between pH values were evaluated using the Wilcoxon rank test. There was no pH effect on the motility of costia. Additional fish were euthanized in unbuffered or buffered tricaine (1000 mg/L) and cross-sectioned starting at the posterior portion of the dorsal fin. Parasites remained attached to the skin of fish that were euthanized in buffered tricaine but detached almost completely from the skin of fish that were euthanized in unbuffered tricaine. Our results indicate that tricaine should always be buffered when clinically evaluating fish for ectoparasites.

49. Inhibition of reproduction in the roach (*Rutilus rutilus*) by the tapeworm *Ligula intestinalis*

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The pseudophyllidean cestode *Ligula intestinalis* is a common parasite that causes, amongst other pathological effects, inhibition of reproduction in its fish intermediate host. We have been investigating one level of this interaction, namely the effect of the parasite on the pituitary gland, in particular, the pituitary gonadotrophs. Previous attempts to identify sex steroids produced by *Ligula*, which could suppress gonadotrophin (GtH-II) production and hence gonadal development, have thus far been unsuccessful. Cytological differences have been reported in pituitary glands of infected and uninfected roach (*Rutilus rutilus*) and radioimmunoassays have shown that infected fish contain significantly less GtH-II than non-infected individuals ($p=<0.0001$). The current study builds on this data in several ways. Firstly, a primary pituitary cell culture was established to study the effects of parasite excretory/secretory (E/S) products on an eel pituitary system. Preliminary data show that *Ligula* E/S are able to affect eel pituitary cells compared to PBS and a related cestode, *Scistocephalus solidus*, which do not have the same inhibitory effects on reproduction. Currently, the effects of parasite E/S products are being investigated by *in vivo* injections. These studies have demonstrated for the first time a direct physiological effect of the parasite on the pituitary-gonadal axis and open up the area for molecular studies on gonadotropin gene expression.

50. Susceptibility and inflammatory response to *Aeromonas hydrophila* in 17α-methyltestosterone-induced sterile common carp, *Cyprinus carpio* (Linn.)

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Susceptibility and inflammatory response to *Aeromonas hydrophila* in 17α-methyltestosterone (MT)-induced sterile common carp was evaluated. At each of the challenged levels, where mortality took place, the time taken to induce mortality was shorter in controls compared to sterile fish. Development of gross ulcerated lesions was consistently quicker in the control than in the sterile fish. Similarly the percentage of fish with completely healed ulcers was more in the sterile fish group than in the control at the end of 10-day experimental period. This difference was very obvious at the cellular level. In controls, at the end of 10^{th} day, the lesion sites were still in the initial stages of resolution and reorganization, while in the sterile fish, the lesion sites were either completely healed or were in advanced stage of resolution. The difference between the two groups was very clear when the sequential inflammatory response was examined daily till 10^{th} day post-challenge with *A. hydrophila*. In controls, up to the 3^{rd} day post-challenge, large numbers of bacteria were visible in between the myotomes at the injection site. Macrophage infiltration to the injection site was slow. Increased macrophage activity, myophagia, vascular activity and reorganization were seen only from 4-6^{th} day post-treatment. At the end of 10^{th} day, the lesion sites were still in the initial stages of healing. However in the sterile fish, there was macrophage activity right from day one post-challenge. The magnitude of macrophage infiltration was much more compared to control. Bacteria were not visible in between the myotomes from 2^{nd} day post-challenge. At the end of the 10^{th} day, the lesion sites were either completely healed or in the final stages of healing.

51. The development of expressed sequence tags and a cDNA microarray for studying the immune responses of Atlantic halibut (*Hippoglossus hippoglossus* L.)

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We are using immunological and molecular techniques to investigate the response of Atlantic halibut to vaccination and pathogen exposure. Analysis of partial cDNA sequences "expressed sequence tags" (ESTs) is a useful approach for gene identification and profiling of expressed genes. Atlantic halibut were immunized with a commercial injectable vaccine against *Vibrio anguillarum* and *Aeromonas salmonicida*. At 2, 7, 14 days post-vaccination, liver, spleen, and kidney tissues were collected. A combined mRNA pool from these tissues was used to construct a cDNA library with a titer of 2×10^7 pfu/µl. Two hundred clones were randomly selected to determine insert sizes. Seventy five percent had inserts that ranged from 600 bp to 1.0 kb and the other 25% had inserts over 1.0 kb. From 600 randomly selected clones that were submitted for sequencing, 51 clones (8.5%) contained immune relevant genes: specific immune system genes (4), complement components (9), acute-phase proteins (7), other immune relevant genes (31) including hepcidin an anti-microbial peptide. These genes and others identified from a normalized full-length cDNA library constructed from the same tissues are being used to construct a microarray that will allow us to determine tissue-specific expression. This microarray will also provide an important tool for further studies of the Atlantic halibut immune system. Supported by AquaNet, Networks of Centres of Excellence.

52. Identification of a single major QTL controlling nonspecific cytotoxic cell (NCC) activity in an OSU x HC rainbow trout model

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Nonspecific cytotoxic cells (NCC) are the teleost equivalent of mammalian natural killer cells. As such, NCC protect against bacteria, protozoan parasites, viruses and tumor growth. We have identified and propagated two clonal lines of rainbow trout that differ in their ability to lyse xenogenic YAC-1 cells *in vitro*. Our HC clonal line exhibits a higher level of NCC activity when compared to our OSU clonal line. To discern the genetic basis of this segregation, HC (YY) and OSU (XX) were crossed to produce an all male (XY) F1 generation whose sperm was used to generate a doubled haploid (DH) population by androgenesis. A total of 106 OSU X HC DH individuals were genotyped at 484 polymorphic AFLP markers to construct a genetic linkage map. Levels of NCC activity for 75 of these fish were also determined in triplicate over periods spanning 3 weeks. Using this genetic map and quantitative trait data, a single major QTL controlling NCC activity was identified. We are currently attempting to further elucidate the genetic basis of NCC activity in rainbow trout using both candidate gene and cDNA-AFLP subtraction techniques. Identifying the actual gene(s) controlling NCC activity in our trout model may discern whether a series of genes analogous to the natural killer gene complex identified on syntenic portions of mouse chromosome 6, human chromosome 12 and rat chromosome 4 exists in rainbow trout. Such information would prove useful for divulging evolutionary relationships concerning innate immunity between higher and lower vertebrates.

53. Isolation and partial characterisation of serum immunoglobulins (IgM) from sterlet (*Acipenser ruthenus*), Russian (*A. güldenstadti*) and Siberian (*A. baeri*) sturgeon

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Serum immunoglobulins (IgM) from three species of sturgeon were purified using affinity chromatography. The fish had been immunized with purified goat IgG, and the specific antibodies from the immune serum collected using a goat IgG-immobilized agarose gel column. The molecular weights of whole IgM molecules were determined by native gel electrophoresis to be approximately 900 kDa for all three species of sturgeon. The molecular weights of the heavy and light chains of the IgM were then determined for the three species using SDS-PAGE. They were composed of single heavy chain, weighting approximately 75 kDa for the Russian sturgeon IgM, 66 kDa for the Siberian sturgeon IgM and 60 kDa for the sterlet sturgeon IgM. Differences were noted in the number of light chains between the three species of sturgeon, with two bands evident at 20 and 14 kDa in the Russian sturgeon IgM, one at 10 kDa in the Siberian sturgeon IgM and one at 16 kDa in the sterlet sturgeon IgM. Mouse polyclonal antisera were produced against IgM purified from the three sturgeon species. The antisera reacted with both the high and the light chains of the IgM molecules in Western blot analysis. All IgM were of a similar tetrameric arrangement, but differences in the molecular weight of their constituent chains suggest some structural heterogeneity.

54. Serum immunoglobulin of hybrid striped bass (*Morone chrysops* × *M. saxatilis*)

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An immunoassay to evaluate the humoral immune response of hybrid striped bass (HSB) would be useful to monitor response to disease and vaccination, as well as general health. A group of hybrid striped bass were given intraperitoneal injections of bovine serum albumin (BSA) at 14 day intervals. Fourteen days after the second injection, the fish were bled and the specific anti-BSA immunoglobulin (Ig) was affinity purified by means of an agarose gel-BSA column. The purified native Ig had an apparent molecular size of 893 kDa, by size exclusion chromatography, and when examined by polyacrylamide gel elctrophoresis under denaturing conditions, produced heavy and light chain fragments of 76 and 27 kDa, respectively. The purified native Ig was used to produce both specific anti-HSB Ig polyclonal and monoclonal antibodies. Data will be presented utilizing these antibodies in an ELISA format to measure specific anti-BSA Ig as well as total Ig.

