Seventh International Symposium on Aquatic Animal Health

August 31 – September 4, 2014 Portland, Oregon, USA

Proceedings

Fish Health Section

ISAAH-7 Program Overview

Sunday Aug 31			Monday Sept 1	Tuesday Sept 2		Wed Sept 3		Thursday Sept 4		Fri. Sept 5
Special Meeting Rooms: Plenary Presentations: Pavilion Room Speaker Ready Room: Executive Suite, 3rd Floor ISAAH-7 Committee Office: Plaza Foyer Designated Poster Sessions: Plaza Foyer Poster Displays (during non-designated poster sessions): Broadway Rooms III / IV		7:00 am	Breakfast Buffet	Breakfast Buffet		Breakfast Buffet		Breakfast Buffet		*Optional Field Trip: Columbia River Gorge*
		8:00			Poster Session # 1		Poster Session # 2		Poster Session # 3	
		8:45-9:00	Welcome and Introductory Remarks							
		9:00-9:30	**Plenary Speaker** Dr. Kevin Lafferty The Ecology of Infectious Diseases in Marine and Freshwater Systems	**Plenary Speaker** Drs. Sam Chan & Jessica Miller Testing the Invasion Process: Marine Biota on the 2011 Japanese Marine Debris Field		**Plenary Speaker** Dr. Thierry Work Investigating disease outbreaks in coral reef ecosystems: Challenges and opportunities		**Plenary Speaker** Dr. Arik Diamant Out of nowhere: novel parasites and a rapidly changing sea		
		9:30-10:00	**Plenary Speaker** Dr. Jim Winton Drivers of Emerging Diseases in Populations of Wild Fish	**Plenary Speaker** Dr. Espin Rimstad ISA and other Emerging Viral Diseases		**Plenary Speaker** Dr. Edmund Peeler All Models are Wrong, but are Some useful in Aquatic Animal Health		**Plenary Speaker:** Dr. Oriol Sunyer Novel mucosal B and T cell immune responses of teleost fish to pathogens and vaccines		
		10:00-10:30	Break	Break		Break		Break		
		10:30 -noon	1) Special Session – Assessing the Impacts of Pathogens on Wild Fishes 2) General Session – Bacteria I 3) Special Session: Amoeba and Tuna Health	10) General Session: Vaccines 11) General Session: Viruses I 12) Special Session: Diseases of Zebrafish		19) Special Session: Myxozoan Origins and Diversity 20) General Session: Diseases of Invertebrates 21) General Session: Outreach and Physiology		28) Special Session: Environmental Contaminants and Fish Health I 29) General Session: Immunology 30) Continuing Ed.: Existing & Emerging Programs, Procedures, and Issues Involving Aquatic Animal Health & Welfare for the Practicing Aquatic Veterinarian		
Noon – 8:00pm: Registration (Plaza Foyer)	2:45 – 4:30 Free Walking Tour: *Secrets of Portlandia*. Meet at 2:45in the Plaza Foyer (wear walking shoes)		12:00-1:30 pm	Group Photograph Hosted Lunch (Skyline Room)	Lunch on your own	Lunch on your own		Hosted Awards Lunch (Pavilion Room)		
			1:30-3:00	4) Special Session – Interactions Between Wild and Aquacultured Fishes 5) General Session – Bacteria II 6) Special Session: Diseases of Ornamental Fishes	13) Special Session: Parasite Life Cycles 14) General Session: Viruses II 15) General Session: Diagnostic Techniques	22) Special Session: Myxozoan Epidemiology & Infection Dynamics 23) Special Session: Shellfish Diseases 24) General Session: Immunostimulants		31) Special Session: Environmental Contaminants and Fish Health II 32) General Session: Bacteria IV 33) Continuing Ed. II		
	3:00-5:30 Student Workshop Effective Writing: Pavilion Rm. East (Plaza Level)	3:00-5:30 AFS-FHS Ex Comm: Senate Suite (3rd Floor)	3:00-3:30	Break	Break	Break		Break		
			3:30-5:00	7) General Session – Diseases in Wild Fishes 8) General Session – Bacteria III 9) Special Session: Selective Breeding for Disease Resistance: Lab and Field Studies	16) General Session: Parasites 17) General Session: Viruses III 18) Special Session: Aquatic Diagnostic Laboratory Quality Assurance	25) Special Session: Myxozoan Pathogenicity, Genomics, and Transcriptomics 26) General Session: Aquaculture / Hatchery Issues 27) General Session: Immunostimulants II		34) Myxozoan Discussion 35) Continuing Ed. III		
	6:00-9:00 Ice Breaker Skyline Room (23rd Floor)		Evening	5:00-6:30: FHS Business Meeting (Pavilion Room) 6:00-9:00: Student / Mentoring Session Skyline Room (23rd Floor)	6:00-7:00: Western Fish Disease Workshop Business Meeting (Senate Suite) 7:00-11:00: *Optional Evening Dining Event - Portland Chinese Gardens*	6:30-9:30 *Portland Spirit* Dinner Cruise				

Welcome from the Organizers

It is our pleasure to welcome you to the Seventh International Symposium on Aquatic Animal Health (ISAAH-7). Every four years, the American Fisheries Society – Fish Health Section expands its annual meeting by inviting international participation in the form of the ISAAH. Stemming from its inauguration as the 'International Fish Health Conference' in Vancouver, BC (1988), the ISAAH has developed into a much anticipated gathering of international fish health professionals. Subsequent host cities have included Seattle (1994), Baltimore (1998), New Orleans (2002), San Francisco (2006), Tampa (2010) and we are proud to present the ISAAH-7 in the beautiful 'Rose City' of Portland, OR. Among approximately 300 attendees at ISAAH-7, we are especially happy to welcome our colleagues from overseas including representatives from 26 countries. Additionally, we extend a warm welcome to approximately 40 students who represent the future of the aquatic animal health field.

The daily scientific program consists of a morning poster session (Tuesday – Thursday), two plenary speakers, and three concurrent sessions of oral presentations throughout the remainder of each day. Plenary speakers will be presenting broad topics that are likely to guide the current and future directions in our field including the ecology of infectious diseases, drivers of emerging diseases, invasive processes associated with the 2011 tsunami in Japan, the emergence of ISAV and other viruses, diseases in coral reefs, disease modelling, emerging parasites in the Mediterranean Sea, and recent advances our understanding of mucosal immunity in fishes. Oral scientific presentations are grouped into either Special Sessions (i.e. presentations that were largely solicited by session leaders) or General Sessions (i.e. presentations that were received through a general call for abstracts). Additionally, through a partnership with the AVMA and WAVMA, we are pleased to offer three Continuing Education sessions on Thursday that provide 4.5 hours of CE credits to American Fisheries Society – Fish Health Section Certified Fish Pathologists, Fish Health Inspectors, and veterinarians.

Special effort has gone into designing a social program that reflects the heart of the host city. Please join us for a humorous and engaging walking tour on Sunday afternoon that will reveal the secrets of Portlandia, a Sunday evening ice breaker with a view of the city, a lovely Tuesday evening at the Portland Chinese Gardens, and a Wednesday evening banquet aboard a riverboat on the Willamette River with live music provided by the incomparable DUFFY BISHOP!

Once again welcome to Portland; it is our hope that the ISAAH-7 provides an intellectually stimulating experience for you and an opportunity to advance our field of aquatic animal health.

- ISAAH Organization and Program Committees

Upcoming Meeting Announcements:

The Next DAFINET Workshop: Fish Models in Research will occur in Copenhagen, Denmark from November 11-13, 2014 (University of Copenhagen). http://www.dafinet.dk/Home.html

The 9th Symposium on Diseases in Asian Aquaculture (DAA9) will occur in Ho Chi Minh City, Vietnam from November 24-28, 2014. http://www.daa9.org/

The 40th Eastern Fish Disease Workshop will occur in Mt. Pleasant, SC from March 2-6, 2015 (Holiday Inn – Mt Pleasant). https://www.facebook.com/events/1428267967445274/

The next American Fisheries Society – Fish Health Section Annual Meeting will occur in Ithaca, NY from 13-15 July 2015

Type in this tiny url and watch our Prezi Presentation!

http://tinyurl.com/ow2apzr

The 2015 Western Fish Disease Workshop will occur in Steamboat Springs, CO from June 2-4, 2015 (Sheridan Steamboat Resort). Organizer: Brandon Taro.

The 9th International Symposium on Fish Parasites will occur in Valencia, Spain from August 31 – September 5, 2015. http://www.9isfp.com/

The 17th International conference on Diseases of Fish and Shellfish will occur in Las Palmas de Gran Canaria, Spain from September 7-11, 2015. A histopathology workshop will occur on September 12. http://eafp.org/eafp-2015-conference/

Table of Contents

ISAAH-7 Organizers

Organization Committee:
Jerri Bartholomew (Chair)
Ray Brunson
Andrew Goodwin
Larry Hanson
Sascha Hallett
Paul Hershberger
Jill Rolland

Program Committee:
Vicki Blazer
Sascha Hallett
Paul Hershberger (Chair)
Ron Thune

Sponsorship and Promotion Committee:
Ray Brunson
Larry Hansen
Andy Kane
Jill Rolland (Chair)

Webpage Committee:
Jerri Bartholomew
Andrew Goodwin
Sascha Hallett

Venue Committee:
Jerri Bartholomew
Ray Brunson
Sascha Hallett
Paul Hershberger

Entertainment Committee:
Jerri Bartholomew
Marcia House
Gael Kurath

Additional Support Provided by:

Special Scientific Sessions organized by:

Mike Kent: Assessing the Impacts of Pathogens on Wild Fishes
Andrew Bridle: Amoeba and Tuna Health
Simon Jones: Interactions between Wild and Cultured Fishes
Tim Miller-Morgan: Diseases of Ornamental Fishes
Greg Wiens: Selective Breeding for Disease Resistance: Lab and Field Studies
Mike Kent: Diseases of Zebrafish
Sarah Poynton: Parasite Life cycles
Dave Groman: Aquatic Diagnostic Laboratory Quality Assurance
Jerri Bartholomew: Myxozoan Origins and Diversity
Myxozoan Epidemiology and Infection Dynamics
Myxozoan Pathogenicity, Genomics, and Transcriptomics
Jerome La Peyre: Shellfish Diseases
Vicki Blazer: Environmental contaminants and Fish Health

Web Page development by Bonnie Johnson
Student activities coordinated by Sarah McConnachie and Amy Long
Student Workshop "Effective Writing" by Sarah Poynton
Continuing Education Sessions organized by David Scarfe – AVMA
Western Fish Disease Workshop planning meeting hosted by Joy Evered
Audio / Visual services provided by Peak A/V
Meeting logistics by Oregon State University Conference Services
ISAAH-7 logo created by Stephen Atkinson
ISAAH-7 banner created by Stephen Atkinson and Marcia House
Cover Image Complements of Portland Travel

Artists Exhibiting at ISAAH-7

Joseph R. Tomelleri has been illustrating fishes since 1985. Joe works in Prismacolor pencil, and his 1,100+ illustrations have been rendered from actual fish to ensure scientific accuracy. He has collected the majority of his specimens in the field so he can study and photograph the fishes and preserve them for use in the illustrations. Joe's work has appeared in more than 1,000 publications, including Trout and Salmon of North America, Fishes of Alabama, Outdoor Life, The In-Fisherman, and Eddie Bauer. His recent projects include research on the native Trout of Mexico, Fishes of the Salish Sea and Puget Sound, Fishes of Texas, and Fishes of Kansas.

A practitioner of the Japanese art of *Gyotaku* (Fish Printing), **Bruce Koike** was always drawn to the aquatic environment. Koike is self-taught in this discipline and likes to represent the animals as they naturally appear. "Fishes are beautifully designed to make a living where they occur, so why deviate from that", stated Koike. Physical details of the fishes as well as behaviors can both be represented in the composition of the fish print. Hand-made rice paper, acrylic or oil paints and the subject itself are essential components to executing each piece of original art. The term *Gyotaku*, literally translates to "Fish Rubbing", thus the need to use the actual fish in the process. Koike earned a Master degree in Fisheries Science (with an emphasis on fish pathology) from Oregon State University. This training led to animal husbandry work at the Aquarium of the Americas (New Orleans, Louisiana) and the Oregon Coast Aquarium (Newport, Oregon). He also developed the nation's first Aquarium Science degree at the Oregon Coast Community College, a program that develops animal husbandry specialists for work in public aquariums, aquaculture and fish research facilities. He will be present at ISAAH-7 for only the first two days but can be contacted in the future at email: koike.bruce@gmail.com.

We Express our Sincere Appreciation to the ISAAH-7 Sponsors

The Animal and Plant Health Inspection Service is a multi-faceted Agency with a broad mission area that includes protecting and promoting U.S. agricultural health, regulating genetically engineered organisms, administering the Animal Welfare Act and carrying out wildlife damage management activities. These efforts support the overall mission of USDA, which is to protect and promote food, agriculture, natural resources and related issues.

In the event that a pest or disease of concern is detected, APHIS implements emergency protocols and partners with affected States to quickly manage or eradicate the outbreak. This aggressive approach has enabled APHIS to successfully prevent and respond to potential pest and disease threats to U.S. agriculture.

APHIS - Veterinary Services protects and improves the health, quality, and marketability of our nation's animals, animal products and veterinary biologics by preventing, controlling and/or eliminating animal diseases, and monitoring and promoting animal health and productivity.

The USGS serves the Nation by providing reliable scientific information to describe and understand the Earth; minimize loss of life and property from natural disasters; manage water, biological, energy, and mineral resources; and enhance and protect our quality of life.

The USGS is a science organization that provides impartial information on the health of our ecosystems and environment, the natural hazards that threaten us, the natural resources we rely on, the impacts of climate and land-use change, and the core science systems that help us provide timely, relevant, and useable information.

The USGS serves the Nation by providing reliable scientific information to describe and understand the Earth; minimize loss of life and property from natural disasters; manage water, biological, energy, and mineral resources; and enhance and protect our quality of life.

As the Nation's largest water, earth, and biological science and civilian mapping agency, the U.S. Geological Survey (USGS) collects, monitors, analyzes, and provides scientific understanding about natural resource conditions, issues, and problems. The diversity of our scientific expertise enables us to carry out large-scale, multi-disciplinary investigations and provide impartial scientific information to resource managers, planners, and other customers.

The National Institute of Food and Agriculture (NIFA) is an agency within the U.S. Department of Agriculture (USDA). NIFA's mission is to lead food and agricultural sciences to create a better future for the Nation and the world by supporting research, education, and extension programs in the Land-Grant University System and other partner organizations. NIFA doesn't perform actual research, education, and extension but rather helps fund it at the state and local level and provides program leadership in these areas. NIFA is one of four USDA agencies that make up its Research, Education, and Economics (REE) mission area. The other three agencies are:

- Agricultural Research Service (ARS)
- Economics Research Service (ERS)
- National Agricultural Statistics Service (NASS)

The USDA-REE agencies provide federal leadership in creating and disseminating knowledge spanning the biological, physical, and social sciences related to agricultural research, economic analysis, statistics, extension, and higher education.

NIFA's mission is to lead food and agricultural sciences to create a better future for the Nation and the world by supporting research, education, and extension programs in the Land-Grant University System and other partner organizations. NIFA doesn't perform actual research, education, and extension but rather helps fund it at the state and local level and provides program leadership in these areas. The broad expectation is that NIFA will enhance the stature and impact of food, agricultural, and natural resource sciences and ultimately grow support for agricultural research, education, and extension.

NIFA's two key mechanisms for accomplishing its mission of "advancing knowledge" are:

- National program leadership. We help states identify and meet research, extension, and education priorities in areas of public concern that affect agricultural producers, small business owners, youth and families, and others.
- Federal assistance. We provide annual formula grants to land-grant universities and competitively granted funds to researchers in land-grant and other universities.

The **Agricultural Research Service (ARS)** is the U.S. Department of Agriculture's chief scientific in-house research agency. Our job is finding solutions to agricultural problems that affect Americans every day, from field to table. Here are a few numbers to illustrate the scope of our organization:

- 800 research projects within 17 National Programs
- 2,100 scientists and post docs
- 6,000 other employees
- 90+ research locations, including overseas laboratories
- $1.1 billion fiscal year budget

ARS conducts research to develop and transfer solutions to agricultural problems of high national priority and provide information access and dissemination to:

- ensure high-quality, safe food, and other agricultural products
- assess the nutritional needs of Americans
- sustain a competitive agricultural economy
- enhance the natural resource base and the environment, and
- provide economic opportunities for rural citizens, communities, and society as a whole.

PACIFIC NORTHWEST FISH HEALTH PROTECTION COMMITTEE

The PNFHPC is an organization of technical and policy representatives from conservation agencies, Tribes, and commercial fish producers from the Pacific Northwest. Participation in the PNFHPC extends far beyond the voting members of the committee and includes valuable input from others in the region, including educational institutions, private and public research laboratories, resource managers, and conservation groups, to name a few.

The Committee originated as a forum, which operates on a consensus basis, to discuss and resolve fish issues, to disseminate research findings/educational material, and to communicate openly on all matters as the relate to production of health fish in the cultured and natural settings.

The PNFHPC holds regular meetings twice a year. Additional meetings are called as needed to address special issues. Many subcommittees operate under the PNFHPC, including one organizing for the symposium *Pathogens and Diseases of Fish in Aquatic Ecosystems: Implications in Fisheries Management*.

The mission of the AVMA is to improve animal and human health and advance the veterinary medical profession through the advancing the science and art of veterinary medicine, including its relationship to public health, biological science, and agriculture.

With the assistance of the Aquatic Veterinary Medicine Committee (AqVMC) and other councils, committees and task forces, the AVMA develops outreach and education programs and initiatives to support and advance the discipline of aquatic veterinary medicine, and ensure a well-skilled aquatic veterinary workforce to service the needs of clients, the aquaculture and other industries, regulators and other service-providing industries. This committee also assists the AVMA in providing input on national and international legislative and regulatory issues related to aquatic veterinary issues, develops programs to ensure the optimal health and welfare of the aquatic animals, a thriving environment, and to support public health and seafood safety. The AqVMC also actively pursues collaboration with other veterinary and non-veterinary organizations on issues of mutual interest.

Our story begins nearly a half-century ago on a frigid January day in Petersburg, Alaska. That's when the town pooled its resources to create Petersburg Fisheries, Inc.—and saved the cannery that had been the heart of the town's economy since the turn of the 20th century.

Today we have become one of the largest and most diversified seafood companies in North America, with facilities throughout Alaska and the Pacific Northwest.

Throughout our history, we have continually looked for new ways to improve our products and operations. Our drive to excel has led us to develop ideas that were new to the Alaska seafood industry some 50 years ago—ideas such as year-round processing facilities and floating processing vessels. These innovative approaches have enabled us to provide stable jobs to our employees, be near fishermen in remote coastal fishing locations to process the catch within hours of harvest, and deliver a variety of seafood products throughout the year to our customers.

Marine Harvest Canada raises salmon in the cold, clean waters of beautiful British Columbia.

Our salmon are three-star certified to the Global Aquaculture Alliance Best Aquaculture Practices and since 2002, Marine Harvest Canada has been certified according to ISO 14001 environmental management standards.

Marine Harvest Canada is proud supplier of Sterling premium brand salmon.

Oregon Sea Grant's mission is to develop and support an integrated program of research, outreach, and education that helps people understand, rationally use, and conserve marine and coastal resources. Our activities respond to the needs of ocean stakeholders and act to stimulate the Oregon economy.

VETERINARY LABORATORY ASSOCIATION

The Veterinary Laboratory Association (VLA) is an international nonprofit veterinary organization in support of diagnostic laboratories. This association was formed in 1989 for the purpose of providing consistency between veterinary diagnostic laboratories, which ultimately improves animal welfare.

The VLA administers the Veterinary Quality Assurance Program (VQAP) in partnership with the Atlantic Veterinary College at the University of Prince Edward Island, Canada, which is the most comprehensive veterinary proficiency program available to laboratories.

Our mission is to provide an external proficiency program specially designed for veterinary diagnostic laboratories and hospitals which delivers a confidential means of comparing your laboratory's internal test results to those of your peers in the veterinary field. The Program I provided by Atlantic Veterinary College (AVC), University of Prince Edward Island, Canada.

Western Fish Disease Workshop

The Western Fish Disease Workshop (WFDW) is hosted every as an opportunity for fish health professionals (Fish Health Inspectors, Pathologists, veterinarians, and researchers) throughout the western United States and Canada to exchange information. In 2014, the WFDW is hosted in conjunction with the ISAAH-7. The 2015 WFDW will occur at the Sheridan Steamboat Resort, Steamboat, CO. The 2016 WFDW will occur at the Snowking Resort, Jackson, WY.

Merck Animal Health is dedicated to preserving and improving the health, well-being and performance of animals through science. We offer veterinarians, farmers, pet owners and governments the widest range of veterinary pharmaceuticals, parasiticides, vaccines and health management solutions. Merck Animal Health, known as MSD Animal Health outside the U.S. and Canada, is the global animal health business unit of Merck.

Clear Springs Foods makes its home on the scenic Snake River Canyon of southern Idaho's Magic Valley. Here, our Rainbow Trout are farm-raised in concrete raceways fed by an abundance of crystal clear spring water. Ideal growing conditions, combined with a commitment to quality and innovation, have made Clear Springs the world's largest producer of Rainbow Trout, processing over 20 million pounds a year.

Skretting is a global leader in providing innovative and sustainable nutritional solutions for the aquaculture industry. We deliver outstanding feeds and services to fish farmers worldwide for the sustainable production of healthy and delicious fish and shrimp.

Recipients of the 2014 Snieszko Student Travel Awards
Provided by the American Fisheries Society – Fish Health Section

Thomas Rosser
Department of Basic Sciences, College of Veterinary Medicine
Mississippi State University
240 Wise Center Drive
Mississippi State, MS, USA

Diem Thu Nguyen
National Centre for Marine Conservation and Resource Sustainability
AMC, University of Tasmania
Locked Bag 1370
Newnham Campus
Launceston TAS 7250, Australia

Carissa Gervasi
Department of Fisheries Science
Virginia Institute of Marine Science, College of William and Mary
1375 Greate Road, Gloucester Point, VA, USA

Megan Kepler
Pennsylvania Cooperative Fish and Wildlife Research Unit
413 Forest Resources Building
Pennsylvania State University
University Park, PA, USA

Bikramjit Ghosh
NCMCRS, AMC
University of Tasmania
Launceston, Tasmania, Australia

Kevin Erickson
School of Medical and Applied Sciences
CQ University, Gladstone, Australia

Monday Morning Scientific Sessions

	Pavilion East 1. Special Session – Assessing the Impacts of Pathogens on Wild Fishes Moderators: Mike Kent & Arik Diamant	**Pavilion West** 2. General Session – Bacteria I Moderators: John Hawke & Tom Wiklund	**Broadway Rooms 1 & 2** 3. Special Sessions - Amoeba and Tuna Health Moderators: Andrew Bridle & Mark Polinski
10:30 – 10:45am	1a. Mollusc pathogen surveillance to inform zonation in the U.S. Pacific Northwest L Gustafson*, R Elston, R Jones	2a. Virulence and immunogenicity of *Edwardsiella ictaluri* ferric hydroxamate uptake mutants H Abdelhamed*, J Lu, ML Lawrence, A Karsi	3a. An optimized real-time PCR for the detection and quantification of viable *Neoparamoeba perurans* using propidium monoazide AR Bridle*, K Tam, P Crosbie, BF Nowak
10:45 – 11:00am	1b. Review on assessing the impacts of chronic parasite infections in wild salmonids JA Ferguson*, PA Rossignol, ML Kent	2b. Investigations into the new taxa *Edwardsiella piscicida* and comparative genomic analysis with *Edwardsiella tarda* and *Edwardsiella piscicida*-like sp. SR Reichley*, HC Tekedar, GC Waldbieser, MM Banes, DJ Wise, TE Greenway, LH Khoo, A Karsi, ML Lawrence, MJ Griffin	3b. Evidence of immune and inflammatory processes in the gills of AGD-affected Atlantic salmon, *Salmo salar* L. Y Pennacchi, MJ Leef, P Crosbie, BF Nowak, AR Bridle*
11:00 – 11:15am	1c. Pathogens and links to prespawning mortality in Chinook salmon from the Willamette River, Oregon. ML Kent*, ME Colvin, JT Peterson, B Dolan, CB Schreck.	2c. Inflammatory Effects of *Edwardsiella ictaluri* lypopolysaccharide modifications in catfish gut J Santander*, J Kilbourne, JY Park, T Martin, I Diaz, D DeNardo, R Curtiss 3rd	3c. Amoebic Infection in the endangered Rio Grande silvery minnow, *Hybognathus amarus* W.K. Vogelbein, T.D. Lewis*, A.M. Hutson, KJ Buhl, DT Gauthier
11:15 – 11:30am	1d. The challenges of studying pathogen effects on juvenile Chinook salmon and coho salmon in the nearshore Pacific Ocean KC Jacobson*, T Sandell, MB Rew	2d. Comparative studies between catfish and zebrafish strains of *Edwardsiella ictaluri* R Wang*, J Kim, N Kim, J Wiles, JP Hawke	3d. SYBR, TaqMan, or both: highly sensitive, non-invasive detection of *Cardicola* blood fluke species in southern bluefin tuna (*Thunnus maccoyii*) M Polinski, DB Hamilton, BF Nowak, AR Bridle*
11:30 – 11:45am	1e. Impact of *Ichthyophonus* sp. on pre-spawn Chinook salmon from the Yukon River RM Kocan*, PK Hershberger	2e. Early intracellular trafficking of *Edwardsiella ictaluri* in channel catfish macrophages. LP Dubytska*, RL Thune	3e. Application of molecular techniques to identify immune / stress associated gene transcripts in bluefin tuna and their subsequent *in vitro* expression M Polinski*, A Bridle, B Nowak
11:45 – noon	1f. Retrospective investigation of fish kill events in Minnesota I Bueno, NBD Phelps*	2f. Studies on the *Edwardsiella ictaluri* Type Three Secretion System Effectors RL Thune*, LP Dubytska	3f. Transcriptional immune response of cage-cultured Pacific bluefin tuna during infection by two *Cardicola* blood fluke species M Polinski*, S Shirakashi, A Bridle, B Nowak
Noon – 1:30pm	**Hosted Lunch Buffet**		

Highlights indicate student presentations

Monday Early Afternoon Scientific Sessions

	Pavilion East 4. Special Session – Interactions between Wild and Cultured Fishes Moderators: Simon Jones & Paul Hershberger	**Pavilion West** 5. General Session – Bacteria II Moderators: Lone Madsen & Ben LaFrentz	**Broadway Rooms 1 & 2** 6. Special Session - Diseases of Ornamental Fishes Moderator: Tim Miller-Morgan
1:30 – 1:45pm	4a. Disease management mitigates risk of pathogen transmission from farmed salmon SRM Jones*, D Bruno, L Madsen, EJ Peeler	5a. *Francisella noatunensis* subps. *orientalis* immunogens detected by experimentally infected Nile tilapia (*Oreochromis niloticus*) E Soto*, M Rogge, J Sauer, J Lawrence	6a. Emerging fish health issues for the global ornamental fish industry TJ Miller-Morgan*
1:45 – 2:00pm	4b. Trafficking of Viral Hemorrhagic Septicemia Virus from wild to farmed fish KA Garver*, J Lovy, PK Hershberger	5b. Disruption of the pathogenicity determinant protein A gene (pdpA) in *F. noatunensis subps. orientalis* results in attenuation and in greater susceptibility to oxidative stress. F Farrell, J Hansen, O Illanes, A Verma, E Soto*	6b. Overview of DNA viruses impacting ornamental aquaculture TB Waltzek*, M Gotesman, N Steckler, S Spears, J Shelley, RPE Yanong
2:00 – 2:15pm	4c. Simulating water-borne pathogen transport in the Broughton Archipelago and Discovery Islands, Canada M Foreman*, D Stucchi, K Garver, M Guo, P Chandler, J Morrison, D Tuele	5c. Vibriosis in aquacultured red snapper DJ Grimes, RB Blaylock*, JM Lotz, EAL Saillant	
2:15 – 2:30pm	4d. Policies and regulations to minimize pathogen transmission between farmed and wild fish EJ Peeler*	5d. The Impact of virulent *Aeromonas hydrophila* (VAh) on Alabama's farm-raised catfish industry (2009-2013) W Hemstreet*	6c. A risk analysis of Australia's marine ornamental value chain focusing on biosecurity (diseases and pathogens) concerns KP Erickson*, ML Campbell, CL Hewitt, OT Nevin, N Flint
2:30 – 2:45pm	4e. Sea lice infestation and climate change effects on marine survival of Atlantic salmon D Jackson*	5e. Florfenicol: Correlation of pharmacokinetics in channel catfish (*I. punctatus*) with minimal inhibitory concentration values against *Aeromonas hydrophila* and the control of associated mortalities in INAD field studies PS Gaunt*, C Langston , C Wrzesinski, B Johnson, L Crouch, D Gao, P Adams, F Sun, R Endris	6d. Identification of B cells as a major site for koi herpesvirus (KHV) latency A Reed*, S Izume, B Dolan, S LaPatra, M Kent, L Jin
2:45 – 3:00pm	4f. Sea Lice – Aquaculture and wild salmon in Norway O Torrissen*	5f. Complete genome sequence of *Aeromonas hydrophila* ML09-119 HC Tekedar*, A Karsi, GC Waldbieser, MR Liles, MJ Griffin, S Vamenta, T Sonstegard, M Hossain, SG Schroeder, L Khoo, ML Lawrence	6e. Buy a fish, save a tree: Fish health management and a sustainable ornamental fishery on the Rio Negro, Brazil TJ Miller-Morgan* S Dowd
3:00 – 3:30pm	**Break**		

Highlights indicate student presentations

Monday Late Afternoon Scientific Sessions

	Pavilion East 7. General Session – Diseases in Wild Fishes Moderators: Rod Getchell & Natalia Sergeenko	**Pavilion West** 8. General Session – Bacteria III Moderators: Diane Elliott & Esteban Soto	**Broadway Rooms 1 & 2** 9. Special Session – Selective Breeding for Disease Resistance: Lab and Field Studies Moderators: Greg Wiens & Masahiro Sakai
3:30 – 3:45pm	7a. *Ceratomyxa shasta* myxospore characteristics in Klamath River Basin adult Chinook carcasses. A poor disease management option. J.S. Foott*, R. Stone, A. Bolick, K. Nichols, K. True, R. Fogerty, S. Hallett, J. Bartholomew	8a. Piscirickettsiosis pathogenesis: Thermolabile exotoxin secretion by *Piscirickettsia salmonis* ME Rojas, M Galleguillos, S Díaz, Á Machuca, PA Smith*	9a. Genetic improvement of disease resistance in salmonid fish using selective breeding: overview of concepts, considerations and limitations. GD Wiens*, TD Leeds
3:45 – 4:00pm	7b. Disease-associated effects on reproductive output of Chesapeake Bay striped bass (*Morone saxatilis*) CL Gervasi*, RJ Latour, WK Vogelbein	8b. New hosts and genetic diversity of *Flavobacterium columnare* isolated from diseased Nile tilapia and native Brazilian species GM Barony, GC Tavares, HCP Figueiredo, CAG Leal*	9b. Is there a genetic correlation between the resistance of channel catfish to *Edwardsiella ictaluri* and *Flavobacterium columnare*, and how do we get there? BR LaFrentz*, BC Peterson, DD Ourth, CA Shoemaker
4:00 – 4:15pm	7c. Lamprey reddening syndrome in Southland rivers, New Zealand – A mystery! A Pande*, C Brosnahan, W McDonald, B Jones, K Walls, N Brangenberg	8c. Effectiveness of florfenicol in controlling *Flavobacterium columnare* infections in channel catfish: comparison between 2 *F. columnare* isolates with high or low florfenicol susceptibility CM Gieseker*, TC Crosby, NR Hasbrouck, ER Evans, CB Stine, AC Kouneski, SR Frobish, PJ Boliek, MW McDonald, SD Rill, OA Chiesa, LR Rodriguez, R Reimschuessel, LC Woods III	9c. Evaluating innate resistance to *Flavobacterium columnare* in rainbow trout (*Oncorhynchus mykiss*) JP Evenhuis*, T Leeds, SE LaPatra
4:15 – 4:30pm	7d. Diseases and biomarkers of marine health in large pelagic sharks from the US Atlantic coast - an overview of data 1996-2013. DH Adams, J Borucinska*, F Bhura, P Bhardwaj, ZJ Grabowski, K Whitburn	8d. Effect of antibiotic treatment during water hardening on *Flavobacterium psychrophilum* and rainbow trout egg survival E Wagner*, R Oplinger, J Baker	9d. Exploring mechanisms of survival in rainbow trout selectively bred for increased resistance to *Flavobacterium psychrophilum* D Marancik*, M Camus, A Camus, T Leeds, G Gao, G Wiens
4:30 – 4:45pm	7e. Geographical prevalence of pathogens in aquatic animals in Kamchatka NV Sergeenko*, TV Ryazanova, TV Gavruseva, EA Ustimenko, EV Bochkova, KV Kozlov, EA Gritskih	8e. The influence of water chemistries on *Flavobacterium columnare* pathogenesis in channel catfish DL Straus*, BD Farmer, BH Beck, BG Bosworth, EL Torrans, CS Tucker	9e. From the laboratory to the field: The performance of a selectively bred line of rainbow trout (*Oncorhynchus mykiss*) under commercial production conditions S LaPatra*, T Leeds, G Wiens
4:45 – 5:00pm	7f. Infectious disease, shifting climates, and opportunistic predators: cumulative factors potentially impacting wild salmon declines KM Miller*, A Teffer, S Tucker, S Li, A Tabata, SG Hinch, DA Patterson, F Juanes, B Riddell	8f. Intragenomic heterogeneity in the 16S rRNA genes of *Flavobacterium columnare* and standard protocol for genomovar assignment BR LaFrentz*, GC Waldbieser, TJ Welch, CA Shoemaker	General Questions and Discussion

Highlight indicates a student presentation

Tuesday Morning Poster Session (Viruses and Bacteria)

P-1. ISA virus of low and high virulence spread differently in Atlantic salmon after infection by immersion challenge
M Aamelfot*, A McBeath, D Christiansen, I Matejusova, K Falk

P-2. Expression and purification of recombinant outer membrane proteins and secreted proteins of *Aeromonas hydrophila* strain ML09-119
H Abdelhamed*, A Karsi, ML Lawrence

P-3. Deletion of TolQ and TolR in *Edwardsiella ictaluri* and effects on virulence
H Abdelhamed*, J Lu, ML Lawrence, A Karsi

P-4. Response of *Piscirickettisa salmonis* to iron limited conditions
O Alamarza, C Segovia, V Maracaja-Coutino, C Sanchez, J. Santander*

P-5[E]. Evaluation of nonlethal sampling techniques for Infectious Hematopoietic Necrosis Virus in steelhead (*Oncorhynchus mykiss*)
DR Burbank*, LV Chiaramonte, TR Fehringer, PM Mamer

P-6. A refined pulsed field gel electrophoresis method to characterize *Flavobacterium columnare*
TC Crosby*, CM Gieseker

P-7. Bacteria from Chilean salmonid farms exhibit growth inhibition of fish pathogens and quorum sensing blocking
M de la Fuente, CD Miranda*, P Jopia, N Guiliani

P-8. Quantification of *F. columnare* in tissues using real-time polymerase chain reaction
GD Gibbs, MJ Mauel*, MJ Griffin, ML Lawrence

P-9. Pox virus related gill disease in Atlantic salmon
M Gjessing*, OB Dale, T Tengs

P-10[E]. The first report of a Hepadnavirus isolated from fishes: evidence of Hepatitis B Virus infection in white sucker (*Catostomus commersoni*) from the Great Lakes Region
CM Hahn*, LR Iwanowicz, VS Blazer, RS Cornman

P-11. Development of an immunochromatographic test kit for rapid detection of fish iridovirus
SM Huang*, TM Huang, C Tu, HJ Tsai

P-12. Determination of *In vitro* antibacterial activity of some plant essential oils on fish pathogenic bacteria
A Kubilay*, P Yıldırım, H Fakir, G Ulukoy