55. Passive immunization of rainbow trout (*Oncorhynchus mykiss*) against *Flavobacterium psychrophilum*

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Flavobacterium psychrophilum, the causative agent of bacterial coldwater disease (CWD) and rainbow trout fry syndrome (RTFS), causes high mortality in cultured salmonids. The present study was designed to determine the role antibody plays in conferring protection to rainbow trout fry (*Oncorhynchus mykiss*) by passive immunization experiments with hyperimmune trout serum, serum from adult rainbow trout immunized with *F. psychrophilum*, and goat anti-*F. psychrophilum* serum. Rainbow trout fry were injected intraperitoneally (i.p.) with antiserum and challenged by subcutaneous injection of a virulent strain of *F. psychrophilum* 24 h post-immunization. Relative percent survival ranged from 9-42 % when rainbow trout fry (mean weight, 1.3 g) were injected with a 1:2 dilution of 25 µL of hyperimmune serum ranging in antibody titers from 1,600-102,400. Rainbow trout fry (mean weight, 1.0 g) passively immunized with 25 µL of serum from immunized adult fish exhibited relative percent survival values of up to 52%. Passive immunization with 25 or 50 µL goat anti-*F. psychrophilum* serum did not confer protection to fry (mean weight, 1.3 g). The results suggest that antibody plays some role in conferring protection to *F. psychrophilum*, however, other components of the immune system may be important to confer complete protection.

56. Some medicinal plants as immunostimulant for fish

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For centuries large number of plants have been used in traditional medicine (Duke, 1987). These traditional uses are dependent on the active constituents, which have several biological activities, such as antimicrobial, antiviral, immunosupressive etc., in the plants. Various extracts of mistletoe (*Viscum album*), nettle (*Urtica dioica*) and ginger (*Zingiber officinale*) have been found to posses immunomodulatory activity. The aim of this work was to investigate the immunostimulant effects of the aqueous exrtracts of these plants on rainbow trout (*Oncorhynchus mykiss*). For this purpose various parameters of non-specific defense mechanisms, including respiratory burst activity, phagocytosis in leukocytes and total protein level of plasma in rainbow trout fed with diet containing plant extracts in different percentages, were examined. Lyophilized extracts were added to fish food, individually in 0.1 % and 1 % percentages. Rainbow trouts were fed with these plant extracts in feed at a rate of 2 % of body weight per day for three weeks. At the end of the period, for the non-specific immune response, extracellular and intracellular respiratory burst activities, phgocytosis and plasma protein levels were determined. Specific growth rates (SGRs) and condition factors (CFs) of the fish were also measured. Among the plant materials tested as immunostimulant as feed additive, ginger was the best one and the rainbow trout fed with the diet containing 1% aqueous extract of powdered ginger roots for three weeks exhibited a significant stimulation on the immune system of rainbow trout and caused an enhanced phagocytosis and respiratory burst activity ($p<0.001$). It needs future studies to include determining the optimal doses, protocols for feeding and the active principle in this plant extract.

57. Nitric oxide production in the culture of head kidney leukocytes of carp (*Cyprinus carpio*) and synergistic effect of recombinant human cytokines

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Nitric oxide (NO) is recognized as an important chemical mediator that controls the immunodefense system in vertebrates. To demonstrate the production of NO from carp leukocytes, head kidney leukocytes were stimulated with lipopolysaccharide (LPS) *in vitro*, and fluorometric measurement and electron spin resonance (ESR) were used for examination on NO production. The supernatant of a leukocyte culture after LPS stimulation showed an increase in fluorescent intensity and this intensity decreased with addition of L-NMMA, a NO synthase inhibitor. ESR analyses revealed that the signal detected in carp leukocyte culture supernatant after LPS stimulation was identified as NO. To clarify the interaction between cytokines and NO production of leukocytes, recombinant human (rh) cytokines and/or LPS were used as stimulants. Cytokines (rhIFNα, rhIFNγ, rhTNFα, rhIL-1α) could not produce NO from leukocyte culture but only rhIL-6 could. When LPS was added together with cytokines, rhIFNα, rhTNFα and rhIL-1α significantly enhanced NO production in the culture. NO production was somewhat suppressed by rhIL-6 in the presence of LPS. These evidences clarified that *in vitro* culture system of carp head kidney leukocytes could produce NO after LPS stimulation and the fluorometric method can be used for the detection of NO. These results suggest that human proinflammatory cytokines biologically act with fish leukocytes and affect NO production.

58. Hypoxia induces HSP 70 production in juvenile Nile tilapia, *Oreochromis niloticus* (L.)

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Enzyme linked immunosorbent assay (ELISA) was used to determine levels of the inducible heat shock protein 70 (hsp70) in response to prolonged anoxic conditions in juvenile Nile tilapia. This protein was detected in brain, liver, muscle and head kidney. A significant difference between treatments ($p>0.01$, $n=30$) was observed between control and hypoxia treatments in brain and muscle tissue. A significant difference was not observed in liver and head kidney tissues. Research on using stress proteins to measure environmental stress in field situations and natural populations is ongoing.

59. The effect of dexamethasone on crucian carp (*Carassius carassius*, L) leukocytes

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This study investigated the effect of a cortisone analogue on the composition and function of leukocytes of the crucian carp (*Carassius carassius*, L). Second-year crucian carps were used in the experiments. Fish were kept in aerated aquaria at 18°C water temperature, and were each injected with 0.2 ml. dexamethasone (KRKA, Novo mesto, Slovenia). Leukocytes composition and function was studied by recording changes in numbers of leukocytes, oxygen-dependent metabolic activity and intensity of lymphocytolysis. Metabolic activity of leukocytes was determined by spontaneous (SPL) and zymosan-stimulated (ZSL) chemiluminescence, lymphocytolysis intensity – by content of live and dead leukocytes. Materials were collected at 1, 3, 7, 15, and 18 days after intra-abdominal injection of the hormone. Fish responded to the hormone injection by de- and restabilization of leukocytes composition and changes of functional state. Inhibition of leukocytes hemiluminescence activity, a 25-60% decrease in the number of lymphocytes and a 30-50% increase of granulocytes in the experimental fish as with the control was registered. Suppressive and destabilizing effects on fish leukocytes were revealed during the first 7 days (of the experiment). The processes of lymphocytes cytolysis are intensified in the presence of the hormone.

60. Pro-opiomelanocortin (POMC) related hormones effect of carp *Cyprinus carpio* phagocytic cells

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The pro-opiomelanocortin (POMC) gene is composed of three exons and two introns that are spliced from the primary transcripts to generate POMC mRNA, this is then translated into a single primary protein product. Subsequent tissue-specific processing by pro-hormone convertases and post-translational modifications generate the regulatory neuropeptides adrenocorticotropin, melanocyte stimulating hormones (α-, β-, γ-MSH), β-lipotropin and β-endorphin. The immuno-modulatory effects of POMC were studied both *in vivo* and *in vitro* in rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*). The production of superoxide anion in kidney phagocytic cells increased significantly, in rainbow trout and carp, when treated with α-MSH and β-endorphin. The chemotaxis and bactericidal activity also significantly increased in kidney phagocytic cells of rainbow trout treated with β-endorphin. The proliferation of carp lymphocytes increased significantly, when treated with β-endorphin. These results suggest that POMC in fish activates the function of phagocytic cells. This indicates the potential role played by POMC to activate the immune response.

61. Oral immunization of salmonids with biodegradable microparticle-based vaccines

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Oral vaccine delivery as a primary or booster immunization would be highly valuable to the aquaculture industry because of the ease of administration, reduced fish handling and it is the only method suitable for the mass immunization of fish of all sizes. However, few oral vaccines are commercially available. To successfully immunize gastric fish by the oral route, antigens must be protected from degradation by the acidic pH of the stomach and be absorbed in the hind gut to induce a protective immune response. Ideally an oral vaccine should also induce long-lasting immunity. In this study, two oral microparticle-based vaccines were evaluated in Atlantic salmon. Killed *Vibrio* was encapsulated into polylactide (PLA) microparticles and for the second vaccine, antigen was adsorbed to polymeric lamellar substrate particles (PLSP). Both vaccines were incorporated into the diet at three doses (1-10%) and feed for ten days. Both microparticle types and all vaccine doses induced high levels of protection with the relative percent survival (RPS) ranging from 83-100%. Protection was equivalent or better than that observed in fish immersed in a commercial vibriosis vaccine (RPS 86%). Negative control mortality was 57%. Thus, oral delivery of microparticle-based vaccines is a feasible and very effective approach to protecting salmonid fish against the bacterial disease vibriosis.

62. Distribution, persistence, and pathological analysis of a DNA vaccine against infectious hematopoietic necrosis virus

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The development of DNA vaccines offers the prospect of a safer and more cost-effective alternative to conventional vaccines. DNA vaccines have been shown to be immunogenic, stimulating cell-mediated immunity as well as antibody. However, to date concerns over the biosafety of such vaccines have limited their use to laboratory settings. Many of the safety issues concerning DNA vaccines involve the biodistribution, persistence, and pathological changes following DNA vaccination. Therefore this study examined the fate of an intramuscularly inoculated plasmid DNA expression vector (pWG) encoding the glycoprotein from infectious hematopoietic necrosis virus (IHNV). The trafficking of DNA after inoculation was evaluated by polymerase chain reaction (PCR) analysis at various time points from 10 minutes to 2 years post vaccination in rainbow trout. After intramuscular admistration, plasmid pWG was rapidly disseminated in all tissues, but at later time points was found primarily in the muscle at the site of injection. Similar tissues and time points were examined to assess possible histopathological changes caused by vaccine administration.

63. Comparative study on inflammatory changes in different species of salmonids vaccinated intraperitoneally with oil-adjuvanted vaccines

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Vaccination against furunculosis in salmonids requires use of adjuvanted vaccines and vaccination by injection, which results in development of injection site reactions in the peritoneal cavity. The underlying cellular mechanisms have not been studied in any detail. We have compared the kinetics of the inflammatory changes over time in different salmonid species, Atlantic salmon (*Salmo salar* L), rainbow trout (*Oncorhynchus mykiss*), and grayling (*Thymallus thymallus*). Fish were injected intraperitoneally with oil-adjuvanted, emulsion vaccines. Fish were sampled at 2, 4, 8, 12, and 16 weeks post vaccination and specimens from pyloric caeca were processed for light microscopic evaluation, where the inflamed area was measured in H&E sections using computer-assisted microscopy. Characterization of leukocytes involved in inflammatory reactions was done on morphological basis. Each leukocyte type was estimated as a percentage of total cells. For Atlantic salmon neutrophil-like cells predominated the early stages followed by a second wave of macrophage-like cells that persists beyond 16 weeks. Mast cells and lymphocyte-like cells increased over time but were found in small numbers. Neutrophil-like cells declined in number beyond 4 weeks. In rainbow trout macrophages predominated the cellular reaction throughout the observation period with a marked decline towards 16 weeks. In grayling macrophage-like cells predominated the cellular reaction from 2 weeks to 4 months and were on an increasing trend at 16 weeks. Neutrophil-like and lymphocyte-like cells were also observed while no mast cells were observed. Marked differences were observed between species, rainbow trout responding quicker and resolving the inflammation quicker. Salmon and grayling were on an increasing trend at 16 weeks.

64. Pathology in weedy sea dragons (*Phyllopteryx taeniolatus*) and leafy sea dragons (*Phycodurus eques*) associated with a mixed bacterial infection

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A mixed population of captive born weedy sea dragons (*Phyllopteryx taeniolatus*) and leafy sea dragons (*Phycodurus eques*) housed at a public aquarium had been experiencing chronic morbidity and mortality over several months. Clinical signs included: lethargy, inappetence, fin erosion, focal areas of skin ulceration, and tail necrosis. Skin scrapes of examined individuals were negative for any obvious external parasites. Bacterial cultures from the ulcerative skin lesions grew *Staphylococcus* sp. A long-term bath immersion of erythromycin (50 mg/liter) was initiated in the more severely affected sea dragons, however, their condition ultimately deteriorated and all treated individuals died. Histopathology of three weedy sea dragons and two leafy sea dragons revealed a severe bacterial septicemia involving at least two different bacteria in multiple organs. Numerous bacterial thrombi were located in several major vessels, the gill, heart, kidney and hepatopancreas. Special stains (Brown-Hopps, Ziehl Neelsen) confirmed that the predominant bacteria was a Gram (+) positive cocci organism which had disseminated to numerous visceral organs, including the heart, gill, hepatopancreas, kidney, intestine, muscle, mesenteric tissues and brain. This Gram (+) positive organism was morphologically consistent with the external isolate of *Staphylococcus* sp. In addition, distributed among various tissues and also in discrete granulomas were less numerous Gram (+) positive, acid-fast positive organisms. The acid-fast organism was cultured on Lowenstein-Jensen media and fit the criteria of a *Mycobacterium* sp. Incidental findings included several metazoan parasites, including mature cestodes in the intestinal tract of one individual and numerous encysted larval nematodes in the mesenteric cavity of several of the sea dragons.

65. Comparative histopathology of *Streptococcus iniae* and *S. difficile*-infected tilapia

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Streptococcus iniae and *S. difficile* infections have similar clinical signs, but different histopathological presentations in tilapia (*Oreochromis* hybrids) during natural infections. *S. iniae* causes pericarditis, epicarditis, myocarditis, meningitis, and macrophages containing intracellular cocci. Inflammatory responses were noted in intestine and skeletal muscle. Similar to *S. iniae*, *S. difficile* also caused pericarditis, epicarditis, myocarditis, meningitis. Additionally, necrotizing splenitis, hepatocellular vacuolar degeneration, nephritis and inflammation in gills were evident in *S. difficile*-affected fish. Large numbers of cocci were present in tissues and the circulation of *S. difficile* but not *S. iniae*-infected fish. In some *S. iniae*-infected tilapia we observed accumulation of eosinophilic substances in the cytoplasm of renal tubular cells, and mild granuloma formation in the spleen and head kidney. Pathology similar to *S. difficile* infection can be reproduced using high dose intraperitoneal injection of *S. iniae*. This suggests that the pathogenesis of both bacteria in tilapia may be similar. Tilapia may have better control of *S. iniae* infection, resulting in a more chronic form of disease compared to that caused by *S. difficile*.

66. The spread of pikes (*Esox lucius*) with tumors in the Chernobyl zone (Kyiv Reservoir, Dnieper River, Ukraine)

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A wide range of anatomic and physiologic defects in fish from radio-contaminated reservoirs has been detected by many authors. 344 pikes from Kyiv reservoir have been tested during 2000-2001. The number of pikes with tumors accounts for 18.1 % (it varies from 11.6 % to 25.8 % in varies parts of reservoir). The size of a tumor is from 0.5-0.7 cm to 3-5 cm. The number of tumors a pike has varies from 1 to 5-6. Sometimes 30-40 % of fish body is covered with tumors. The largest number of pikes with tumors has been registered during spring (the spawning period) – 19.5 % less in autumn – 6.4 %. The correlation of ectoparasites and tumor pike contamination has been detected. Such ectoparasites as *Henneguya psorospermia, Trichodina esocus, Tetraonchus monenteron, Ergasilus sieboldi, Argulus foliaceus* have been found in 2.4-5.6 times more often in pikes with tumors. Map-scheme of the spread of pikes with tumors and the recommendation on the limitation of the number of pikes with tumors in the Kiev reservoir are enclosed.

67. Physiological indices of pikes (*Esox Lucius*) affected by tumors within Chernobyl Catastrophic (Kyiv Reservoir, Dnieper River, Ukraine)

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Two series of experiments have been performed. 1st series: Erythrocytes amount of healthy *E. lucius* (standard) fluctuates from 1,35 to 2,5 million/mm^3, leucocytes number – 43-70 thousands/mm^3, hemoglobin level – 5-6 %, erythrocyte sedimentation rate (ESR) – 1 mm/h. Fishes affected with 1-2 tumors have erythrocytes and hemoglobin amount on 20-30 % below and leucocytes amount and ESR on 40-50 % above accordingly. Erythrocytes, hemoglobin, leucocytes concentrations of fishes affect by 3-5 tumors decrease on 60-70 % and ESR increase on 100-120 % in contrast with standard. 2nd series: The level of water amylolytic and proteolytic activity in muscles of healthy *E. lucius* (standard) is lower on 30-80 % in contrast with tumor tissues. The differences in protein, acid and alkaline phosphatases concentrations in muscles affected by tumors and in standard are not registered. Physiological data indexes deep pathological processes concomitanting by general intoxication and leading lethal outcome.

68. Methods of diagnostics of fishes' illnesses

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The original methods of lifetime diagnostics of fishes' illnesses in aquiculture are developed and modified. These methods allow performing repeated and chronic experiments on the same individual fish without breaking its vital signs. Physical and chemical methods: definition of nitrates, nitrites and ammonia in slime of a fishes skin by the test reagents; definition of a fishes body temperature by means of the quick-response thermometer; definition of a condition of ill and healthy fishes by electroresistance. Biochemical methods: definition of alkaline phosphatase activity in slime of fishes by molybdenum a reagent; definition of pH, protein, glucose, ketone and hemoglobin in fishes' tissues by means of diagnostic stripe; definition of blood coagulability time. Immunological methods: definition of natural heterohemagglutinins and hemolysins titer; revealing of antibodies to parasites by means of passive hemagglutination reaction (PHAR); revealing of antibodies to bacterial antigens of bacterial hemorrhagic septicemia activators by PHAR an estimation of a blood monocytes to macrophages transformation level; registration of morphological changes blood erythrocytes at pathogens influence; revealing of the toxins with antigenic properties. Examples, circuits of experiments statement and tables with each of described physical and chemical, biochemical and immunological methods are represented.