P-13. Whole genome sequencing and diversity analysis of *Francisella noatunensis* subsp. *orientalis* isolated from Nile tilapia
CAG Leal*, LA Gonçalves, SC Soares, FL Pereira, VAC Azevedo, HCP Figueiredo

P-14. Novel Chinook salmon Bafinivirus isolations from Ontario fish health monitoring
SD Lord*, MJ Raymond, PJ Krell, AM Kropinski, RMW Stevenson

P-15. The relationship of infectious dose and lethal dose for two strains of the salmonid rhabdovirus IHNV that differ in virulence
DG McKenney*, Gael Kurath, ARWargo

P-16. Pathogenicity of *Vibrio splendidus* associated with massive mortalities of reared larvae of scallop *Argopecten purpuratus* (Lamarck, 1819)
CD Miranda*, R Rojas, R Opazo, J Romero

P-17[E]. Inhibition of *Flavobacterium psychrophilum* adhesion *in vitro*
A Papadopoulou*, A Howell, T Wiklund

P-18[E]. Antibiotic resistance testing of a genetic sublineage of *Renibacterium salmoninarum*
LD Rhodes*, AM Wargo Rub

P-19. Transcriptomics of temperature inducible chromosomal recombination in *Aeromonas salmonicida*
C Segovia, M Ayala, K Valderrama, M Moreno, M Astete, C Sanchez, J Santander *

P-20. Phenotypic and molecular study of *Edwardsiella piscicida* isolated from farmed whitefish *(Coregonus lavaretus)* in Finland
S Shafiei, K Sundell, S Heinikainen, T Abayneh, S Viljamaa-Dirks, Tom Wiklund*

P-21. Culture and characterization of *Flavobacterium branchiophilum* from bacterial gill disease in Ontario fish
IG Skulska*, MJ Raymond, SD Lord, RMW Stevenson

P-22. Assessing genetic markers for discerning serovars and strains of *Yersinia ruckeri*
O Tremblay, MJ Raymond, C Ostrowski, SD Lord, IG Skulska, RMW Stevenson*

P-23. *Aeromonas salmonicida* cyclic adenosine 3', 5'-monophosphate receptor protein (Crp) mutants in fish host.
K Valderrama, O Almarza, J Santander*

P-24. Mixed mycobacterial infections in farmed sturgeons
D Zhang, C Ji, X Zhang, T Li, A Li*, X Gong

[E]Indicates E-poster

Highlights indicate student presentations

Tuesday Morning Scientific Sessions

	Pavilion East 10. General Session – Vaccines Moderators: Scott LaPatra & Larry Hanson	**Pavilion West** 11. General Session – Viruses I Moderators: Jim Winton & Kyle Garver	**Broadway Rooms 1 & 2** 12. Special Session – Diseases of Zebrafish Moderator: Mike Kent
10:30 – 10:45am	10a. Evaluation of botulinum neurotoxin-E heavy chain expressing recombinant channel catfish virus as a potential vaccine for visceral toxicosis of catfish K Chatla*, PS Gaunt, T Greenway, D Kunec, LM Ford, LA Hanson	11a. Control and eradication of viral hemorrhagic septicemia in Danish aquaculture NJ Olesen*, HF Skall, BB Jensen, NH Henriksen, S Mellergård, H Korsholm	12a. Transmission of microsporidia in the zebrafish, *Danio rerio* JL Sanders*, ML Kent
10:45 – 11:00am	10b. Immersion vaccination of Atlantic salmon (*Salmo salar*) against *Yersinia ruckeri* TD Nguyen*, AR Bridle, BF Nowak	11b. Recurrence of VHS outbreaks in the Lower Great Lakes RG Getchell*, ER Cornwell PR Bowser	12b.. Activity of antibiotics against Mycobacterium species commonly found in zebrafish CT Chang*, CM Whipps
11:00 – 11:15am	10c. Effect of age and temperature at vaccination on immunization and protection conferred by a live attenuated *Francisella noatunensis* immersion vaccine in red hybrid tilapia N Brown*, ZO Gardenfors, S Yount, F Revan, S Francis, A Camus, E Soto	11c. Evidence for differential virulence among sequence types of viral hemorrhagic septicemia virus genotype IVb ER Cornwell*, SM Imanse, RG Getchell, PR Bowser	12c. *Pseudoloma neurophilia*: A retrospective and descriptive study S Spagnoli*, L Xue, K Murray, F Chow, M Kent
11:15 – 11:30am	10d. A new method of intestinal epithelial passage of betanodavirus vaccine with the aid of natural inflammatory substances for the development of oral vaccine AY Gaafar*, H Yamashita, T Nakai	11d. Host susceptibility and viral fitness as potential drivers of IHN virus emergence and displacement events in steelhead trout G Kurath*, R Breyta, AM Kell, AR Wargo	12d. Interpretive challenges in zebrafish histopathology studies JC Wolf*
11:30 – 11:45am	10e. Ultrasound-mediated delivery of DNA vaccines for non-invasive, mass immunization in commercially important fish TT Wong*, N Zmora, V Frenkel, S LaPatra, Y Zohar	11e. The role of Chinook salmon (*Oncorhynchus tshawytscha*) in the ecology of Infectious Hematopoietic Necrosis Virus (IHNV) in the Columbia River Basin DG Hernandez*, TP Quinn, G Kurath	12e. Biosecurity and diagnostics at the Zebrafish International Resource Center; identifying and minimizing risks in an evolving research environment KN Murray*
11:45 – noon		11f. Preliminary risk assessment of fish hosts experimentally challenged with a North American strain of Spring Viremia of Carp Virus E Emmenegger*, G Sanders, F Binkowski, J Winton, G Kurath	12f. An update on detection and control of *Edwardsiella ictaluri* infections in zebrafish. JP Hawke*, E Desonier, R Wang
Noon – 1:30pm			

Highlights indicate student presentations

Tuesday Early Afternoon Scientific Sessions

	Pavilion East 13. Special Session – Parasite Life Cycles Moderators: Sarah Poynton & Andrew Bridle	**Pavilion West** 14. General Session – Viruses II Moderators: Gael Kurath & Takashi Aoki	**Broadway Rooms 1 & 2** 15. General Session - Diagnostic Techniques Moderators: Roselynn Stevenson & Kevin Snekvik
1:30 – 1:45pm	13a. Life cycles of marine parasites: insights from rescued and exhibit animals in public aquaria BR Whitaker*	14a. Genetic analysis of two novel Orthomyxoviruses from rainbow trout and koi WN Batts*, MSO Brieuc, SE LaPatra, MK Purcell, TB Waltzek, JR Winton	15a. Fluorescent imaging of *Edwardsiella ictaluri* infection in zebrafish (*Danio rerio*) B Grillis, W Baumgartner*, C Hohn, L Petrie-Hanson, M Lawrence
1:45 – 2:00pm	13b. Managing indirect life cycle parasites in aquaculture LM Pote, MJ Griffin, LH Khoo, DJ Wise, TG Rosser* , TE Greenway, MC Yost, CM Doffitt, MM O'Hear, TD King, BS Dorr, SMA Quiniou	14b. Observations on age dependency of Viral Nervous Necrosis and implications for disease control D. Jaramillo*, P. Hick, S. Fielder, R. Whittington	15b. Turning to a "higher power" in pathology for investigating fish and wildlife disease J Lovy*, LL Coffee, SE Friend
2:00 – 2:15pm	13c. Interrupting life cycles: considerations for evaluating effectiveness of antiparasitic drugs ED Landis*, CM Gieseker, JA Matysczak, SR Gore	14c. Effects of temperatures on polyI:C inducible viral resistance status in sevenband grouper K Thanasaksiri, N Sakai, H Yamashita, I Hirono, H Kondo*	15c. Use of matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) for the speciation of pathogenic *Vibrio* in fish CB Miller, S Nydam, GK Hammac, T Maddox, T Besser, D Diaz, K Snekvik*
2:15 – 2:30 pm	13d. Transforming trophozoites: life cycle adaptions in the diplomonad flagellate *Spironucleus* SL Poynton*, MFS Fard, E Sterud, A Jørgensen	14d. Isolation and molecular characterization of a novel calicivirus from baitfish in the USA SK Mor*, NBD Phelps, AG Armien, R McCann, C Puzach, T Waltzek, SM Goyal	15d. POCKIT™ system: a field-deployable tool for rapid, specific, and sensitive on-site diagnosis of aquaculture animal diseases C Su*, LJ Ma, PH Chou, YC Lin, SH Weng, YL Tsai, PY Lee, HFG Chang
2:30 – 2:45pm	13e. How DNA sequencing helps us elucidate parasite life cycles SD Atkinson*	14e. Experimental infection of Salmonid alphavirus 1 in Atlantic salmon fry TK Herath*, A Ashby, NS Jayasuriya, JE Bron, RH Richards, HW Ferguson, A Adams, M Weidmann, JF Taylor, H Migaud, M Fordyce, KD Thompson	
2:45 – 3:00pm	13f. The worm has turned: models could help us manage the invertebrate host (*Manayunkia speciosa*) of the myxozoan salmon parasite *Ceratonova shasta* JD Alexander*, KA Wright, NA Som, NJ Hetrick, JL Bartholomew	14f. Does gill have a role in salmonid alphavirus infection? TK Herath*, JE Bron, JB Taggart, KD Thompson, HW Ferguson, M Weidmann, S Adams, BL Jimena, NS Jayasuiriya, RH Richards	
3:00 – 3:30pm	**Break**		

Highlights indicate student presentations

Tuesday Late Afternoon Scientific Sessions

	Pavilion East 16. General Session - Parasites Moderators: Kym Jacobson & Sascha Hallett	**Pavilion West** 17. General Session – Viruses III Moderators: Maureen Purcell & Espen Rimstad	**Broadway Rooms 1 & 2** 18. Special Session – Aquatic Diagnostic Laboratory Quality Assurance Moderators: Dave Groman, Patricia Gaunt, Marilyn (Guppy) Blair
3:30 – 3:45pm	16a. Persistence of ichthyophoniasis external signs in Pacific herring *Clupea pallasii* LM Hart*, C Conway, D Elliott, PK Hershberger	17a. Phylogenetic analysis of IPNV isolates from Atlantic Canada DR Mateo*, SJ Greenwood, DB Groman, C Yason	18a. Inter-laboratory proficiency tests on detection of notifiable fish diseases NJ Olesen*, N Vendramin, A Ojala, SS Mikkelsen
3:45 – 4:00pm	16b. Co-infection dynamics of *Nanophyetus salmincola* and two bacterial diseases in Chinook salmon SR Roon*, M Jakaitis, JD Alexander, JL Bartholomew	17b. Development and diagnostic validation of a reverse transcription quantitative PCR (RT-qPCR) assay for detection of infectious pancreatic necrosis virus (IPNV) S Clouthier*, T Schroeder, C McClure, M Lindsay, S Khatkar, C Collette-Belliveau, L Gaudet, E Johnsen, J Allen, A Zetner, E Anderson	18b. Veterinary Laboratory Association Quality Assurance Program (VLAQAP) Virtual Microscopy Module – A model for aquatic histopathology proficiency testing DB Groman*
4:00 – 4:15pm	16c. *Mikrocytos*: An extremely divergent eukaryotic genus of microcell parasites and the changing landscape of current research introduced by modern molecular techniques. GJ Lowe*, GR Meyer, CL Abbott	17c. Risk assessment of Piscine Reovirus (PRV) infection in Pacific salmon and trout MK Purcell*, K.A Garver, JR Winton	18c. Quality Assurance according to ISO 17025 at fish diagnostic laboratories in Europe: Practice and status OLM Haenen*, N Vendramin, A Ajola, NJ Olesen[2]
4:15 – 4:30pm	16d. Re-evaluation of the myxosporean family Ortholineidae MA Freeman*, T Laoprasert, P Brown, PE Lim	17d. Isolation and genomic identification of a novel Aquareovirus detected in the endangered fountain darter, *Etheostoma fonticola* LR Iwanowicz*, DD Iwanowicz, TD Lewis, T Brandt	18d. Improvements are needed in reporting of accuracy studies for diagnostic tests used for detection of finfish pathogens IA Gardner*, T Burnley, C Caraguel
4:30 – 4:45pm	16e. Development of monoclonal antibodies for polar filaments and valves of *Myxobolus honghuensis* (Myxosporea: Bivalvulida) L Jia*, D Li, Y Zhai, J Yuan, Z Gu	17e. Development and analytical validation of quantitative PCR assays for detection of sturgeon nucleocytoplasmic large DNA viruses (NCLDVs) SC Clouthier*, EJD VanWalleghem, ED Anderson	18e. Influences of government regulators on aquatic laboratory quality assurance: A global perspective KC Klotins, DB Groman*
4:45 – 5:00pm			18f. Discussion on improvements with recommendations with a summary to be published in JAAH as a commentary (hosted by D Groman)

Highlights indicate student presentations

Wednesday Morning Poster Session (Parasites)

P-25. Systematic infection of *Ethynnus affinis* by cestode of the order Callitetrahynchus in Omani waters: Pathological aspects.
S Al Jufaili*, UK Al Kindi, V Machkevskyi, N Al Mazrooei

P-26. Investigations into the possible impacts of the digenetic trematode *Drepanocephalus spathans* on catfish aquaculture in the southeastern United States
NR Alberson*, MJ Griffin, LH Khoo, LM Pote, SM Quiniou, TE Greenway, MM O'Hear

P-27. Prospecting for myxozoan infections in marine annelid worms to find the alternate host of *Kudoa inornata*, a pathogen of spotted seatrout
SD Atkinson*, I de Buron, D Diaz-Morales, SL Hallett, JL Bartholomew

P-28. Two trematode species, one snail host: comparative patterns of cercarial maturation
A Born-Torrijos, T Kamiya, JA Raga, AS Holzer*

P-29[E]. The non-native monogenea *Thaparocleidus caecus* in India on its introduced host, *Pangasianodon hypophthalmus*: About two decades of unnoticed presence
A Chaudhary, HS Singh, C Székely*

P-30[E]. The treatment of some protozoan and monogenean diseases using bath with high concentration of sodium chloride solution
CD Cojocaru*, CL Ardelean

P-31[E]. The prevalence and sanitary implications of *Eustrongylides excisus* (larvae) infection in the region of Iron Gates Dam from Danube River, Romania
CD Cojocaru*, CL Ardelean

P-32. Detection of *Ichthyophonus* by chromogenic in situ hybridization
CM Conway*, MK Purcell, Diane G Elliott, PK Hershberger

P-33[E]. The influence of gender distribution on the reproduction and transmission dynamics of the parasitic salmon louse *Lepephtheirus salmonis* on wild salmon in British Columbia, Canada.
R Cox*, M Groner, G Gettinby, CW Revie

P-34[E]. Development and validation of a molecular diagnostic assay for detection of the swimbladder nematode *Anguillicoloides crassus* in *Anguilla rostrata* glass eels
SJ Greenwood, DB Groman*, SR Ault, R. Heun, R Threader

P-35. A case of systemic scuticociliatosis in a scorpionfish (Scorpaenidae)
F Magunda*, CM Davitt, KR Snekvik

P-36. Growth of the fish microsporidian parasite, *Loma salmonae*, within cell culture
SH McConnachie*, J Sheppard, G Wright, DJ Speare

P-37. Influence of temperature and fish stock on progression of *Ichthyophonus* infections in Chinook salmon (*Oncorhynchus tshawytscha*)
CL McKibben*, PK Hershberger, MK Purcell, CM Conway, DG Elliott

P-38. Methodologies for the isolation of free-living amoeba and identification of amoeba-resistant bacteria by co-culture with environmental amoeba
J McLean, LM Mutharia*

P-39. Molecular phylogeny and ultrastructure of *Myxobolus cuneus*, a parasite of patinga hybrid, and *Henneguya pseudoplatystoma*, a parasite of pintado hybrid
T Milanin*, AAM Maia, MRM Silva, MM Carriero, EA Adriano

P-40. Ultrastructure of two myxosporean species parasites of *Phractocephalus hemioliopterus* from the Amazon region
J Naldoni, AAM Maia, EA Adriano*

P-41. Predicting the effects of climate change on myxozoan disease: a case study of *Ceratonova* (syn *ceratomyxa*) *shasta*.
RA Ray, JD Alexander*, JL Bartholomew

P-42. Effects of coinfection of channel catfish (*Ictalurus punctatus*) with *Edwardsiella piscicida* and two digeneans, *Bolbophorus damnificus* and *Drepanocephalus spathans*
SR Reichley*, MJ Griffin, C Ware, TE Greenway, LH Khoo, ML Lawrence, DJ Wise

P-43. A molecular and morphological survey of myxozoan actinospores isolated from *Dero digitata* in commercial channel catfish ponds in the Mississippi Delta
TG Rosser*, MJ Griffin, LH Khoo, DJ Wise, TE Greenway, SMA Quiniou, LM Pote

P-44. A novel species of *Henneguya* from the gills of farm-raised channel catfish (*Ictalurus punctatus*)
TG Rosser*, MJ Griffin, SMA Quiniou, LH Khoo, LM Pote

P-45. New primers for DNA barcoding of digeneans and cestodes (Platyhelminthes).
NV Steenkiste, SA Locke, M Castelin, GJ Lowe*, DJ Marcogliese, CL Abbott

P-46[E]. Parasitological investigations of the Asian seabass (*Lates calcarifer*) cultured in a fish farm in Setiu Lagoon, Malaysia
C Székely*, MH Borkhanuddin, G Cech, F Shaharom, K Mohamed, M Shukri, A Embong, K Molnár

P-47. Taxonomic status of *Ichthyobodo* spp., ectoparasitic flagellates causing high mortality of Pacific salmon
S Urawa*, M Freeman, S Mizuno

P-48. The effect of fluorescent dyes on the survival and infectivity of larval trematodes: Development of a methodology for determining the portals of entry of *Cardiocephaloides longicollis* into *Sparus aurata*
GS van Beest, M Villar-Torres, AS Holzer*, FE Montero, JA Raga, A Born-Torrijos

P-49. Light microscopic study of *Myxobolus* sp. (Myxozoa) parasitic kidney of *Leporinus friderici* Bloch, 1794 (Characiformes: Anostomidae) from Brazil
LGP Vidal*, JL Luque

P-50. Host-parasite relationship and phylogeny of *Myxobolus* sp. parasite of *Brycon orthotaenia* from São Francisco River, Brazil
SA Zatti, J Naldoni, KRH Capodifoglio, T Milanin, AAM Maia, MRM Silva, EA Adriano*

[E]Indicates E-poster
Highlights indicate student presentations

Wednesday Morning Scientific Sessions

	Pavilion East 19. Special Session – Myxozoan Origins and Diversity Moderators: Jerri Bartholomew & Edit Eszterbauer	**Pavilion West** 20. General Session – Diseases of Invertebrates Moderator: Andy Kane	**Broadway Rooms 1 & 2** 21. General Session – Outreach and Physiology Moderators: Janet Warg & Chris Wilson
10:30 – 10:45am	19a. Myxozoans as cnidarians B Okamura*	20a. Microparasites causing reduced commercial value of northern shrimp, *Pandalus borealis* Á Kristmundsson*, MA Freeman	21a. US Food and Drug Administration's Phish-Pharm Database - A searchable database of pharmacokinetics data in Fish – 2014 update NR Hasbrouck*, TC Crosby, R Reimschuessel
10:45 – 11:00am	19b. *Bipteria* sp. – old parasite in an old host: Tracing the origin of myxosporean parasitism in the vertebrates A Kodádková, P Bartošová-Sojková, I Fiala*	20b. Targeting essential genes utilizing RNA interference to elucidate shrimp-WSSV interaction MBB Maningas, DAV Guanzon*, JMS Lazarte, RRR Alenton, MVR Tare, H Kondo, I Hirono	21b. Aquatic animal health courses: converting to online and the infusion of one health and interdisciplinary views ILV Larkin*, HTD Maness
11:00 – 11:15am	19c. Environmental DNA reveals novel myxosporean diversity H Hartikainen*, D Bass, B Okamura	20c. Functional elucidation of *Mr*C20 by RNA interference DAV Guanzon*, FG Bolinao IV, AJI Salvador, JCV Vergel, H Kondo, I Hirono, MBB Maningas	21c. An examination of job market and valued knowledge and skills for veterinarians in aquatic animal health HTD Maness*, S Galindo-Gonzalez, ILV Larkin, TG Roberts, R Francis-Floyd
11:15 – 11:30am	19d. Is there a future for the order Multivalvulida in myxosporean systematics? MA Freeman*, I Fiala, A Kodádková, Á Kristmundsson	20d. Apicomplexan infection of the Atlantic sea scallop *Placopecten magellanicus* Á Kristmundsson*, S Inglis, K Stokesbury, MA Freeman	21d. Exogenous insulin modulated the cellular accumulation in the exudate of tilapia during infectious aerocistite MAA Belo*, EJR Prado, AC Moraes, EP Foz, R Barbuio, VP Faria
11:30 – 11:45am	19e. Freshwater myxozoans in China: epidemiology, taxonomy and prospects Z Gu* , Y Liu, L Jia, M Huang, Q Guo		21e. Pharmacokinetics and effects on thromboxane production by cownose ray (*Rhinoptera bonasus*) whole blood cells following single intramuscular injection of carprofen CL Field*, HK Kynch
11:45 – noon	19f. *Ceratonova shasta*: Evolution of (how we perceive) a parasite JL Bartholomew*, SD Atkinson		
Noon – 1:30pm	**Hosted Lunch Buffet**		

Highlights indicate student presentations

Wednesday Early Afternoon Scientific Sessions

	Pavilion East 22. Special Session – Myxozoan Epidemiology and Infection Dynamics Moderators: Beth Okamura & Ivan Fiala	**Pavilion East** 23. Special Session – Shellfish Diseases Moderators: Jerome La Peyre & Charlotte Corporeau	**Broadway Rooms 1 & 2** 24. General Session – Immunostimulants I Moderators: Ken Cain & Charles McGurk
1:30 – 1:45pm	22a. Invertebrate hosts and the epidemiology of proliferative kidney disease I Fontes*, H Hartikainen, N Taylor, B Okamura	23a. Evaluation of the role of a novel plasma iron binding protein in eastern oyster host defense against the protozoan parasite *Perkinsus marinus* J La Peyre*, S Casas, J Lee, J Gauthier, JP Beguel	24a. Immunostimulant effects of recombinant cytokine administration in the Japanese pufferfish *Takifugu rubripes* R Nagamine*, G Biswas, J Hikima, M Sakai, T Kono
1:45 – 2:00pm	22b. Long-term surveillance of a fish pathogen by molecular quantification of waterborne stages in river samples SL Hallett*, GR Buckles, CN Hurst, RA Ray, JL Bartholomew	23b. Metabolic changes during the herpesvirus OsHV-1 µVar infection in the oyster. C Corporeau*, D Tamayo, F Pernet, C Quéré, S Madec	24b. Oral immunoprophylaxis of finfish using alginate microencapsulation B Ghosh*, KD Cain, BF Nowak, AR Bridle
2:00 – 2:15pm	22c. Seasonal fluctuation of myxozoa infection in rohu, *Labeo rohita*, in Myanmar KL Tun*, HH Htay, JM Maung, H Yokoyama, T Yoshinaga	23c. Skulking behind an MSX smokescreen: SSO prevalence in Maine and Massachusetts C Giray*, D Murphy, ML Nelson	24c. Haematological, histopathological changes and antimicrobial residue in sub-adult *Clarias gariepinus* (Burchell, 1822) infected with multidrug resistant *Pseudomonas aeruginosa* exposed to some selected medicinal plant extracts OM Amrevuawho, AA Akinyemi, OGN Ezeri*, OM Bankole, M Agbaje, PA Akinduti
2:15 – 2:30pm	22d. Blebbing around: motility of *Ceratonova shasta* (Myxozoa) in rainbow trout G Alama-Bermejo*, AS Holzer, JA Raga, J Bartholomew	23d. Development of a TaqMan real-time PCR assay for the detection of *Perkinsus olseni* in Australian abalone DM Cummins*, BJ Jones, MS Crane, N Gudkovs	24d. Effect of *Tetracera potatoria* and *Psidium guajava* on growth and haematology of cultured *Clarias gariepinus*. BO Oyebanji*, OU Eyenre, OL Olatunji
2:30 – 2:45pm	22e. Emerging numbers of motile myxozoan blood stages in common carp – A closer look at *Sphaerospora molnari*, a parasite on the rise AS Holzer*, A Hartigan, S Patra, E Eszterbauer	23e. Impact of the protozoan *Perkinsus olseni* on wild Manila clam populations in Japan T Waki, T Yoshinaga*, M Takahashi, T Eki, J Shimokawa	24e. Functional genomics studies of the impact of diets containing camelina oil and/or camelina meal on Atlantic cod and Atlantic salmon immune responses M Booman, Q Xu, ML Rise*
2:45 – 3:00pm	22f. Preliminary attempts to reveal the life cycle of *Sphaerospora molnari* (Myxozoa) S Patra*, A Hartigan, AS Holzer	23f. Biodiversity reduces disease risk in aquatic systems: if only it were so simple! JE Welsh*, C Brussaard, J van der Meer, DW Thieltges	
3:00 – 3:30pm	**Break**		

Highlights indicate student presentations

Wednesday Late Afternoon Scientific Sessions

	Pavilion East 25. Special Session – Myxozoan Pathogenicity, Genomics, and Transcriptomics Moderators: Astrid Holzer & Mark Freeman	**Pavilion East** 26. General Session – Aquaculture / Hatchery Issues Moderators: Doug Munson & Dave Jackson	**Broadway Rooms 1 & 2** 27. General Session – Immunostimulants II Moderators: Oriol Sunyer & Scott LaPatra
3:30 – 3:45pm	25a. Development of *Henneguya ictaluri* in the channel catfish, blue catfish, and their hybrid cross TG Rosser*, MJ Griffin, LM Pote, TE Greenway, DJ Wise	26a. Land-based aquatic practices in New Zealand – Building our awareness to improve biosecurity response preparedness J Fischer*	27a. Dietary effects on immunity, stress, and efficacy of a live attenuated *Flavobacterium Psychrophilum* vaccine KD Cain*, S Ponnerassery
3:45 – 4:00pm	25b. Within-host parasite competition in a myxozoan-fish system C.N. Hurst*, J. L. Bartholomew	26b. Risks of pathogen entry and amplification at three hatcheries in the Willamette River Basin, Oregon, U.S.A M. Jakaitis*, S.R. Roon, S.L. Hallett, R.A. Holt, A Amandi, JL Bartholomew	27b. Antimicrobial activity of *Origanum vulgare* L. on protection against *Lactococcus garvieae* and *Vibrio anguillarum* in rainbow trout (*Oncorhynchus mykiss*, Walbaum) O Diler*, O Gormez, A Diler
4:00 – 4:15pm	25c. The effect of inbreeding on the susceptibility of brown trout (*Salmo trutta* m. *fario*) to the whirling disease parasite *Myxobolus cerebralis* E Eszterbauer*, B Forró, CF Guti, DM Kallert	26c. First nationwide survey documenting and analysing causes of loss of fish and its predisposing factors in Norwegian salmonid aquaculture H Bleie*, A Skrudland, M Stormoen, RI Krontveit	27c. The external administrated fish cytokine will increase the survival in bacterial and virus challenge JHY Lin*, CC Lin, SJ Lin, WC Kuo, YF Foung, HT Lin
4:15 – 4:30pm	25d. Pathogenicity studies of *Myxobolus honghuensis* (Myxosporea: Bivalvulida) using suckling mice model Q Guo*, Z Gu, L Jia, J Qin, H Li	26d. A farm-level view of furunculosis in salmonids and challenges faced for natural resource management of wild trout in New Jersey J Lovy*, SE Friend, S Crouse, D DiCarlo-Emery	27d. A probiotic provides significant protection against *Flavobacterium psychophilum* in rainbow trout after injection by two different routes S LaPatra*, T Feringer, K Cain
4:30 – 4:45pm	25e. Insights into the genome of *Ceratonova shasta*, a myxozoan parasite of salmonids SD Atkinson*, ST O'Neil, E Meyer, SL Hallett, JL Bartholomew	26e. Naturally infected catfish concurrently transmit *Ichthyophthirius multifiliis* and *Edwardsiella ictaluri* to naive catfish DH Xu *, C Shoemakerr, QZ Zhang	27e. Optimizing the efficacy of a live attenuated *Flavobacterium psychrophilum* vaccine for coldwater disease S Ponnerassery*, KD Cain
4:45 – 5:00pm	25f. Transcriptome analysis of *Sphaerospora molnari* (Myxozoa: Myxosporea) and putative peptidase characterization. A Hartigan*, M Kašny, AS Holzer	26f. Summary and treatment effectiveness of cases examined at the Kentucky State University Fish Disease Diagnostic Laboratory from 2009 through June 2014 R Durborow*, J Kelso, J Ma, C Frederick, T Ogunsanya, A Redden, K Campbell, W Kahill	

Highlights indicate student presentations

Thursday Morning Poster Session

P-51. A new strategy to characterize different cell types from fish leukocytes using the bio-imaging technology of the confocal Raman microspectroscopy
T Aoki*, M Ando, H Hamaguchi, JI Hikima, M Sakai, T Moritomo, T Nakanishi, H Takeyama

P-52[E]. FISHPATHOGENS.NET – A richly visual fish pathogen database
SD Atkinson*, SL Hallett, CP Dinsmore, C Banner, JL Bartholomew

P-53. The effects of a sublethal dose of botulinum serotype E on the swimming performance of channel catfish (*Ictalurus punctatus*) fingerlings
R Beecham*, T Thomas, DX Gao, PS Gaunt

P-54. Insulin treatment affects carbohydrate, protein and fat metabolism in tilapia during acute inflammation
MAA Belo*, EJR Prado, AC Moraes, EP Foz, R Barbuio, VP Faria

P-55. Immunostimulant effects of the Mongolian dairy product derived lactic acid bacteria in the Japanese pufferfish (*Takifugu rubripes*)
G Biswas, T Kono, JI Hikima, M Sakai*

P-56. Analysis of the kinome of the Pacific oyster *Crassostrea gigas* for the identification of signals in response to the environement.
C Corporeau*, Y Epelboin, L Quintric, E Guévélou, V Pichereau

P-57. Integrative analysis of the effects of microplastics in Pacific oyster *Crassostrea gigas*.
C Corporeau*, C Laot, R Sussarellu, P Soudant, C Lambert, C Fabioux, I Paul-Pont, N Le Goïc, V Quillien, H Hegaret, AL Cassone, MEJ Arsenault-Pernet, M Boulais, C Mingant, J Robbens, M Suquet, A Huvet

P-58. Novel cytokine homologue gene, IL-17, in Kuruma shrimp *Marsupenaeus japonicus*
M Inada*, M Sakai, T Itami

P-59. Characterization of cytokine homologue genes, VEGF, MIF and astakine, in Kuruma shrimp *Marsupenaeus japonicus*
M Inada, T Yui, M Sakai*, T Itami

P-60. Cytokine of TGF-β family, myostatin, in Kuruma shrimp *Marsupenaeus japonicus*
M Inada, T Yui, M Sakai*, T Itami

P-61. Molybdoflavo enzyme gene, xanthine dehydrogenase, in Kuruma shrimp *Marsupenaeus japonicus*
M Inada, D Shigeyoshi, M Sakai*, T Itami

P-62. Oyster restoration research in Apalachicola Bay, Florida: Engaging academic and community partnerships to address environmental and fishery challenges
AS Kane*, KE Havens, S Hartsfield, J Taylor, A Lindsey, T Irani, JG Morris

P-63. Splenic macrophage aggregates as potential biomarker of exposure in red snapper sampled from the northern Gulf of Mexico Post-DWH Oil Spill
AS Kane*, J Pine, IC Romero, DJ Hollander, WF Patterson III

P-64. The relative proteome analysis of olive flounder cultured at different temperature
JO Kim, WS Kim, J Kwon, D Kim, MJ Oh*

P-65. Immune-related gene expression profiling in Pacific bluefin tuna (*Thunnus orientalis*) during larval stage
K Kobayashi*, T Sakurai, G Kato, M Yasuike, Y Nakamura, A Fujiwara, K Kumon, H Nikaido, H Kondo, I Hirono

P-66. Cytokine-induced CD4 positive T cell differentiation in the Japanese pufferfish, *Takifugu rubripes*
T Kono*, H Korenaga, R Nagamine, G Biswas, JI Hikima, M Sakai

P-67[E]. Validation of MALDI-TOF Mass Spectrometry for Rapid Identification of *Yersinia ruckeri*
C Ramey, J Lewis, J Giles*, B Despres, A Muckle, D Groman

P-68[E]. Regulation pattern of IRF4 by STAT6 and c-Rel in zebrafish
S Li, LF Lu, YA Zhang*

P-69. Comparative anesthetic effects of eugenol and benzocaine in juveniles of red tilapia (*Oreochromis sp.*)
G Santiago Rucinque, G Polo, J Borbón, JF González*

P-70. Evaluation of five commercial kits for isolation of quantifiable, polymerase chain reaction-quality genomic DNA from commercial catfish ponds
C Ware, MJ Griffin, MJ Mauel*

P-71. Non-lesions, misdiagnoses, missed diagnoses, and other interpretive challenges in fish histopathology studies
JC Wolf*, WA Baumgartner, VS Blazer, AC Camus, JA Engelhardt, JW Fournie, S Frasca Jr., DB Groman, ML Kent, LH Khoo, JM Law, ED Lombardini, C Ruehl-Fehlert, HE Segner, SA Smith, JM Spitsbergen, K Weber, MJ Wolfe

P-72[E]. Fish Deformities in African Catfish *Heterobranchus bidorsalis* in relation to Chromosome set manipulation techniques
OT Agbebi*, SO Otubusin, SO Olufeagba

[E]Indicates E-poster

Highlights indicate student presentations

Thursday Morning Scientific Sessions

	Broadway 1 & 2 28. Special Session – Environmental Contaminants and Fish Health I Moderators: Vicki Blazer & Jeff Wolf	**Broadway 3 & 4** 29. General Session – Immunology Moderators: John Hansen & Ron Thune	**Salon Room (Executive Tower)** **30. Continuing Ed:** Existing & Emerging Programs, Procedures, and Issues Involving Aquatic Animal Health & Welfare for the Practicing Aquatic Veterinarian I Moderator: David Scarfe
10:30 - 10:45am	28a. Evaluation of potential disease causing agents in young of the year smallmouth bass in the Chesapeake Bay Watershed MV Kepler*, V Blazer, T Wagner, H Walsh, G Smith	29a. Transcriptome response associated with protective immunity in T and B cell deficient zebrafish A Krishnavajhala, A Zhao, XF Wan, L Hanson*, L Petrie-Hanson	30a. Weissellosis – An important emerging disease in farmed rainbow trout TJ Welch, DP Marancik, CM Good*
10:45 – 11:00am	28b. Potential risk factors for skin and liver tumors of white sucker and brown bullhead VS Blazer*, HL Walsh, CM Hahn, RP Braham, LR Iwanowicz	29b. Effect of temperature on innate immune response of Japanese flounder (*Paralichthys olivaceus*) N Kaneshige*, H Kondo, I Hirono	30b. Contact zoonotic risks for aquaculture professionals in warm water aquaculture OLM Haenen*, J Evans, F Berthe
11:00 – 11:15am	28c. An evaluation of biological markers as indicators of exposure to genotoxic and mutagenic compounds in the Great Lakes Basin, United States RP Braham*, VS Blazer, HL Walsh, CM Hahn, PM Mazik	29c. Differential mortality of wild-type and T and B lymphocyte deficient zebrafish infected with a novirhabdovirus suggest that lymphocytes mediate age and temperature associated resistance. DN Nguyen*, L Ford, L Petrie-Hanson, L Hanson	30c. Histopathology for aquatic cases in practice: When should I use it, what samples do I take and what's it going to tell me. KR Snekvik*
11:15 – 11:30am	28d. Biological effects of environmental contaminants on gene expression endpoints in largemouth bass (*Micropterus salmoides*) and smallmouth bass (*Micropterus dolomieu*) from Great Lakes areas of concern CM Hahn*, LR Iwanowicz, VS Blazer, HL Walsh, RP Braham, PM Mazik	29d. Cytosolic sensor, DDX41 activates antiviral and inflammatory immunity in response to stimulation with dsDNA in Japanese flounder, *Paralichthys olivaceus* J Hikima*, NT Quynh, FF Fagutao, M Sakai, TS Jung, T Aoki	
11:30 – 11:45am	28e. Contaminant-associated health effects in fishes from the Ottawa and Ashtabula Rivers, Ohio LR Iwanowicz*, VS Blazer, H Walsh, C Hahn, DS DeVault, JA Banda	29e. Discovery of the nasopharynx-associated lymphoid tissue of rainbow trout I Salinas*, L Tacchi, R Musharrafieh, ET Larragoite, S LaPatra	30d. Florfenicol: Correlation of pharmacokinetics in channel catfish (*I. punctatus*) with minimal inhibitory concentration values against *Aeromonas hydrophila* and the control of associated mortalities in INAD field studies PS Gaunt*, C Langston, C Wrzesinski, B Johnson, L Crouch, D Gao, P Adams, F Sun, R Endris
11:45 – noon	28f. The pollution sentinel *Fundulus heteroclitus*: Application to sediment remediation efforts WK Vogelbein*, M Unger, J Rieger	29f. Nasal vaccines for use in aquaculture I Salinas*, S LaPatra	
Noon – 1:30pm	**Hosted Awards Lunch**		