69. Sanitary condition of marine mollusks cultured in México

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Marine mollusks aquaculture in México is an increasing industry; it is carried out in the Pacific and Atlantic coast of the country. In the Gulf of México the American oyster *Crassostrea virginica* is cultured using extensive methods, while several mollusks species such as the pacific oyster *Crassostrea gigas*, blue mussel *Mytilus galloprovincialis*, lion clamp scallop *Lyropecten subnodosus*, catarina scallop *Argopecten circularis*, pearl oyster *Pteria sterna* and abalone species *Haliotis* spp. are cultured in the Northwest of the Pacific coast using extensive and intensive methods. Several infectious diseases in mollusks have been recorded around the world, some of them has been considered subject of certification by international regulations and their control is necessary. Since 1994, the Laboratorio de Biología y Patología de Organismos Acuáticos from CICESE has been carried out different studies on the parasitic load of cultured mollusks in the country. Different methods have been used, including parasitology, bacteriology, histopathology and molecular tools (*In situ* hibridization, PCR). Results have shown the presence of different parasites and diseases, including a wide spectrum of species, since metazoans to virus. Among them are marine mites in mussels, copepods such as *Pseudomyicola spinosus*, the protozoan *Margolisiella haliotis* in abalone, intracellular bacteria (Rickettsiales) in oysters and abalone, and viruses in the pacific oyster. A summary of these results and sanitary recommendations to producers and sanitary authorities are presented in this work.

70. An East Coast estuary without dermo or MSX? The presence of *Perkinsus* or *Haplosporidium* pathogens is undetectable in bivalves from Delaware's inland bays

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A standard polymerase chain reaction assay targeted at ITS domains between rRNA genes in both *Perkinsus marinus* and *Haplosporidium nelsoni* was used to screen DNA extracted from hemolymph samples taken from wild and cultured bivalves around Indian River and Rehoboth Bays, two shallow coastal lagoons in southern Delaware. Over 75 oysters *(Crassostrea virginica)*, hard clams (*Mercenaria mercenaria*), and ribbed mussels (*Geukensia demissa*) from a variety of locations were sampled to assess the regional distribution of these pathogens within this mid-Atlantic estuary. Remarkably, in only one individual (oyster) was the presence of *P. marinus* detectable, and this at barely distinguishable levels. No individuals evidenced the presence of *H. nelsoni*. In contrast, oysters collected from the nearby Choptank River, Maryland, evidenced high body burdens of both pathogens in parallel assays. Given the ubiquitous distribution of these protozoans along the Atlantic seaboard, it is surprising to find an estuary that does not evidence any significant presence of these pathogens. An oyster aquaculture demonstration project has experienced more than three years of excellent growth with low mortality and no incidence of either MSX or Dermo disease. Some possible explanations for the relative absence of *Perkinsus* or *Haplosporidium* pathogens are presented.

71. A novel lysozyme purified from the plasma of the eastern oyster, *Crassostrea virginica*

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Lysozyme is the common name for 1, 4-β-acetylmuramidases which cleave the glycosidic bond between N-acetylmuramic acid and N-acetylglycosamine. Several types of lysozymes which differ in biochemical properties and amino acid sequences have been identified in a large variety of organisms. Lysozymes purified from a few bivalve species showed molecular weights in the 11-18 kDa range. Their N-terminal amino acid sequences indicated that they belonged to i-type lysozyme. We have purified a lysozyme from the plasma of eastern oysters (*Crassostrea virginica)* by ion exchange and gel filtration chromatography. The molecular weight of the purified lysozyme was 18.4 kDa and its isoelectric point was higher than 10 as determined by iso-electric focusing. Its N-terminal amino acid sequence (43rd residues) did not show any homology with i-type lysozyme nor was there any sequence similarity with other proteins in available databases. The optimal activity for the purified lysozyme was in the pH range of 5.5-6.0, ionic strength (*I*) range of 0.180-0.200, and a temperature of 45°C. Its activity remained high at low temperature and it was stable below 60°C. Na^+ showed no specific effects while Ca^{2+} and Mg^{2+} significantly enhanced its enzymatic activity. Seawater at 2 ppt increased its activity but higher salinity was inhibitory. This is the first lysozyme purified from oysters, and also the first lysozyme purified from bivalve hemolymph. Its biochemical property and N-terminal sequence indicate that this lysozyme is novel.

72. Extracellular enzyme activities of *Perkinsus atlanticus* in culture and comparison with *Perkinsus marinus*

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Perkinsus-like parasites are widespread in clam populations along the European and North African coasts. *Perkinsus atlanticus* has been occasionally associated with mass mortalities of the clam species *Tapes decussatus* and *Tapes philippinarum*. Recently, continuous *in vitro* cultures of *P. atlanticus* have been established. A study of the extracellular proteins (ECP) of *P. atlanticus* cultures is ongoing to better understand host-parasite interaction. As part of the study, ECP enzyme activities of *P. atlanticus* cultures initiated from three different sources (infected clam gill, infected clam hemolymph and parasite hypnospores) were investigated using the commercial kit ApiZYM. The following enzymatic activities were detected: esterase, esterase lipase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-glucosidase, α-mannosidase, alkaline phosphatase, lipase, leucine arylamidase, valine arylamidase, β-galactosidase and α-glucosidase. Significant differences in enzymatic activities were found in ECP of cultures initiated from different sources. Surprisingly, no activity of the serine proteases, trypsin and α-chymotrypsin, were detected in ECP of *P. atlanticus* cultures. This is in contrast to the ECP of cultures of the eastern oyster parasite *P. marinus* which has high α-chymotrypsin activity and low trypsin activity. This difference may be important considering the suggested relationship between protease production and parasite pathogenicity. The lack (or very low) production of serine proteases by *P. atlanticus in vitro* and the typical host defense response of the clam, whose hemocytes encapsulate the parasite, may explain the suggested lower virulence of *P. atlanticus* to *T. decussatus* in contrast to the high virulence of *P. marinus* to eastern oysters (*Crassostrea virginica)*.

73. Effects of growth factors, hormones and lectins on Eastern oyster cells in primary cultures

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No oyster cell line has been developed despite numerous attempts. A major difficulty is that proliferation of oyster cells cannot be maintained *in vitro*. Moreover while numerous procedures have been used to prepare and maintain primary oyster cell cultures, the viability and survival of oyster cells obtained from dissociated tissues have been disappointing. We recently developed a defined culture medium LA-3 that increased the survival and metabolic activity of oyster cells in primary cultures. This defined medium allowed investigations on the effects of 27 potential mitogens on oyster cells. These mitogens included growth factors, hormones and lectins. These potential mitogens were initially tested individually by measuring their effects on the metabolic activity of oyster ventricle cells using AlamarBlue™ cell proliferation assay. This extremely sensitive assay allows monitoring of cells over extended periods because AlamarBlue™ is not toxic. Results indicated that ß-fibroblast growth factor (ß-FGF), insulin and endothelial cell growth supplement (ECGS) significantly increased oyster cell metabolic activity. Based on these observations, a final experiment was conducted to determine the effects of these chemicals in combination on the metabolic activity and, DNA and RNA content of oyster cells cultured for one, three and seven days. Insulin (100 µg/ml) with ECGS (500 µg/ml) was found most effective in increasing cell metabolic activity and RNA content. Although cell DNA content decreased in all treatments, the decrease was less in cells treated with a combination of insulin and ECGS. Results from this experiment will be used to aid in the development of an oyster cell line.

74. Development of serological techniques to study host induced proteins of the oyster parasite *Perkinsus marinus*

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The protistan oyster parasite, *Perkinsus marinus*, produces multiple extracellular products (ECP), including both constitutive and inducible serine proteases, *in vitro*. Quantification of *in vivo* expression of these products, and correlation with virulence would be facilitated by specific serological detection and molecular characterization. To facilitate the production of the requisite probes, ECP protein and, in specific, serine protease expression was modulated *in vitro* using products from the host, *Crassostrea virginica*. The modulated ECP pattern continues to be expressed after transfer to a chemically defined medium, thus providing a source of *P. marinus* antigens devoid of potentially immunogenic medium-derived proteins. Serine proteases and other extracellular products produced in this manner are concentrated by ultrafiltration for use as immunogens in rabbits and mice. Polyclonal immune serum from rabbits was routinely found to be of low titer, while ECP appeared to be toxic in mice. Immunization with ECP in Freund's complete adjuvant was lethal, and immunization with incomplete adjuvant was associated with progressive necrosis of distal tail and ear tissues, as well as deleterious immunological effects. Co-administration of oyster hemolymph with ECP caused a diminution in the anti-hemolymph titer, even when administered 12 weeks after the ECP. Mice immunized with ECP demonstrated a restricted response by Western blot. ECP appeared to destabilize the adjuvant emulsion, and further investigation revealed that a surfactant present in a media component was co-concentrated with the ECP. Ion-exchange chromatography was used to separate the surfactant from the ECP. This purified ECP has proven to be less toxic, and is associated with a broader recognition of ECP components.