Highlights indicate student presentations

Thursday Early Afternoon Scientific Sessions

	Broadway 1 & 2 31. Special Session – Environmental Contaminants and Fish Health II Moderators: Vicki Blazer & Jeff Wolf	**Broadway 3 & 4** 32. General Session – Bacteria IV Moderators: Reg Blaylock & Pedro Smith	**Salon Room (Executive Tower)** **33. Continuing Ed:** Existing & Emerging Programs, Procedures, and Issues Involving Aquatic Animal Health & Welfare for the Practicing Aquatic Veterinarian II Moderator: Chris Good
1:30 – 1:45pm	31a. 17α-Ethinylestradiol dysregulates both mRNAs and miRNAs leading to impaired immune responses in zebrafish SR Das, JC Woodson, R Bessire, DE Tillitt, J Winton, JD Hansen*	32a. Non-lethal samples for detection of *Renibacterium salmoninarum* in juvenile Chinook salmon (*Oncorhynchus tshawytscha*)—an update DG Elliott*, CL McKibben, CM Conway, DM Chase, MK Purcell, LMJ Applegate	33a. Business and Marketing 101 for the well run, profitable and enjoyable private aquatic animal practice J Questen*
1:45 – 2:00pm	31b. Pathology working group review of histopathologic specimens from three laboratory studies of Diclofenac in trout JC Wolf*, C Ruehl-Fehlert, HE Segner, K Weber, JF Hardisty	32b. Bacterial kidney disease in a captive and endangered Chinook salmon broodstock: Did we manage it or did it manage us? M Peters*, S Gutenberger, C Risley, P Long, J Rockowski, S Doulos	33b. Global initiatives to advance aquatic veterinary education D Palić*, AD Scarfe, C Walster, LD Urdes
2:00 – 2:15pm	31c. Analytical toxicology of seafood and communications post-DWH Oil Spill: Bridging environmental and public health concerns AS Kane*, MKS Charles, RM Brooks, B Brumback, AB Lindsey, T Irani, A Mathews, LD Stuchal, SM Roberts, JG Morris	32c. Phenotypic and genotypic heterogeneity amongst *Streptococcus iniae* isolates recovered from cultured and wild fish in North America, Central America and the Caribbean Islands L Chou, M Griffin, T Fraites, C Ware, H Ferguson, N Keirstead, J Brake, J Wiles, JP Hawke, RG Getchell, P Gaunt, E Soto*	33c. Aquatic veterinary education opportunities at AVMA-COE accredited schools J Jeffries*, AD Scarfe
2:15 – 2:30pm	31d. Toxicology of sodium cyanide in four aquacultured fish species of importance in Colombia JF González*, DM Ochoa, JF Borbón, V Rodríguez, AL León, S Marroquín, PD Jiménez, RA Díaz, M Muñoz, ML Correal	32d. Phylogenomic analysis of *Weissella ceti* isolated from diseased rainbow trout (*Oncorhynchus mykiss*) and comparison with other *Weissella* species HCP Figueiredo, SC Soares, FL Pereira, CAG Leal*	33d. National Veterinary Accreditation Program (NVAP) – aquatics modules LH Creekmore*, CL Speekmann, KH Hartman
2:30 – 2:45pm	Discussion Hosts: Vicki Blazer & Jeff Wolf	32e. Rainbow trout fed diets with varying content of marine and plant origin; how does that influence the outcome of experimental infections of the fry with *Flavobacterium psychrophilum* and *Yersinia ruckeri*? L Madsen*, HC Ingerslev, M Boye, I Dalsgaard	33f. Components of a model certificate of veterinary inspection useful for ensuring fish are not infected with priority diseases AD Scarfe*
2:45 – 3:00pm		32f. Identification of O-antigen biosynthetic genes specific to serotype 01 *Yersinia ruckeri:* Role in virulence and protective immunity TJ Welch*, SE LaPatra	
3:00 – 3:30	**Break**		

Thursday Late Afternoon Scientific Sessions

		Broadway 3 & 4 **34. Myxozoan Discussion** Hosts: Stephen Atkinson & Astrid Holzer	**Salon Room (Executive Tower)** **35. Continuing Ed:** Existing & Emerging Programs, Procedures, and Issues Involving Aquatic Animal Health & Welfare for the Practicing Aquatic Veterinarian III Moderator: Pat Gaunt
3:30 – 3:45pm 3:45 – 4:00pm			35a. The trials and tribulations of a wild-caught ornamental fish: A veterinarian's perspective on the collection, transport and sustainability of wild-caught ornamentals T Miller-Morgan*, J Heidel
4:00 – 4:15pm 4:15 – 4:30pm			35b. Principles of biosecurity for the ornamental fish industry - How you can help your clients improve fish quality and health T Miller-Morgan*, J Heidel
4:30 – 4:45pm 4:45 – 5:00pm			35c. Developing & implementing practical aquatic veterinary biosecurity programs to meet international (OIE) standards & national regulations D Palić*, AD Scarfe, C Walster

Abstracts for Plenary Speakers

The ecology of infectious diseases in marine and freshwater systems

Kevin Lafferty*

Infectious diseases have major economic impacts on fisheries and aquaculture, and can sometimes impact wild species of conservation concern. This often leads us to consider infectious diseases as problems that need fixing. The backdrop to these problems is a range of infectious agents in the wild that are parts of natural functioning ecosystems. These infectious agents comprise massive amounts of biomass, add complexity to food webs and regulate host populations. Understanding infectious as normal components of healthy ecosystems helps put disease problems and challenges in their proper perspective.

Drivers of emerging diseases in populations of wild fish

James R. Winton*

USGS Western Fisheries Research Center, 6505 NE 65th Street, Seattle, WA 98115 USA; jwinton@usgs.gov

Infectious disease is an important cause of mortality among both wild and cultured finfish in freshwater, estuaries or the open ocean and, where populations of wild and cultured species overlap, many of the same pathogens will be shared. In recent years, there has been a remarkable increase in new or emerging diseases in fish, some caused by previously undescribed pathogens while others characterized by major host or geographic range expansions or significant changes in virulence or pathology. Although partially driven by increased surveillance activities using improved diagnostic tools, many of the emerging diseases affecting both cultured and wild populations of fish are due to the activities of man. Here, I review some of the factors that may drive the emergence or severity of infectious diseases in populations of wild fish to include: the rapid expansion of commercial aquaculture, increased global trade, habitat alteration, commercial fishing, environmental contamination, introduction of non-native species and climate change. Among these factors, the rapid growth of commercial aquaculture has resulted in the introduction of exotic pathogens, amplification of endemic pathogens, changes in the susceptibility of host species and modifications of pathogen virulence in ways that can increase the impact of disease on both hatchery and wild populations. Emerging diseases affecting populations of wild fish have also resulted from increased global trade including the unregulated movement of live fish, bait fish or fish products and the introduction of non-native species and their pathogens by fisheries agencies, anglers or ballast water. Although most infectious diseases affecting fish are endemic among free-ranging or wild populations, factors such as temperature, water quality and nutritional status are known to affect the host-pathogen relationship. However, research is needed to better understand how broad-scale anthropogenic changes to the aquatic environment may act synergistically with infectious diseases to alter levels of natural mortality among populations of wild fish.

Testing the invasion process: Marine biota on the 2011 Japanese tsunami marine debris field

Jessica A. Miller[1]*, James T. Carlton[2], John W. Chapman[1], Jonathan B. Geller[3], Gregory M. Ruiz[4]

[1]Oregon State University, Hatfield Marine Science Center, Department of Fisheries and Wildlife, Newport, OR 97365
[2]Williams College, Mystic, CT 06355
[3]Moss Landing Marine Laboratories, Moss Landing, CA 95039
[4]Portland State University, Portland, OR 97207

As of April 2014, biological samples have been acquired from >160 items that were classified as JTMD based on the presence of characteristic Japanese biota. The most common items include docks, buoys, skiffs, post and beam timber, and pallets. The majority of the items were collected from Oregon (76), followed by the Hawaii and the northwest islands (48), Washington (19), British Columbia (10), California (4), and Alaska (2). In collaboration with taxonomic experts, >200 taxa have been identified, including >160 animal and >40 algal taxa. At least 15 invertebrate species are known to have been successfully introduced outside of Japan, including the barnacle *Megabalanus rosa*, the crab *Hemigrapsus sanguineus*, the mussel *Mytilus galloprovincialis*, the amphipod *Jassa marmorata*, the seastar *Asterias amurensis*, and the sea squirt *Didemnum vexillum*. Several species were reproductive upon arrival, including *M. galloprovincialis* and *J. marmorata*. There was geographic variation in the occurrence of reproductive mussels: 100% of the individuals collected in Oregon contained gametes whereas an average of 80% and 28% of the individuals on debris items collected in Washington and Hawaii, respectively, contained gametes. We continue to collect data on species identity, population structure, reproductive condition, growth histories, genetics, and parasite/pathogen presence from JTMD biota, while also quantifying spatial and temporal variation and biodiversity attrition over time. The continued delivery of debris with living, adult coastal species from the NW Pacific provides an opportunity to our advance understanding of invasion biology.

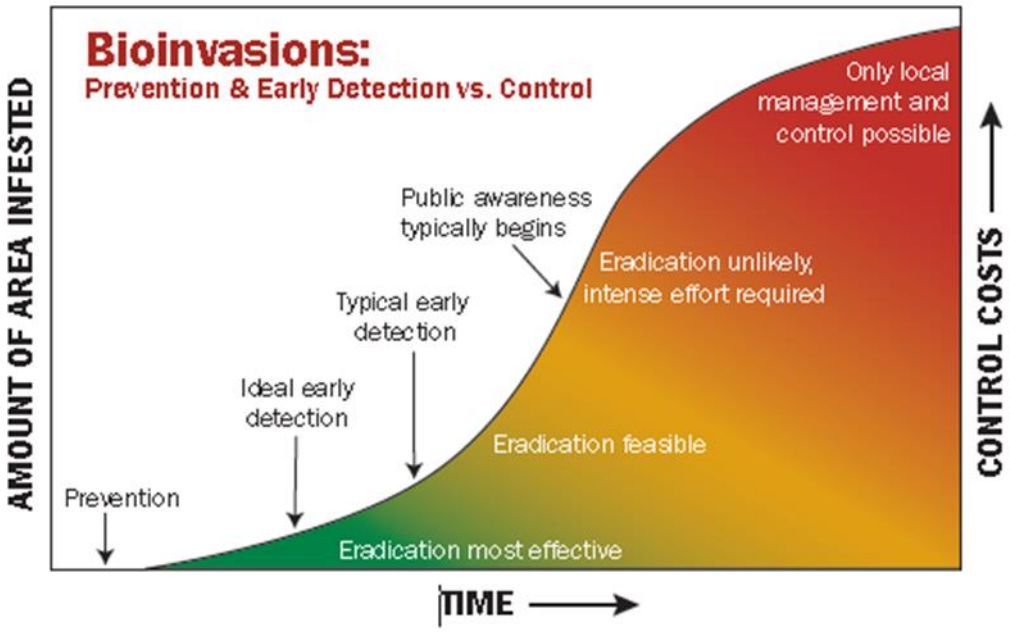
Educational and engagement perspective to risks of bioinvasions caused by natural disasters: The case of tsunami marine debris from the 2011 Japan tsunami

Sam Chan*

Assistant Professor, Fisheries and Wildlife and Applied Economics, Sea Grant Extension, Oregon State University, Corvallis, OR

Sea Grant College Programs have a common theme: to integrate research, outreach/education and communications as part of a planned project design from conception to completion. My role has been to work with cross disciplinary teams to integrate these elements. The beaching of a large dock at Agate Beach on June 4th 2012, from Misawa, Japan, set adrift for ~15 months by the 2011 Japanese tsunami was the first documented instance where large communities of living organisms had rafted, perhaps mostly intact, from the western shores of the Pacific to the eastern Pacific shores of North America. This first of numerous rafting events that followed, generated much national and international scientific, media, public and political interest in tsunami hazards, tsunami debris and marine invasive species. Rapid action was needed to learn of the potential invasive species threats and to have consistent protocols in place for monitoring and responding to sightings. Soon after the arrival of the Misawa dock, Oregon Sea Grant funded projects led by Drs. Jim Carlton, Miller, Chapman and Hansen to catalog, identify and collaborate to develop outreach strategies and products on the organisms found with Japanese tsunami marine debris (JTMD) Japan. These efforts evolved into an NSF Rapid Response grant on JTMD comparative species taxonomy and community composition in Japan and the USA and work on JTMD species age and energetics project led by Dr. Carlton (will be reported on my Dr. Miller). The arrival of invasive species on JTMD revealed not just large research needs (e.g. in taxonomy, community composition, community dynamics, life history, bioenergetics, drift patterns, colonization substrates, risk analysis) but also critical gaps in monitoring, outreach and communication response protocols**.** Some of these critical gaps were addressed by convening a workshop where research, monitoring, management, policy, outreach and communications experts from the region worked through hypothetical case studies (which later proved close to reality) to evolve rapid response protocols that were published as "Response Protocols for Bio fouled Debris and Invasive Species Generated by the 2011 Japan Tsunami".
http://www.anstaskforce.gov/Tsunami/FINAL%20JTMD%20Biofouling%20Response%20Protocol_19%20Oct%202012.pdf
Points for Consideration and associated key questions

1) It is currently unknown if species transported by JTMD are established and reproducing in the Eastern Pacific Coastlines of North America. Detection of invasive species in marine environments is challenging and oftentimes may not be detected until years or even decades after the initial invasions. From an invasive species and cost efficiency Best Management Practices angle, EDRR (Early detection and rapid response) while eradication is still economically possible is essential for minimizing the impacts from invasive species.
    a. As the abundance of JTMD and their sightings decline, how can we build a sustainable long term program for monitoring invasive species that drifted over on JTMD?



Chan et al. 2014. On the Lookout for Aquatic Invaders: An Identification Guide for the West. Oregon Sea Grant. 92 pages. In press (expected by end of May).

2) There are insufficient resources for monitoring the vast coastlines of North America for marine invasive species. Need information to develop a credible JTMD Invasive Species Risk Analysis. Interactions based on where JTMD drifts, its frequency (propagule pressure), timing, species ecology, species life histories, tolerances and preferences and habitat suitability
    a. Which habitats and locations are more likely to be at risk of invasion from the species identified so far on JTMD?
    b. When JTMD invasive species is detected in nearshore habitats, what are the protocols for decisions on deciding between eradication and containment?
3) Leveraging momentum from JTMD transported organisms into a more integrated, effective and sustainable invasive species and marine debris mitigation program. Coastal hazards, JTMD and their transport of invasive species have elevated public awareness, interest and desire to take action.
    a. A need to integrate social science research and the social sciences.
    b. How to leverage the relatively high public and political interest over the Japanese tsunami and JTMD into long-term actions and momentum for more effective action on prevention, early detection and rapid response associated with bioinvasions entering our oceans and estuaries.
    c. A need to span disciplines. The trans-Pacific transport of organisms following a natural disaster in areas of high human activity highlights the potential for future events. Increased knowledge of risks and having a response plan that acknowledges and engages a wider spectrum of experts can help communities better prepare .

ISA and other emerging viral diseases

Espen Rimstad*

Norwegian University of Life Sciences

Viruses may thrive in environments with high-density of hosts, numerous routes of transmission and suboptimal possibilities for protection; all of which are available in aquaculture. Infectious diseases do not respect national boundaries and may have detrimental effects both on the production and on the possibilities to export aquaculture products. The key to control viral diseases is to block the transmission of infection. Properties of the viruses and their spread should determine the set of rules made by fish health authorities to control disease. Many of these properties are not known in sufficient details and estimations are used. This includes knowledge about reservoirs, susceptibility of infection for the different species of fish, the pattern of shedding of virus and survival of viral infectivity outside the host. Effective vaccines are available for a limited number of serious fish viral diseases, often leaving compulsory stamping-out eradication as the official approach. The development of effective vaccines offers another way of preventing and controlling future risks.

Investigating disease outbreaks in coral reef ecosystems: Challenges and opportunities

Thierry M. Work*

US Geological Survey, National Wildlife Health Center, Honolulu Field Station, Honolulu, Hawaii, 96850, USA.

Tropical coral reefs are some of the most diverse ecosystems on the planet and provide myriad ecosystem and other economic services to human communities globally. However, with increasing coastal urbanization, tropical marine ecosystems face numerous threats. Warming temperatures will exacerbate bleaching events where corals lose their symbiotic algae. Ocean acidification is already impairing the ability of many marine organisms to deposit calcium, and acidification's effect on corals is potentially dire. Overfishing and land based pollution are also imposing increasing burdens on coral reefs. Given these threats, disease is likely to play an increasingly important role in decline of coral reefs. In the Western Atlantic, coverage of corals has been reduced by 80%, mainly because of losses resulting from disease. Yet, in spite of over 40 years of research, our knowledge of coral anatomy, physiology, and causes of disease is rudimentary at best. A principal reason for this is that standard biomedical approaches used to investigate diseases in other animals have not been systematically applied to coral reefs, and we have little idea of what occurs at the cellular and tissue level in coral disease. There is, then, a compelling argument to be made to involve more animal health specialists in investigation of diseases in tropical marine organisms. Pathology should play a central role, because it provides a logical framework to guide subsequent laboratory confirmations, gives concrete evidence at the cellular level of potential causes of disease and host response, and serves as a reference point to relate experimental studies to disease in the wild. Many opportunities exist for animal health professionals working jointly with coral ecologists to develop novel approaches and make exciting new discoveries simply by applying basic histopathology judiciously supplemented by molecular and other approaches. Unlike other animal disease systems where the loss of a species seldom leads to massive ecological changes, corals, as ecosystem engineers, are the environment. Animal health professionals working in tandem with coral biologists could potentially play a pivotal role in helping us understand causes and drivers of coral reef health. Developing this knowledge could go a long way to helping recover these amazing ecosystems, or at the very least, allowing us to make them more resilient.

All models are wrong, but are some useful in aquatic animal health?

E.J. Peeler*

Centre for Environment, Fisheries and Aquaculture Science, Weymouth, DT4 7QN, UK.
ed.peeler@cefas.co.uk

Model construction is a conceptual reduction of a complex biological process into a simplified, more easily understood sequence of events. Thus models are always 'wrong' (George Box) because they are not a comprehensive, accurate representation of reality, but they can be useful as the reductionist approach defines processes and identifies a system's most important components. We review examples of statistical, network, epidemic and risk models in aquatic animal health management and ask whether they have been useful. Statistical models have identified risk factors for a range of infectious and non-infectious aquatic animal diseases. Infectious salmon anaemia is the best studied example; risk factors, which have highlighted spread from processing plants, infected sites, and mechanically via boats, and management factors (e.g. size of smolt at transfer) have underpinned control programs. Analysis of networks of connections of farms through, for example, water or live fish movements, has been used to identify high risk sites which should be targeted in surveillance programs. Compartment-based epidemic models have used these networks to assess the dynamics of, for example, infectious pancreatic necrosis (IPN) epidemics (in Scotland and Ireland) and the spread of koi herpesvirus (KHV). Models of KHV spread were built to support decision making about control and specifically eradication in the UK. Both KHV and IPN models identified how key processes driving transmission changed during the epidemic. Hydrodynamic and epidemic models of the dispersal of viruses and sea lice have identified the high level of connectivity between farms at a large spatial scale in the marine environment, with consequences for disease control and spatial planning. Within aquatic animal health, risk modelling has focused on assessment of the likelihood of the introduction of exotic pathogens through trade – import risk analysis (IRA), e.g. the import of shrimp to Australia. The World Trade Organization requires the use of IRA to justify trade barriers because it is transparent and defensible. More broadly IRA can be used to support aquatic animal health management. An IRA of the imports of fish carcasses to the UK for processing highlighted potential weaknesses in current biosecurity. Risk models have been used to study disease emergence in aquaculture and due to climate change. The models discussed are inter-dependent. Epidemic model use the outputs from statistical modelling. Risk models draw on a wide range of evidence including outputs from epidemic models. Economic models of disease are underpinned by assumptions based on epidemic models. Further integration of modelling approaches will considerably increase their value in decision making. Specifically the development of IRA depends on integration of epidemiological and economic models within a risk framework. Integration of hydrodynamic with epidemic models could produce tools to predict disease spread during an outbreak, and allow comparison of competing control strategies. However, increased modelling sophistication needs to be matched by robust underpinning data for models to remain useful.

Out of nowhere: emerging fish parasites in a rapidly changing sea

Arik Diamant*

Israel Oceanographic and Limnological Research Institute, National Center for Mariculture, Eilat, Israel

The largely landlocked Mediterranean Sea's history incorporates centuries of human influence. The last few decades in particular have had an unparalleled deep footprint on the sea's biodiversity. The massive influx of biota coming in from the Red Sea through the Suez Canal (termed "Lessepsian Invasion"), as well as relentless overfishing, habitat modification and pollution have resulted in serious decline of indigenous fish populations, altering the regional coastal ecosystems of the Levant Basin (eastern Mediterranean). The influx of alien species is believed to have been accelerated by rising sea surface temperatures and continuous widening and deepening of the Suez Canal. The generation of mixed species assemblages consisting of native and invasive biota has in effect transformed the Levant Basin into a "pseudo-province" of the neighboring tropical Red Sea.

As important components of trophic webs and as agents of disease that regulate host populations, parasites can have significant impact on unstable, changing communities. Nonetheless, the study of Lessepsian Invasion has hitherto rarely included parasites, and their roles in this unfolding, large-scale biogeographical phenomenon are still poorly understood. In view of the rising incidence of diseases in the world's oceans and their profound effects on marine communities, we assume that the persistent influx of alien species and new pathogens is likely to continue to impact fish assemblages of the unstable Levant Basin. The results of a study on two native and alien species infected with two pathogenic microsporidians resulting in variable impact at the host individual and population levels will be discussed.

Novel mucosal B and T cell immune responses of teleost fish to pathogens and vaccines

J. Oriol Sunyer*

School of Veterinary Medicine, Department of Pathobiology, University of Pennsylvania. Philadelphia, PA19104, USA, sunyer@vet.upenn.edu.

Most pathogens enter and infect fish through mucosal sites, including the skin, gill and gut. Thus, understanding how immunity operates in the fish mucosa will provide the rationale for designing effective vaccines and immunostimulants that induce mucosal protective immune responses. In 2010 we reported that IgT is a teleost immunoglobulin class specialized in gut mucosal immunity. Based on this finding, we have hypothesized that IgT is also a key player in other fish mucosal sites, including the skin and gills. To address this hypothesis, we analysed the specific abundance of IgT, IgM, IgD and B cells in skin and gills of rainbow trout, and we assessed their modulation by *Ichthyophtirius multifiliis* (Ich), a salmonid pathogen known to prevalently infect skin and gill tissues. Similar to what we have previously reported in the gut, flow cytometric analysis of skin and gill leukocyte suspensions showed that IgT^+ B cells represented the prevalent B cell subset in these organs. Comparable to what we showed for the gut mucus, the IgT/IgM ratio of the skin and gill mucus was several fold higher than that of serum. Moreover, skin and gill mucus IgT is present mainly in polymeric form as opposed to serum IgT which is found in monomers. We found that fish that survived infection with Ich showed large accumulations of IgT^+, but not IgM^+ B cells, in the skin and gill mucosal associated lymphoid tissues (MALTs). Along with these substantial increases in IgT^+ B cells, we detected large increases of IgT protein in the skin and gill mucus, while IgM levels remained unchanged. More critically, fish that survived Ich infection had significant Ich-specific IgT titers in the skin and gill mucus, whereas most fish showed undetectable or very low IgM titers in the same mucus samples [3, 4]. Conversely, we detected significant Ich-specific IgM titers in the serum, while those of IgT were negligible. Interestingly, no IgD-specific titers against the parasite could be found neither in the serum nor in the skin or gill mucus. To further support the idea that IgT is involved in skin and gill mucosal homeostasis, we tested the hypothesis that similar to gut IgT, skin and gill IgT may also coat microbiota from those tissues. In support of this hypothesis we found that a majority of skin and gill microbiota were coated with IgT, and to a much lesser degree with IgM and IgD. The aforementioned dominant IgT responses in the skin and gill mucosal areas provide further support that IgT is an immunoglobulin specialized in mucosal immunity at all fish mucosal sites.

The above mentioned results led us to hypothesize that stimulation of fish mucosal areas by vaccination may prevalently elicit IgT-specific immune responses. To test this prediction, we vaccinated fish by immersion with a formalin-killed *Vibrio* bacterin expecting that the skin and gill areas exposed to the vaccine would stimulate IgT-mediated immunity. After three months vaccination we found significant *Vibrio*-specific IgT titers in the skin and gill mucus, while very low IgM-specific titers could be detected in the same samples. Conversely, *Vibrio*-specific IgM titers where prevalently found in the serum, while negligible IgT-specific titers could be detected in those samples. This is the first time in which immersion vaccination is shown to mainly induce IgT-specific responses in the skin and gill areas, a result that points to the critical need to assess IgT responses upon delivery of fish with vaccines that may prevalently stimulate mucosal areas (i.e., immersion and oral vaccination).

While our findings on B cell responses demonstrate the dominant role of IgT^+ B cells in mucosal immunity, very little is known with regards to the role of CD4-T cell responses in mucosal sites. Trout contain two distinct CD4 molecules, CD4-1 and CD4-2. We have recently generated the first monoclonal antibodies that specifically recognize these two molecules. These antibodies have enabled us to characterize two main lymphocyte populations of $CD4^+$ T cells, the double positive CD4-1/CD4-2 subset, which constitutes the vast majority of $CD4^+$ T cells in systemic and mucosal

lymphoid sites, and the single positive CD4-2 subset. The latter subset, while minor, is more prevalent in mucosal than in systemic lymphoid tissues. Importantly, we found that the % of lymphocytes representing $CD4^+$ T cells in mucosal sites is very large (~30-50% of all lymphocytes) and it is significantly higher than that of systemic organs where $CD4^+$ T cells comprise ~3-25% of all lymphocytes. The large number of $CD4^+$ T cells observed in mucosal areas suggest an important role of these cells in mucosal immunity. We are currently analysing the modulation of rainbow trout B and T cell populations in mucosal and systemic organs upon infection and vaccination with the goal to better understand how immunity is induced in systemic and mucosal sites.

In conclusion, our findings on IgT, including the newly developed strategies for measuring IgT responses in gut, skin and gills, will greatly facilitate the evaluation and understanding of the protective effects of fish vaccines and immunostimulants. In turn, this will have an impact in the future design of more effective vaccines and immunostimulants that stimulate not only systemic, but also mucosal protective immune responses. Moreover, our unique ability to evaluate simultaneously rainbow trout B and T-cell subsets is expected to produce groundbreaking data on the dynamics of integrated B- and T-cell responses and their regulation during the course of infection and vaccination. In turn, this will deliver much needed knowledge on how immunity is induced both in systemic and mucosal sites, thus providing further rationale for the development of more effective vaccines and immunostimulants.

Abstracts for Oral Presentations

1) Special Session: Assessing the Impacts of Pathogens on Wild Fishes

1a. Mollusc pathogen surveillance to inform zonation in the U.S. Pacific Northwest

Lori Gustafson[1]*, Ralph Elston[2], Rebecca Jones[1]

[1]USDA APHIS Veterinary Services, Fort Collins, CO 80526 USA lori.l.gustafson@aphis.usda.gov, rebecca.d.jones@aphis.usda.gov
[2]AquaTechnics Inc., Sequim, WA 98382 USA ralph@aquatechnics.com

Mollusc aquaculture practices often rely to some extent on open systems, whether for growing beds, water source, broodstock or feed. As such, the health of the farmed animals is interwoven with the health of their surroundings; and facility disease freedom status is dependent, in part, on the health status of the surrounding zone. Consequently, zoning by pathogen status can greatly facilitate mollusc health management and trade, as decision makers aim to ensure, and protect, the health status of both farm and wild environments. However, disease freedom determination in wild populations is often complicated by the coordination required between co-managers (jurisdictions or agencies), limited resources for wild population surveillance, or pathogen introduction risks that may limit the longevity of disease freedom claims through time. We present here the results of a collaborative effort between Federal, State, private laboratory and industry partners to compile and analyze surveillance data, expert opinion on pathogen introduction risk, and ecologic knowledge to inform zoning for mollusk pathogens in the NW Pacific United States. Assessed pathogens include *Bonamia exitiosa*, *Bonamia ostreae*, *Haplosporidium nelsoni*, *Marteilia refringens*, *Marteilia sydneyi*, *Marteiliodes chungmuensis*, *Mikrocytos mackini*, ostreid herpesvirus-1 µvar (OsHV-1 µvar), *Perkinsus marinus*, *Perkinsus olseni*, and *Vibrio tapetis*. Recommendations for ongoing surveillance and Federal-State-industry partnerships to maintain zonation status are described.

1b. Review on assessing the impacts of chronic parasite infections in wild salmonids

Jayde A. Ferguson[1*], Philippe A. Rossignol[2], Michael L. Kent[3]

[1]Alaska Department of Fish and Game, Commercial Fisheries Division, Fish Pathology Laboratory, 333 Raspberry Road, Anchorage, AK 99518 USA jayde.ferguson@alaska.gov
[2]Department of Fisheries and Wildlife, Oregon State University, 104 Nash Hall, Corvallis, OR 97331 USA rossignolconsulting@yahoo.com
[3]Department of Microbiology, Oregon State University, 220 Nash Hall, Corvallis, OR 97331 USA michael.kent@oregonstate.edu

Parasites may be a significant source of mortality in wild fish populations, but this assessment has several challenges. Impacts tend to be density dependent, which can be difficult to estimate due to the typically aggregated distribution of parasites among hosts. In 1984, R.J.G. Lester reviewed the common methods used for estimating parasite-associated mortality in wild fishes, many of which require temporal observations of the same host population. Fish are often inaccessible and the most impacted fish are likely to die without detection. Nevertheless, there have been studies reporting that wild fish with higher parasite intensities have a higher mortality rate. Studying host–parasite systems in wild salmon presents two additional challenges: (i) many populations are listed as threatened, making it difficult to obtain large sample sizes, and (ii) parr grow and develop usually as separate, multiple, sub-populations in freshwater, and then migrate to the ocean as a randomly mixed population of smolts, making temporal observations of the same cohort problematic. An alternative technique is to conduct a retrospective analysis by predicting the parasite distribution in host populations based on observed data from lightly infected animals, as originally proposed by H.D. Crofton in 1971. He showed how analyzing the negative binomial distribution can estimate mortality associated with macroparasitism. When the observed parasite burden is lower than predicted, a likely explanation is that heavily infected hosts are more predisposed to mortality and are lost from the population. Crofton's technique relies on approximating the distribution, but has become widely accepted and used in theoretical and empirical models. However, it is descriptive, having at best indirect biological interpretation and is somewhat arduous to perform. We recently published on a new parsimonious mathematical model, the congestion rate, which was originally proposed by our co-author W. Koketsu in his 2004 M.Sc. thesis. This technique improves some of the limitations of Crofton's model and is based on Malthusian parameters rather than probability theory. An entirely different, yet complimentary approach is to conduct controlled laboratory studies on infected fish to evaluate various fitness endpoints linked to survival under natural conditions. Here, we provide a review on assessing the impacts of chronic parasite infections in wild salmonids.

1c. Pathogens and links to prespawning mortality in Chinook salmon from the Willamette River, Oregon.

Michael L. Kent [1,3]*, Michael E. Colvin[2], James T. Peterson[2,4], Brian Dolan[3], Carl B. Schreck[2,4]

[1]Department of Microbiology, Oregon State University, 220 Nash Hall, Corvallis, Oregon 97331, USA
[2]Department of Fisheries and Wildlife, Oregon State University, 104 Nash Hall, Corvallis, Oregon 97331, USA
[3]Department of Biomedical Sciences, Oregon State University, 104 Nash Hall, Corvallis, Oregon 97331, USA
[4]Oregon Cooperative Fish and Wildlife Research Unit, U.S.G.S. Department of Fisheries and Wildlife, Oregon State University, 104 Nash Hall, Corvallis, Oregon 97331, USA

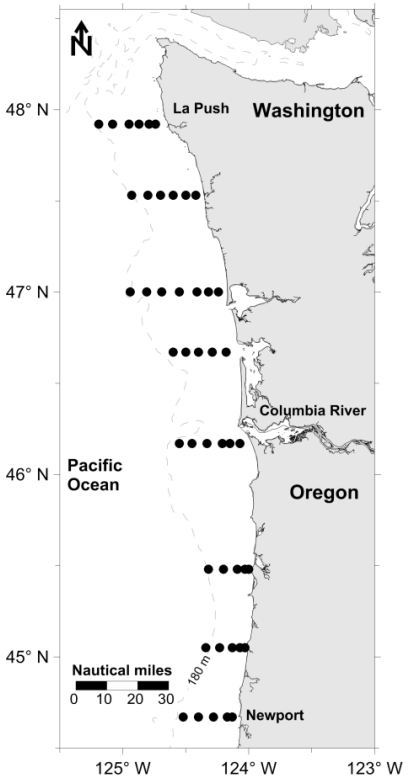
Up to 90 percent of adult Chinook salmon, *Oncorhynchus tshawytscha* die before spawning after transport past dams in the Willamette River, Oregon. We have documented numerous pathogens and lesions in these prespawn mortalities (PSM) for the last four years, and compared these to patterns to those seen in healthy fish collected in the summer, and with post-spawned fish from the river and the Willamette Hatchery. Given the variety of pathogens and associated lesions in found in adult salmon, our primary diagnostic method has been histopathology as this does not a priori designate specific diseases. We have examined approximately 500 Chinook salmon representing the following categories: 1) PSM summer fish, 2) healthy summer fish, 3) fish that survived to spawn in the autumn in either the river or at the Willamette Hatchery. The most common infections observed were *Nanophyetus salmincola*, gill metacercariae (*Apophallus* or echinostomes), *Parvicapusla minibicornis, Ceratomyxa shasta, Aeromonas salmonicida,* and *Renibacterium salmoninarum*. Based on four years of data, we see some general trends emerging: 1) PSM fish consistently have heavier infections than healthy midsummer fish for some pathogens, but 2) PSM fish from summer show patterns similar to post-spawned fish from the river or the Willamette Hatchery in the fall. One explanation for the latter is that is that PSM fish perhaps return earlier than survivors, become infected at a faster rate, and thus die in the summer rather than after spawning. Alternatively, the high pathogen burdens could be caused by the PSM fish being more immune compromised than corresponding healthy fish at the same times in the summer. Co-infecting pathogens are also hypothesized to contribute to prespawning mortality. We used a hierarchical multi pathogen co-infection model to evaluate if co-infections occur randomly or not at the host and organ levels. Preliminary model results indicate that *N. salmincola* and gill metacercariae infections occur together more often than expected early in the run.

1d. The challenges of studying pathogen effects on juvenile Chinook salmon and coho salmon in the nearshore Pacific Ocean

Kym C. Jacobson[1]*, Todd Sandell[2], Mary Beth Rew[3]

[1]NOAA Fisheries, Northwest Fisheries Science Center, Newport, OR 97365 USA Kym.Jacobson@noaa.gov
[2]Wild Fish Conservancy, Duvall, WA 98019 USA todd@wildfishconservancy.org
[3]Cooperative Institute of Marine Resources Studies, Oregon State University, Newport, OR 97365 USA

In 1998 NOAA Fisheries' Northwest Fisheries Science Center began a program to study the early ocean ecology and potential causes of mortality of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) and coho salmon (*O. kisutch*) during early marine residence. Sampling occurred in May, June and September off the coasts of Oregon and Washington. Stations generally sampled in May, June and September are shown on the map below. As part of the collaborative project we conducted an analysis of pathogens of juvenile salmon during early marine residence from 1999 through 2012. We focused on pathogens common in freshwater and estuaries that could affect juvenile Pacific salmon during their initial months of marine residency. The research platform, the fish, and the target pathogens bring different challenges to studying the potential effects of pathogens on these juvenile salmon; during this critical period of their life cycle. This presentation will cover the challenges of working with fish that are listed as endangered or threatened, are of natural and hatchery origin, as well as sampling at sea, and documenting effects of pathogens on migrating fish.



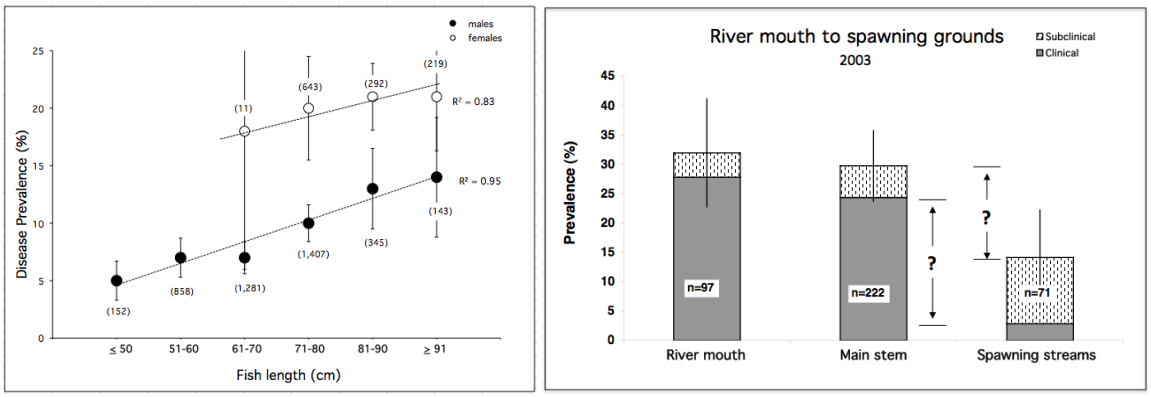
1e. Impact of *Ichthyophonus* sp. on pre-spawn Chinook salmon from the Yukon River

Richard M Kocan[1]*, Paul K Hershberger[2]

[1]School of Aquatic & Fishery Science, University of Washington, Seattle, WA USA kocan@uw.edu

[2]U.S. Geological Survey, Western Fisheries Research Center, Marrowstone Marine Field Station, 616 Marrowstone Point Road, Nordland, Washington USA pkhershberger@usgs.gov

Chinook salmon returning to the Yukon River on their annual spawning migration offered a unique opportunity to study the progression of ichthyophoniasis in a wild population over a 12-year period. Because infections occurred prior to fish entering the River, fish did not feed during adult freshwater migration, and no new recruits joined the population during the migration, we were able to track changes in infection and disease prevalence without the variables of reinfection and transmission to new naïve recruits. We focused on temporal and zoogeographical changes in infection and disease during the annual migration. Infection prevalence remained relatively unchanged in the Yukon River and Tanana River (a major Alaskan tributary), however clinical disease increased steadily throughout the migration. Annually, infection and disease prevalence was higher in females than males, and there was a direct correlation between fish size and infection/disease prevalence for both sexes. Conversely, post-spawn fish showed a significant decrease in both infection and disease relative to cohorts sampled in the mainstem, presumably due to case-related mortality as fish approached the spawning grounds; this same phenomenon was also observed at a third site at Whitehorse, Yukon Territory.



From 2001-2003 60% of *Ichthyophonus*-infected Chinook salmon from the Yukon and Tanana Rivers died prior to spawning, representing 20% of all Chinook salmon returning to the during those 3 years. Because pre-spawn mortalities do not contribute to the next generation, losses of this magnitude represent a serious impediment to the recovery of this rapidly declining resource.

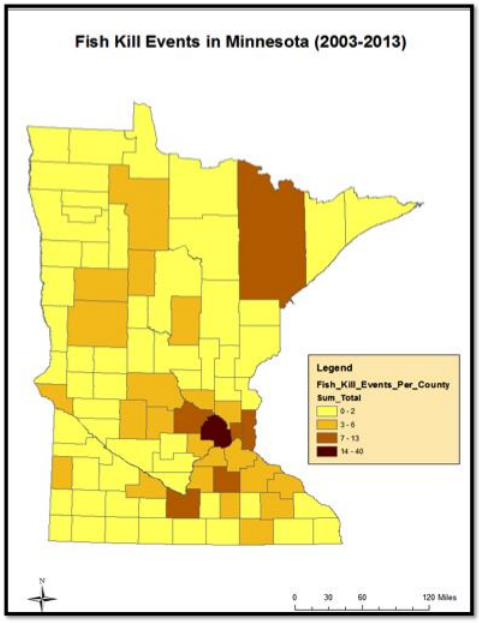
1f. Retrospective investigation of fish kill events in Minnesota

Irene Bueno[1], Nicholas B. D. Phelps[2]*

[1]Veterinary Medicine Graduate Program, College of Veterinary Medicine, University of Minnesota, St. Paul, MN USA

[2]Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN USA, phelp083@umn.edu

Fish kills in Minnesota are widespread and result in localized accumulation of dead fish, leading to public health concerns, costly cleanup, and potentially significant declines in fish populations. While some fish kill events are the result of natural processes, they can serve as one potential marker for emerging threats, such as environmental degradation, impaired immune function, infectious diseases, and climate change. Understanding trends overtime can provide proactive management strategies to mitigate these risks. To that end, a retrospective analysis, from 2003-2013, was performed of all reported wild fish kills in two databases managed by the MN Department of Natural Resources. From 2003-2013, a total of 341 unique fish kill events were reported. There was 10% overlap (n=24) between the two databases. Approximately 17% of the events were missing critical information, such as species affected or location. The most common species in the fish kill events were within the Centrarchidae (44.15%), Cyprinidae (18.71%), and Ictaluridae (13.74%) families and a significant peak in June. The leading reported cause of death was environmental factors (i.e. summer kill, stress, low DO, etc). Fish kill events were not equally distributed across the state (Figure 1). Based on statistical analysis of the available data, reported fish kill events were correlated with higher human population density (Moran's Index = 0.21, $p = 0.02$). There was no correlation with lake density (Moran's Index = -0.07, $p < 0.05 = 0.58$). This retrospective study was highly informative, showing the potential of these data to monitor trends overtime. However, several gaps regarding fish kill investigations were identified, including underreporting of fish kill events, incomplete and inconsistent data collection, potential reporting bias, and few laboratory-confirmed causes for the fish kill events. Due to these limitations, interpretation of retrospective results must be done with caution. The development of an online user-friendly and standardized database would have the potential to streamline data entry, increase reporting, allow for real-time monitoring and response, and future research. Increasing the frequency of fish kill reporting will be a way to increase confidence in the results and correlate fish kill events with potential risk factors for improved fish health management.



2) General Session: Bacteria I

2a. Virulence and immunogenicity of *Edwardsiella ictaluri* ferric hydroxamate uptake mutants

Hossam Abdelhamed*, Jingjun Lu, Mark L. Lawrence, Attila Karsi

Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762-6100, USA hossam_amr8@yahoo.com, jlu@cvm.mssate.edu, lawrence@cvm.msstate.edu, karsi@cvm.msstate.edu

Edwardsiella ictaluri is a Gram-negative pathogen causing enteric septicemia in fish. Ferric iron is an essential micronutrient for bacterial survival, and bacterial pathogens use secreted hydroxamate type siderophores to chelate iron in host tissues. Siderophore-iron complexes are taken up by bacteria via the ferric hydroxamate uptake (Fhu) system. In *E. ictaluri*, the Fhu system consists of *fhuC, fhuD, fhuB,* and *fhuA* genes. However, the importance of the Fhu system in *E. ictaluri* virulence has not been investigated completely. Here, we present construction of *E. ictaluri fhuD* and *fhuB* mutants (*Ei*Δ*fhuD* and *Ei*Δ*fhuB*) by in-frame gene deletion and evaluation of the mutants' virulence and immunogenicity in channel catfish fingerlings. Our results indicated that deletion of the *E. ictaluri fhuD* and *fhuB* genes did not affect growth of *E. ictaluri* in both iron-replete and iron-depleted media. Using an experimental infection model by bath immersion, *Ei*Δ*fhuD* showed no attenuation while *Ei*Δ*fhuB* was highly attenuated compared with the parent wild type strain. Catfish immunized with *Ei*Δ*fhuD* and *Ei*Δ*fhuB* mutants and challenged with wild type *E. ictaluri* displayed high relative percent survival (100% and 93.1%, respectively). Our data indicates that the *fhuB* gene, but not the *fhuD* gene, contributes to *E. ictaluri* virulence.

2b. Investigations into the new taxa *Edwardsiella piscicida* and comparative genomic analysis with *Edwardsiella tarda* and *Edwardsiella piscicida*-like sp.

Stephen R. Reichley[1,2]*, Hasan C. Tekedar[2], Geoffrey C. Waldbieser[3], Michelle M. Banes[2], David J. Wise[1], Terrence E. Greenway[1], Lester H. Khoo[1,2], Attila Karsi[2], Mark L. Lawrence[2], Matt J. Griffin[1,2]

[1]Thad Cochran National Warmwater Aquaculture Center, P.O. Box 197, Stoneville, MS 38776 USA sreichley@cvm.msstate.edu, dwise@drec.msstate.edu, greenway@drec.msstate.edu, khoo@cvm.msstate.edu, griffin@cvm.msstate.edu

[2]College of Veterinary Medicine, Mississippi State University, P.O. Box 6100, Mississippi State, MS 39762 USA tekedar@cvm.msstate.edu, banes@cvm.msstate.edu, karsi@cvm.msstate.edu, lawrence@cvm.msstate.edu

[3]USDA-ARS Warmwater Aquaculture Research Unit, P.O. Box 38, Stoneville, MS 38776 USA geoff.waldbieser@ars.usda.gov

Edwardsiella spp. cause significant losses in cultured and wild fish throughout the world. Recent investigations into the genotypic and phenotypic variability of *Edwardsiella tarda* led to the adoption of a new species, *Edwardsiella piscicida*. Further phylogenetic analysis suggests the existence of yet another taxonomic group (*Edwardsiella piscicida*-like sp.), which clusters independently of other *Edwardsiella* spp. based on 16S, *gyrB* and other gene sequences. In addition, research suggests *E. piscicida* is more prevalent in Mississippi catfish aquaculture than *E. tarda*. The goal of this project was to identify differences between the *Edwardsiella* spp. that can be exploited for more rapid phenotypic and genotypic differentiation. To this end, genomic DNA libraries from *E. piscicida* (S11-285), *E. piscicida*-like (LADL05-105) and *E. tarda* (FL95-01) were shotgun sequenced using Illumina technology at greater than 50X coverage. Sequence reads were trimmed and *de novo* assembled at 99% similarity using CLC Workbench 6.5 and Sequencher 5.2. The contigs were submitted to the Rapid Annotation using Subsystem Technology (RAST) prokaryotic genome annotation service of the National Microbial Pathogen Data Resource (http://rast.nmpdr.org). One hundred eighteen functional differences were identified between *E. piscicida* and *E. tarda*. Of these differences, 18 were related to carbohydrate metabolism, 4 were related to virulence, disease, and defense and 2 were related to iron acquisition and metabolism. The specific functional differences will be discussed in detail and additional comparisons between genomic characteristics will be discussed. Further studies will be directed at developing a differential media that will provide rapid and simple discrimination of the species as an alternative to the molecular techniques currently required to discriminate between these closely related pathogens.

2c. Inflammatory effects of *Edwardsiella ictaluri* lypopolysaccharide modifications in catfish gut

Javier Santander[1,2,3*], Jacquelyn Kilbourne[1], Jie-Yeun Park[1], Taylor Martin[1,3], Ignacia Diaz[1,4], Dale DeNardo[3], Roy Curtiss 3rd[1,3]

[1]Microbiology and Immunity Laboratory, Faculty of Sciences, Universidad Mayor, Huechuraba, Chile 8580745
[2]Center for Infectious Diseases and Vaccinology, The Biodesign Institute, Arizona State University, Tempe, AZ 85287
[3]School of Life and Sciences, Arizona State University, Tempe, Arizona 85287
[4]Department of Physics, Master Program in Nanoscience, Arizona State University, Tempe, Arizona 85287.

Bacterial lipopolysaccharides (LPS) are structural components of the outer membranes of Gram-negative bacteria and also are potent inducers of inflammation in mammals. Higher vertebrates are extremely sensitive to LPS but lower vertebrates, like fish, are resistant to their systemic toxic effects. However, LPS effects on the fish intestinal mucosa remain unknown. *Edwardsiella ictaluri* is a primitive member of the *Enterobacteriaceae* family that causes enteric septicemia in channel catfish (*Ictalurus punctatus*). *E. ictaluri* infects and colonizes deep lymphoid tissues upon oral or immersion infection. Both gut and olfactory organs are the primary sites of invasion. At the systemic level *E. ictaluri* pathogenesis is relatively well characterized, but our knowledge about *E. ictaluri* intestinal interaction is limited. Recently, we observed that *E. ictaluri* oligo-polysaccharide (O-PS) LPS mutants have differential effects on the intestinal epithelia of orally inoculated catfish. Here we evaluate the effects of *E. ictaluri* O-PS LPS mutants using a novel catfish intestinal loop model and compared it to the rabbit ileal loop model inoculated with *Salmonella* Typhimurium LPS. We found evident differences in rabbit ileal loop and catfish ileal loop responses to *E. ictaluri* and *S.* Typhimurium LPS. We determined that catfish respond to *E. ictaluri* LPS, but not to *S.* Typhimurium LPS. We also determined that *E. ictaluri* inhibits cytokine production and induces disruption of the intestinal fish epithelia in an O-PS dependent fashion. *E. ictaluri* wild type and Δ*wibT* LPS mutant caused intestinal tissue damage and inhibited pro-inflammatory cytokine synthesis in contrast to *E. ictaluri* Δ*gne* and Δ*ugd* LPS mutants. We concluded that the *E. ictaluri* O-PS subunits play a major role during pathogenesis, since that they influence the recognition of the LPS by the intestinal mucosal immune system of the catfish. The LPS structure of *E. ictaluri* mutants is need to understand the mechanism of interaction.

2d. Comparative studies between catfish and zebrafish strains of *Edwardsiella ictaluri*

Rui Wang[1]*, Joohyun Kim[2], Nayong Kim[2], Judy Wiles[1], John P. Hawke[1]

[1]Department of Pathobiological Sciences, School of Veterinary Medicine, 1909 Skip Bertman Drive, Louisiana State University, Baton Rouge, Louisiana, 70803 USA
[2]Center for Computation and Technology, 2054 Digital Media Center, Louisiana State University, Baton Rouge LA, 70803 USA

Edwardsiella ictaluri is known as the causative agent of enteric septicemia of catfish (ESC) in channel catfish, causing severe losses annually. It is known to comprise a homogenous group. However, recently identified *E. ictaluri* from zebrafish *Danio rerio* showed different characters, including autoagglutination in broth, different sizes of plasmids, different API codes and lacking of lipopolysaccharide recognition with monoclonal antibody Ed9. In addition, the catfish and zebrafish strains of *E.ictaluri* also exhibit their own host specificity. To study the mechanisms of host specificity of *E.ictaluri* from the catfish and zebrafish, the lipopolysaccharides (LPS) from both catfish and zebrafish strain were first purified and results showed that LPS of zebrafish strain lacked many of the bands present in LPS of catfish strain by SDS-PAGE. Secondly, gas chromatography/mass spectrometry (GC/MS), applied to analyze the glycosyl composition of these LPS samples, indicated that zebrafish isolates have more galactose and glucose while the catfish strain has more ribose, mannose and heptose; the glucuronic acid exists in the LPS sample of zebrafish strain but not in LPS sample of catfish strain. Meanwhile, genome sequencing of two strains LADDL 11-100 and LADDL 11-194 isolates from zebrafish were done to reveal the differences at the gene level. The genome sequences of both zebrafish strains were compared to a typical catfish strain LADL 93-146 and the single nucleotide polymorphisms (SNPs) analysis identified 8,501 and 8,708 SNPs respectively. Among these, 2,224 of non-synonymous SNPs in the coding regions, shared by both strains, were selected for further study. The non-synonymous SNPs were identified in the virulence factors, including LPS synthesis genes, flagella structural genes, et al. Further, protein structures were predicted and the differences were found in important virulence factors, eg. LPS synthesis proteins, outer membrane protein YaeT, proteins in type III secretion systems. Obviously, the results of SNPs analysis and the structural changes in virulence factors indicated that the mutations on the gene level may give rise to the host specificity. To sum up, we discovered the differences in the composition of LPS and the non-synonymous SNPs between catfish and zebrafish strains of *E. ictaluri*, and these findings provide important clues for illuminating the mechanisms of host specificity.

2e. Early intracellular trafficking of *Edwardsiella ictaluri* in channel catfish macrophages.

L.P. Dubytska[1]*, R. L. Thune[1,2]

[1]Department of Pathobiological Sciences Louisiana of Veterinary Medicine
ldubyt1@lsu.edu and thune @vetmed.lsu.edu
[2]School of Animal Science LSU Agricultural center, Louisiana State University Baton Rouge, LA

Edwardsiella ictaluri is a facultative intracellular Gram-negative bacterium causing enteric septicemia of catfish, a serious pathogen of farm-raised catfish in world-wide. We previously reported that *E. ictaluri* enters and multiplies rapidly within head kidney-derived channel catfish macrophages (HKDM) in a unique an *Edwardsiella* containing vacuole (ECV). Because, little is known about the development of the ECV, we investigated the recruitment of several vacuolar membrane markers to the ECV membrane. Shortly after bacterial uptake, ECVs acquired the early endosomal marker EEA1 and late endosomal marker Rab7, but not the lysosomal marker Lamp1. Our previous work demonstrated that the ECV was acidified by vacuolar ATPases and subsequently neutralized by the *E.ictaluri* urease enzyme (Baumgartner at al. 2014 Infection and Immunity). The absence of Lamp1 and the neutral pH of the ECV indicates that Rab7 recruitment to the ECV is not sufficient to promote their maturation to phagolysosomes. Shortly after acquiring EEA1 and Rab7, ECVs also contained the ER-resident protein calnexin and the golgi marker giantin. The presence of ER and golgi-associated material in the ECV likely involves recruitment of membranes for ECV growth and provides nutrition for bacterial replication.

Although 80% of the ECVs developed as described above, approximately 20% of the ECVs contained the autophagosomal marker LC3. The signal(s) that trigger autophagy of *E.ictaluri* are not clear, but it is possible that some dead bacteria are present in the culture used for infection of the macrophages or that a small fraction of bacteria damaged the ECV and activated the lysosomal repair system. This hypothesis is supported by the data showing that approximately 20% of the ECV also contained Lamp1.

2f. Studies on the *Edwardsiella ictaluri* Type Three Secretion System Effectors

Ronald L. Thune[1,2]*, Lidiya P. Dubytska[1]

[1]Department of Pathobiological Sciences, School of Veterinary Medicine
thune@vetmed.lsu.edu and ldubyt1@lsu.edu
[2]School of Animal Sciences, Louisiana State Agricultural Center,
Louisiana State University, Baton Rouge, LA 70803

We previously identified a Type III secretion system (T3SS) in *Edwardsiella ictaluri* that is essential for intracellular replication and virulence. We also identified seven T3SS effector proteins encoded in the *E. ictaluri* genome, and demonstrated active translocation to the host cell cytoplasm. In this work, the translocated effectors of the T3SS were evaluated for their role in pathogenesis. Individual effector mutants were constructed by over-lap extension PCR and allelic replacement. Briefly, the amino and carboxyl termini of each effector were amplified with linkers, then combined and used as a template for PCR that amplified Δeffector constructs. The deleted amplicon was cloned into pRE107, transformed into wild-type *E. ictaluri*, and selected on ampicillin for a single crossover integration of the plasmid into the chromosome. Resulting colonies were replica-plated to BHI-sucrose, inducing expression of *sacB* on pRE107, which is lethal in Gram negative bacteria. This resulted in selection of colonies in which a double crossover event excised the plasmid, which produced both wild-type and deleted genotypes. Mutant constructs were identified by PCR and confirmed by DNA sequencing. One mutant was significantly more virulent than the wild-type, while one was statistically the same as the wild-type. Four mutants were moderately attenuated and not significantly different from each other. The one most attenuated mutant was not significantly different from the media only controls or the T3SS knock-out. There was no significant difference in initial uptake of the mutants into HKDM or invasion into catfish. Only three mutants were significantly reduced in their ability to replicate in HKDM. All of the most attenuated mutants persisted at least nine days in the head-kidney, and some for at least 13 days.

3) Special Session: Amoeba and Tuna Health

3a. An optimized real-time PCR for the detection and quantification of viable *Neoparamoeba perurans* using propidium monoazide

Andrew R Bridle*, Kinglsey Tam, Philip Crosbie, Barbara F Nowak

National Centre for Marine Conservation and Resource Sustainability, University of Tasmania, Launceston, TAS, AUS Andrew.Bridle@utas.edu.au

The protozoan parasite *Neoparamoeba perurans* is the causative agent of amoebic gill disease (AGD) and an emerging threat to the aquaculture of marine finfish species worldwide. Despite recent advances by our research group, culturing this pathogen from sources other than the host remain problematic. Therefore current environmental detection methods rely on molecular techniques namely, polymerase chain reaction (PCR) and in situ hybridization (ISH). Previously we developed a qPCR assay able to detect a single 18S rRNA gene copy and readily detect a single *N. perurans* trophozoite filtered from a volume of water. Herein, we describe an optimized DNA extraction technique and quantitative real-time PCR that reduces the chance of acquiring a false negative from water samples containing large amounts of naturally occurring PCR inhibitors. Furthermore, we describe a modification of the assay allowing the discrimination of viable *N. perurans* by real-time PCR using propidium monoazide.

The optimized qPCR method was applied to seawater samples collected from both an experimental AGD infection tank and a variety of environmental sites including those used to culture Atlantic salmon (*Salmo salar* L.) in Tasmania, Australia. Amoebae were detected and quantified from sites in and closely surrounding cage culture of Atlantic salmon.

3b. Evidence of immune and inflammatory processes in the gills of AGD-affected Atlantic salmon, *Salmo salar* L.

Ylenia Pennacchi, Melanie J Leef, Philip Crosbie, Barbara F Nowak, Andrew R Bridle*

National Centre for Marine Conservation and Resource Sustainability, University of Tasmania, Launceston, TAS, AUS Andrew.Bridle@utas.edu.au

Amoebic gill disease (AGD) is a disease caused by the ectoparasite *Neoparamoeba perurans* which affects several cultured marine fish worldwide. The characterization of pro-inflammatory and immune related genes at the mRNA level in AGD-affected Atlantic salmon gills was performed at 10 days post-inoculation using 2D quantitative RT-PCR, a method of mapping transcriptional responses in tissues. This method is a variant of traditional quantitative RT-PCR whereby RNA was extracted and reverse transcribed from gill samples obtained using a biopsy punch from designated areas of the gill from fish. In theory this method could be used to map the transcriptional responses throughout the entire gill with the resolution of the mapped changes dependent on the number and size of gill subsamples. In the present study we restricted our subsample area to eight 2 mm biopsy punches from the dorsal portion of the second left gill arch of each individual fish.

The genes of interest were IL-1β, TNF-α, TCR-α chain, CD8, CD4, MHC-IIα, MHC-I, IgM and IgT. A significant increase in expression of the mRNA of all the genes was observed in the gills of AGD-affected fish. Furthermore, a correlation analysis between CD8-α and TCR-α showed that the mRNA expression of these two genes was strongly positively correlated ($r = 0.9$). Contrary to previous studies, our data suggest that the parasite, *N. perurans*, elicits a classical inflammatory response in the gills of AGD-affected fish and indicates that the mRNA expression of immune genes within gill lesions misrepresents the cellular immune response in the gills during AGD.

3c. Amoebic infection in the endangered Rio Grande silvery minnow, *Hybognathus amarus*

Wolfgang K. Vogelbein[1], Teresa D. Lewis[2]*, Alison M. Hutson[3], Kevin J. Buhl[4], David T. Gauthier[5]

[1]Department of Environmental and Aquatic Animal Health, Virginia Institute of Marine Science, College of William and Mary, Rt. 1208, Gloucester Point, VA 23062 USA wolf@vims.edu
[2]U.S. Fish and Wildlife Service, Southwestern Native Aquatic Resources and Recovery Center, P.O. Box 219, Dexter, NM 88230 USA teresa_lewis@fws.gov
[3]Los Lunas Silvery Minnow Refugium, New Mexico Interstate Stream Commission, 1000 Main St. NW, Building H, Los Lunas, NM 87031 USA alison.hutson@state.nm.us
[4]U.S.G.S. Columbia Environmental Research Center, Yankton Field Station, 31247 436th Avenue, Yankton, SD 57078 USA kevin_buhl@usgs.gov
[5]Department of Biological Sciences, Old Dominion University, Norfolk, VA 23529 USA dgauthie@odu.edu

The Rio Grande silvery minnow (RGSM), *Hybognathus amarus*, is an endangered fish, currently found in only ~5% of its historic range in the southwestern United States. Three captive propagation facilities in New Mexico culture RGSM to meet U.S. Fish and Wildlife Service recovery goals for the species and additional facilities culture RGSM for research purposes. In 2012, staff from the Los Lunas Silvery Minnow Refugium (LLSMR) observed abnormal spinning behavior and low level chronic mortalities in RGSM at their facility. Necropsy identified no gross clinical signs and standard disease diagnostic tests (AFS Fish Health Section Blue Book) were negative for viruses culturable on EPC, CHSE-214, and FHM cell lines. No pathogenic bacteria or other microbial pathogens were isolated and no internal or external protozoan or metazoan parasites were observed in any of the fish. Whole body histological exam was negative for pathogens or significant pathology. Additional fish were processed in a second attempt to identify a cause of the behavioral signs and mortalities, as there was considerable concern regarding the suitability of these fish for stocking purposes. Diagnostic assays for microbial pathogens were negative but histopathology indicated significant granulomatous inflammation associated with putative histozoic amoebic infection in the loose connective tissues, primarily surrounding the brain. In 2014, abnormal spinning behavior and mortality was observed in a research population of RGSM at the USGS Columbia Environmental Research Center, Yankton Field Research Station. Whole body histology exam confirmed these fish were also infected with histozoic amoebae, although the host inflammatory response was greatly diminished compared to that of fish reared at the LLSMR. The identity, sources, and adverse health impacts of this amoeba are unknown and currently under investigation.

3d. SYBR, TaqMan, or both: highly sensitive, non-invasive detection of *Cardicola* blood fluke species in southern bluefin tuna (*Thunnus maccoyii*)

Mark Polinski, Dylan Belworthy Hamilton, Barbara F Nowak, Andrew R Bridle*

National Centre for Marine Conservation and Resource Sustainability, University of Tasmania, Launceston, TAS, AUS Andrew.Bridle@utas.edu.au

Three species of blood fluke from the genus *Cardicola* are known to parasitize and cause disease in Bluefin Tunas – *C. forsteri*, *C. orientalis*, and *C. opisthorchis*. Although initially believed to be separated by geography and host specificity, recent identification of at least two *Cardicola* spp. concurrently present within all three Bluefin species has raised questions concerning pathogenicity, relative abundance, and distribution of these parasites within Bluefin populations.

Here, we present sensitive and differential real-time qPCR nucleic acid detection of these *Cardicola* spp. by targeting the ITS2 region of the parasite rDNA for PCR amplification. A limit of sensitivity was achieved to be between 1-5 genome copy equivalents for each of the three *Cardicola* species tested without cross-species or host genomic amplification. Similar sensitivity was further achieved in the presence of up to 20 ng/µL non-target host gDNA using SYBR Green chemistry alone, or in the presence of up to 160 ng/µL host gDNA through the utilization of a TaqMan probe common-reporter detection system. These methods were subsequently used to positively identify both *C. forsteri* and *C. orientalis* DNA in preserved samples of serum, gill, and heart from ranched Southern Bluefin Tuna Thunnus maccoyii. Both methods were more sensitive for positively and differentially identifying the presence of *Cardicola* spp. than either histological or heart-flush microscopy techniques previously employed, and also possess the ability to be applied in non-lethal blood sampling of these highly-valued fish.

This is the first report for rapid and differential molecular quantitative detection of *Cardicola*, and opens the potential for effective monitoring of infection in cultured bluefin populations. Further, it is anticipated that the use of SYBR Green for melt-curve analyses in conjunction with a common-reporter TaqMan assay will present a flexible, accurate, and cost-effective approach for differential detection of a variety of other pathogens in future.

3e. Application of molecular techniques to identify immune / stress associated gene transcripts in bluefin tuna and their subsequent *in vitro* expression

Mark Polinski*, Andrew Bridle, Barbara Nowak

National Centre for Marine Conservation and Resource Sustainability, Australian Maritime College, University of Tasmania, Launceston, Tasmania, Australia mark.polinski@utas.edu.au

Bluefin tuna (*Thunnus* spp.) are a globally threatened genus of fish that currently constitutes one of the most economically important food fisheries in the world. Intensive culture efforts have intensified in recent years to maximize fishery profitability and mitigate market dependency on wild stock; yet little is currently known concerning health and immune functions of these highly specialized organisms and best culture practices continue to be investigated. In an attempt to aid future aquaculture interests and to provide general tools for identifying immune responses and disease status for this group of fishes, we have investigates a number of immune and disease-identifying aspects regarding bluefin tuna through the use of molecular laboratory techniques centering on quantitative PCR. In this presentation, we will focus on our identification of 20 immune, stress, or growth related mRNA sequences of bluefin and subsequent *in vitro* explorations regarding the utility of selected immune and stress associated genes as biomarkers in bluefin through gene expression analysis. Our findings suggest that temperature exerts influence in the timing but not the degree of an innate inflammatory response in bluefin tuna and that different cell populations have differential responsiveness to heat shock in this heterothermic species. Further, *E. coli* LPS stimulation of Southern bluefin leukocytes failed to induce host Hsp70; yet, increased inflammatory signaling was observed in following a combined heat shock/LPS stimulation, suggesting a time specific 'cross-talk' between these signaling pathways during immune and stress associated responses. A strong correlation between Hsp70 and IL-8 transcriptional expression was also observed following LPS/heat shock stimulation of leukocytes and five potential heat shock response elements were subsequently identified on the gene promoter region of IL-8 indicating that heat shock co-activation of this chemokine previously identified in mammals is also likely present in fish.

3f. Transcriptional immune response of cage-cultured Pacific bluefin tuna during infection by two *Cardicola* blood fluke species

Mark Polinski[1]*, Sho Shirakashi[2], Andrew Bridle[1], Barbara Nowak[1]

[1]National Centre for Marine Conservation and Resource Sustainability, Australian Maritime College, University of Tasmania, Launceston, Tasmania, Australia mark.polinski@utas.edu.au
[2]Fisheries Laboratory, Kinki University, Wakayama, Japan

Infections by two blood fluke species, *Cardicola orientalis* and *Cardicola opisthorchis*, currently present the greatest disease concern for the sea-cage culture of Pacific bluefin tuna (PBT) - a species of high global economic importance and ecological concern. In this presentation, we describe our methods for rapidly, quantitatively, and differentially identifying infections by these two parasite species in cultured PBT as well as associated host immune responses. Using real-time qPCR, we were successful in quantitatively detecting parasite specific DNA from within host blood, gill, and heart tissues; positively identifying parasitic infections 44 days earlier than microscopy methods previously employed. Parasite-specific DNA of *C. orientalis* and *C. opisthorchis* became prevalent in both heart and gill of PBT within two months of sea-cage culture, which was only mitigated by the administration of anthelmintic Praziquantel. Nevertheless, fish were observed to mount an organ specific transcriptive immune response during infection that mirrored the relative quantity of pathogenic DNA load. In heart, significant (3-6 fold) increases in IgM, MHC2, TCRβ, and IL-8 transcription was observed in infected fish relative to uninfected controls; whereas in the gills only IgM transcription was observed to be induced (11 fold) by infection. Interestingly, the relative quantity of IgM transcription in gill was highly correlated to the relative abundance of *C. orientalis* but not *C. opisthorchis* DNA, even though this organ showed high prevalence of DNA from both parasite species. Taken together, these findings indicate that although ineffective at combating infection during primary exposure, a cellular immune response is mounted in PBT as a potential rejoinder to future *Cardicola* exposure, particularly against *C. orientalis*. Additionally, the preliminary evidence for mechanism of action of Praziquantel as an anthelmintic in bluefin tuna, as well as its role as an immunostimulant and importance in treating primary parasite exposure during tuna culture will be discussed.

4) Special Session: Interactions between Wild and Cultured Fishes

4a. Disease management mitigates risk of pathogen transmission from farmed salmon

Simon R M Jones[1,*], David Bruno[2], Lone Madsen[3], Edmund J Peeler[4]

[1] Fisheries and Oceans Canada, Pacific Biological Station, 3190 Hammond Bay Road, Nanaimo, British Columbia, Canada V9T 6N7 simon.jones@dfo-mpo.gc.ca
[2]Marine Scotland Science, 375 Victoria Road, Aberdeen AB11 9DB, Scotland david.bruno@scotland.gsi.gov.uk
[3]Technical University of Denmark, National Veterinary Institute, Bülowsvej 27, 1870 Frederiksberg C, Denmark loma@vet.dtu.dk
[4]Cefas, Barrack Road, Weymouth, Dorset, DT4 8UB UK ed.peeler@cefas.co.uk

Open marine net pens facilitate virus and sea lice transfer occasionally leading to infections and outbreaks of disease in farmed salmon. A review of three salmon diseases (infectious salmon anaemia, pancreas disease and salmon lice) shows that increased risk of exposure to neighbouring farms is negatively related to distance from and positively related to biomass at the source of infection. Epidemiological techniques integrating data from oceanography, diagnostics, pathogen shedding rates and viability contribute to improved understanding of pathogen transmission pathways among farms and permit the designation of areas of risk associated with sources of infection. Occupation of an area of risk by susceptible fish may increase the likelihood of exposure, infection and disease. Disease mitigation in mariculture occurs at two scales: area-based (coordinated stocking, harvesting and fallowing) and farm-based (vaccination, early pathogen detection, veterinary prescribed treatments and depopulation or early harvest in the event of viral disease). Collectively, implementation of mitigation measures results in virus disease outbreaks of shorter duration with lower mortality and therefore reduce the likelihood of pathogen transmission. In contrast, the mitigation of sea lice transmission is less likely to be effective in some areas due to the loss of parasite sensitivity to therapeutants and to the absence of treatment when parasites occur below management thresholds. Risk of pathogen spillback to wild populations is estimated from epidemiological data however validation efforts using direct surveillance of wild populations require ongoing commitment.