75. Post capture muscle necrosis in the Norway lobster, *Nephrops norvegicus*, and its physiological consequences

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A post-capture, abdominal muscle necrosis of rapid onset has been identified in Norway lobsters, Nephrops norvegicus (L.), captured off the West coast of Scotland. Affected animals show signs of this condition within hours of capture, and usually die within days. The time-course of this necrosis, from the whitening of individual fibre bundles of the abdomen to a complete opacity of the abdominal musculature has been characterised using an index of progression. The pathology, which involves a condensation of myofibrils and an infiltration of necrotic regions by granulocytes, causes a loss of the normal tail flip swimming in this species. The condition most closely resembles idiopathic or spontaneous muscle necrosis, a pathology previously reported from both wild and cultured crustaceans. Damage to the integument in conjunction with exposure to various stressors both during and immediately after capture is its most likely cause. The physiological responses of the lobsters to development of muscle necrosis have been determined through changes in abdominal muscle glycogen, blood glucose, and lactate concentrations. The relative effects of trawling stress and post capture aerial emersion time on these parameters are also being examined. The rapid onset of the pathology has implications for the post-capture handling procedure for N. norvegicus and their subsequent vivier (live well) transportation to market. It may also be partially responsible for the high mortality rate of undersized N. norvegicus returned to the sea (discards) following their capture and aerial emersion.

76. Proteomic analysis of hemolymph from *Litopenaeus vannamei* infected with white spot syndrome virus

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Bacterial or viral infections can be catastrophic not only for shrimp aquaculture farms but also for natural stocks, therefore a better understanding of the shrimp immune system is needed for prevention and control of diseases. Although penaeidins, small bacteriostatic and antifungal peptides, have been characterized, little is known about shrimp viral defenses. The objective of this study is to employ proteomics to understand shrimp responses to viral infection. *Litopenaeus vannamei* hemolymph proteomes were analyzed before and after White Spot Syndrome Virus (WSSV) challenge. Several analytical methods (SDS Page gels, 2D gels, gel filtration and liquid chromatography) were used to separate plasma or hemocyte proteins from control and WSSV-challenged animals over time. Differences in protein expression between infected and control animals were then analyzed using mass spectrometry. Several plasma proteins were identified as hemocyanin cleavage products. Peptides cleaved from the hemocyanin C-terminus have recently been shown to have antifungal activity, so the presence of these fragments may be significant for shrimp immunity. In the challenged and unchallenged hemocytes, high levels of histones H2A, H2B, H3 and H4 were found. *Litopenaeus vannamei* histone H2A was sequenced and its N-terminus is highly homologous to parasin I and buforin I, antimicrobial peptides found in a variety of aquatic organisms. The role of these proteins in shrimp viral defense mechanisms is being further elucidated.

77. **Putative attenuated infectious hypodermanal and hematopoetic necrosis (IHHN) virus: case study with *Litopenaeus vannamei***

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Pacific white shrimp *Litopenaeus vannamei* (~25g) cultured in an indoor recirculation system tested positive by dot blot for Infectious Hypodermal and Hematopoetic Necrosis Virus (IHHNV), although they were asymptomatic. A second tank of shrimp (~5g) connected to the same system tested negative and were moved to a new system. Three months later these shrimp tested positive for IHHNV with no clinical signs of the disease. Therefore, a study was perfomed to determine if IHHNV was the pathogen. Six control and experimental groups of 30 SPF juvenile *L. vannamei* (3.56±0.54g) each were placed in aquaria and reared for six weeks. A cell free tissue homogenate was prepared from the IHHNV positive shrimp. Experimental groups were injected with a 1:1 w/v shrimp tissue:0.75% saline. Controls were injected with saline. Shrimp were monitored weekly using four diagnostic techniques: dot blot hybridization, PCR, histology and in situ hybridization. At the end of week one, all four diagnostic tests indicated some degree of infection in the experimental groups, although results varied with diagnostic test: 17% by histology to 100% with dot blot. At termination, all tests indicated that 100% of the shrimp examined were IHHNV positive. Shrimp did not develop clinical signs of infection. This indicates that shrimp were infected by a putative attenuated strain of IHHNV. Confirmation of attenuation needs to be undertaken by bioassay with a susceptible strain (e.g. *P. stylirostris)* or by comparison to a known virulent strain of IHHNV using molecular techniques.

78. **Horseshoe crab (*Limulus polyphemus)* hemolymph biochemical and immunological parameters**

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The horseshoe crab (*Limulus polyphemus*) is one of four extant species remaining worldwide, and can be found along the western Atlantic coast from Maine to the Gulf of Mexico. While the characteristics of the crab's "blue blood" and the composition of LAL have been studied in detail, basic information on the horseshoe crab's physiological parameters are completely lacking. To better evaluate the health of wild populations of horseshoe crabs, biochemical parameters of the horseshoe crab's hemolymph were determined from a collection of fifty wild adults (29 male and 21 female). Results of the biochemistry parameters (mean values) for the hemolymph of the horseshoe crab were: total protein (8.15 g/dl), glucose (58.5 mg/dl), creatinine (0.7 mg/dl), cholesterol (0.8 mg/dl), sodium (389.5 mEq/l), potassium (12.5 mEq/l), chloride (445.1 mEq/l), calcium (39.0 mg/dl), magnesium (96.1 mg/dl), phosphorus (3.4 mg/dl), triglycerides (5.3 mg/dl), amylase (9.3 U/l)), lipase (32.7 U/l), alkaline phosphatase (12.1 U/l), aspartate aminotransferase (5.4 U/l) and gamma glutamyl transferase (0.92 U/l). In addition, the taxonomic position of horseshoe crabs in the Phylum Arthropoda has been debated, specifically their systematic proximity to crustaceans and arachnids. Using SDS-PAGE, Western blotting and agarose gel immunoprecipitation techniques, hemolymph proteins were compared between a horseshoe crab (*L. polyphemus*); representatives of crustaceans: the American lobster (*Homarus americanus*) and blue crab (*Callinectes sapidus*); insects: the Madagascar hissing cockroach (*Gromphadorina portentosa*); and arachnids: the emperor scorpion (*Pandinus imperator*) and the Mexican red-legged tarantula *(Bracypelma emilia)*. Our immunological results support the conclusion that horseshoe crabs are more closely related to arachnids than crustaceans and insects.

79. Histological techniques and manifestations of abnormal protrusions in copepods from Michigan lakes

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Crustacean zooplankton in the Great Lakes region have been reported with gross abnormalities (copepods) or reduced numbers (amphipods). Protruding lesions or tumor-like abnormalities (TLAs) have been observed on copepod species since 1995 and *Diporeia* amphipod populations have been declining since 1992. Two separate investigations assayed histologic characteristics of protrusions in copepods and disease in amphipods. Protrusions on copepods had diverse histological characteristics; over 50% of TLAs contained necrotic tissue; some were composed of acellular, granular, or non-staining material. Some TLAs had hyaline crystal-like structures either on the surface or embedded within the TLA. Nearly 40% of TLAs appeared to be herniated host tissue, usually muscle, hemocytes, or lipid, but occasionally gonad or gut. In histological sections, elongate transparent TLAs resembled ellobiopsid parasites containing granular material and eosinophilic round bodies. Initially the population decline in *Diporeia* spp. was thought to be due to zebra mussels *Dreissena polymorpha* intercepting food material before it settled to the bottom, but sampling efforts have shown there is sufficient food available to the amphipods. An alternative explanation for the amphipod population decline may be pathogens. Surveys revealed numerous parasites in amphipods including rickettsia-like microorganisms, yeast-like organisms, a haplosporidian-like organism, a microsporidian-like organism, external ciliates, gregarines, and worms. No one etiologic agent has been identified as causing the amphipod population decline but several parasites identified during this investigation likely result in amphipod mortalities.

80. Detection of white spot syndrome virus (WSSV) genome in frozen commodity shrimp sold at Massachusetts supermarkets

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One of the most damaging viral diseases affecting the shrimp industry is White Spot Syndrome Virus (WSSV), which causes high morbidity and mortality rates in penaeid shrimp and other crustaceans. The rapid spread of WSSV within wild and cultured stocks of shrimp may be caused by unregulated processing, disposal of infected imported shrimp, or the use of contaminated broodstock. The risk of introducing this virus to cultured and wild shrimp and other native species of crustaceans in the United States warrants investigation. The aim of this study was to examine the prevalence of WSSV in frozen commodity shrimp sold at four stores in the Boston area belonging to different supermarket chains. Samples from two size classes were collected in two different batches a month apart. Polymerase chain reaction was used to amplify a portion of the WSSV genome using a commercial PCR kit (*ShrimpCare*, DiagXotics). WSSV positive samples were visualized by electrophoresis and amplified product of selected samples was sequenced. Results showed a range of 0-38.7% for WSSV prevalence rates, with an overall prevalence of 4.7%. Significant ($P<0.001$) differences in WSSV prevalence were observed between shrimp from the two batches, the two size classes, and the four test stores. DNA sequence confirmed the presence of WSSV genome in PCR-positive samples. The results provide preliminary evidence that an appreciable proportion of the shrimp sold in Massachusetts' supermarkets are carrying WSSV. Further investigation is necessary to determine the risk of release of this virus into the aquatic environment in Massachusetts and across the United States.