4b. Trafficking of Viral Hemorrhagic Septicemia Virus from wild to farmed fish

Kyle A. Garver*[1], Jan Lovy[2], Paul K. Hershberger[3]

[1]Fisheries and Oceans Canada, Pacific Biological Station, Nanaimo, British Columbia, Canada, Kyle.Garver@dfo-mpo.gc.ca.
[2]New Jersey Fish & Wildlife, Fish & Wildlife Health & Forensics, Oxford, New Jersey, USA Jan.Lovy@dep.state.nj.us
[3]U.S. Geological Survey, Marrowstone Marine Field Station, Nordland, Washington, USA. phershberger@usgs.gov

Viral hemorrhagic septicemia virus (VHSV) is an aquatic rhabdovirus that is highly pathogenic and can infect a diverse range of fish species in marine, estuarine, and freshwater environments of the Northern Hemisphere. In the eastern North Pacific Ocean, VHSV genotype (IVa) has been reported for over 25 years where it is extremely virulent in populations of Pacific herring *Clupea pallasii* (Valenciennes) and sardine *Sardinops sagax,* yet also infects wild Pacific salmon and trout species often without disease. Additionally in this geographic area, VHSV-IVa has been identified in cultured Atlantic *Salmo salar* and Chinook *Oncorhynchus tshawytscha* salmon where net-pen aquaculture sites share coastal waters with virus endemic species. Typing of virus isolates from farmed fish revealed a close genetic linkage with VHSV-IVa from wild Pacific herring, providing evidence for virus trafficking from wild to farmed fish. Further, virus transmission between species was simulated empirically when Atlantic salmon and Pacific herring were exposed to VHSV through cohabitation studies. These laboratory studies will be discussed in the context of viral transmission, adaptation, and trafficking among populations of farmed salmonids and wild marine reservoirs.

4c. Simulating water-borne pathogen transport in the Broughton Archipelago and Discovery Islands, Canada

Mike Foreman[1]*, Dario Stucchi[2], Kyle Garver[3], Ming Guo[4], Peter Chandler[5], John Morrison[6], Darren Tuele[7]

[1]Institute of Ocean Sciences, Fisheries and Oceans Canada, Sidney BC, V8L 4B2 Canada mike.foreman@dfo-mpo.gc.ca
[2]Institute of Ocean Sciences, Fisheries and Oceans Canada, Sidney BC, V8L 4B2 Canada dario.stucchi@dfo-mpo.gc.ca
[3]Pacific Biological Station, Fisheries and Oceans Canada, Nanaimo BC, Canada kyle.garver@dfo-mpo.gc.ca
[4]Institute of Ocean Sciences, Fisheries and Oceans Canada, Sidney BC, V8L 4B2 Canada ming.guo@dfo-mpo.gc.ca
[5]Institute of Ocean Sciences, Fisheries and Oceans Canada, Sidney BC, V8L 4B2 Canada peter.chandler@dfo-mpo.gc.ca
[6]Institute of Ocean Sciences, Fisheries and Oceans Canada, Sidney BC, V8L 4B2 Canada john.morrison@dfo-mpo.gc.ca
[7]Institute of Ocean Sciences, Fisheries and Oceans Canada, Sidney BC, V8L 4B2 Canada darren.tuele@dfo-mpo.gc.ca

High resolution ocean circulation and particle tracking models have been developed for the Broughton Archipelago and Discovery Islands and used to simulate the water-borne transport and mortality of sea lice (*Lepeophtheirus salmonis*) and the *infectious hematopoietic necrosis virus* (IHNV) emanating from Atlantic salmon (*Salmo salar*) net-pen farms in those regions. Historical simulations have been carried out for variable river discharge, wind, solar/UV radiation, and air temperature conditions to determine their direct impact on near-surface currents and water properties, and subsequent impact on pathogen dispersion and survival. Sea lice model outputs include time-varying concentration fields, dispersion footprints for individual farms, and farm-to-farm connectivity matrices. The IHNV model produces analogous concentration fields and, when combined with lab-determined shedding rates and minimum infective dosages, it can also estimate virus transmission among farms and to wild salmon. Applications to aquaculture management and farm siting issues will be briefly discussed.

4d. Policies and regulations to minimize pathogen transmission between farmed and wild fish

E.J. Peeler*

Centre for Environment, Fisheries and Aquaculture Science, Weymouth, DT4 7QN, UK.
ed.peeler@cefas.co.uk

There is an inherent risk of pathogen interaction between farmed and wild fish populations in open water aquaculture systems, including the risk of disease emergence, for example, infectious salmon anemia (ISA) and viral hemorrhagic septicemia (VHS) have their origin in wild fish populations. Both legislation and regulation have a role to play in minimizing these risks. In open water systems farmed and wild stocks little can be done to reduce pathogen transmission through water but the risk of disease emergence declines if farmed fish are healthy and unstressed. This is primarily achieved through Industry Codes of Practice (COP). VHS and pilchard herpesvirus emerged through the feeding of discarded marine fish to farmed salmonids (in Europe) and wild populations of *Sardinops sagax* (in Australia); the practice is now not permitted under many COP. Wild stocks are at risk of pathogen spillback from farmed populations and from pathogens carried by farmed fish released into the wild for angling. The scope of European Union (EU) legislation on aquatic animal health (Council directive 2006/88/EC) includes wild fish but is primarily concerned with regulating the movement of species susceptible to listed diseases. It allows MS to insist on a high health status for fish being stocked into the wild, irrespective of the infection status of the receiving water. EU Member States (MS) have implemented national measures to maintain freedom from diseases which could spill over from aquaculture and negatively impact wild fish, notably bacterial kidney disease (BKD) and *Gyrodactylus salaris* (Gs). However, the EU gave up its claim freedom from epizootic ulcerative syndrome (EUS), primarily a threat to wild, not farmed, species in Europe because the restriction on trade in ornamental fish was considered too onerous. Trade in ornamental aquatic animals clearly provides an important route of spread of EUS and other pathogens which may negatively affect wild populations (e.g. the spread of chytrid fungus and ranaviruses in amphibians). *Anguillocoloides crassus* is arguably the freshwater parasite that has caused the most significant impact on a European wild fish populations: eels (*Anguilla anguilla*). In UK, the Environment Agency seek to restrict the spread of *A. crassus,* and other introduced parasites, by authorizing and health checking movements of fish into the wild; but the level of enforcement is questionable. Sealice are an important threat to wild Atlantic salmon. Governments have attempted to minimize spillback from farmed salmon through both COP and regulation that requires monitoring and treatment when levels exceed a threshold (e.g. an average of 0.5 lice per adult female in Norway). Arguably these measures have not been entirely successful and more sophisticated approaches, applied at larger geographic scales and that take account of seasonal migration are needed. European legislation provides the basis for controls to maintain freedom from listed diseases which may be introduced with farmed fish and spread to wild populations. Industry codes of practice and domestic regulations are used to minimize disease interaction between wild and farmed populations.

4e. Sea lice infestation and climate change effects on marine survival of Atlantic salmon

Dave Jackson*

A fall in Atlantic salmon marine survival has prompted debate as to potential causes for this decline including the potential role of salmon aquaculture. Analysis of available published data from locations in Norway and Ireland was carried out to assess the impact of early infestation with sea lice on marine mortality of Atlantic salmon in the northeast Atlantic. The results of the study show that a mortality of approximately 0.5% is attributable to sea lice, over and above mortality from other sources. These results are in line with a long term study on an index stock in the west of Ireland.

Over the course of the study period from 1996 to 2009 changes in the climate of the Northeast Atlantic as measured by reference to the Atlantic Multi-decadal Oscillation (AMO) have mirrored changes in salmon survival. Over the period 1929 to 2009 salmon catch statistics have co-varied with the AMO.

While sea lice infestation does not seem to be linked to declining levels of survival in migrating salmonids over the study period, variations of climatic conditions in the North Atlantic, as measured by the AMO, show a strong relationship with variations in salmon catch and by inference with marine survival over nine decades.

4f. Sea Lice – Aquaculture and wild salmon in Norway

Ole Torrissen[1,2]

Institute of Marine Research, Bergen, Norway; Faculty of Biosciences and Aquaculture, University of Nordland, Bodø, Norway

Sea lice (*Lepeophtheirus salmonis salmonis*) infections and genetic impact of escaped farmed salmon is considered the most problematic risk factors associated with salmon farming in Norway, and concern about negative impacts on Atlantic salmon (*Salmo salar*) and sea trout (*Salmo trutta trutta*) has resulted in the postponement of new salmon licenses and lower output growth in the existing concessions.

Approximately 300 million Atlantic salmon and rainbow trout (*Oncorhynchus mykiss*) smolts are annually stocked in 1000 sites along the Norwegian coast giving an annual production of 1.3 million tons. The treatment threshold is an average of 0.5 female lice per fish based on weekly counting (t> 4 C).

Despite awareness of the possible population-reducing effects of lice infection of wild anadromous salmonids since the early 1990s, it is still unclear what effect specific infection pressures have on populations of salmon, sea trout or Arctic char (*Salvelinus alpinus*). However, there are evidence that lice can give negative population effects on the salmon
and sea trout and, based on the national sea lice monitoring program, it has been estimated a salmon or trout population-reducing effects some years.

5) General Session: Bacteria II

5a. *Francisella noatunensis* subps. *orientalis* immunogens detected by experimentally infected Nile tilapia (*Oreochromis niloticus*)

Esteban Soto[1]*, Matt Rogge[2], Jon Sauer[3], Jim Lawrence[3]

[1]Department of Biomedical Sciences, Ross University-School of Veterinary Medicine, St. Kitts, West Indies esoto@rossvet.edu.kn
[2]Department of Biology, University of Wisconsin-Stevens Point, Stevens Point, WI 54481 Matt.Rogge@uwsp.edu
[3]Department of Chemistry, University of Wisconsin-Stevens Point, Stevens Point, WI. 54481 Jon.S.Sauer@uwsp.edu, Jim.Lawrence@uwsp.edu

Francisella noatunensis subps. *orientalis* (*Fno*) is an emergent pathogen in the food and ornamental fish industry. It has a worldwide distribution, and no commercial vaccines are currently available. In order to gain a better understanding of immunodominant *Fno* antigens, proteomic analyses were performed to investigate possible alterations in the proteomes of *Fno* wild type or an *iglC* mutant that result from cultivation at different temperatures (25 or 30°C) or in different growing phases (early exponential, late exponential, early stationary). Pooled sera from five adult Nile tilapia experimentally infected with *Fno* by immersion were used in this study. Western blots revealed consistent detection of antigens between 20-30 kDa regardless of the bacterial strain, temperature or growth phase. Antigenic proteins were immunoprecipitated from *Fno* whole cell lysates using tilapia serum and separated by SDS-PAGE. Dominant protein bands were excised from SDS-PAGE gels and analyzed by using liquid chromatography-mass spectrometry. Over twenty proteins, including both housekeeping and virulence proteins, were identified by mass spectrometry. These data give insights into the adaptive immune response of tilapia upon *Fno* infection, and have potentially important implications for the rational design of non-living vaccines for piscine francisellosis.

5b. Disruption of the pathogenicity determinant protein A gene (pdpA) in *F. noatunensis subps. orientalis* results in attenuation and in greater susceptibility to oxidative stress.

Fionn Farrell[1], John Hansen[2], Oscar Illanes[1]; Ashutosh Verma[1], Esteban Soto[1]*

[1]Ross University School of Veterinary Medicine, P.O Box 334, West Farm, Basetterre, St. Kitts. ffarrell@rossvet.edu.kn, OIllanes@rossvet.edu.kn, AVerma2@Rossu.edu , ESoto@rossvet.edu.kn,

[2] U. S. Geological Survey-Western Fisheries Research Center, 6505 NE 65th St., Seattle, WA, 98115-5016 Seattle, Washington, United States of America. jhansen@usgs.gov

Francisella noatunensis subsp. *orientalis* (*Fno*) is an emergent warm-water fish pathogen and the causative agent of francisellosis in tilapia (*Oreochromis* sp). Previous studies have found that the intracellular growth loci (*iglC*) gene located within the *Fno* Pathogenicity Island (*Fno*PI) is important for both virulence and intramacrophage growth. The *Fno pdpA* gene encodes the *F. tularensis* pathogenicity determinant protein A-homologue. In *F. tularensis*, *pdpA* has been shown to be necessary for intracellular growth and virulence; however, the role of the *Fno pdpA* gene in the pathogenesis of piscine francisellosis is unknown. In this project, the virulence of two different marker-based *Fno pdpA* mutants (Δ*pdpA*-1 and Δ*pdpA*-2) generated in opposing polarity and its wild type parent strain was investigated following immersion challenges in hybrid red tilapia (*Oreochromis* sp.). Fingerlings were challenged with 6 different concentrations of each strain in 10 gallons of static fresh water at 25°C for 1h. Mortalities were recorded twice daily for a period of 21 days, and bacterial concentrations in the spleen were evaluated by bacterial plate counts in survivor fish 21 days post-challenge. Both mutant strains were highly attenuated when compared to the wild type parent strain. The Lethal Dose $(LD)_{50}$ in both mutants was greater than $2x10^6$ CFU per ml of water; whereas the LD_{50} in the WT strain was 891 CFU per ml of water. The polarity in the mutation caused greater attenuation in the Δ*pdpA*-2 strain when compared to the Δ*pdpA*-1. No Δ*pdpA*-2 bacterium was recovered from any of the surviving fish; resulting in a Infective Dose $(ID)_{50}$ greater than $1x10^7$ CFU/ml of water. On the other hand, the infective dose of the *Fno* Δ*pdpA*-1 and WT strain was estimated to be $6.8x10^6$ and <200 CFU/ml. Additionally, oxidative mediated killing was investigated utilizing hydrogen peroxide (at concentrations of 0 to 32 mM). Compared to the Δ*pdpA*, the wild type parental strain was more resistant to oxidative killing. This data identifies the *pdpA* gene product as an important virulence factor in *Fno*.

5c. Vibriosis in aquacultured red snapper

D. Jay Grimes, Reginald B. Blaylock*, Jeffrey M. Lotz, Eric A.L. Saillant

Gulf Coast Research Laboratory, The University of Southern Mississippi, Ocean Springs, MS USA
jay.grimes@usm.edu, reg.blaylock@usm.edu, jeff.lotz@usm.edu, eric.saillant@usm.edu

The red snapper (*Lutjanus campechanus*) is among the most popular recreational and commercial fishes in the Gulf of Mexico. As such, it is considered overfished by the National Marine Fisheries Service. USM's GCRL is developing the technology for culturing red snapper for use in stock enhancement as part of a comprehensive fisheries management strategy and as a foundation for future commercial culture of the species. Our focus is on intensive culture in closed, recirculating systems. Since 2007, we have experienced several outbreaks of *Vibrio vulnificus* that resulted in mortality in juvenile red snapper, and in all cases the strains were the clinical (C) type [1]. Unlike environmental types, C types contain an antiphagocytic capsule, survive in human serum, produce powerful siderophores, produce powerful tissue-degrading enzymes and produce a powerful endotoxin. In the 2007 and 2008 outbreaks, the fish exhibited a classic vibriosis, but in the recent 2013 outbreak the symptoms were very different. The only gross pathology was bile in the peritoneal cavity and leaking from the vent. Fish were emaciated posterior to the vent, but there was no distended abdomen or ascites. One of the moribund juveniles presented with bleeding from an eye socket. Antibiotic sensitivity testing was conducted and Baytril (enrofloxacin) was used to successfully treat the remaining juveniles.

[1] Rosche T.M., Y. Yano and J.D. Oliver (2005) A rapid and simple PCR analysis indicates there are two subgroups of *Vibrio vulnificus* which correlate with clinical or environmental isolation. Microbiol. Immunol. 49:381-389.

5d. The impact of virulent *Aeromonas hydrophila* (VAh) on Alabama's farm-raised catfish industry (2009-2013)

William (Bill) Hemstreet*
Auburn University, Alabama Fish Farming Center, 529 Centreville St., Greensboro, AL 36744 USA hemstwi@ auburn.edu

In the summer of 2009, Alabama's farm-raised catfish industry experienced severe losses due to Motile Aeromonas Septicemia (MAS). It was caused by what appeared to be a virulent strain of *Aeromonas hydrophila* that had been previously un-documented in the U.S. catfish industry. In the winter of 2010, the bacteria appeared to go dormant but re-emerged in late spring. It has continued to spread to almost all catfish farms in west Alabama and east Mississippi causing severe annual losses. Because historically MAS had not been a major problem in the catfish industry and because of the severity of the losses, a joint task force of land grant university and USDA researchers from the major catfish producing states pooled research efforts and determined that indeed *Aeromonas hydrophila* was the primary etiological agent of these extensive fish kills. It was also found to be very similar genetically to a strain of *Aeromonas hydrophila* documented in Asia. How and when it came to be in the U.S. is not known. In 2010 the task force expanded its research efforts to develop a genetic diagnostic test (PCR) to be used as a confirmative test to identify the virulent *Aeromonas hydrophila* (VAh). Since then a myo-inositol media (M-9I) has been developed that has been useful as a diagnostic as well as determinative test for this strain. VAh has become a major threat to an already fragile farm-raised catfish industry. Events leading up to the current situation as well as challenges facing the industry from this bacterium will be presented.

5e. Florfenicol: Correlation of pharmacokinetics in channel catfish (*I. punctatus*) with minimal inhibitory concentration values against *Aeromonas hydrophila* and the control of associated mortalities in INAD field studies

Patricia S. Gaunt[1]*, Cory Langston[1], Christopher Wrzesinski[2], Bonnie Johnson[3], Louis Crouch[2], Dana Gao[1], Paul Adams[2], Fangshi Sun[2], Richard Endris[2]

[1]Mississippi State University, College of Veterinary Medicine, 240 Wise Center Road, MS State, MS 39762 USA gaunt@cvm.msstate.edu, Langston@cvm.msstate.edu, xgao@cvm.msstate.edu
[2]Merck Animal Health, 556 Morris Avenue, Summit, NJ 07901 USA Christopher. wrzesinski@merck.com, Louis.crouch@merck.com, paul.adams@merck.com, fangshi.sun@merck.com, richard.endris@merck.com
[3]US Fish and Wildlife Service, 4050 Bridger Canyon Road, Bozeman, MT 59715 USA bonnie_johnson@fws.gov

Florfenicol (FFC) is currently approved to control mortality associated with enteric septicemia of catfish (ESC) and columnaris disease in catfish (*Ictalurus punctatus*) at a dose rate of 10 mg/kg one time a day for 10 days. Efforts are underway to gain its approval for control of mortality associated with *Aeromonas hydrophila* in catfish. The objective of this report is to demonstrate with a multidose pharmacokinetic study that the minimal steady state concentration (Css (min)) of FFC in plasma after administration one time a day for 10 days exceeds the FFC minimal inhibitory concentration 90 (MIC_{90}) values for *A. hydrophila* and therefore indicates susceptibility of these bacteria to FFC. These values were then correlated with the clinical performance of FFC in controlling mortality associated with *A. hydrophila* in catfish in Investigational New Animal Drug (INAD) field trials.

A multidose oral pharmacokinetic study was conducted at a dose rate of 10 mg/kg one time a day for 10 days to assess the plasma concentration of FFC in channel catfish. At various time points ranging from predose to 120 hours after the final administration, blood was sampled from individual fish (10 fish per time point). The plasma was assayed for FFC using an LC-MS/MS method. The pharmacokinetic modeling of the results was performed using the computer program WinSAAM.

Catfish in commercial ponds experiencing field outbreaks of *A. hydrophila* were treated under INAD permits with FFC-medicated feed at a dose rate of 10 mg/kg body weight. The MIC values of FFC were determined for *A. hydrophila* isolated in these studies by the broth microdilution assay using commercially-prepared plates.

Results of the pharmacokinetic studies demonstrated that after administration of FFC to catfish, the mean terminal half-life (t1/2), maximum concentration at steady-state (Css(max)), minimal concentration at steady-state (Css(min)), time of Css(max) Tmax, and Vc/F were 9.0 h, 9.72 µg/mL, 2.53 µg/mL,8 h, and 0.653 L/kg, respectively. In the INAD field studies, catfish ate the medicated feed, and mortality diminished after initiation of treatment. The MIC_{90} of FFC against *A. hydrophila* from the field outbreaks was 0.5µg/mL.

The FFC concentration vs. time curve demonstrated that for the 10 day treatment period the Css (min) was above the MIC_{90} against *A. hydrophila*. These values along with the clinical performance in the INAD field studies support a FFC label claim for control of mortality associated with *A. hydrophila* in catfish.

.

5f. Complete genome sequence of Aeromonas hydrophila ML09-119

Hasan C. Tekedar[1]*, Attila Karsi[1], Geoffrey C. Waldbieser[2], Mark R. Liles[3], Matt J. Griffin[1], Stefanie Vamenta[1], Tad Sonstegard[4], Mohammad Hossain[3], Steven G. Schroeder[4], Lester Khoo[1], Mark L. Lawrence[1]

[1]College of Veterinary Medicine, Mississippi State University, P.O. Box 6100, Mississippi State, MS 39762, USA tekedar@cvm.msstate.edu, karsi@cvm.msstate.edu, griffin@cvm.msstate.edu, bibiow@gmail.com, khoo@cvm.msstate.edu, lawrence@cvm.msstate.edu

[2]USDA-Warmwater Aquaculture Research Unit, P.O. Box 38, Stoneville, MS 38776 USA geoff.waldbieser@ars.usda.gov

[3]Department of Biological Sciences, 101 Rouse Life Science Bldg., Auburn University, Auburn, AL 36849, USA lilesma@auburn.edu, mjh0007@tigermail.auburn.edu

[4]Bovine Functional Genomics Laboratory, Agricultural Research Service, United States Department of Agriculture, 10300 Baltimore Ave, Bldg 306 Barc-East, Beltsville, MD 20705, USA tad.sonstegard@ars.usda.gov, steven.schroeder@ars.usda.gov

Aeromonas species are Gram-negative facultative anaerobes that are ubiquitous in aquatic environments and cause infections in several host species, including humans, invertebrates, reptiles, and amphibians. In particular, many *Aeromonas* species are pathogenic to fish, causing septicemia in carp, tilapia, perch, salmon, catfish, and other species. In channel catfish aquaculture, *Aeromonas hydrophila* is historically considered an opportunistic pathogen. However, since 2009 a clonal group of *A. hydrophila* isolates have been causing large-scale disease outbreaks in Alabama and Mississippi. Strain ML09-119 is an isolate from a disease outbreak on a commercial catfish farm, and it is representative of this clonal group. The genome sequence of *Aeromonas hydrophila* ML09-119 was completed using a combination of Illumina Genome Analyzer IIx next-generation sequencing and the 454 GS-FLX titanium platform. Scaffolded gaps were closed by Sanger sequencing of PCR amplicons. Unscaffolded gaps were closed by sequencing single-primer PCR amplicons. rRNA operons and other repeat regions were amplified and sequenced to resolve misassemblies. The final closed-circle version of the *A. hydrophila* ML09-119 genome sequence was submitted to the NCBI's Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) for annotation, followed by submission to GenBank. The total *A. hydrophila* genome comprises 5,024,500 bp with 60.8% GC content. It contains 4,577 predicted genes, of which 4,434 are protein-coding sequences. A total of 112 tRNAs and 10 rRNA operons were predicted. We identified 21 genomic islands and 24 insertion elements as well as genes involved in secretion systems, flagellar assembly, and TCA cycle genes. Taken together, the completed genome sequence of *Aeromonas hydrophila* ML09-119 will enable future functional genomics research and accelerate hypothesis-driven research on this pathogen.

6) Special Session: Diseases of Ornamental Fishes

6a. Emerging fish health issues for the global ornamental fish industry

Timothy J. Miller-Morgan*

Aquatic Animal Health Program, Oregon Sea Grant, College of Veterinary Medicine, Oregon State University, tim.miller-morgan@oregonstate.edu

Ornamental fish comprise a very large and diverse global industry with trading in over 4500 species of freshwater fish, 1450 species of marine fish, and over 650 species of corals and other marine invertebrates. Exports originate more than 100 countries. Europe, North America and Japan are the major importers of ornamental fish.

More than 50% of the ornamental fish supply originates in Asia. Eighty percent of these are farm-raised freshwater fish, while 15% and 5% are wild-caught marine and freshwater species, respectively. A number of Asian and South American countries export these wild-caught species while a number of other Asian and European countries also specialize in farming freshwater ornamental fish.

As the industry continues to develop and diversify there are three emerging areas that all sectors of the industry must address in the next few years:

1. **The need for improved biosecurity** throughout all sectors of the industry. This is being driven by new emerging diseases as well as re-emerging diseases that not only pose a threat to the ornamental fish trade but also to the aquaculture industry for food fish and invertebrates. Consequently there is increased scrutiny by the regulatory bodies for national and international trade.
2. The need to address the issue of **aquatic invasive species**. There are many animals traded that could have significant invasive potential in many countries. Many of these are banned for import but are often included due to poor quality control at packing or a lack of awareness of the specific regulations and/or risks on the part of the exporters and importers. There is a need for more research characterizing the specific invasive pathways as well as improved outreach and education at all levels when it comes to aquatic invasive species.
3. There is emerging pressure to develop specific guidelines that ensure adequate concern for **animal welfare** throughout all sectors of the industry. This may include: optimal fish densities in holding systems, specific handling procedures, collection techniques, disease screening, or recommended or non-recommended species lists.

These are all weighty issues that will not be addressed overnight. However, it is very important to continue discussions among industry, academia and regulatory bodies. Further, it is important to continue developing industry solutions, based upon sound science, and to maintain contact and educate key regulatory bodies about the industry. It is important to remain proactive. The alternative is regulatory requirements developed and implemented with little industry input.

Each of these issues is addressed at some level in papers given throughout this conference. A number of papers will be presented in this special session on ornamental fish disease that will directly address these issues.

6b. Overview of DNA viruses impacting ornamental aquaculture

Thomas B Waltzek[1]*, Michael Gotesman[1], Natalie Steckler[1], Staci Spears[1], Johnny Shelley[2], Roy PE Yanong[3]

[1]Department of Infectious Diseases and Pathology, 2015 SW 16th Avenue, University of Florida, Gainesville, FL 32611 USA tbwaltzek@ufl.edu, mgotesman@ufl.edu, steckler@ufl.edu, smspears@ufl.edu

[2]5D Tropical Inc., 6507 Bob Head Road, Plant City, FL 33565 USA ext205@5dtropical.com

[3]Tropical Aquaculture Laboratory, Fisheries and Aquatic Sciences Program, School of Forest Resources and Conservation, IFAS, University of Florida, 1408 24th St. SE, Ruskin, FL 33570 USA rpy@ufl.edu

Large double-stranded DNA viruses within the families *Alloherpesviridae* and *Iridoviridae* are recognized as significant pathogens of ornamental fishes. For example, the alloherpesviruses *Cyprinid herpesvirus 1*, *2*, and *3* continue to negatively impact koi (CyHV1, CyHV3) and goldfish (CyHV2) aquaculture. Iridoviruses within the genera *Ranavirus*, *Megalocytivirus*, and *Lymphocystivirus* have been described from ornamental fishes. However, *Infectious Spleen and Kidney Necrosis Virus* with the genus *Megalocytivirus* is the most important impacting a wide variety of freshwater and marine ornamental fishes. The discovery, genetic characterization, and epidemiology of emerging alloherpesviruses and iridoviruses within the international ornamental fish industry will be discussed. Finally, the discovery of the first poxvirus in fish will be discussed in reference to recent case observed in koi imported from Malaysia.

6c. A risk analysis of Australia's marine ornamental value chain focusing on biosecurity (diseases and pathogens) concerns

Kevin P Erickson[1*] Marnie L Campbell[2], Chad L Hewitt[1,2], Owen T Nevin[1], Nicole Flint[3]

[1]School of Medical and Applied Sciences, CQ University, Gladstone, QLD, 4680, Australia
K.Erickson2@cqu.edu.au, o.nevin@cqu.edu.au
[2]School of Science, University of Waikato, Hamilton 3240, New Zealand
mcampbel@waikato.ac.nz, chewitt@waikato.ac.nz
[3]School of Business and Law, CQ University, Rockhampton, QLD, 4702, Australia
n.flint@cqu.edu.au

This presentation shows the progress of ongoing PhD research focusing on the management risk prevention efforts that Australia's implementing to insure imported marine ornamental animals do not establish a permanent while foothold within Australia. Additionally, consideration and monitoring of these imported organisms as pathways of foreign disease transmission is being carried out. Supply/value chain analysis is being used to establish an Australian current practices baseline for the marine aquarium trade. This occurs via a survey of the marine ornamental organisms that are available in Australia to identify the species that are non-native. Determination of the marine species status (native, introduced or cryptogenic) occurs using an established protocol.

From there, the methods of how these imported species enter the country are investigated in the importers current biosecurity measures are recorded. These imported organisms are then followed to the distributor who sends them to various stores around Australia who then passes them on to individual hobbyists. While the organisms are being followed, they are monitored for potential diseases and parasites that may have been imported with them, or that they may have been exposed to when they were in quarantine facilities.

6d. Identification of B cells as a major site for koi herpesvirus (KHV) latency

Aimee Reed[1,2]*, Satoko Izume[1], Brian Dolan[1], Scott LaPatra[3], Michael Kent[1,2], Ling Jin[1,2]

[1]Department of Biomedical Sciences, College of Veterinary Medicine, Oregon State University, Corvallis, OR 97331. reeda@onid.orst.edu brian.dolan@oregonstate.edu strawberry4one.0829@gmail.com

[2]Department of Microbiology, College of Science, Oregon State University, Corvallis, OR 97331. michael.kent@oregonstate.edu ling.jin@oregonstate.edu

[3]Research Division, Clear Springs Foods, Inc, Buhl, ID 83316 scott.lapatra@clearsprings.com

Koi herpesvirus (KHV) is a member of the *Alloherpesviridae* and is an emerging herpesvirus that is highly pathogenic for koi and common carp. In this study, KHV latency was investigated in IgM^+ WBC, the primary subset of peripheral B lymphocytes. The presence of the KHV genome in IgM^+ WBC was about 20-fold greater than in IgM^- WBC. To determine if KHV expressed genes during latency, transcription from all 8 ORFs in the terminal repeat was investigated in IgM^+ WBC from koi with latent KHV infection. Only a spliced ORF6 transcript was found to be abundantly expressed in IgM^+ WBC from KHV latently infected koi. The spliced ORF6 transcript was also detected *in vitro* during productive infection as early as 1 day post-infection. The ORF6 transcript from *in vitro* infection begins at -127 bp upstream of the ATG and ends +188 bp downstream of the stop codon, +20 bp downstream of the polyadenylation signal. The hypothetical protein of ORF6 contains a consensus sequence with homology to a conserved domain of EBNA-3B and ICP4 from Epstein Barr Virus and Herpes simplex virus 1, respectively, both members of the *Herpesviridae*. This is the first report of latent KHV in B lymphocytes as well as identification of gene transcription during latency for a member of the *Alloherpesviridae*.

6e. Buy a fish, save a tree: Fish health management and a sustainable ornamental fishery on the Rio Negro, Brazil

Tim Miller-Morgan[1,3]*, Scott Dowd [2,3]

[1]Aquatic Animal Health Program, Oregon Sea Grant, College of Veterinary Medicine, Oregon State University, Newport, Oregon, tim.miller-morgan@oregonstate.edu
[2]New England Aquarium, Boston, Massachusetts, sdowd@neaq.org
[3]Project Piaba, Bio-Amazonia Conservation International, Weymouth, Massachusetts

The ornamental fishery is the principal subsistence activity for the riverine communities in the municipality of Barcelos (Amazonas state, Brazil). The trade in ornamental fish now contributes at least 60% of the income revenues in the municipality. Fluctuations in fish production, market demand, mortality rates and price are the main constraints on the fishers' subsistence. When fishers are asked what they would do if they could not sell fish, the most common answers are: timber harvest, cattle ranching, gold mining, or urban migration. Fortunately, the annually inundated, floodplain habitats of ornamental fishes have remained largely intact. Many forest fishes have a short life cycle (less than 2 years), and fish populations can be quickly replenished. Thus, it may be possible through proper management to protect the habitat from degradation, while maintaining adequate harvests for the local fishing communities.

For nearly 25 years, Project Piaba has been researching the ornamental fishery of the Rio Negro. Very early on it was discovered that the ornamental fishery was not only sustainable, but it was the principal driver for creating value for the environment. Every year a small group of international fish health specialists, trade stakeholders, public aquarium biologists, and fish enthusiasts participate in an annual expedition to Barcelos and the fishing grounds. The outcomes of this program have led to a much better understanding of the role of this fishery and project members are helping the fishery adapt to changes in global markets.

The industry and the business climate in which the fishery operates have changed significantly in recent years and this fishery is increasingly in competition from native Brazilian species being farm-raised in Asian countries. In the past, customers in the import countries have been willing to expend resources to acclimate, manage minor health issues and condition these wild-caught fish in preparation for sale to customer. Today customers expect a high quality and healthy wild-caught fish that requires little in the way of post-shipment health management and conditioning. In order to stay competitive one key area in which the Brazilian industry must focus is improved health management of these fish throughout the chain of custody, from collection to export.

Key factors that appear to impact fish health and quality include: post-collection handing and transport, feed quality and feeding regimes, dramatic shifts in water quality, poor knowledge of disease identification and management and inadequate pre-export acclimation and conditioning. We will discuss some of the current strategies being undertaken by Project Piaba, and our partners, the local fishers cooperative and three Brazilian ministries, to improve the overall fish quality and health within this unique, sustainable fishery.

7) General Session: Diseases in Wild Fishes

7a. *Ceratomyxa shasta* myxospore characteristics in Klamath River Basin adult Chinook carcasses. A poor disease management option.

J. Scott Foott*[1], Ron Stone[1], Anne Bolick[1], Ken Nichols[1], Kim True[1], Ryan Fogerty[2], Sascha Hallett[3], Jerri Bartholomew[3]

[1]US Fish and Wildlife Service , CA-NV Fish Health Center, 24411 Coleman Hatchery Road, Anderson CA 96007 Scott_Foott@fws.gov, Ron_Stone@fws.gov, Anne_Bolick@fws.gov, Ken_Nichols@fws.gov, Kimberly_True@fws.gov,

[2]US Fish and Wildlife Service, Yreka Fish and Wildlife Office, 1829 Oregon Street, Yreka CA 96037 Ryan_Fogerty@fws.gov

[3]Department of Microbiology, 220 Nash Hall, Oregon State University, Corvallis, OR 97331 halletts@science.oregonstate.edu, bartholj@science.oregonstate.edu

A four year data set on *Ceratomyxa shasta* myxospore prevalence, intensity, host and spore characteristics was obtained from adult Chinook salmon carcasses surveyed in the Klamath, Shasta, and Trinity Rivers. Annual prevalence of myxospore infection, detected within the intestine, ranged from 22 – 52% while spore concentration per intestinal scraping ranging from 394 to 14.7 million. A mean of 7.5% of the carcasses produced $\geq$ 500,000 spores and these "high" spore carcasses contribute 76 – 95% of the total spores in a given season. QPCR analysis of non-detected scrapings showed that 45 – 87% of these samples had either low spore numbers or more likely non-spore stages of the parasite. Myxospores are rarely found in freshly spawned adults but are common in decomposed carcasses of either sex. Date of collection or age (fork length) did not influence detection. Longer residence time, of Trinity R. Spring run compared to Fall-run Chinook carcasses, was associated with higher spore loads. Using dye exclusion (intact spore indicator), there was an inverse trend in spore "intactness" (viability?) and initial spore load. Some refrigerated myxospores remained intact over the entire winter. The question of myxospore viability as well as quantity must be better understood in order to inform life cycle models. A 2 year carcass- removal pilot project failed to influence *C. shasta* DNA quantity in targeted waters. This labor intensive method is not a viable management option to reduce *C. shasta* infectivity in the Klamath River. Reports on the subject can be found at: http://www.fws.gov/canvfhc/reports.asp

7b. Disease-associated effects on reproductive output of Chesapeake Bay striped bass (*Morone saxatilis*)

Carissa L Gervasi[1]*, Robert J Latour[1], Wolfgang K Vogelbein[2]

[1]Department of Fisheries Science, Virginia Institute of Marine Science, College of William and Mary, 1375 Greate Road, Gloucester Point, VA 23062 cgervasi@vims.edu, latour@vims.edu
[2]Department of Environmental and Aquatic Animal Health, Virginia Institute of Marine Science, College of William and Mary, 1375 Greate Road, Gloucester Point, VA 23062 wolf@vims.edu

A thorough reproductive study on the Chesapeake Bay Striped Bass, an economically and ecologically vital population, has not been conducted since the early 1990s, just after the stock had crashed but before it rebounded. Due to management efforts, the population has grown tremendously, necessitating an update to fecundity and maturity schedules. Additionally, the emergence of mycobacteriosis in the Chesapeake Bay Striped Bass population in the late 1990s has prompted much research, and the current prevalence of greater than 50% raises questions about population level effects of the disease. The objectives of this study are to update current knowledge on maturation and fecundity of Striped Bass in the bay and examine disease-associated effects of mycobacteriosis on reproductive metrics through egg-per-recruit analysis. Reproductive data were obtained from female Striped Bass collected (n=469) during spring 2012-2013 in the York, James, and Rappahannock Rivers of the Chesapeake Bay. Fish ranged in age from 2-17 years and disease prevalence across rivers and years was 64%, with prevalence increasing with age to age-7 and then decreasing in the older fish. Model results indicate total lifetime egg production of disease-positive fish is substantially reduced, which has important implications for management.