81. Heavy metals in wild and cultured marine shrimp from different geographic regions and in frozen commodity shrimp sold in Massachusetts supermarkets: preliminary results

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Previous research revealed a potential negative association between genetic differentiation and IHHNV prevalence in wild *Penaeus monodon* of the Philippines. It is possible that other variables and their interactions may affect disease prevalence, such as chemical pollutant load and host susceptibility. Moreover, no information is available on safety of frozen commodity shrimp. Thus, a database on the genetic structure, disease prevalence, and pollutant load in wild and cultured populations of major shrimp-producing countries is being constructed. In this pilot study, trace concentrations of seven heavy metals were examined in wild and cultured shrimp from various geographic regions and shrimp sold in Massachusetts supermarkets. Preliminary results show that levels of heavy metals in commodity shrimp appear to be lower than those present in wild and cultured shrimp. Though a larger sample size needs to be analyzed before final analysis and conclusions are made, the high levels of some of these metals are of potential concern to animal health.

82. Estrogenic potential and vitellogenin induction in fish related to sewage plants effluents

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It is well documented that sewage treatment plant effluents (STPEs) include numerous xenobiotic compounds and xenoestrogens. Their discharge in the aquatic environment can produce endocrine disrupting effects on fish populations living in the receiving rivers. These findings demonstrate the necessity of studying these effluents and determine their environmental impact. In this work, 11 STPEs were studied to determine (1) their intrinsic estrogenic potential, (2) the presence of natural and synthetic estrogens and xenoestrogens and (3) the vitellogenin induction in common cyprinid freshwater fish captured in rivers receiving sewage treatment effluents. Both 24 h and punctual samples were taken, concentrated using solid phase extraction C_{18} cartridges and eluted with methanol. To assess estrogenic potential, the extracts were tested using the yeast-assay. They were also analysed by GC/MS (SIM mode) to detect 4-t-octylphenol, 4-nonylphenol, bisphenol A, 17ß-estradiol, 17α-ethynilestradiol and estrone. Additionally the presence of other estrogenic compounds was confirmed. A field study was carried out in order to assess estrogenic effects by vitellogenin assay in *Cyprinus carpio*. After injection of 17α- ethynilestradiol, vitellogenin induction was measured. At each of three different rivers, 10-29 carps were captured. Plasma vitellogenin was determined by Western blot and ELISA analyses, other blood parameters (hematocrit value and total plasma protein), and somatic indexes. Results show that river effluents have estrogenic compounds that are biologically active. INIA SC00-040.

83. Chronic effects of bromodichloromethane on medaka

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Bromodichloromethane (BDCM), a drinking water disinfection by-product, is found in the drinking water supply in the USA. The purpose of this study was to determine the chronic toxic effects of BDCM in an alternative animal model, Japanese medaka fish (*Oryzias latipes*). Seven-day-old fry (N=30 per concentration) were exposed to 10 or 25 mg/L BDCM in DMSO for 10 days. Spring water control and a DMSO carrier solvent control were also used in this study. At the end of the exposure period fry were grown out for six months, killed by giving an overdose of anesthesia, and evaluated histologically. No toxic effects were noted in the liver, kidney, brain, pancreas, eye, gills, heart, intestine, muscles, olfactory organ, pseudobranch, and skin. An increased prevalence of thyroid follicular proliferation was observed in fish exposed to 10 mg/L BDCM. Gonadal abnormalities observed were ovo-testis (10mg/L BDCM), and the male/female ratio was skewed towards more males at 10 mg/L BDCM and towards more females at 25 mg/L BDCM and DMSO control. Other lesions observed in the gonads of these fish were cystic ovaries, fluid-filled follicles, cysts and infiltration of adipose tissue in the testis. The results from this study have biological significance, raising the question of potential endocrine disrupting effects and that further studies are indicated.

84. Hepatic and gonadal lesions in medaka (*Oryzias latipes*) exposed to trichloroacetic acid as embryos

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Trichloroacetic acid is one of the haloacetic acids found in drinking water disinfection by-products. Based on epidemiological studies, the toxic effect of drinking water disinfection by-products is of major concern. The purpose of this study was to determine the effects of trichloroacetic acid at environmentally relevant levels, in medaka. The major endpoints evaluated were hepatic and gonadal pathology. Medaka embryos (N=250 per concentration) at early high blastula stage were exposed to 0, 7, 12, and 50 µg/L and 50 mg/L trichloroacetic acid for 10 days and then transferred to contaminant-free water and allowed to hatch. Fry were grown-out for six months then evaluated histologically. Data analyses are currently being conducted. Based on the preliminary evaluation, the hepatic lesions observed were cysts and spongiosis hepatis. The gonadal lesions observed were testicular cysts, testicular hypoplasia, cystic ovary and immature gonads. The preliminary results from this study suggest that trichloroacetic acid, at environmentally relevant concentrations, has impacts on the health of developing medaka.

85. The use of immunological parameters of water animals as a criterion of environmental condition

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The object to maintain biodiversity can be attained by using reliable criteria of environmental conditions for organisms, populations and communities. One fruitful modern approach to assessing such criteria is comparative investigation of immuno-defense reactions in mass species of water animals in norm, pathology and under man-caused influence. We have shown that stresses and adverse environmental conditions change innate and adaptive immunity (concentration of total protein and lysozyme in blood serum and blood cells, titer of specific Igs in blood serum) in rainbow trout (*Oncorhynchus mykiss*) therefore low-pathogenic bacterial symbionts of fishes can cause diseases in aquaculture. Invasion of White Sea navaga (*Eleginus navaga*) and cod (*Gadus morhua maris-albi*) by *Echinorhynchus gadi* decreases concentration of blood cells and haemoglobin, raises concentration of total protein and lysozyme in blood serum, and causes the appearance of new protein fractions in blood serum. Environmental attacks and injected or added antigens (BSA, LPS, HRBC) induce immune reaction in White Sea starfish (*Asterias rubens*): amoebocytes concentration increases 3-fold during the first 24 hours, protein concentration increases 2-fold, lysozyme concentration increases 3-fold. Injection of various antigens (BSA, HRBC, India ink) into White Sea mussel (*Mytilus edulis*) under laboratory conditions raises concentration of haemocytes (3-fold) and total protein (2-fold) during the first 24 hours. The existence of short-term immunological memory in mussels after immunization with HRBC has been shown for the first time. Parameters of innate and adaptive immunity of fishes, starfishes, and mussels reflect the status of water animals and consequently environmental conditions.

86. White cachama (*Piaractus brachypomus*) as a bioindicator of cadmium-polluted waters

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Cadmium as a hazardous water contaminant can induce drastic effects on fish health populations and humans. This work was conducted to provide information that could suggest the use of cachama (a characin fish from Orinoco and Amazonas rivers basins) as a bioindicator of Cd-polluted waters. Two bioassays were performed in a static-renewal exposure system. In the first bioassay, 58 cachamas were randomly distributed in 6 treatments (factorial experiment: 2 water hardness levels: 67, 115 ppm $CaCO_3$) and 3 Cd concentrations (0.0, 0.1, 0.32 ppm Cd; 96 h exposure). Changes in behavior and Cd bioaccumulation in kidney, liver and gills were used as endpoints. The gills had the highest Cd bioaccumulation, as assessed by flame atomic absorption analysis (X= 55.1 +/- 11.4 ppm) followed by kidney (x= 24.0 +/- 10.0) and liver (x= 8.9 +/- 1.7). Significant differences were found when looking for interaction of hardness upon Cd concentrations. Changes in swimming patterns (motionless fish) and reduced feed intake were features of the highest Cd concentrations-exposed fish. In the second bioassay, 20 cachamas were distributed into controls and Cd-exposed (0.38 ppm) and tested during 216 h. Hardness levels in waters were (ppm $CaCO_3$): 32.2 (control) and 34.4 (Cd-exposed). No Cd accumulation in muscle dissected from fish was revealed after experimental time. Easy laboratory handling of specimens, rapid body weight gain and prominent renal tissue easily dissectible, make cachama a good bioindicator of Cd-polluted waters, offering the possibility for testing, surveillance and quality control of effluents from industrial sources.

87. Waterborne nitrite exposure in white cachama (*Piaractus brachypomus*)

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Unique physicochemical properties of Amazonas River waters have led to adaptive mechanisms in indigenous fish. White cachamas (N=120) were used to evaluate the response to waterborne nitrite (NO_2). Percent methemoglobin (MetHb), liver and blood nitrite concentrations and gross lesions were evaluated as endpoints. First phase experiments were conducted testing 4 N-NO_2 concentrations (ppm) (T1=0, T2=35, T3=50, T4=65) (15 fish/treatment) in a static-renewal exposure system (96h). MetHb values were significantly different between exposed and control (T1= 0.0 ± 0.0, T2= 51.3 ± 21.9, T3= 56.8 ± 11.1, T4= 57.3 ± 20.8) (no differences were found among NO_2 concentrations). Liver N-NO_2 (ppm) (T1=1.8 ± 2.2, T2= 14.4 ± 9.4, T3= 19.3 ± 9.6, T4= 17.5 ± 6.9) and blood N-NO_2 (ppm) (T1= 0.1 ± 0.26, T2= 32.1 ± 13.7, T3= 39.3 ± 9.7, T4= 46.8 ± 20.0) increased as [NO_2] in waters was higher. Interestingly, there was no NO_2 bioconcentration in exposed cachamas in contrast to NO_2 toxicokinetics followed by most fish species. Eye opacity was a significant feature in some of the NO_2-exposed fish. In the second phase, 12 fish per treatment (T1= control, T2= 24h exposure, T3= 48h, T4= 72h, T5= 96h) were exposed to 35 ppm N-NO_2. No major changes in measured endpoints were found despite differences in exposure time. White cachama showed a remarkable resistance to NO_2 exposure in comparison to other fish species such as rainbow trout and tilapia. Further research is needed to investigate specific adaptive mechanisms to explain this particular resistance.

88. Quantitative analysis of fish swimming and startle behaviors in response to low level stressors

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Behavioral alterations serve as important sublethal endpoints of stress, toxicant exposure and disease. However, the effects of sublethal stressors on behavior have historically been difficult to quantify. To meet this challenge, we designed and developed a video analysis system to quantitatively investigate changes in fish movement in response to controlled stress exposures. Ten fish were individually housed in 14 liter exposure arenas and automatically videotaped at multiple, discrete intervals during exposures to MS222, a fish anesthetic, and brevetoxin (PbTx2, a harmful algal bloom toxin). Analog video was digitized, converted into (x, y) coordinates, and fish paths were generated and quantified using software developed at the UM Aquatic Pathobiology Center. Quantitative endpoints discerned from control and stress-treated fish included velocity, distance traveled, angular change, percent movement, space utilization, and fractal dimension (i.e., path complexity) and startle response. Analysis of movement data from fish exposed to MS222 indicated that swimming behaviors and startle responses could be characterized accurately through the use of our behavioral analysis system. Specifically, percent movement, movement duration, velocity, distance from center of the arena, and burst acceleration were significantly increased in MS222-treated fish. In contrast, a significant decrease in path complexity occurred in MS222-treated fish versus control fish. Fish treated with the anesthetic displayed reduced startle response behaviors, i.e., decreased velocity, distance traveled, and % responders. We have found that each of the movement endpoints contribute specific insights into fish locomotion and response to stimuli during stressful events. Sublethal behavioral alterations provide a valuable tool for quantifying changes in reproductive behaviors, predator-prey interactions, alterations associated with parasitic or infectious agents, and evaluating changes across environmental gradients.

89. Toxicokinetics of two classes of contaminants in shrimp

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The toxicokinetics of oxytetracycline (OTC) and copper, representing two classes of anthropogenic contamination (pharmaceutical/pesticide and heavy metal), were studied using shrimp as a crustacean model. Inputs of OTC to the environment include crop production, livestock farming, aquaculture, and treated sewage. Copper, measured in national monitoring programs, has been increasingly discharged to the environment from industrial, agricultural, and residential sources. Basic toxicokinetic properties of OTC in the subadult white shrimp, *Litopenaeus setiferus*, and copper in the adult grass shrimp, *Palaemonetes pugio*, were derived to be used in risk assessment modeling of estuarine contamination. Toxicokinetic studies help assess potential for environmental impact by determining those parameters that determine chemical uptake, bioavailability, tissue accumulation, depuration time, and persistence. OTC studies included intravascular dosing and feeding studies; intravascular toxicokinetics allow parameters to be obtained independent of the rate and extent of absorption. Copper studies compared waterborne and oral exposure routes. OTC was analyzed by HPLC; copper determinations were by ICP-MS with operating conditions optimized. Both OTC and copper were bioavailable to shrimp. OTC concentration versus time profiles following intravascular administration were well described with a biexponential equation, suggesting a two compartment pharmacokinetic model. Whole body accumulation of copper from water by shrimp was well described with a monoexponential equation, suggesting one-compartment toxicokinetic disposition resembling constant-rate intravenous infusion. In addition to assessing the possibility of impact from environmental contaminants, kinetic information can be useful for determining the minimum efficacious pharmaceutical treatment regimen in farmed animals to minimize input to the environment.

90. Initial assessment of sediment quality and benthic condition within the Lower St. Johns River estuary

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A large, multi-disciplinary research program was initiated in October 1999 to evaluate potential relationships between water- and sediment- quality variables, occurrences of harmful algal blooms, and the incidence of fish diseases and other biological impacts within the Lower St. John's River estuary (LSJR), Florida. One aspect of the overall program is the monitoring of benthic macroinfaunal communities and associated analysis of chemical contaminants and other stressors (e.g., ammonia, sulfide) in sediments, thereby providing the overall program with a basis for interpreting potential biological impacts in the LSJR in relation to multiple stressor inputs. Potential pollution inputs include shipping, marinas, commercial shipbuilding and repair, military bases, pulp and paper manufacturing, petroleum storage facilities, power generation, urban and high-density residential development, and water-based recreation. The river also supports commercial and recreational fishing, a wide variety of agricultural activities, and includes several superfund sites. Reported here are the results from the initial sampling event of July 2000. Benthic macroinfaunal communities at five of the seven sites sampled in the LSJR show indications of stress that could be associated with anthropogenic activities. Four sites are dominated by species indicative of polluted environments and one additional site shows degraded condition based on values of several benthic community measures. Chemical contamination of sediments likely to have adverse effects on benthic fauna was observed at three stations. However, concentrations of man-made pesticides or other chemical substances typically associated with human activities (e.g., PCBs) were detectable at all stations.

91. Pathogenesis of the Acute Ulceration Response (AUR)

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Previously, we discovered that acute confinement stress causes rapid ulceration of the fins of hybrid striped bass (*Morone chrysops* female X *Morone saxatilis* male). In the present study, hybrid striped bass were confined for 2 hr and tissues were examined in a time-course response by light and electron microscopy. The earliest detectable change was swelling of the outermost layers of the epidermis and loss of microridges. Within 30 min, the epidermis sloughed off and progressed rapidly leading to ulceration of all fins. Both necrotic and apoptotic cells were found in the epidermis. The basal epidermal layer developed severe spongiosis, in which intercellular spaces were widened, and the dermis became edematous. Melanophores aggregated near the basement membrane and dermis, which caused fin blanching. This pathology, which we have named the Acute Ulceration Response (AUR), caused fin rays (lepidotrichia) to split and the fins to become ragged at the distal tips. In severely damaged areas, the epidermis was completely ulcerated and the basement membrane was exposed to the environment. In addition, corneal ulceration of the eye was present. Skin at the head and operculum was not affected. Also, we reproduced the AUR in the guppy (*Poecilia reticulata*), angelfish (*Pterophyllum scalare*) and channel catfish (*Ictalurus punctatus*) after 2 hr confinement stress. This study indicates that acute confinement in many fish can rapidly cause significant epidermal damage. The Acute Ulceration Response might be a primary cause of morbidity in many acutely stressed fish.

92. Evolution of microbial quality of *Merlucius merlucius* during the storage in ship using coolers with ice

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Seafood is still predominately a wild-caught flesh food that must be harvested under frequently difficult conditions and at varying distances -often quite significant- from processing, transport, and retail facilities. These conditions, distances, and duration of fishing trips can tax any system of controls designed to assure safety and prevent spoilage. Microorganisms are found on all the outer surfaces (skin and gills) and in the intestines of live and also caught fish. The total number of organisms varies enormously (a normal range of 10^2-10^7 cfu/cm^2 on the skin surface and between 10^3 and 10^9 cfu/ in the gills and the intestines) nevertheless fish tissue is almost sterile. When the fish dies, the immune system stops functioning and bacteria are allowed to proliferate freely. On the skin surface, the bacteria to a large extent colonize the scale pockets. During storage, they invade the flesh by moving between the muscle fibres. It was found that only a very limited number of bacteria invaded the flesh during iced storage. The aim of this work was to determinate the evolution of microbial quality of *Merluccius merlucius* from Ireland fisheries during the storage in ships. Samples were taken at different times, starting at the time of capture, and after 24, 48 and 72 h of storage in coolers with ice. The final samples were taken when the ship arrived in port.

93. Evaluation of the microbiota present in ice used in the freezing of captured fish

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After fish are captured, they must be quickly cooled to freezing temperatures, since fish are a perishable product. Ice allows a fast cooling of the product. The freezing is a conservation system that allows the maintenance of this excellent source of nutrients, and to have fish during times of shortage. The conservation with ice becomes by simple mixture of this one with the fish deposited in boxes, which are generally of plastic, and take in its base orifices that allow that the water that takes place when being worn away the ice, slips easily. The ice is added the most crushed possible, with the purpose of obtaining the Maxima faying surface with the body of the animal and, not to produce traumatic effects, it is deposited in proportions from the 10 to 15% in layers alternated of about 8 centimeters in thickness. If the ice is sufficient to cover all the organisms, the conservation can last up to 10 days. It is important to make the process of freezing of a correct form to avoid the alteration of the fish (the temperature and the speed of cooling it is important to avoid the contamination of captured products), but also is important the quality of the ice used during the freezing because it can influence in microbiota final present in the fish. In this study microbiological analyses of different used ice samples have been made during the freezing from captured fish on the high seas. The samples were collected after once being in contact with the fish and were preserved in sterile containers. Once the samples arrived at the laboratory, they were analyzed following official methods of microbiological water analyses.