7c. Lamprey reddening syndrome in Southland Rivers, New Zealand – A mystery!

Anjali Pande[1]*, Cara Brosnahan[1], Wendy McDonald[1], Brian Jones[1], Kathy Walls[2], Naya Brangenberg[1]

[1]Ministry for Primary Industries, Investigation and Diagnostic Centre, Wallaceville, Upper Hutt
anjali.pande@mpi.govt.nz, brian.jones@mpi.govt.nz, wendy.mcdonald@mpi.govt.nz, cara.brosnahan@mpi.govt.nz, naya.brangenberg@mpi.govt.nz
[2]Ministry for Primary Industries, 25 The Terrace, PO Box 2526, Wellington 6011.
katherine.walls@mpi.govt.nz

In September 2011 the Ministry for Primary Industries (MPI) Pest and Disease hotline received a call about mass mortalities of lamprey in the Mataura River in Southland, New Zealand. The affected lampreys have focal to coalescing areas of hyperemia of the dermis primarily on the ventrum, external gills and at the base of the fins. Since the first report, affected lamprey have been found in other Southland rivers, although mortalities did not seem as prevalent. An investigation was undertaken to identify the cause of what has been termed Lamprey Reddening Syndrome (LRS). Diagnostic testing returned a positive result to *Aeromonas salmonicida* by PCR, which triggered a biosecurity response by MPI. However, the strain was subsequently identified as atypical and exotic strains were ruled out. In 2012, more lamprey were tested (both affected and unaffected) for a wide range of pathogens to exclude *Yersinia ruckeri*, *Pseudomonas* spp., the exotic to NZ viruses including Viral Haemorrahagic Septicaemia virus (VHS), Infectious Haemorrhagic Necrosis virus (IHN), iridovirus and infectious pancreatic necrosis virus (IPN) as well as conducting general bacterial and viral culture and histopathology. Histopathology is not consistent with infectious agents or pollutants, but a robust histopathological description of the actual lesions has been developed.

LRS is currently still being seen in Lamprey in Southland rivers and a case definition was developed, to underpin further surveillance efforts. Monitoring continues in the Southland river systems in order to gain a better understanding of the epidemiological picture of LRS, but risk factors and causal components are still a mystery. This paper presents the progression of the investigation into LRS over the last three years.

7d. Diseases and biomarkers of marine health in large pelagic sharks from the US Atlantic coast - an overview of data 1996-2013

Douglas H. Adams[1], Joanna Borucinska[2]*, Faiza Bhura[2], Puja Bhardwaj[2], Zbigniew J. Grabowski[3], Katelyn Whitburn[2]

[1]Cape Canaveral Scientific 220 Surf Road Melbourne Beach, FL 32951 USA, research@capecanaveralscientific.com,
[2]Department of Biology, University of Hartford, 200 Bloomfield Ave., W. Hartford, CT 06117 borucinsk@hartford.edu, fabhura@hartford.edu, bhardwaj@hartford.edu, whitburn@hartford.edu
[3]Department of Environmental Science, Institute for Sustainable Solutions, Portland State University, PO Box 751, Portland, OR 97207, z.j.grabowski@pdx.edu

Most shark species are long-lived, migratory top predators, and thus constitute effective sentinels to monitor the health of marine ecosystems. Surprisingly few studies have used them in such capacity, and little is known about diseases occurring in free-ranging sharks in general. We have opportunistically collected health data from large pelagic sharks landed during fishing tournaments along the US coast of north-western Atlantic between 1996-2013, as well as several sharks captured or stranded along the south-eastern US coast. In addition, we collected data on selected morphological biomarkers of environmental health, including hepatic melanomacrophage cells and thyroid histology. We used standard brightfield microscopy to examine all collected tissues. The most frequently encountered pathological conditions included lesions associated with external and internal parasites and injuries due to retained fishing hooks. The parasite-induced lesions varied from gastric ulceration and enteritis to granulomatous oophoritis and metritis. Hook-induced trauma included transmural gastritis with gastric perforation and peritonitis, hepatic laceration with hepatitis and neoplastic lesions, proliferative peritonitis with intralesional bacteria and/or algae, necrotizing myocarditis and pericarditis with thrombotic arteritis and systemic emboli resulting in multiple organ infarcts. We recorded a few cases of neoplasia including testicular and pericardial mesotheliomas and fibropapillomas, hepatic tumors, and a gingival epulis. In addition peculiar, most likely physiological changes in visceral pericardium and coronary arteries were recorded. Our studies of shark health biomarkers yielded baseline data on species differences in thyroid morphology and morphometric characteristics of hepatic melanomacrophages, including their size/species/gender properties and temporal changes.

7e. Geographical prevalence of pathogens in aquatic animals in Kamchatka

Nataliia V. Sergeenko*, Tatyana V. Ryazanova, Tatyana V. Gavruseva, Elens A. Ustimenko, ElenaV. Bochkova, Kirll V. Kozlov, Evgeniy A. Gritskih

Kamchatka Research Institute of Fisheries and Oceanography, Naberesnaja 18, Petropevlovsk-Kamchatsky, 683000, Russia nvsergeenko@gmail.com

The spectrum of pathogens associated diseases and their prevalence in the fish and crab populations of the seas of the Russian Far East have been insufficiently studied and their influence on aquatic populations remains elusive. This paper presents data on the prevalence of pathogens in Pacific salmon and crabs from Kamchatka, derived from the analysis of studies conducted between 2001 and 2013.

Two important threats to the Pacific salmon populations are infectious hematopoietic necrosis virus (IHNV) and the bacterium *Aeromonas salmonicida*. IHNV (prevalence up to 70%) and *Aeromonas salmonicida* (infection rates reaching 33%) have been identified in sockeye salmon in the three major spawning rivers of Kamchatka and their tributaries — Ozernaya, Bolshaya (West coast) and the Kamchatka River (East Coast).
Regarding parasites, pathological effects from the protozoa *Ichthyobodo necator* (prevalence 1-19,2%), *Trichodina trutta* (3,8-40%), *Apiosoma conicum* (7,7-50%), *Ichthyophthirius multifiliis* (13,4-26,7%) and the crustaceans *Ergasilus auritus* (6,7-30%) in lakes Azabachye (East Coast), Nachikinskoye and Kurilskoe (West coast) caused changes in the gills of juvenile salmon. In sockeye smolts from Lake Kurilskoe the nematode *Philonema oncorhynchi* caused degenerative changes of oocytes (up to 20%) and plerocercoids of cestodes *Diphyllobothrium* sp. (prevalence up to 100%), causing changes in the gastrointestinal tract.

The most serious threat for crab populations is likely a herpes-viral infection, detected in blue crab in the northern part of the West Kamchatka shelf and in the eastern Bering Sea. We discovered Rickettsia-like organisms affecting hepatopancreas blue crab in the Okhotsk Sea. On the West Kamchatka shelf and in the Bering Sea we identified abscess-like necrosis of hepatopancreas in six species of crab as a result of infection by the bacteria genus *Vibrio*. We found microsporidia genus *Thelohania* in five species and the genus Ameson in three species of crabs in the Okhotsk Sea. «Bitter Crab Syndrome» caused by dinoflagellate genus *Hematodinium* was found in four species of crabs in the West Kamchatka shelf, and in three species in the Bering Sea. In the western Bering Sea an invasion of trematode metacercariae in snow crabs was observed.

7f. Infectious disease, shifting climates, and opportunistic predators: cumulative factors potentially impacting wild salmon declines

Kristina M. Miller[1,2*], Amy Teffer[3], Strahan Tucker[1], Shaorong Li[1], Amy Tabata[1], Scott G. Hinch[2], David A. Patterson[4], Francis Juanes[3], Brian Riddell[5]

[1]Pacific Biological Station, Fisheries and Oceans Canada, Nanaimo, BC, Canada Kristi.miller@dfo-mpo.gc.ca; Shaorong.li@dfo-mpo.gc.ca; amy.tabata@dfo-mpo.gc.ca; Strahan.tucker@dfo-mpo.gc.ca

[2]Forest and Conservation Sciences, University of British Columbia, Vancouver, BC, Canada scott.hinch@ubc.ca

[3]Biology Department, University of Victoria, Victoria, BC, Canada amy.kathryn.kevin@gmail.com; juanes@uvic.ca

[4]Fisheries and Oceans Canada, School of Resource and Environmental Management, Simon Fraser University, Science Branch, Burnaby, BC, Canada david.patterson@dfo-mpo.gc.ca

[5]Pacific Salmon Foundation, Vancouver, BC, Canada briddell@psf.ca

Emerging diseases are impacting animals under high-density culture, yet few studies assess their importance to wild populations. In this presentation, we suggest ways in which modern technologies merged with novel ecological approaches can improve our knowledge by elucidating the microparasites of greatest potential import to wild migrating salmon. The foundation of this modernized approach is the development of a microfluidic system for microbe monitoring being developed through the Strategic BC Salmon Health Initiative that is capable of quantitatively assessing presence and load of >45 microbe in 96 samples at once. We present three case studies that utilize this platform to resolve microparasite impacts on adult salmon migration success, impact of river warming on microparasite replication, and infection status on susceptibility to predation. Future health of wild salmon must be considered in a holistic context that includes the cumulative or synergistic impacts of multiple stressors. These approaches will identify populations at greatest risk, critically needed to manage and potentially ameliorate the shifts in current or future trajectories of wild populations.

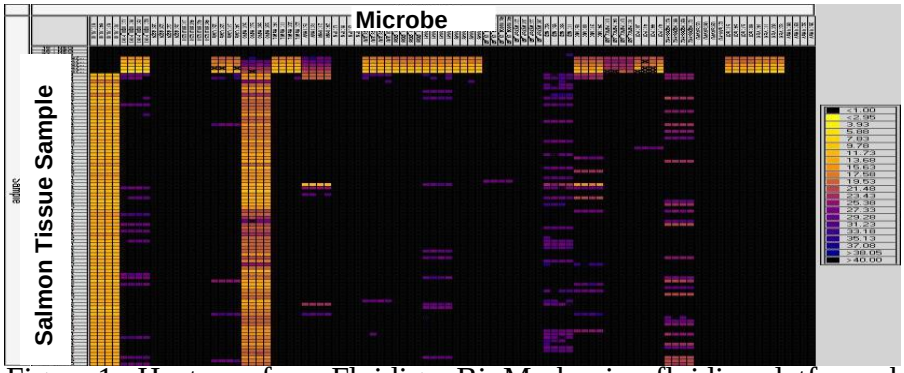


Figure 1. Heatmap from Fluidigm BioMark microfluidics platform showing RT-PCR results in quadruplicate for 23 microbes and one house-keeping gene. Scale-bar at the right indicates brighter colors for higher load samples.

8) General Session: Bacteria III

8a. Piscirickettsiosis pathogenesis: Thermolabile exotoxin secretion by *Piscirickettsia salmonis*

María E Rojas, Marco Galleguillos, Soraya Díaz, Álvaro Machuca, Pedro A Smith*

Department of Animal Pathology. Faculty of Veterinary Sciences. University of Chile, Avenida Santa Rosa 11735, La Pintana, Santiago, Chile mestelarojas@gmail.com, mgallegu@uchile.cl, diazquezada.soraya@gmail.com, alvmachuca@uchile.cl, psmith@uchile.cl

Piscirickettsia salmonis is the causative agent of piscirickettsiosis, a disease affecting a variety of teleost species and that has been particularly severe in salmonid fish reared in Chilean sea waters for many years. Extracellular products (ECPs), obtained from cultures of three isolates of *P. salmonis*, were inoculated in Atlantic salmon (*Salmo salar*) and in three continuous cell lines. Although steatosis was found in some liver samples, no mortalities or clinical signs occurred in the inoculated fish. Clear cytotoxicity was observed in the cell lines CHSE-214 and ASK, derived from salmonid tissues, but not in MDBK, which has a mammal origin. The degree of cytotoxicity of the ECPs was different among the *P. salmonis* isolates tested. The isolate that evidenced the highest cytotoxicity in its ECPs exhibited only an intermediate virulence after challenging fish with whole bacterial suspensions of the three *P. salmonis* isolates. Inhibition of the cytotoxic activity of ECPs was seen after proteinase K treatment, indicating their peptidic nature. Cytotoxicity was also inhibited after ECP incubation at 50 °C for 30 min. Results show that *P. salmonis* ECPs contain thermolabile exotoxins that probably play a role in the pathogenesis of piscirickettsiosis.

8b. New hosts and genetic diversity of *Flavobacterium columnare* isolated from diseased Nile tilapia and native Brazilian species

Gustavo M Barony[1], Guilherme C Tavares[2], Henrique C P Figueiredo[3], Carlos A G Leal[4]*

[1]AQUAVET – Laboratory of Aquatic Animal Diseases, Department of Preventive Veterinary Medicine, Veterinary School, Federal University of Minas Gerais, MG, Brazil
carlosleal@vet.ufmg.br
[2]AQUAVET – Laboratory of Aquatic Animal Diseases, Department of Preventive Veterinary Medicine, Veterinary School, Federal University of Minas Gerais, MG, Brazil
gui.ichijoji@hotmail.com
[3] AQUAVET – Laboratory of Aquatic Animal Diseases, Department of Preventive Veterinary Medicine, Veterinary School, Federal University of Minas Gerais, MG, Brazil
figueiredoh@yahoo.com
[4]AQUAVET – Laboratory of Aquatic Animal Diseases, Department of Preventive Veterinary Medicine, Veterinary School, Federal University of Minas Gerais, MG, Brazil
carlosleal@vet.ufmg.br

Flavobacterium columnare is one of the most important bacterial pathogens for freshwater farm-raised fish. This bacterium causes outbreaks with high mortality rates, affecting mainly fry and fingerlings. Several Brazilian native fish species have been raised in aquaculture systems in the last years, including Amazon catfish (*Leiarius marmoratus* x *Pseudoplatystoma corruscans*) and pacamã (*Lophiosilurus alexandri*). The principal pathogens of these fish are poorly characterized. The present study aimed to identify the etiologic agent of outbreaks in Amazon catfish and pacamã hatcheries. Also, to address the genetic diversity of *F. columnare* isolated from these fish species and Nile tilapia (*Oreochromis niloticus* L.) in Brazil. Two outbreaks of columnaris in a hatchery of Amazon catfish and pacamã were accompanied in 2010 and 2011, respectively. Four *F. columnare* isolates were obtained from diseased Amazon catfish (n=2) and pacamã (n=2). Together with 11 strains from Nile tilapia, their genomovar were determined by RFLP. The genetic diversity of isolates was evaluated by phylogenetic analysis of 16S rRNA and REP-PCR. The discriminatory power of genotyping techniques was calculated by Simpon's diversity index. The majority of Brazilian strains were belonged in genomovar II (n=13) with just two isolates from Nile tilapia classified as genomovar I. There was no relation between fish host and genomovar. In phylogenetic analysis of 16S rRNA and REP-PCR, the isolates were clustered in four distinct genotypes with 100% of congruence between two assays. These methods showed a moderated discriminatory power. The present results demonstrated an intra-genomovar diversity, showing that this method may be unable to predict some genetic or evolutionary events in *F. columnare*. This is the first report of *F. columnare* infection in hatcheries of Amazon catfish and pacamã. In addition, Brazilian strains *F. columnare* showed a moderate genetic diversity, and REP-PCR was shown to be a feasible method for genotyping this bacterium.

8c. Effectiveness of florfenicol in controlling *Flavobacterium columnare* infections in channel catfish: comparison between 2 *F. columnare* isolates with high or low florfenicol susceptibility

CM Gieseker[1*], T C Crosby[1], NR Hasbrouck[1], ER Evans[1], CB Stine[1], AC Kouneski[1], SR Frobish[1], PJ Boliek[1], MW McDonald[1], SD Rill[1], OA Chiesa[1], LR Rodriguez[1], R Reimschuessel[1], LC Woods III[2]

[1]US Food and Drug Administration, Center for Veterinary Medicine, Office of Research, 8401 Muirkirk Road, Laurel, MD 20708
[2]Department of Animal and Avian Science, University of Maryland, College Park, MD 20742

We evaluated the effectiveness of florfenicol in controlling infections induced in channel catfish, *Ictalurus punctatus*. We compared 2 *Flavobacterium columnare* isolates that differed in their florfenicol susceptibility based on *in vitro* antimicrobial susceptibility testing. Our goal was to test whether the isolate with less susceptibility as indicated by the *in vitro* test could resist treatment. One hundred eighty catfish were exposed to either: 1) a *F. columnare* isolate with high susceptibility to florfenicol, 2) a *F. columnare* isolate with relatively low susceptibility, or 3) uninoculated media (negative control) in a 2 hour static bath at 28±1º C. After exposure, the fish were randomly distributed among 6 tanks per exposure (10 fish/tank, 18 tanks total) and held at 25±1º C to observe for signs of morbidity. We fed the fish an approved dose (10 mg drug/kg fish body weight) of florfenicol-medicated catfish feed or a non-medicated feed beginning 4 hours post-exposure once a day. After 10 days, all of the fish were fed the non-medicated feed for an additional 8 days. We observed the fish daily for morbidity. Any fish that had at least 2 of 3 defined signs of morbidity (lesions, lethargy, and listing) were sacrificed and necropsied. We repeated the experiment 2 more times for a total of 3 replicates. The medicated feed successfully controlled infections induced with the more susceptible isolate, but not infections induced with the less susceptible isolate. The cumulative percent survival of fish exposed to the more susceptible *F. columnare* isolate was 84% for the fish fed the medicated feed and 17% for the fish fed the non-medicated feed. The cumulative percent survival of fish exposed to the less susceptible *F. columnare* isolate was 18% for the fish fed the medicated feed and 10% for the fish fed the non-medicated feed. All of the non-exposed fish survived regardless of which feed they received. Long filamentous bacteria were found in wetmounts of skin lesions and *F. columnare* colonies were cultured from the skin, spleen, or posterior kidney of all moribund fish sampled. To our knowledge, this is the first report of *F. columnare* that resist the approved florfenicol treatment.

8d. Effect of antibiotic treatment during water hardening on *Flavobacterium psychrophilum* and rainbow trout egg survival

Eric Wagner*, Randall Oplinger, Jared Baker

Fisheries Experiment Station, Utah Division of Wildlife Resources, Logan, Utah.
ericwagner@utah.gov

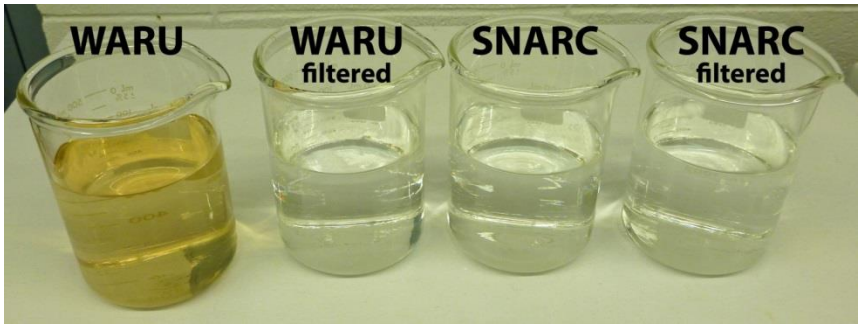
This study evaluated a mixture of penicillin and streptomycin for treatment of rainbow trout eggs during fertilization and water hardening to prevent vertical transmission of *Flavobacterium psychrophilum*. A few different experiments were conducted at small scale and production scale to determine the safety of the treatment to eggs. An additional test used eggs inoculated with the bacterium to evaluate the bactericidal effects of treatment. For the small scale safety trial, treatments (3 reps/treatment) were 1) antibiotics added to the diluent, 2) antibiotics added during the first 15 minutes of water hardening, 3) antibiotics during the first 60 minutes of water hardening, 4) antibiotics added to both the diluent and during the first 15 minutes of water hardening, 5) antibiotics added to both the diluent and during the first 60 minutes of water hardening, and 6) control, i.e., no antibiotics added. Percent eye-up, hatch, and the cripple rate among the eggs did not vary among treatments. The same antibiotic mixture (0.079 g/L penicillin + 0.125 g/L streptomycin; dosage based in previous in-vitro tests) was tested at a production scale, treating over a million rainbow trout eggs during both fertilization and water hardening for 20 min. Egg survival to eye-up of the heat-shocked eggs (for triploidy induction) was 74 to 80%. In the spiked egg experiment, treatments were 1)spiked (10,000 CFU/ml *F. psychrophilum*), then treated with antibiotics up to 20 min after fertilization, 2) spiked, then treated with antibiotics up to 60 min after fertilization, 3) negative control, 4) spiked, not treated with antibiotics (positive control). There were 4 replicates for each treatment; 25 eggs/rep were homogenized with a pestle in TYES broth in a microcentrifuge tube. The tubes were incubated overnight at 16 C, then 100 ul plated on TYES. No *F. psychrophilum* was found on the eggs treated with antibiotic for either 20 or 60 min, but the bacterium was found on both negative (present in Mantua Hatchery brood) and positive controls. So, 0.079 g/L penicillin + 0.125 g/L streptomycin in the diluent and water hardening solutions effectively and safely controlled vertical transmission of *F. psychrophilum* in rainbow trout eggs.

8e. The influence of water chemistries on *Flavobacterium columnare* pathogenesis in channel catfish

David L. Straus[1,3*], Bradley D. Farmer[1,4], Benjamin H. Beck[1,5], Brian G. Bosworth[2,6], E. Les Torrans[2,7], Craig S. Tucker[2,8]

[1]USDA/ARS, Harry K. Dupree - Stuttgart National Aquaculture Research Center, Stuttgart, AR 72160, USA
[2]USDA/ARS, Warmwater Aquaculture Research Unit, Thad Cochran National Warmwater Aquaculture Center, Stoneville, MS 38776 USA
[3]SNARC, P.O. Box 1050, Stuttgart, AR 72160, USA dave.straus@ars.usda.gov
[4]SNARC, P.O. Box 1050, Stuttgart, AR 72160, USA bradley.farmer@ars.usda.gov
[5]SNARC, P.O. Box 1050, Stuttgart, AR 72160, USA benjamin.beck@ars.usda.gov
[6]WARU, P.O. Box 38, Stoneville, MS 38776 USA brian.bosworth@ars.usda.gov
[7]WARU, P.O. Box 38, Stoneville, MS 38776 USA les.torrans@ars.usda.gov
[8]WARU, P.O. Box 38, Stoneville, MS 38776 USA craig.tucker@ars.usda.gov

Columnaris disease can cause tremendous losses of freshwater fish. While it has been studied exhaustively, little is known about its affinity to specific water chemistries that affects attachment. Recent studies in our labs have illuminated this subject. In the first experiment, two waters were used: unfiltered well waters from the Stuttgart National Aquaculture Research Center (SNARC; Stuttgart, Arkansas) and from the Warmwater Aquaculture Research Unit (WARU; Stoneville, Mississippi). Fingerling channel catfish (*Ictalurus punctatus*) were exposed to an *F. columnare* suspension in aquaria for 4 days; each aquarium contained 10 L of water and ½ was replaced daily. No fish died in the WARU water, but 100% of the fish died in SNARC water. Using qPCR, we found that there were ~1900 times more *F. columnare* attached to the gills of the fish in SNARC water ($P = 0.0001$). In the second experiment, four waters were used: the above waters, WARU water filtered through a carbon bed to remove tannins and SNARC water filtered through a water softener to remove hardness. No fish died in the WARU or filtered waters, but 17% of the fish died in SNARC water. Again using qPCR, we found that there were ~1600 times more *F. columnare* attached to the gills of the fish in SNARC water ($P = 0.0001$). Filtered SNARC water had less *F. columnare* than unfiltered SNARC water ($P = 0.0809$) and filtered WARU water had more *F. columnare* than unfiltered WARU water ($P = 0.6191$). Results suggest tannins have minimal involvement, and water analyses suggest that calcium and hardness are two parameters influencing bacterial attachment and ultimately pathogenesis. Results of ongoing tests will be discussed.



8f. Intragenomic heterogeneity in the 16S rRNA genes of *Flavobacterium columnare* and standard protocol for genomovar assignment

Benjamin R. LaFrentz[1*], Geoffrey C. Waldbieser[2], Timothy J. Welch[3], Craig A. Shoemaker[1]

[1]United States Department of Agriculture-Agricultural Research Service (USDA-ARS), Aquatic Animal Health Research Unit, 990 Wire Road, Auburn, AL 36832-4352 USA benjamin.lafrentz@ars.usda.gov; craig.shoemaker@ars.usda.gov
[2]USDA-ARS, Warmwater Aquaculture Research Unit, Thad Cochran National Warmwater Aquaculture Center, PO Box 38, Stoneville, MS 38776 USA geoff.waldbieser@ars.usda.gov
[3]USDA-ARS, National Center for Cool and Cold Water Aquaculture, 11861 Leetown Road, Kearneysville, WV 25430 USA tim.welch@ars.usda.gov

Genetic variability in 16S rRNA gene sequences has been demonstrated among isolates of *Flavobacterium columnare* and a restriction fragment length polymorphism (RFLP) assay is available for genetic typing this important fish pathogen. Interpretation of restriction patterns can be difficult due to the lack of a formal description of the expected number and sizes of DNA fragments generated for each of the described genomovars. In this study, partial 16S rRNA gene sequences (ca. 1250 bp fragment) from isolates representing each described genomovar and isolates generating unique restriction patterns were cloned and sequenced. The results demonstrated that some isolates contained up to three different 16S rRNA genes whose sequences generate different RFLP patterns due to intragenomic heterogeneity within *Hae*III restriction sites. The occurrence of *Hae*III restriction sites within the portion of the 16S rRNA gene used for typing the *F. columnare* isolates and intragenomic heterogeneity within these sites explained the restriction patterns observed following RFLP analyses. This research provides a standard protocol for typing isolates of *F. columnare* by RFLP and a formal description of the expected restriction patterns for the previously described genomovars I, II, II-B, and III. Additionally, we describe a new genomovar, I/II.

9) Special Session: Selective Breeding for Disease Resistance: Lab and Field Studies

9a. Genetic improvement of disease resistance in salmonid fish using selective breeding: overview of concepts, considerations and limitations

Gregory D. Wiens*[1], Timothy D. Leeds[1]

[1]National Center for Cool and Cold Water Aquaculture, USDA/ARS Kearneyville, WV 25430
Greg.wiens@ars.usda.gov

In aquaculture, endemic infectious diseases constitute a considerable economic burden due to direct losses as well as indirect impacts on growth and animal welfare. In response to infectious disease, it is well documented that host genetic variation is present in most animal populations, especially aquatic animals due to short domestication history and outbred origin. Recent progress in high-throughput animal health phenotyping combined with quantitative genetic analysis has demonstrated the feasibility of improving disease resistance through family-based selective breeding. However, there are only a few examples of successful application of this disease control strategy. Salmonid fish are uniquely suitable for selective breeding as reproduction can be controlled, pedigree is easy to track, large numbers of offspring are generated from each family, and egg development can be temperature manipulated to synchronize hatching thus disease resistance phenotyping can be performed using animals with similar body weight. Herein, we review progress made toward breeding for disease resistance, using as an example, results generated from the National Center for Cool and Cold Water Aquaculture selective breeding program.

9b. Is there a genetic correlation between the resistance of channel catfish to *Edwardsiella ictaluri* and *Flavobacterium columnare*, and how do we get there?

Benjamin R. LaFrentz[1]*, Brian C. Peterson[2], Donald D. Ourth[3], Craig A. Shoemaker[1]

[1]United States Department of Agriculture-Agricultural Research Service (USDA-ARS), Aquatic Animal Health Research Unit, 990 Wire Road, Auburn, AL 36832-4352 USA benjamin.lafrentz@ars.usda.gov; craig.shoemaker@ars.usda.gov
[2]USDA-ARS, Warmwater Aquaculture Research Unit, Thad Cochran National Warmwater Aquaculture Center, PO Box 38, Stoneville, MS 38776 USA brian.peterson@ars.usda.gov
[3]Department of Biology, The University of Memphis, Life Sciences Building, Memphis, TN 38152-3560 USA ddourth@memphis.edu

Two major problems in the channel catfish (*Ictalurus punctatus*) aquaculture industry have been high disease losses to enteric septicemia of catfish (ESC), caused by *Edwardsiella ictaluri* and columnaris disease, caused by *Flavobacterium columnare*. Methods to control and prevent these diseases include antibiotic therapy, vaccinations, and management strategies. Another approach may include selective breeding. The objective of this study was to obtain baseline information on the susceptibility of channel catfish families to *E. ictaluri* and *F. columnare*

Four families of channel catfish were used: family A was randomly chosen from spawns of catfish that had not been selectively bred for resistance; families B, C, and D were families obtained following selection for resistance to *E. ictaluri*. All four families were challenged by immersion with both bacterial pathogens. Mean cumulative percent mortality (CPM) of the families following *E. ictaluri* challenge ranged from 4 to 33%. Families A and B were more susceptible to *F. columnare* (mean CPM of three independent challenges, 95 and 93%) than families C and D (45 and 48%), demonstrating that there is genetic variation in resistance to *F. columnare*. An interesting observation was the two families that exhibited the highest CPM following the *F. columnare* challenges had the lowest CPM following *E. ictaluri* challenge.

Further research on larger numbers of families is needed to determine if there is any genetic correlation between resistance to *E. ictaluri* and *F. columnare*; however, such research is currently limited by the lack of a reliable columnaris disease model using well water at the Warmwater Aquaculture Research Unit. Research that has been conducted to address this limitation will be discussed.

9c. Evaluating innate resistance to *Flavobacteruim columnarae* in rainbow trout (*Oncorhynchus mykiss*)

Jason P. Evenhuis*[1], Tim Leeds[1], Scott E. LaPatra[2]

[1]National Center for Cool and Cold Water Aquaculture, USDA/ARS Kearneyville, WV 25430
[2]Research Center Clear Springs Foods, Inc., Buhl, ID 83316 jason.evenhuis@ars.usda.gov

Flavobacterium columnare (Fc) is the causative agent for columnaris disease and this pathogen infects both warm water and cool water aquaculture species. Recently, columnaris has become an emerging problem in rainbow trout farmed within southern Idaho. Herein, we investigate whether selective breeding has the potential to be part of an integrated approach for controlling columnaris disease. The objectives of this study were to 1) develop a reproducible immersion challenge model, 2) investigate whether NCCCWA selectively bred rainbow trout families and lines exhibit variation in survival following challenge, 3) determine heritability of disease resistance, and 4) examine whether there is a genetic correlation between bacterial cold water disease resistance and columnaris resistance. A 21 day immersion challenge using *F. columnare* strain CSF298-10 was developed. Two challenges were conducted: the first challenge included 44 families from a bacterial cold water disease resistant line (ARS-Fp-R), and the second challenge included 21 families each from the ARS-Fp-R line and a bacterial cold water disease susceptible line (ARS-Fp-S). There was considerable variation in survival between families ranging from 87% to 18% in the ARS-Fp-R line and from 73% to 3% for ARS-Fp-S line. The average percent survival in the ARS-Fp-R line was 56% while the average percent survival for the ARS-Fp-S line was 38%. The heritability of innate resistance against Fc was 0.17 and was identical to the heritability estimate for innate resistance against Fp in this population of rainbow trout. The genetic correlation between Fp and Fc innate resistance was 0.40 as determined from a linear model applied in MTDFREML. There was an unfavorable, albeit weak, genetic correlation (-0.09) between innate Fc resistance and 9-month body weight. In summary, we have developed an immersion challenge model suitable for high-throughput phenotyping for columnaris resistance. We have identified considerable phenotypic variation between families and importantly, there was a positive genetic correlation between bacterial cold water disease resistance and columnaris resistance indicating that it should be possible to generate a genetic line with improved resistance against both pathogens.

9d. Exploring mechanisms of survival in rainbow trout selectively bred for increased resistance to *Flavobacterium psychrophilum*

David Marancik[1]*, Melinda Camus[2], Alvin Camus[2], Timothy Leeds[1], Guangtu Gao[1], Gregory Wiens[1]

[1]NCCCWA-ARS-USDA, 11861 Leetown Rd., Kearneysville, West Virginia 25430
david.marancik@ars.usda.gov, tim.leeds@ars.usda.gov, Guang.gao@ars.usda.gov, greg.wiens@ars.usda.gov
[2]Dept. of Pathology, College of Vet. Med., University of Georgia, Athens, GA 30602
mscamus@uga.edu, camus@uga.edu

A challenge for selective breeding programs is to better understand how artificial selection alters host pathophysiologic and immunologic response following pathogen exposure. The National Center for Cool and Cold Water Aquaculture is exploring this in rainbow trout bred for increased survival (ARS-Fp-R line) and susceptibility (ARS-Fp-S line) to *Flavobacterium psychrophilum* by comparing responses within the context of host "resistance" and "tolerance". Resistance and tolerance are assessed by quantifying the level at which host health is affected by infection intensity. Surrogate measures of host health were developed in ARS-Fp-R and ARS-Fp-S line fish by measuring packed cell volume (PCV) and plasma biochemistry analytes and correlating changes in analyte levels with qPCR-derived splenic bacterial loads. Results suggest that selective breeding has increased host resistance with little change in tolerance in the ARS-Fp-R line. Current efforts to elucidate mechanisms of resistance include proteomic profiling of plasma of ARS-Fp-R and ARS-Fp-S line fish following experimental challenge. Changes in plasma protein levels were analyzed at six days post-infection using iTRAQ and 2D-LC-MS/MS analysis and searching resultant peptide fragment sequences against the published rainbow trout genome (https://www.genoscope.cns.fr/trout). Proteins demonstrating significant differential abundance over time and between genetic lines are being categorized through bioinformatic analyses. We anticipate that identified immunologic and physiologic pathway differences between ARS-Fp-R and ARS-Fp-S line fish will reveal how selective breeding has altered disease pathogenesis and host survival.