94. Evidence for immune-regulated transgene expression in channel catfish, *Ictalurus punctatus*

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Diseases effecting aquaculture species continue to be a source of lost revenue for farmers. In channel catfish farming, *Edwardsiella ictaluri* and *Flavobacterium columnare* continue to cause high mortality rates with little recourse for fighting these pathogens. Only two FDA approved antibiotics are available to eliminate these pathogens and resistant strains of each have been identified. In addition, development of vaccines to these bacteria has been slow with the only commercial vaccine in the near future being to *E. ictaluri*. An alternative method for enhancing resistance to pathogenic agents is incorporation of a gene encoding disease resistance into the catfish genome. For maximum effect, this gene should be under control of the fish immune system so expression occurs only when a pathogen or stressing agent is present. To accomplish this, genes encoding lytic peptides have been cloned downstream of an acute phase promoter that is regulated by interleukin-1. Using a transposon-based vector for gene delivery, lines of transgenic fish were made to each of the lytic peptides and the efficacy of each in preventing disease was tested by challenge with *E. ictaluri*. Using this approach, mRNA corresponding to lytic peptides was demonstrated only after challenge with *E. ictaluri*. A reduction in mortality was seen in channel catfish carrying either the lytic peptide for cecropin B or phor21. The data presented here suggests a transgenic approach to preventing disease caused by *E. ictaluri* is feasible and could be a viable alternative for broad spectrum disease resistance.

95. Development of methods to genetically sterilize transgenic fish

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Research in our laboratory has demonstrated successful generation of transgenic fish with enhanced disease resistance. However, to obtain FDA approval for using transgenic fish, one of the hurdles that must be overcome is proof that the animals are sterile. The most common method used in aquaculture to date has been creation of triploid lines that are not capable of reproduction. Production of triploids though, is labor intensive, time consuming, and requires certification as triploid, thus making their use in aquaculture cost prohibitive. To overcome this problem, we have begun developing a method for disrupting the reproductive cycle by expressing a peptide that will target gonadal tissue and destroy the reproductive cells. By delivering a gene encoding a fusion peptide consisting of a lytic peptide linked to fragment of leutinizing hormone that binds a receptor on the gonads, we have been able to destroy gonads in both male and female goldfish.

96. Fish diseases education: use of a DVD-ROM for teaching producers, students, and colleagues about fish pathogens

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Use of a fish diseases educational DVD-ROM for teaching fish producers, students and colleagues in the fish health field will be demonstrated. The videos *Diseases of Warmwater Fish* and *Trout Diseases* produced by Kentucky State University and collaborators from Rangen Feeds, Hagerman, Idaho: North Carolina State University, Fletcher, NC; University of Idaho, Twin Falls, ID; Clear Springs Foods, Inc., Buhl, Idaho: Stuttgart National Aquaculture Research Center, Arkansas; Mississippi State University College of Veterinary Medicine; and University of Kentucky Animal Disease Diagnostic Laboratory, Lexington, KY were produced in DVD format to be more effective as a teaching tool. In this DVD, specific viral, bacterial, and parasitic pathogens can be selected from a menu and played by viewers, rather than having to fast-forward or rewind through a linear videotape to locate certain pathogens. Also included in this DVD-ROM are 53 aquaculture fact sheets published by the Southern Regional Aquaculture Center (SRAC), the booklet *Catfish Farming In Kentucky* (2000), and Web Links to aquaculture and fish health sites on the Internet.

97. Fishdisease.net—an online home for aquatic animal health professionals

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The global community of aquatic animal disease professionals does not have a centralized web presence at present. We have established *Fishdisease.net* to provide just such a web resource. The two main purposes for *Fishdisease.net* are: **To provide aquatic disease-related content** to aquatic animal health professionals and students including images, links to researchers and institutions, and data and articles about aquatic diseases; and **To improve communication among aquatic animal health professionals** by providing a web forum for promoting conferences, societies, job opportunities and events. Also, through an email list, *Fishdisease.net* seeks to facilitate discussion of topics relevant to aquatic disease research and management. Through these two purposes, *Fishdisease.net* aims to draw together experts from all the disparate disciplines that take part in aquatic disease research and to provide them a better web focus. *Fishdisease.net* is particularly interested in supporting students by providing them with useful content and a forum in which to promote their research to other aquatic disease professionals. The database behind *Fishdisease.net* will allow users to add their own content, edit their own profiles and promote courses, discussions, conferences or any aquatic disease related content themselves in real time; thus the success of *Fishdisease.net* will be greatly enhanced by users submissions. *Fishdisease.net* is a not-for-profit site.

98. Interdisciplinary training in aquatic animal health at the University of Florida

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The University of Florida has offered veterinary, graduate, and continuing education courses in aquatic animal health for many years. Increasingly, university administrators recognize that this highly specialized field provides an excellent opportunity to develop interdisciplinary programs that use resources from diverse areas within the university community. At the University of Florida, existing programs are being expanded, and new ones planned, that will use facilities and faculty within three distinct units to create a comprehensive program that will enhance the education of veterinary students, non-veterinary graduate students, and graduate veterinarians. Significant collaboration with specialists in the private sector, and within other state agencies, will further enhance these educational programs. Barriers within the university itself have been removed due to willing collaboration, and effort, from all participating units. Administrative barriers to development of these programs can be challenging to overcome, but our experience indicates that the benefit of shared resources and productivity outweighs the costs of a more traditional approach.

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United States Department of Agriculture
Agricultural Research Service

Aquaculture Research at the Agricultural Research Service

Mission

The mission of the ARS national program in aquaculture research is to conduct high quality, relevant, basic and applied aquaculture research and technology transfer to improve efficiency, profitability, and sustainability of U.S. aquaculture, and reduce dependence on imported seafood and threatened ocean fisheries.

Vision

The vision is to establish a globally competitive, sustainable Aquaculture industry in the U.S.

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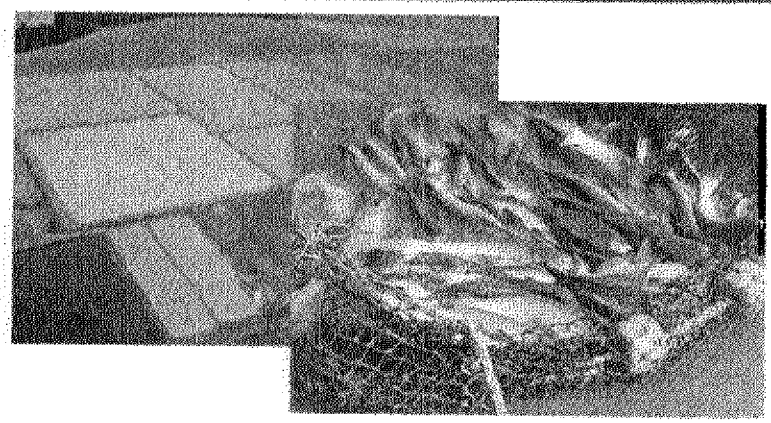

The Agricultural Research Service's aquaculture research program includes marine shrimp and shellfish in the Pacific region; cold, freshwater species in the Pacific Northeast and Northwest Regions; and warm, freshwater species in the Mid-South region of the United States. Research is conducted on genetics, breeding, nutrition, disease diagnostics and control, water quality and use, and production systems to increase production capacity. Improved product quality and marketing are supported with research on processing, off-flavors, food texture and taste, packaging, food safety, and value-added products.

Research programs are conducted at the Aquatic Animal Health Unit, Auburn, AL; Harry K. Dupree National Aquaculture Research Center, Stuttgart, AR; Aquaculture Systems Research Unit, Pine Bluff, AR; Food Processing and Sensory Quality Unit, New Orleans, LA; Catfish Genetics Research Unit, Thad Cochran National Warmwater Aquaculture Center, Stoneville, MS; and the National Center for Cool and Cold Water Aquaculture, Leetown, WV.

ARS carries out cooperative research programs with the University of Arkansas, Pine Bluff, AR; Harbor Branch Oceanographic Institution, Ft. Pierce, FL; the Oceanic Institute, Waimanalo, HI; Mississippi State University; the University of Mississippi; the University of Idaho, Oregon State University; the Freshwater Institute, Shepherdstown, WV; West Virginia University, the University of Connecticut and the Canaan Valley Institute, WV; and Kodiak and Fairbanks, AK.

ARS Aquaculture National Program Web Site:
http://www.nps.ars.usda.gov/programs/programs.htm?NPNUMBER=106