9e. From the laboratory to the field: The performance of a selectively bred line of rainbow trout (*Oncorhynchus mykiss*) under commercial production conditions

S. LaPatra[1]*, T. Leeds[2], G. Wiens[2]

[1]Clear Springs Foods, Inc., Research Division, Buhl, ID USA Scott.LaPatra@clearsprings.com
[2]National Center for Cool and Cold Water Aquaculture, USDA/ARS, Kearneysville, WV USA Greg.Wiens@ars.usda.gov; Tim.Leeds@ars.usda.gov

Selective fish breeding programs for disease resistance comprise an increasingly important role in aquaculture production and offer an additional management tool for reducing bacterial-caused disease losses. Bacterial cold water disease (BCWD) is one of the most frequent causes of elevated mortality in juvenile salmonids, and a genetic line of rainbow trout has been selectively bred by the USDA-ARS for resistance to BCWD. This line is designated ARS-Fp-R (resistant) and has been shown to be significantly less susceptible than randomly-mated control and industry reference lines following standardized laboratory challenges with the causative agent of BCWD, *Flavobacterium psychrophilum*. However, this line was shown to potentially have increased susceptibility to infectious hematopoietic necrosis (IHN) virus in a laboratory evaluation. In 2013, a production lot farm trial utilizing 300,000 eyed-eggs obtained from the ARS-Fp-R line was initiated at Clear Springs Foods (CSF), Inc. and survival was monitored from hatching through harvest. Survival during hatch-house rearing was high and fish were vaccinated utilizing standard CSF protocols. Feeding and mortality were normal until day 189 post-hatch. At this point, elevated mortality occurred and systemic or hematopoietic IHN was diagnosed concomitantly with a minor flavobacterial gill infection. Fish were treated with chloramine-T three times during one week. Subsequently, over the course of 25 days an estimated 19% of the fish were lost to IHN virus (n=14,284) before mortality returned to baseline. No additional mortality events were recorded following this outbreak through harvest (~385 days post-hatch). Feed conversion rate and fillet yield were judged to be favorable and within typical production limits. Based on results from this farm trial and historical experience with IHN virus mortality at CSF, the ARS-Fp-R line appears to potentially have increased susceptibility to IHN, however, a more robust vaccination regime could minimize this problem. Additional production trials are planned or in progress with this and other selected USDA-ARS lines of rainbow trout that could significantly benefit the commercial rainbow trout industry.

10) General Session: Vaccines I

10a. Evaluation of botulinum neurotoxin-E heavy chain expressing recombinant Channel Catfish Virus as a potential vaccine for visceral toxicosis of catfish

Kamalakar Chatla[1]*, Patricia S Gaunt[2], Terry Greenway[2], Dusan Kunec[3], Lorelei M Ford[1], Larry A Hanson[1]

[1]Department of Basic Science, College of Veterinary Medicine, Mississippi State University, MS state, MS-39759 kc418@msstate.edu, ford@cvm.msstate.edu, hanson@cvm.msstate.edu
[2]ThadCochran National Warm Water Aquaculture Center, College of Veterinary Medicine, Mississippi State University, Stoneville, MS gaunt@cvm.msstate.edu, greenway@drec.msstate.edu
[3]Institute of Virology, Free University Berlin, Philippstr.13, 10115 Berlin, Germany dusan.kunec@fu-berlin.de

Visceral toxicosis of catfish (VTC) is a sporadic, often devastating disease in catfish aquaculture. VTC is caused by botulinum neurotoxin serotype/E (BoNT/E). BoNT/E is synthesized by theanaerobic, gram positive bacterium, *Clostridium botulinum* as a single 150 kDa polypeptide chain. This product is cleaved by bacterial or host proteases to produce the activated toxin consisting of a 100 kDa heavy chain (HC) and a 50 kDa light chain (LC) linked by a disulfide bond. The HC binds the cell receptor and helps transport the LC into the cytosol of the neuron, where LC (Zn^{2+}-endoprotease) cleaves SNAP 25, one of the SNARE (soluble N-ethylmaleimide sensitive fusion protein attachment protein receptors) proteins. This cleavage blocks the SNARE complex formation, which is needed for synaptic vesicle exocytosis. The lack of synaptic vesicle exocytosis leads to blockage of signaling molecules transfer which ultimately blocks the signal transfer between neurons and muscles causing paralysis. The HC of botulinum neurotoxin is a non-toxic immunogen which is capable of inducing strong BoNT neutralizing antibody responses in mice and rabbits. To evaluate HC immunogenicity, rBoNT/E/HC vaccine produced by USAMRIID was used to vaccinate channel catfish. This vaccine was unable to induce a robust antibody response in channel catfish but western blot analysis demonstrated specific antibody production in 3 of 11 vaccinated fish. We then developed four channel catfish virus (*Ictauluridherpesvirus* 1, CCV) recombinants to express BoNT/E/HC to determine if the virus vector could improve the response. We produced the recombinants by inserting synthetic HC genes into our established Gateway CCV recombination system. These recombinants expressed BoNT/E/HC; the protein concentrations and expression patterns were similar in both lactocystein (proteasome inhibitor) treated cell lines and non-treated cell line. The western blots results showed c-terminal of HC expression. The four recombinants differed in promoters and gene sequence. These recombinants were used to vaccinate channel catfish but no significant protective immunity or BoNT/E antibodies presence was observed. In comparison, a control vector that expressed *Escherichia coli* betagalactosidase induced a strong antibody in the same group of fish. These results suggest that BoNT/E HC has low immunogenicity in channel catfish and deviates from the high immunogenicity previously observed in mouse and rabbit studies. In order to develop a better vaccine it will be necessary to understand BoNT/E /HC immunogenicity and channel catfish immune response against HC.

10b. Immersion vaccination of Atlantic salmon (*Salmo salar*) against *Yersinia ruckeri*

Thu D Nguyen*, Andrew R Bridle, Barbara F Nowak

National Centre for Marine Conservation and Resource Sustainability, AMC, University of Tasmania, Locked Bag 1370, Newnham Campus, Launceston TAS 7250, Australia
Diem.Nguyen@utas.edu.au

Yersinia ruckeri is a pathogen which causes yersiniosis and significant losses in farmed Atlantic salmon (*Salmo salar*) in the Southern Hemisphere. Currently, Yersinivac-B, a commercial bacterin-based vaccine manufactured by MSD Animal Health and prepared from formalin killed whole-cells, is used for immersion vaccination against *Y. ruckeri* for most Tasmanian Atlantic salmon. Until recently, these fish were vaccinated once by bath immersion at 5g. However, half a million vaccinated juvenile Atlantic salmon died of yersiniosis in a single Tasmanian hatchery in 2007. In this PhD study, we evaluated different inactivation methods of *Y. ruckeri* on the efficacy of single dip vaccines including formalin inactivation, ammonium sulphate inactivation, and pH-lysed then formalin inactivation. Additionally, hyperosmotic infiltration was combined with an ammonium sulphate inactivated bacterin in immersion vaccination to assess the protection of these vaccines. The relative percent survival (RPS) afforded was 93.4% for the fish vaccinated with ammonium sulphate inactivated vaccine, 85.4% for fish vaccinated with formalin inactivated vaccine and 81.3% for fish vaccinated with pH-lysed then formalin inactivated vaccine. The results of this study have shown that ammonium sulphate can be used to inactivate *Y. ruckeri* and can replace formalin inactivation.

10c. Effect of age and temperature at vaccination on immunization and protection conferred by a live attenuated *Francisella noatunensis* immersion vaccine in red hybrid tilapia

Nicholas Brown[1*], Zackarias O. Gardenfors[1], Shaun Yount[1], Floyd Revan[1], Stewart Francis[1], Alvin Camus[2], Esteban Soto[1,3]

[1]Center for Conservation Medicine and Ecosystem, School of Veterinary Medicine, Ross University, Main Island Road, West Farm, St. Kitts, West Indies nicholasbrown@students.rossu.edu; ZackariasGardenfors@students.rossu.edu; SYount@rossvet.edu.kn; frevan@rossvet.edu.kn; SteFrancis@rossvet.edu.kn; esoto@rossvet.edu.kn

[2]Department of Pathology, College of Veterinary Medicine, University of Georgia Athens, Athens, GA, USA camus@uga.edu

[3]Department of Biomedical Sciences, Ross University, School of Veterinary Medicine, Ross University, Main Island Road, West Farm, St. Kitts, West Indies

Francisella noatunensis subsp. *orientalis* (*Fno*) is a pleomorphic, facultative intracellular, Gram-negative emerging bacterial pathogen of marine and fresh water fish with worldwide distribution. In this study, we evaluated the efficacy of an attenuated mutant (insertion mutation in the *Fno* intracellular growth loci C (*iglC*)) to be used as a live immersion vaccine when administered to hybrid tilapia at two different stages of growth (fry and fingerlings), and at two different temperatures, 25°C and 30°C. Mortality, days to first death, and *Fno* genome equivalents (GE) in the spleens of survivors, as well as serum and mucus antibody levels were evaluated 30 d post-challenge and compared those produced by a wild type virulent strain to determine vaccine efficacy. Both size and temperature at vaccination played an important role in immunization and protection. Fry (5 g fish) vaccinated at 25°C were not significantly protected compared to non-vaccinated fish at 25°C ($p=0.870$), whereas 5 g fish vaccinated at 30°C were significantly protected compared to non-vaccinated fish at 30°C ($p=0.038$). Although presenting lower mortality, 10 g fish vaccinated at 25°C were not significantly protected compared to non-vaccinated fish at 25°C ($p=0.328$); whereas 10 g fish vaccinated at 30°C were significantly protected compared to non-vaccinated fish at 30°C ($p=0.038$). Additionally, overall mortality of 5 g fish was significantly higher than in 10 g fish. Mortality was also significantly higher in fish subjected to a temperature change (30 to 25°C one week prior to challenge) than in fish subjected to the same temperature at vaccination and challenge.

10d. A new method of intestinal epithelial passage of betanodavirus vaccine with the aid of natural inflammatory substances for the development of oral vaccine

Alkhateib Y. Gaafar[1]*, Hirofumi Yamashita[2], Toshihiro Nakai[3]

[1]National Research Centre, El Buhouth St., Dokki, 12311 Cairo alkhateibyg@yahoo.com
[2]Fisheries Research Center, Ehime Research Institute of Agriculture, Forestry and Fisheries, Ehime 798-0104, Japan
[3]Graduate School of Biosphere Science, Hiroshima University, Hiroshima 739-8528, Japan nakaitt@hiroshima-u.ac.jp

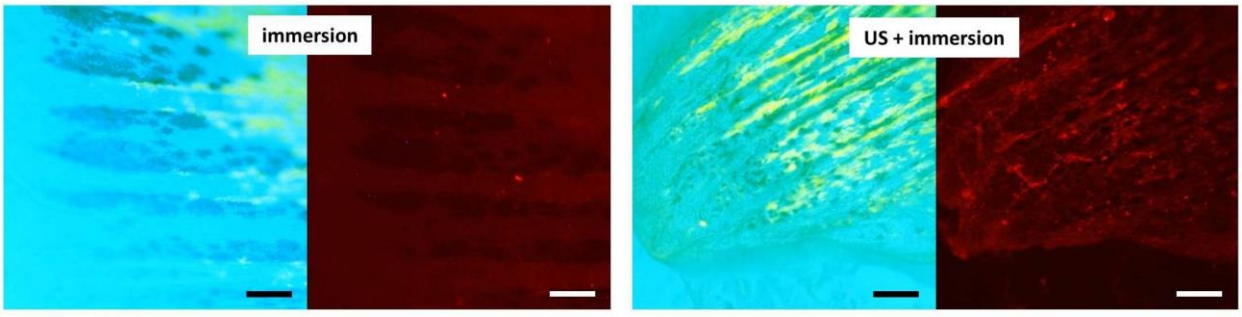
In the last decade, piscine nodavirus (betanodaviruses) infections have emerged as major constraints on the culture of marine fish all over the world causing severe economic losses. This worldwide finfish disease has primarily been associated with juvenile stages in many marine fish species. The most successful vaccination trials were injectable vaccines, which need large sized fish to be applicable. While most epizootics and losses occurs in fish at juvenile or larval stage. So another method of vaccination rather than parenteral route should be investigated. A new trend is appointed toward the oral vaccination in some terrestrial animals, in which some toxins that modulate the passage of large vaccine particles through intestinal epithelial barrier are applicable; zonula occludens toxin (Zot), an enterotoxin expressed by Vibrio cholerae that reversibly opens the intercellular tight junctions. Following a similar concept, some inflammatory substances which cause reversible pathology to the intestinal epithelium may be employed for the same purpose. In this study, sevenband grouper (*Epinephelus septemfasciatus*) and inactivated striped jack nervous necrosis virus (SJNNV) were used as test fish and vaccine, respectively, and the natural inflammatory substances used were a-capsaicin (methyl vanillyl nonenamide) which is naturally produced from hot chili pepper (1mg/fish), b-piperine (1-[5-(1,3-Benzodioxol-5-yl)-1-oxo-2,4-pentadienyl] piperidine) which is naturally produced from black pepper (2mg/fish), and c-okadaic acid (9,10-Deepithio-9,10-didehydroacanthifolicin) which is naturally produced algal toxin and accused for toxic shellfish syndrome (1µg/fish). Both inflammatory substances and vaccine were introduced via anal intubation to fish. As a result, capsaicin proved to be effective to aid the transepithelial passage of vaccine particles more than piperine, while okadaic acid had no detectable effect. These natural inflammatory substances might be used as oral vaccine adjuvants.

10e. Ultrasound-mediated delivery of DNA vaccines for non-invasive, mass immunization in commercially important fish

Ten-Tsao Wong[1]*, Nilli Zmora[1], Victor Frenkel[2], Scott LaPatra[3], Yonathan Zohar[1]

[1]Institute of Marine and Environmental Technology, University of Maryland Baltimore County, MD twong@umbc.edu; nzmora@umbc.edu; zohar@umbc.edu
[2]Biomedical Engineering, Catholic University of America, Washington, DC frenkel@cua.edu
[3]Clear Springs Foods, Inc., Research Division, Buhl, ID scott.lapatra@clearsprings.com

Mass administration of beneficial agents into farmed fish continues to be a challenge, where there are presently no efficient delivery methods for fry and fingerlings. We have been developing non-invasive ultrasound technology for temporarily permeabilizing the skin of fish to enable these compounds to be taken up by immersion. To date we've demonstrated enhanced uptake of soluble compounds such as hormones (e.g. GnRH) and fluorescent marking compounds (e.g. Calcein and Dextrans, see figure below). We are presently focusing our efforts on vaccines, where the delivery of particulate material has proven to be more challenging. We've evaluated two complimentary delivery strategies, where ultrasound exposures were given either during or prior to immersions in the agents to be delivered. Ultrasound effects in the skin were characterized using transmission electron microscopy. Uptake of fluorescently labeled surrogate agents was evaluated using fluorescent microscopy. Our specific interest in DNA vaccines has lead us to look at gene expression levels of reporter genes (e.g. luciferase and □-gal), in the form of naked plasmid, plasmid coated onto polycationic nanoparticles, and nanoparticle coacervates comprised of deacetylated chitosan and DNA. This presentation will summarize our efforts to date to develop a commercially viable, noninvasive, mass administration procedure for delivering DNA vaccines into commercially important fish. This includes results from preliminary, large-scale challenges of rainbow trout using live infectious hematopoietic necrosis virus (IHNV), an important pathogen in commercial aquaculture in the North West region.



Representative brightfield (left) and fluorescent (right) micrographs showing red-fluorescent dextrans (500 kDa) in the skin of zebrafish (0.5 gr). Images were captured in the caudal fins. Greater amounts of the fluorophore were consistently found when ultrasound exposures were given during immersions in the agent compared to immersions alone. Scale bar = 100 μm.

11) General Session: Viruses I

11a. Control and eradication of viral hemorrhagic septicemia in Danish aquaculture

Niels J. Olesen*[1], Helle F. Skall[1], Britt B. Jensen[2], Niels H. Henriksen[3], Stig Mellergård[4], Henrik Korsholm[5]

[1]National Veterinary Institute, DTU, Copenhagen, Denmark, njol@vet.dtu.dk
[2]Norwegian Veterinary Institute, Oslo, Norway, britt-bang.jensen@vetinst.no
[3]Danish Aquaculture Association, Silkeborg, Denmark, niels@danskakvakultur.dk
[4]Danish Veterinary and Food Administration, Glostrup, Denmark STIM@fvst.dk
[5]Danish Veterinary and Food Administration, Vejle, Denmark HEKOR@fvst.dk

Viral haemorrhagic septicaemia (VHS) virus was first isolated in Denmark in 1962, when more than half of the approximately 800 Danish fish farms were considered to be infected. In November 2013, 50 years later, the terrestrial parts country obtained status as EU approved VHS free zone. In the years in between very significant resources have been used to control and eradicate the disease. The control program included strict biosecurity and preventative measures, trade regulations, zoning and intensive inspections and laboratory testing.

During the first decades of control and eradication programs the number of infected farms was significantly reduced while the curve flattened the last 20 years. It was only after a large and costly coordinated action in 2009-2013 including all affected areas that the country managed to free itself totally from VHS. It is the first time that VHS has been eradicated from an endemically infected country. Among the causes of the success are a close collaboration between industry, stakeholders, veterinary authorities and scientists. Also the reduction of the number of farms and novel farming strategies account for the success. Furthermore, in Denmark rainbow trout farming would not survive in the international competition being endemically infected with this serious disease providing a strong incitement for the fish farmers.

Molecular tracing of the origin of VHSV isolates revealed that despite strict trade regulations and a ban on introduction of live salmonids into the country VHSV seemed to have crossed the boarders into Denmark in a couple of cases. Molecular tracing also showed that the numerous VHS outbreaks in marine fish farms were due to stocking these with VHS infected rainbow trout in the incubation phase and not to infection with VHSV from the marine environment. From sequencing more than 400 VHSV isolates from Denmark it appears that evolution of low virulent VHSV from marine fish species is a very rare event and is most likely related to feeding with fresh fish which is now prohibited in rainbow trout farming.

Vaccination was not included in the control in Denmark but if licensed, vaccines would have been useful in order to reduce virus load before stamping-out.

This project has been funded by the European Fisheries Fund: Denmark and EU invests in sustainable fisheries

11b. Recurrence of VHS outbreaks in the Lower Great Lakes

Rodman G Getchell*, Emily R Cornwell, Paul R Bowser

Aquatic Animal Health Program, Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853 USA rgg4@cornell.edu; erc58@cornell.edu; prb4@cornell.edu

Over the last two years viral hemorrhagic septicemia outbreaks have recurred in the Lower Great Lakes. VHS outbreaks diagnosed in Irondequoit Bay, an embayment off of Lake Ontario east of Rochester, NY, and at the mouth of Chautauqua Creek on Lake Erie in Western New York in the spring of 2013 were the first fish kills caused by viral hemorrhagic septicemia virus type IVb (VHSV) in New York State since 2007. The Irondequoit Bay kill involved thousands of gizzard shad (*Dorosoma cepedianum*), as well as several yellow perch (*Perca flavescens*) and freshwater drum (*Aplodinotus grunniens*). The Chautauqua Creek kill involved hundreds of white perch (*Morone americana*). This year, an ongoing gizzard shad kill that lasted over a period of weeks was documented in March 2014 in the harbor at Dunkirk, NY on Lake Erie. For all three outbreak investigations, kidney/spleen/liver/heart homogenates were prepared from individual fish and the filtered homogenate was inoculated onto EPC cells. Cytopathic effect was observed after several days from the majority of samples. Frozen aliquots of cell culture supernatant were shipped to the Western Fisheries Research Laboratory in Seattle for partial sequencing of the glycoprotein gene. A quantitative RT-PCR test specific for VHSV was also performed on combined kidney, spleen, liver, and heart homogenates and brain tissue samples. Both types of samples were positive. The important message from these recurrences is that VHSV is still present in the Great Lakes and capable of killing substantial numbers of fish.

11c. Evidence for differential virulence among sequence types of viral hemorrhagic septicemia virus genotype IVb

Emily R. Cornwell*, Sierra M. Imanse, Rodman G. Getchell, Paul R. Bowser

Aquatic Animal Health Program, Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853-6401

Viral hemorrhagic septicemia (VHS) has been known as a devastating disease of cultured rainbow trout in Europe since 1938. Since that time, the known host and geographic range of this disease has expanded dramatically. The causative agent, VHS virus (VHSV) was first detected in the Laurentian Great Lakes in 2005. To date, all isolates recovered from Great Lakes fish have fallen within genotype IVb. Phylogenetic analyses of a region of the central glycoprotein gene have revealed over 60 sequence types of VHSV present in the Great Lakes that have a differential spatial distribution throughout the basin. This differential spatial distribution appears to be relatively stable over time. We hypothesized that there would be a difference in virulence between the two most common VHSV sequence types: vcG001 and vcG002 and tested this using *in vitro* and *in vivo* approaches. *In vitro*, vcG001 had a faster rate of growth and replicated to a higher titer than vcG002 in two of three cell lines tested. However, there was no significant difference in the quantity of viral RNA produced between the two sequence types. *In vivo*, round gobies (*Neogobius melanostomus*) exposed to vcG001 or vcG002 by immersion experienced a higher mortality due to vcG001 than vcG002. These results suggest that there are some phenotypic differences between different VHSV IVb sequence types and may explain some of the geographic variation in prevalence observed in the Great Lakes. Knowledge of the distribution and phenotypic characteristics of VHSV IVb sequence types will be a useful tool to manage VHSV in the Great Lakes. The phenotypic characteristics of VHSV IVb are not spatially homogeneous and prevention and recovery strategies should differ based the dominant characteristics of the virus in each region.

11d. Host susceptibility and viral fitness as potential drivers of IHN virus emergence and displacement events in steelhead trout

Gael Kurath[1,2]*, Rachel Breyta[1,2], Alison M. Kell[1,2], Andrew R. Wargo[1,3]

[1]USGS Western Fisheries Research Center, 6505 NE 65th St., Seattle, WA 98115 USA kurath@usgs.gov
[2]University of Washington, Pathobiology Graduate Program and School of Aquatic and Fishery Sciences, Seattle, WA 98195 USA rbjmax@uw.edu, kella@uw.edu
[3]Virginia Institute of Marine Science, Gloucester Point, VA 23062 USA arwargo@vims.edu

The salmonid rhabdovirus infectious hematopoietic necrosis virus (IHNV) is a well-known pathogen that has been a subject of research for many decades. Phylogeography of over 2400 IHNV field isolates from the Pacific Northwest of North America has defined distinct geographic ranges of three major genogroups and revealed an intriguing history of IHNV emergence and displacement events. In the first stages of a large project aimed ultimately at developing a landscape epidemiological model of IHNV transmission in the Pacific Northwest we have explored two factors hypothesized to be drivers of IHNV emergence and displacement in the field. The first is variation in susceptibility of host populations of steelhead trout, *Oncorhynchus mykiss*. Controlled laboratory challenge experiments confirmed that different steelhead trout populations vary significantly in susceptibility, even within one watershed, and that host populations that are naive to IHNV are significantly more susceptible than those with a history of exposure and disease due to the virus. The second factor explored is viral fitness, as measured by controlled *in vivo* co-infection and superinfection fitness assays using four IHNV strains that represent three successive viral displacement events observed in the field. Detailed studies defining multiple fitness measures found the four virus strains did not differ in most analyses, and where they did differ the displaced strain had higher fitness than the strain that replaced it. This does not support the hypothesis that viral fitness is correlated with IHNV displacements. Taken together these studies suggest that variation in susceptibility of host populations is a likely contributing factor in IHNV emergence and displacement, but viral fitness, as measured in laboratory assays, is not. Further work will examine additional factors to inform a mathematical model of IHNV transmission that we anticipate may serve as a reference model for aquatic pathogens.

11e. The role of Chinook salmon (*Oncorhynchus tshawytscha*) in the ecology of Infectious Hematopoietic Necrosis Virus (IHNV) in the Columbia River Basin

Daniel G. Hernandez[1,2]*, Thomas P. Quinn[1], Gael Kurath[2]

[1]School of Aquatic and Fishery Sciences, University of Washington 1122 NE Boat Street, Seattle, WA 98105, dh38@washington.edu, tquinn@u.washington.edu
[2]U.S. Geological Survey, Western Fisheries Research Center, 6505 NE 65the Street Seattle, WA 98115 gkurath@usgs.gov

Infectious hematopoietic necrosis virus (IHNV) is an acute viral pathogen that causes significant disease and mortality in wild and cultured salmonids. Genetic sequencing of hundreds of virus isolates has identified three genetic subgroups of IHNV designated U (upper), M (middle) and L (lower) for their relative geographic occurrence in Western North America. Each genogroup of IHNV contains many individual isolates, and genogroup-specific patterns of host specificity have been observed. The U (most northerly distributed) genogroup of IHNV is primarily virulent for sockeye salmon (*Oncorhynchus nerka*), whereas the M (middle or central) genogroup of IHNV is primarily virulent for rainbow and steelhead trout (*O. mykiss*). The L (lower or southerly) genogroup of IHNV is almost exclusively virulent in juvenile Chinook salmon (*O. tshawyscha*) of California where disease outbreaks of L group IHNV have occurred since the 1940's and have resulted in mortality of up to 90%.

In the Columbia River Basin (CRB), IHNV has been detected regularly in Chinook salmon since 1973 but primarily in the form of asymptomatic infection of adult fish with the U and M group viruses. The general lack of disease in juvenile Chinook salmon in the CRB led to the belief that Chinook were refractory to disease caused by both the U and M genetic subgroups of IHNV, thus their potential role in the epidemiology of the virus throughout the CRB had gone unexplored. The consistent presence of IHNV in adult Chinook salmon has led us to hypothesize that this species may function as a reservoir and/ or vector of IHNV to other susceptible salmonid hosts. Within the CRB, Chinook salmon are diverse with differences in juvenile life history (stream-type and ocean-type) and evolutionary lineage (interior and coastal). Having developed a controlled laboratory model of Chinook salmon infection with select IHNV isolates, our current investigation aims to describe the infection kinetics of the most commonly detected isolates of U and M IHNV in four populations of juvenile Chinook representative of the genetic & life history diversity present in the CRB. Using viral plaque assay and an IHNV glycoprotein gene Reverse Transcription – Quantitative Polymerase Chain Reaction (RT-qPCR) assay, we have confirmed infection and quantified viral load from the tissues of fish experimentally challenged with U and M IHNV. Our approach and methodology has made it possible to begin discerning the role of Chinook salmon in the ecology of IHNV in the CRB. The information gleaned from this investigation will also make it possible to better understand recent IHNV epizootic events in juvenile Chinook salmon of the CRB.

11f. Preliminary risk assessment of fish hosts experimentally challenged with a North American strain of Spring Viremia of Carp Virus

Evi Emmenegger[1*], George Sanders[2], Fred Binkowski[3], Jim Winton[1], Gael Kurath[1]

[1]Western Fisheries Research Center, Dept. of the Interior, US Geological Survey, 6505 NE 65th Street, Seattle, Washington 98115 USA evi_emmenegger@usgs.gov
[2]University of Washington, School of Medicine, Dept. of Comparative Medicine, T-160 Health Sciences Center, Seattle, Washington 98195 USA gsander@uw.edu
[3]University of Wisconsin–Milwaukee, School of Freshwater Sciences, 600 E. Greenfield Ave., Milwaukee, Wisconsin 53204 USA sturgeon@uwm.edu

Spring viremia of carp virus (SVCV) is a rhabdoviral pathogen associated with disease outbreaks in cultured and wild fish worldwide, including Europe, Asia, Middle East, South America, and North America. It is one of only 10 notifiable fish pathogens listed by the World Organization for Animal Health. Common carp (*Cyprinus carpio carp*), koi (*C. carpio koi*), and other carp species suffer the highest mortalities from SVCV infections, while other cyprinid fish species have varying susceptibility. Although salmonid fish typically are considered refractory to infection by SVCV, there have been a few reports suggesting infection has occurred in rainbow trout (*Oncorhynchus mykiss*), but there have been no reports to our knowledge of Percid fish being infected with SVCV. Since the first outbreak of SVCV at a North Carolina koi farm in 2002 there have been eight subsequent detections or outbreaks of SVCV among fish species from the families of *Cyprinidae* and *Centrarchidae* within the US and Canada. Thus, this exotic virus is considered a potential threat to native and cultured fish populations in North America. We performed multiple experimental challenges with fish species from three families (*Salmonidae*, *Cyprinidae*, and *Percidae*) to identify the potential risk associated with SVCV exposure of resident fish populations in North America.

Three salmonid species, rainbow trout and steelhead (*O. mykiss*), chinook salmon (*O. tshawytscha*), and sockeye salmon (*O. nerka*), were challenged by immersion or injection with the North Carolina SVCV isolate. Two cyprinid species, koi and fathead minnows (*Pimephales promelas*) and one percid species, yellow perch (*Perca flavescens*) were also challenged. Virus challenged salmonid fish had cumulative percent mortalities ranging from 0 to 100%, with sockeye salmon fry being the most vulnerable. SVCV challenged cyprinids had mortalities ranging from 11% to 100% in koi and 29% in fathead minnows. Yellow perch had cumulative mortalities of 2% to 33%. A sub-sample of mortalities and survivors were screened for virus by plaque assay and reverse transcription polymerase chain reaction (RT-PCR). In general, all mortalities tested positive for SVCV with high viral titers while the survivors had variable persistence of SVCV with overall lower virus titers. The age of fish tested in addition to host species vulnerability appear to be key risk factors for infection by SVCV. The results of these preliminary challenges will be presented.

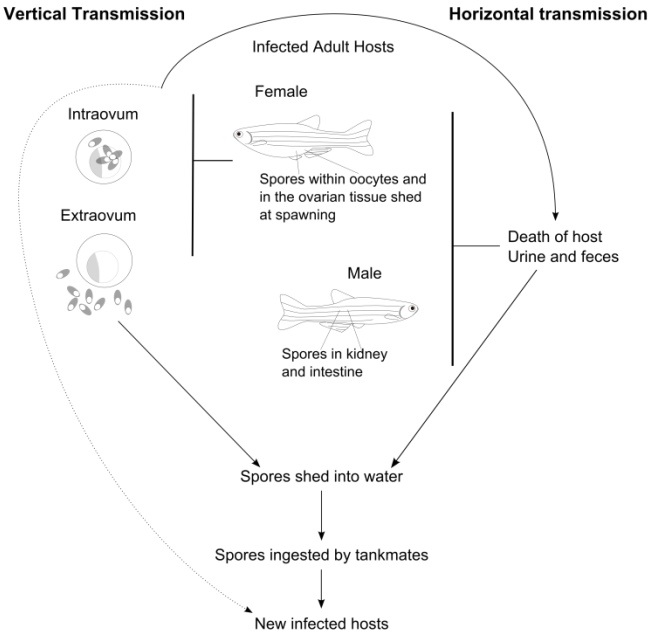
12) Special Session: Diseases of Zebrafish

12a. Transmission of microsporidia in the zebrafish, *Danio rerio*

Justin L. Sanders[1]*, Michael L. Kent[1,2]

[1]Department of Microbiology, Oregon State University, Corvallis, OR, USA
[2]Department of Biomedical Sciences, Oregon State University, Corvallis, OR, USA

The microsporidian parasite, *Pseudoloma neurophilia*, is the most commonly diagnosed infectious disease in laboratory populations of the zebrafish, *Danio rerio.* We describe here the transmission characteristics of *P. neurophilia* in laboratory colonies of zebrafish and its importance in the context of control of the pathogen. Intraovum transmission of *P. neurophilia* was directly observed by histological screening of larval fish spawned from infected adults and by analyzing eggs using a transmitted light dissecting microscope. The prevalence of intraovum transmission was determined by screening surface-decontaminated eggs from paired spawns using a real-time PCR based assay. Intraovum transmission was detected in 4 of the 27 spawns and the prevalence of intraovum *P. neurophilia* in the eggs from these spawns was determined to be approximately 1%. Parasite DNA was also detected in the spawning water from 11 of the 27 spawns, highlighting the potential for extraovum transmission of *P. neurophilia.* The early stages of *P. neurophilia* infection were observed in experimentally infected larval zebrafish using a combination of standard histological staining and an *in situ* hybridization probe specific to the small-subunit ribosomal DNA gene of *P. neurophilia.* The distribution of the early stages of *P. neurophilia* and the lack of mature spores until 96 hours post exposure suggests that the parasite gains access to organs distant from the initial site of entry, likely by penetrating the intestinal wall with the polar tubule, and that autoinfection does not occur at any detectable frequency during the initial stages of infection.



12b. Activity of antibiotics against *Mycobacterium* species commonly found in zebrafish

Carolyn T Chang[1]*, Christopher M Whipps[2]

SUNY-ESF, State University of New York College of Environmental Science and Forestry
Department of Environmental and Forest Biology, 1 Forestry Drive, Syracuse, NY 13210 USA
[1]ctchang@syr.edu
[2]cwhipps@esf.edu

The zebrafish (*Danio rerio*) is a popular vertebrate model organism. With increased usage and investment in wild-type and mutant zebrafish strains, considerable value is placed on preserving zebrafish health. Mycobacteriosis is a common bacterial infection of laboratory zebrafish caused by *Mycobacterium* species. Current recommendations for control of mycobacteriosis include removal of infected fish and in more severe outbreaks, depopulation. Despite the effectiveness of these control measures, less disruptive management recommendations should be investigated. Little is known regarding the efficacy of antibiotic treatment for zebrafish mycobacteriosis; however, treatment of infected zebrafish may be appropriate in order to maintain valuable strains. We investigated the susceptibility of both rapid and slow growing zebrafish *Mycobacterium* species to antibiotic treatments *in vitro*. Testing of antibiotics was carried out using a commercially available 96-well microtiter plate format. Human infections of rapidly growing mycobacteria are generally susceptible and treated with clarithromycin, amikacin, and cefoxitin. Slow growing *M. marinum* is susceptible and treated with rifampin, rifabutin, ethambutol, as well as clarithromycin. It is hypothesized that *Mycobacterium* isolates from zebrafish will exhibit a similar susceptibility to antibiotics. Results will assist in the development of remediation techniques that all zebrafish research facilities can use to improve fish health. Additionally, because some *Mycobacterium* species have the potential to infect humans this study may also benefit fish handler health in the case of zoonotic transmission.

12c. *Pseudoloma neurophilia*: A retrospective and descriptive study

*Sean Spagnoli[1], Lan Xue[2], Katrina Murray[3], Fidelis Chow[4], Michael Kent[5]

[1]Department of Environmental Health Sciences; Oregon State University: 526 Nash Hall, Corvallis, OR 97331. sean.spagnoli@oregonstate.edu
[2]Department of Statistics; Oregon State University; 82 Kidder, Corvallis, OR 97331. xuel@science.oregonstate.edu
[3]Zebrafish International Resource Center Pathology and Health Services; 5274 University of Oregon. Eugene, OR 97403. katy@zebrafish.org
[4]Department of Microbiology; Oregon State University: 526 Nash Hall, Corvallis, OR, 97331. fidelis.chow@oregonstate.edu
[5]Department of Microbiology. 526 Nash Hall, Corvallis, OR, 97331. michael.kent@oregonstate.edu

Pseudoloma neurophilia (PN) is a microsporidium of zebrafish (*Danio rerio*) that preferentially infects neural tissue. The most commonly reported gross lesions are emaciation, axial skeletal deformities, and death. From the years 2006 to 2013, the Zebrafish International Resource Center (ZIRC) diagnostic service (Eugene, OR) diagnosed PN in an average of 50% of submitting facilities (range 19-74%). 559 fish from the ZIRC archives were evaluated via histopathology for parasite cluster number and anatomic location, meninxitis/meningitis, encephalitis/myelitis, myositis, and the presence or absence of other diseases. These histopathologic criteria were then compared with historical data in order to determine if significant differences existed between fish submitted with and without clinical disease. Microscopically, parasite clusters PC occurred in distinct axonal swellings, frequently with no associated inflammation. Neuron cell bodies in the spinal cord gray matter and the reticular formation frequently displayed marked satellitosis with or without neuronophagia even though they were generally distant from any observable PC. When inflammation was directly associated with PC, it appeared that it was due to rupture of the axon containing the microsporidium. PC occurred most frequently in the ventral white matter of the spinal cord and the spinal nerve roots. Within the hind brain white matter, PC were found most commonly in the dorsal and ventral medial lateral fascicle as well as in the commissura ventralis rhombencephali . Within the hind brain gray matter, PC occurred most frequently in the RF and the griseum centrale . The presence of myositis was significantly correlated with higher numbers of spore clusters in nerve roots. The presence of high numbers of PC within brain and spinal cord structures mediating startle responses and fear memory suggests that related behaviors could be altered by PN infection. These behavioral endpoints are an important part of many fish behavioral studies, which suggests that PN infection could introduce unacceptable variation. It may, therefore, be important to screen zebrafish for PN before using them in sensitive behavioral studies.

12d. Interpretive challenges in zebrafish histopathology studies

Jeffrey C. Wolf*

Experimental Pathology Laboratories, Inc., Sterling, Virginia, USA

Contrary to popular rumor, zebrafish histopathology is not dead. Despite myriad new cutting edge technologies that are currently being implemented to take advantage of this versatile animal model, histopathology continues to be a valuable tool for investigating the morphologic features and extent of both naturally-occurring and experimentally-induced disease. Not only does it serve as a key link between apical (i.e., population relevant) and subcellular (e.g., molecular) endpoints, histopathology remains one of the most reliable, sensitive, and comprehensive assays for identifying and characterizing a vast array of physical disorders. In an experimental capacity, histopathologic results are used frequently to determine potential treatment effects, offer insights into mechanisms of action, and provide phenotypic mapping of genetically altered animals. Additionally, if sufficient sampling is performed, the presence of confounding subclinical disease(s) may also be detected in experimental animals or broodstock. However, histopathology is the proverbial double-edged sword. Differentiating salient histopathologic changes from normal anatomic features or tissue artifacts can be decidedly challenging, especially for the novice fish pathologist. As a consequence, findings of questionable accuracy may be reported inadvertently, and the potential negative impacts of publishing inaccurate histopathologic interpretations are not always fully appreciated. Analogous to erroneous conclusions in other scientific disciplines, histopathologic misdiagnoses and misinterpretations can have negative consequences that reverberate far beyond the scope of a single study. The goal of this presentation is to illustrate a number of specific morphologic findings in commonly examined tissues of zebrafish and other fish species that are frequently either misdiagnosed or underdiagnosed, and to address related issues involving the interpretation of histopathologic data. Additionally, general recommendation for improving the accuracy and precision of histopathology results will be provided.

12e. Biosecurity and diagnostics at the Zebrafish International Resource Center; identifying and minimizing risks in an evolving research environment

Katrina N. Murray*

Zebrafish International Resource Center, 5274 University of Oregon, Eugene, OR 97403
katy@zebrafish.org

The Zebrafish International Resource Center (ZIRC) is a repository and distribution center for wild-type, mutant, and transgenic zebrafish. ZIRC has also been providing a diagnostic pathology service to the zebrafish research community since 1999. In 2013 the service examined 2313 fish, representing 128 diagnostic cases from 43 zebrafish facilities. The most common pathogens that we detect are *Pseudoloma neurophilia*, mycobacteria (*M. chelonae* or *M. haemophilium*), and *Pseudocapillaria tomentosa*. Infections with *Edwardsiella ictaluri* and *M. marinum* have been relatively uncommon, but are of great concern because of their virulence. At the ZIRC facility, our daily health monitoring, sampling strategy, and sentinel programs are designed to identify pathogens and minimize transmission and spread. To date, *Pseudoloma neurophilia* and *Mycobacterium chelonae* have been identified in our main fish facility. Cleaning, personal protective equipment, and husbandry protocols are emphasized in our strategy to control these pathogens. Preventing entry of new pathogens is a high priority. ZIRC is in the midst of importing over 100,000 new zebrafish lines generated in large-scale mutagenasis projects. These lines are imported as cryopreserved sperm and regenerated based on customer orders. Sperm samples bypass the quarantine room, which has traditionally been the first defense against new pathogens in zebrafish facilities. We have devised a plan in which the relative risk of samples is predicted based on submitting facility health data. These data will prompt variable strategies for regenerating the lines. For example, the first generation of a line submitted from a facility with high virulence pathogens will be raised in the quarantine room. The line will then be cryopreserved from this generation and all contributing males screened for pathogens. Minimizing the biosecurity risk posed by imported sperm samples must be balanced against the need to fulfill customer orders in a time frame that facilitates research advancement.

12f. An update on detection and control of *Edwardsiella ictaluri* infections in zebrafish.

John P. Hawke*, Elise Desonier, Rui Wang

Department of Pathobiological Sciences, LSU School of Veterinary Medicine 1909 Skip Bertman Drive, Baton Rouge, LA 70803, Jhawke1@lsu.edu

In recent years there has been a dramatic increase in the use of zebrafish in biomedical research. Zebrafish in research laboratories are raised indoors, either on recirculating or flow-through water systems with UV sterilizers. Fish stocks are generally housed in separate tanks according to genetic background (wild-type, mutant, and transgenic lines) and generation. The closed nature of these systems facilitates tracking of morbidities and mortalities and disease monitoring. The source of zebrafish for research laboratories ranges from pond-reared fish from the aquarium trade to laboratories such as the Zebrafish International Resource Center (ZIRC), in which pathogens are documented and controlled. Most research facilities introduce new fish into their main facilities as second generations derived from eggs that are surface disinfected with chlorine.
From 2011-2014, multiple cases of Edwardsiellosis in zebrafish have been reported by the Louisiana Aquatic Diagnostic Laboratory at Louisiana State University, School of Veterinary Medicine. The acute nature of this bacterial disease resulted in high mortality rates and the loss of potentially valuable stocks. Therefore, developing rapid diagnostic and screening methods and effective treatment for this disease is necessary. Two laboratories affected by the disease used florfenicol and enrofloxacin in an attempt to control the infection. In a laboratory research trial, twenty tanks of zebrafish were exposed to the bacteria by immersion and treated using feed medicated with florfenicol 15mg/kg/day or enrofloxacin 15/mg/kg/day. Mortality was recorded for 21 days post-challenge. The results of the trials and the ability to detect carriers post infection are discussed.

13) Special Session: Parasite Life Cycles:

13a. Life cycles of marine parasites: Insights from rescued and exhibit animals in public aquaria

Brent R Whitaker[1,2]*

[1]National Aquarium, 501 E Pratt Street, Baltimore, MD 21202 USA bwhitaker@aqua.org
[2]Institute of Marine and Environmental Technology, 701 E Pratt St, Baltimore, MD 21202 USA

Large public aquaria, as the custodians of a great diversity of aquatic animals, under comprehensive veterinary care, afford an excellent opportunity to study parasites. Public aquaria acquire most of their exhibit animals (fishes, amphibians, reptiles, birds, marine and terrestrial mammals), and the parasites they carry, from both wild and non-wild sources around the globe. Furthermore, many aquaria rescue, rehabilitate and release marine animals, providing further opportunity to discover novel organisms and speculate as to the role that they play in the animal's life. The holding of marine species in public aquaria offers a particularly important opportunity to study their parasites fauna, since: (i) observing and taking samples from these hosts – particularly the cetaceans - in the wild may be difficult or impossible, and (ii) the extended periods for which the hosts are held often allows comprehensive study of their parasite fauna, and possible pathogenicity. Today's modern aquaria typically have advanced quarantine and health monitoring programs, under the scrutiny of trained aquatic animal veterinarians and husbandry professionals, and thus parasites are often recognized, investigated, and treated, in advance of introducing the animal to the established collection. On occasion, in large complex exhibits, parasitic outbreaks occur that are significantly challenging to treat, for example outbreaks of the ciliate *Cryptocaryon* and of the monogenean *Neobenedinia*. In other instances, seemingly unique and isolated infections, such as several recent mortalities caused by the blood fluke *Cardicola chaetodontis* in butterfly fish *Chaetodon spp*., are reported with increased frequency among aquariums, suggesting the possibility of an emerging pathogen or increased presence due to the collection of animals from a specific wild population where the infection is endemic, such as may have occurred with coccidia in the cownose rays *Rhinoptera bonasus* in the late 1990's. Rescued animals provide a rare opportunity to observe, record and describe novel organisms such as the trypanoplasm-like flagellate, *Jarrellia atramenti*, and ciliates that were found in the blowhole of a stranded pygmy sperm whale *Kogia breviceps*. Partnering of aquarium staff with specialists at research laboratories, government agencies, universities and other aquaria is often essential in order to identify the parasites, understand their life cycles, predict potential impact to the collection, identify control strategies including options for effective treatment, and interpret the conservation implications for wild and captive marine animals.

13b. Managing indirect life cycle parasites in aquaculture

Linda M Pote[1], Matt J Griffin,[1,2] Lester H Khoo[1,2], David J Wise[2], Thomas G Rosser[1]* ,Terrence E Greenway[2], Marlena C Yost[1], Cynthia M Doffitt[1], Mary M O'Hear[1], Tommy D King[3], Brian S Dorr[3], Sylvie M A Quiniou[4]

[1]College of Veterinary Medicine, Mississippi State University, 240 Wise Center Drive, Mississippi State, MS 39762 lpote@cvm.msstate.edu, griffin@cvm.msstate.edu, khoo@cvm.msstate.edu, tgr49@msstate.edu, myost@pvcc.edu, doffitt@nsula.edu, maryohear@gmail.com

[2]Thad Cochran National Warmwater Aquaculture Center, Mississippi State University, PO Box 197, Stoneville, MS 38776 dwise@drec.msstate.edu, greenway@drec.msstate.edu

[3]USDA/ARS/Wildlife Services/Starkville, MS 39759 Tommy.King@aphis.usda.gov, brains.dorr@aphis.usda.gov

[4]Warmwater Aquaculture Research Unit, Agricultural Research Service, United States Department of Agriculture, PO Box 38, Stoneville, MS 38776 sylvie.quiniou@ars.usda.gov

With the increased consumption of fish worldwide and the continual decline of wild fish stocks, aquaculture has continued to expand globally to meet these needs. Many of these intensively managed outdoor aquaculture systems are ideal for the introduction and propagation of fish parasites with indirect life cycles. These aquaculture systems have all of the components for these parasites to thrive. Many of these systems have open ponds in which a diverse population of wildlife are present, often heavily infected with numerous parasites that also infect fish; there is an abundance of aquatic invertebrate hosts in these ponds to serve as hosts; and a concentrated population of cultured fish are readily available to serve as intermediate or final hosts, thus creating an ideal environment for the completion of these life cycles. An example of this scenario is the husbandry of pond raised catfish in the United States, the largest warm water commercial fish industry in the U.S. In this industry mixed-aged fish are housed densely in large open ponds, there are high numbers of snail and aquatic oligochaetes present in these ponds and there is a constant influx of fish-eating birds loafing and feeding at these ponds. The control and management of these parasites, in particular the myxozoans and digenetic trematodes, has been a continual challenge. This presentation will address the obstacles encountered and the approaches taken to address this challenge in the development and implementation of management schemes to control these parasites in the commercial catfish industry.

13c. Interrupting life cycles: considerations for evaluating effectiveness of antiparasitic drugs

E. D. Landis[2]*, C. M. Gieseker[1], J. A. Matysczak[2], S.R. Gore[2]

[1]US Food and Drug Administration, Center for Veterinary Medicine, Office of Research, 8401 Muirkirk Road, Laurel, MD 20708 USA charles.gieseker@fda.hhs.gov

[2] US Food and Drug Administration, Center for Veterinary Medicine, Office of New Animal Drug Evaluation, 7500 Standish Place, Rockville, MD 20855 USA eric.landis@fda.hhs.gov, jennifer.matysczak@fda.hhs.gov, stacey.gore@fda.hhs.gov

In the relatively short history of pre-market drug approvals for aquaculture in the US, most effectiveness trials for proposed therapeutic uses have focused on the reduction of mortality as a primary study endpoint. Infestations of parasites are a significant problem in fish culture systems and can do significant harm to a population without inducing acute mortality. Therefore assessment of parasite treatments typically rely on more nuanced endpoints associated with reduction of parasite numbers. There are a number of scientific considerations that can influence the assessment of antiparasitic effectiveness in fish. These considerations include the diversity of fish; the diversity of parasite species and their life cycles; and the selection of appropriate clinical endpoints. This presentation will discuss these considerations as well as how *in vitro* assays can help determine a parasite's drug susceptibility. We will also discuss the drug approval process and the Minor Use and Minor Species (MUMS) Animal Health Act of 2004 so the audience learns what information is required and what options are available to generate the scientific data needed to achieve regulatory approval.

13d. Transforming trophozoites: life cycle adaptions in the diplomonad flagellate *Spironucleus*

Sarah L. Poynton [1,2]*, Mohammad Feza Saghari Fard[2], Erik Sterud[3], Anders Jørgensen[3]

[1]Department of Molecular and Comparative Pathobiology, Johns Hopkins University School of Medicine, 8th Floor Edward D. Miller Research Building, 733 North Broadway, Baltimore, MD 21205, USA spoynton@jhmi.edu
[2]Leibniz Institute for Freshwater Ecology and Inland Fisheries, Müggelseedamm 310, Berlin D 12587, Germany (former affiliation)
[3]National Veterinary Institute, PO Box 8156 Dep., 0033 Oslo, Norway (former affiliation)

Successful life cycles require that parasites be adapted morphologically and physiologically to their hosts, and to the external environment, as is evident by the ubiquity of parasites. Among parasites of fish, a common strategy for flagellates with direct life cycles, is to transform from motile trophozoite in the host to resistant cyst for transmission through the external environment. In such parasites, reproduction can occur by binary fission of trophozoite, and division within the cyst. Although the trophozoite – cyst life cycle is exemplified in the common diplomonad flagellate *Spironucleus* spp., which infects salmonids, gadids and cichlids, key aspects of the transformation of trophozoites to cysts have remained poorly known. For example: (i) which factors trigger encystment, (ii) what are the adaptions for endurance in the water, (iii) how does the parasite maximize the chance of infecting a new host? We have been able to address these questions during our *in vitro* culture of *Spironucleus salmonis* from juvenile rainbow trout, *Oncorhynchus mykiss*. We initially established cultures of *S. salmonis* in two different media, while developing plasma incubation tests to investigate the innate immunity of different host species. In one of the media, single trophozoites transformed into single cysts, as commonly reported. However, we were intrigued to observe that in the other medium, the trophozoites typically clustered together before encysting, resulting in clusters of cysts. The elaborate process was mediated by the posterior flagella, and we hypothesize that this occurred via up-regulation of adhesins and intraflagellar transport. Electron microscopy showed that in *S. salmonis*, encystment was asynchronous within a cluster, and that the trophozoite could divide within the cyst. We propose that: (i) clustering is triggered by changes in the environment, such as critical water temperatures and death of the fish, (ii) the cyst wall and abundant glycogen deposits in the cyst are adaptions for endurance in the water, and (iii) clusters of cysts will be more buoyant than individual cysts, and will exceed the minimum infective dose required to initiate an infection, thus increasing the chance of a successful oral transmission and establishment of a new infection.

13e. How DNA sequencing helps us elucidate parasite life cycles

Stephen D Atkinson

Department of Microbiology, Oregon State University, Corvallis, Oregon, USA

Many parasites have life cycles that involve multiple hosts. The task of matching up alternate life stages can be difficult to impossible, given the different morphological forms a parasite usually has in those different hosts. Problems with life cycle elucidation are exemplified by the Myxozoa - a common, speciose, yet persistently enigmatic group of parasitic Cnidaria, which can cause problems in wild and cultured fish. The relatively few known myxozoan life cycles involve alternation of a vertebrate host (typically a fish) and an invertebrate host (typically an oligochaete or polychaete worm, or bryozoan). A given myxozoan species has multiple morphologies within each host and different infectious waterborne spore stages, thus conspecific stages are impossible to determine from morphology. Theoretically, if the hosts are known (or suspected) and available for study, parasite life cycles could be replicated in the laboratory. Practically, often only the economically important host is known, and infection experiments would require too much trial and error to determine the alternate host/s.

An exceedingly more practical approach to determining conspecific parasite stages (and thus the life cycle) has been to match DNA sequences from different hosts. Unlike phenotype, the fidelity of a parasite's DNA is maintained throughout its life cycle. DNA can be used to screen pools of putative hosts, and then parasite stages discovered by follow-up direct examination of hosts or infection trials. DNA sequences can be used to identify and compare myxozoan DNA from different sources, which include hosts (developmental and cryptic stages in vertebrates and invertebrates), parasite spores (released or purified from hosts), environmental samples (mud, water, etc.) and different geographic regions.

Problems associated with DNA sequencing include: co-amplification of parasite and host or other contaminating organisms when general PCR primers are used; ambiguous identification if multiple parasites are present in a sample, especially if present as cryptic stages, and especially as PCR or primer bias may preferentially amplify one taxon (not necessarily the target). Incorrectly identified reference sequences in the NCBI database can lead to false conclusions about which life cycle stages are conspecific.

I will illustrate how DNA sequencing has been used to elucidate parasite life cycles by recounting several case studies, including our lab's recent efforts to discover the first life cycle of a *Kudoa* species (Myxozoa) as a model for this group of particularly troublesome marine fish parasites.

13f. The worm has turned: models could help us manage the intermediate host (*Manayunkia speciosa*) of the myxozoan salmon parasite *Ceratovova shasta*

J.D Alexander[1]*, K.A. Wright[2], N.A. Som[3], N.J. Hetrick[4], J.L. Bartholomew[5]

[1]alexanju@science.oregonstate.edu, Oregon State University Department of Microbiology, 220 Nash Hall, Corvallis, OR, 97330.
[2]katrina_wright@fws.gov, U.S. Fish and Wildlife Service, 1655 Heindon Road, Arcata, CA, 95521.
[3]nicholas_som@fws.gov, U.S. Fish and Wildlife Service, 1655 Heindon Road, Arcata, CA, 95521.
[4]nick_hetrick@fws.gov, U.S. Fish and Wildlife Service, 1655 Heindon Road, Arcata, CA, 95521.
[5]bartholj@science.oregonstate.edu, Oregon State University Department of Microbiology, 220 Nash Hall, Corvallis, OR, 97330.

Many emerging infectious diseases are caused by heteroxenic parasites that infect multiple obligate hosts. Enteronecrosis (syn Ceratomxyosis) is an emerging infectious disease of Klamath River salmonids (California). The causative parasite, *Ceratonova* (syn *Ceratomyxa*) *shasta*, alternately infects *Manayunkia speciosa* (freshwater polychaete). Demand for disease management solutions for Klamath River salmon has generated interest in using flow manipulation to reduce *M. speciosa* populations. However, evaluating the potential efficacy of such an action is constrained by lack of data on factors that limit the distribution of *M. speciosa*. We present a modeling approach for predicting the distribution of *M. speciosa* and evaluating the effects of flow manipulation. Two-dimensional hydraulic models (2DHMs) were built to describe hydraulic variation during peak discharge in three sections of the Klamath River. Polychaete sampling was stratified across depth-velocity gradients within substrate classes predicted from the 2DHMs. A statistical model describing the relationship between physical habitat characteristics and *M. speciosa* distribution was built using data collected in July 2012 and validated using an independent dataset collected in 2013. We then used the 2DHMs and statistical model in tandem to examine the effects of flow manipulation scenarios on polychaete distribution. The best fitting statistical model demonstrated that in summer, *M. speciosa* distribution is associated with substrate, as well as depths and velocity conditions during peak discharge (predicted from the 2DHMs) during the immediate water year. The statistical model predicted the (independent) 2013 data with high accuracy (AUC=0.87) and preliminary results from flow manipulation scenarios suggest high magnitude peak discharge result in lower probabilities of polychaetes than low magnitude peak discharge, which provides compelling evidence that that manipulating the hydrograph could influence distribution of polychaete hosts.

14) General Session: Viruses II

14a. Genetic analysis of two novel Orthomyxoviruses from rainbow trout and koi

William N Batts[1*], Marine S O Brieuc[2], Scott E LaPatra[3], Maureen K Purcell[1], Thomas B Waltzek[4], James R Winton[1]

[1] Western Fisheries Research Center, USGS, 6505 NE 65th Street, Seattle, WA 98115 USA bbatts@usgs.gov, mpurcell@usgs.gov, jwinton@usgs.gov
[2] School of Aquatic and Fishery Sciences, University of Washington, Box 355020, Seattle, WA 98195 USA mbrieuc@uw.edu
[3] Research Division, Clear Springs Foods, Inc., P O Box 712, Buhl, ID 83316 USA scott.lapatra@clearsprings.com
[4] College of Veterinary Medicine, University of Florida, 2015 SW 16th Avenue, Gainesville, FL 32608 USA tbwaltzek@ufl.edu

Infectious salmon anemia virus (ISAV) is the type species and currently the only described member of the *Isavirus* genus in the family *Orthomyxoviridae*; however, two novel fish orthomyxoviruses have been recently discovered. The first was isolated from koi in a California pond in 2007 while using KF-1 cells to screen for koi herpesvirus. The second was isolated from commercially-reared rainbow trout in Idaho in 1997 and 2000 using the CHSE-214 cell line. The entire PB1 RNA polymerase gene of each virus was amplified and sequenced, providing approximately 2200 nucleotides (about 700 amino acids) to analyze. The amino acid sequences of the PB1 genes of the viruses from koi and rainbow trout were 45.2% identical to each other and 41.4 to 43.8% identical to ISAV isolates. In a phylogenetic analysis, the new isolates diverged from the other orthomyxoviruses (like influenzaviruses) to cluster with ISAV as possible new species within the genus *Isavirus*. The detection systems to optimize isolation and growth of these viruses will be discussed. In addition, a set of primers was designed using conserved regions of the PB1 gene to produce amplicons that can be readily sequenced to distinguish among the viruses or to identify novel fish orthomyxoviruses in the future. Recent sequence analysis efforts of the other viral segments will be discussed.

14b. Observations on age dependency of viral nervous necrosis and implications for disease control

Diana Jaramillo[1]*, Paul Hick[1], Stewart Fielder[2], Richard Whittington[1]

[1]Department of Farm Animal Health, Faculty of Veterinary Science, University of Sydney, 2570 NSW Australia diana.jaramillo@sydney.edu.au, paul.hick@sydney.edu.au, richard.whittington@sydney.edu.au

[2]Port Stephens Fisheries Research Institute, NSW Department of Primary Industries, 2316 NSW Australia stewart.fielder@dpi.nsw.gov.au

Viral Nervous Necrosis (VNN) also known as Viral Encephalopathy and Retinopathy (VER) is a globally distributed disease that affects a large number of finfish species, causing significant economic losses on affected farms. Clinical signs include abnormal behavior and high mortality associated with histopathological findings of necrosis in the nervous tissue. Nevertheless, the prognosis of infection with the causative agent, Nervous necrosis virus, (NNV) is highly variable. Longitudinal testing done in Australian fish farms reveals that the virus is often detected and can be isolated from apparently healthy barramundi (*Lates calcarifer*) and Australian bass (*Macquaria Novemaculeata*) in absence of clinical signs or pathological lesions (Hick, 2010).

Field observations of barramundi and Australian bass in aquaculture facilities suggest that the disease is highly age dependent, as noted for some fish species, but apparently not for others like sea bass and groupers (Nakai et al., 2009). Because no clinical disease has been recorded in barramundi and Australian bass that are older than 35 days of age in affected farms, an experimental model was used to test the importance of age of the host on the infectivity and pathogenesis of NNV. Barramundi and Australian bass larvae were challenged by immersion (10^4 $TCID_{50}$) at 4 different ages from weeks 3 to 9 post hatch using the same viral dose, water temperature and husbandry conditions such that age was the only variable. The outcome and course of infection was followed using RT-qPCR on tissue and water samples while pathogenesis was studied using histopathology, IHC, VI and serology.

Although evidence was found of the replication of the virus in the nervous tissue of fish at all ages tested, the typical clinical presentation of the disease only occurred in fish challenged at 5 weeks of age or younger. Afterwards, all fish challenged seemed refractory to the disease as no clinical signs or histopathological lesions were observed. From this, we believe that there is a clear age threshold for resistance to the disease in the species tested. We consider that the best prevention practice for the disease would involve the isolation of the larvae from viral reservoirs using ozonation of eggs and disinfection of water until they reach the age threshold. Vaccination on the other hand would be of use only if developed for young larvae, but these may or may not be immunocompetent. Vaccination of fish older than the threshold may be of no practical benefit.

HICK, P. 2010. *Detection and epidemiology of Betanodavirus in Australian finfish.* Doctor of Philosophy, University of Sydney.

NAKAI, T., MORI, K., SUGAYA, T., NISHIOKA, T., MUSHIAKE, K. & YAMASHITA, H. 2009. Current knowledge of viral nervous necrosis (VNN) and its causative betanodaviruses. *The Israeli Journal of Aquaculture – Bamidgeh,* 61**,** 198-207.

14c. Effects of temperatures on poly I:C inducible viral resistance status in Sevenband grouper

Kittipong Thanasaksiri[1], Nichika Sakai[1], Hirofumi Yamashita[2], Ikuo Hirono[1], Hidehiro Kondo[1]*

[1]Laboratory of Genome Science, Tokyo University of Marine Science and Technology, Minato, Tokyo, Japan h-kondo@kaiyodai.ac.jp
[2]Fisheries Research Center, Ehime Research Institute of Agriculture, Forestry and Fisheries, Uwajima, Ehime, Japan

Temperature is one of the most important factors influencing the physiological status of fish. Many studies report that temperature influences the immune responses of eurythermal fish. Polyinosinic: polycytidylic acid (polyI:C) is a strong inducer of type I interferon (IFN), which induces anti-viral state by increasing mRNA levels of IFN-inducible genes such as Mx gene in many animals including fish. Some researchers demonstrated that Mx gene expression after polyI:C treatment are differentially regulated at different rearing temperatures. In this study, we evaluated the effects of temperature on viral resistance status induced by polyI:C in Sevenband grouper *Epinephelus septemfasciatus*. Fish were kept at 15, 20, 25 and 30 ˚C for 2 weeks prior to the experiment. Fish were intramuscularly injected with polyI:C at 100 μg /fish. The head kidney samples were collected at 3 hours post injection (hpi) and 1-, 3- and 7-days post injection (dpi), and then used for Mx gene expression analysis. The fish on 3- and 7 dpi were challenged with red-spotted grouper nervous necrosis virus (RGNNV), and their mortalities and viral titers in brain were measured. At 25 and 30 ˚C, Mx gene mRNA levels of 3 hpi group were higher than those on the other time points. On the other hand, at 15 and 20 ˚C, the 1 dpi group showed higher Mx mRNA levels than those on the other time points. Significant up-regulation of Mx gene expression on 3 dpi was only observed at 15 ˚C. Unfortunately, almost all fish injected with polyI:C survived after challenge with RGNNV, while 60-95 % of control died. Although the fish survived, viral titers in the fish of 7 dpi at 25 and 30 ˚C were higher than those of the other polyI:C treated group. These results indicate that the effects of polyI:C at 15 and 20 ˚C were maintained longer than those at 25 and 30 ˚C.

14d. Isolation and molecular characterization of a novel calicivirus from baitfish in the USA

Sunil K. Mor*[1,2], Nicholas B. D. Phelps[1,2], Anibal G. Armien[1,2], Rebekah McCann[3], Corey Puzach[3], Thomas Waltzek[4], Sagar M. Goyal[1,2]

[1]Minnesota Veterinary Diagnostic Laboratory, 1333 Gortner Avenue, St. Paul, MN 55108
[2]University of Minnesota, Department of Veterinary Population Medicine, 1365 Gortner Avenue, St. Paul, MN 55108 kumars@umn.edu, phelp083@umn.edu, goyal001@umn.edu
[3]US Fish and Wildlife Service, La Crosse Fish Health Center, 555 Lester Avenue, Onalaska, WI 54650
[4]University of Florida, College of Veterinary Medicine, University of Florida, Building #1379, Mowry Road, Gainesville, FL, 32610 tbwaltzek@ufl.edu

During regulatory and routine surveillance sampling of apparently healthy baitfish from the states of Minnesota and Wisconsin, a novel calicivirus was isolated from kidney/spleen of fathead minnows (*Pimephales promelas*). Homogenates of kidney/spleen were inoculated into epithelioma papulosum cyprini (EPC) cells followed by incubation at 20°C. Infected cell cultures exhibiting CPE were tested by polymerase chain reaction (PCR) and reverse transcription (RT)-PCR for common and/or reportable fish pathogens, including viral hemorrhagic septicemia virus, spring viremia of carp virus, infectious pancreatic necrosis virus, largemouth bass virus, bluegill picornavirus, golden shiner virus, and fathead minnow nidovirus. Total extracted RNA was submitted for Illumina HiSeq next generation sequencing. The sequences were analyzed by *de novo* assembly method and obtained contigs were identified by tBLASTx analysis, which gave match with mammalian calicivirus. The complete polyprotein of this novel calicivirus is 2114 aa in size. The amino acid identity of complete polyprotein showed a maximum of 20.97% identity with Atlantic salmon calicivirus followed by 10.98% to 14.19% identity with mammalian caliciviruses. Specific primers were designed from this sequence followed by the development of a gel based RT-PCR for screening of field samples. This calicivirus seems to be a novel species under the family *Caliciviridae*.

14e. Experimental infection of Salmonid alphavirus 1 in Atlantic salmon fry

Tharangani K. Herath[1]*, Angela Ashby[1], Nilantha S Jayasuriya[1], James E Bron[1], Randolph H Richards[1], Hugh W Ferguson[2], Alexandra Adams[1], Manfred Weidmann[1], John F Taylor[1], Herve Migaud[1], Mark Fordyce, Kim D Thompson[1]

[1]Institute of Aquaculture, University of Stirling, Stirling, UK t.k.herath@stir.ac.uk
[2]School of Veterinary Medicine, St George's University, Grenada, West Indies
[3]Marine Scotland, 375 Victoria Road, Aberdeen, UK

Salmonid alphavirus (SAV) (family *Toga viridae*) induced salmon pancreas disease (PD) has become one of the main viral disease concerns in commercial salmon farming. The clinical disease, characterized by severe degenerative lesions in the pancreas, heart, and skeletal muscle of infected fish has been reported in commercial stocks of Atlantic salmon in Scotland, Ireland and Norway. The susceptibility of fresh water reared *parr* to SAV has been demonstrated under experimental conditions, but information is lacking on susceptibility of Atlantic salmon fry for SAV. To investigate their susceptibility to the virus, the present experiment exposed Atlantic salmon fry to SAV1 using different routes of infection (*i.e.* intraperitoneal (IP), co-habitation (co-hab) and bath challenges) together with an un-exposed group. Mortalities commenced in fish infected by IP injection 11 days post- infection (d.p.i.), while mortalities in the co-hab and bath groups started from 12 and 13 d.p.i., respectively. The final cumulative mortality of IP challenged fish was significantly higher than the two other groups (P value < 0.01). Fish were randomly selected from each tank on Day 17, when the experiment terminated and fry were fixed and processed for routine histopathology. Of the main target organs following SAV infection, histological changes could be seen in the pancreas and heart, and these were generally more severe than those typically seen in older fish exposed to SAV1 under similar conditions, while the lesions observed in the skeletal muscle were mild. Degenerative changes were also observed in the liver and kidney of these fry, which are not typically reported in older fish exposed to SAV1. Furthermore, histopathological changes appeared to be most severe in the IP group, followed by the bath and co-hab groups. In summary, these results suggest that SAV can cause severe systemic infection in fry, an age group that is not normally exposed with this pathogen in the field. Investigations are currently underway to further characterize the pathology and immune response of these fish.

14f. Does gill have a role in salmonid alphavirus infection?

Tharangani K. Herath[1]*, James E Bron[1], John B. Taggart[1], Kim D. Thompson[1], Hugh W Ferguson[2], Manfred Weidmann[1], Sandra Adams[1] Benjamin Lopez Jimena[1], Nilantha S Jayasuiriya[1], Randolph H Richards[1]

[1]Institute of Aquaculture, University of Stirling, Stirling, UK t.k.herath@stir.ac.uk
[2] School of Veterinary Medicine, St George's University, Grenada, West Indies

Salmonid alphavirus (SAV), a single-stranded positive sense, enveloped RNA virus of the *Togaviridae* family, is one of the main viral disease concerns in commercial salmonid aquaculture in Europe. Of the tissues that have been reported to harbour the virus, the gill appears to be the tissue where virus persist the longest. The present study was performed to give an insight into the role of the gill in SAV pathogenesis.

In the first experiment, tissue samples were collected from Atlantic salmon parr (n=8) injected intra-peritoneally (i.p.) with CHSE-214 cell supernatant and from fish (n=8) injected i.p. with SAV1 on 4 and 11 days post-infection (d.p.i). The results obtained from a custom 15K probe Agilent salmon oligo microarray analysis (2-way ANOVA, FDR off, p=0.05) for factors State (infection vs control) and State x Time (*i.e.* interaction of infection with time points 4 and 11 found 1738 significantly differentially regulated genes in the gill. Most of these were significantly differentially expressed in virus-challenged fish, with high-fold up- or down-regulation at 4 d.p.i compared to control fish, but these had decreased to around a fold-change of ±1 by 11 d.p.i. Interestingly, most of the interferon and interferon-associated genes were down-regulated in the gill at 11 d.p.i. compared to 4 d.p.i., suggesting that this lack of interferon-induced antiviral activity may allow the virus to persist in the gills.

The same experiment was repeated to observe ultrastructure changes associated with SAV infection, sampling only gill, heart and kidney. Under TEM it was hard to see any free viruses in the gill however; numerous cytoplasmic vacuoles and structures similar to alphavirus replication complexes were observed in the branchial epithelial cells. Gill samples collected from infected fish on both 4 and Day 11 d.p.i (n=12/day) were positive for virus on CHSE-214 cells. The absence of visible free virus-like particles in the gill suggests that virus normally isolated from this tissue might not be derived from the tissue itself, but rather from the blood remaining in the gill. To confirm these findings further *in vitro* and *in vivo* experiments are underway.

15) General Session: Diagnostic Techniques

15a. Fluorescent imaging of *Edwardsiella ictaluri* infection in zebrafish (*Danio rerio*)

Bayard Grillis[1], Wes Baumgartner[2]*, Claudia Hohn[1], Lora Petrie-Hanson[1], Mark Lawrence[1]

[1]Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS, 39762. bsg71@msstate.edu, hohn@cvm.msstate.edu, lora@cvm.msstate.edu, lawrence@cvm.msstate.edu
[2]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS, 39762. baumgartner@cvm.msstate.edu

Edwardsiella ictaluri is the cause of enteric septicemia of catfish (ESC), an economically important disease in channel catfish. Recently, zebrafish have been established as a model for studying ESC. However, the natural route of entry and early dynamics of *E. ictaluri* infection in the zebrafish model is unknown. Bioluminescence imaging (BLI), fluorescence *in situ* hybridization (FISH), and histopathology were therefore used to characterize infection. Adult zebrafish were exposed to fluorescent *E. ictaluri* (93-146) pAK*gfplux1* via gastric gavage, bath immersion, and intraperitoneal injection. At 0, 0.5, 6, 24, 48, 72, 96, 120, and 144 hours post-infection, zebrafish were anesthetized, imaged (BLI), sacrificed, and processed for FISH using a non-specific eubacterial probe. BLI revealed bacterial signal from unique locations for each treatment, showing increasing signal in injected fish up to 72 hours post-infection, at which point most fish succumbed to infection. In contrast, gavage and immersion treatments showed increasing signal through 72 and 24 hours, respectively, followed by decreasing signal through 144 hours. FISH enabled pathogen detection for gavage (intestinal mucosa/submucosa), immersion (nasal bulbs, gills), and injection (abdominal viscera, peritonitis) treatments, respectively. In conclusion, these results indicate that: 1.) *E. ictaluri* possesses multiple routes of entry in the zebrafish model; 2.) BLI data is accurate in localizing zebrafish infection; and 3.) FISH is a useful technique for visualizing *E. ictaluri* histologically.

15b. Turning to a "higher power" in pathology for investigating fish and wildlife disease

J Lovy[1]*, LL Coffee[2], SE Friend[1]

[1]Office of Fish & Wildlife Health & Forensics, N.J. Division of Fish & Wildlife, NJ 07863, USA
Jan.Lovy@dep.state.nj.us, Sarah.Friend@dep.state.nj.us
[2]Animal Health Diagnostic Laboratory, N.J. Department of Agriculture, NJ 08628, USA
lauracoffeedvm@gmail.com

Transmission electron microscopy (TEM) is a useful method for diagnostic virology, particularly when a virus cannot be successfully isolated and propagated on available cell lines, or other diagnostic methods have not been developed. Often the need to do TEM may arise after samples have been collected and fresh tissues are no longer available. A useful method in diagnostic virology is to reprocess routine histology tissues for TEM, although this can come at the expense of morphology. Using tissue samples from a fish kill attributed to cyprinid-herpesvirus-2 in a New Jersey lake, we assessed the efficacy and limitations of TEM for characterizing viral morphology from tissues initially processed for routine histology. A TEM comparison of viral ultrastructure was done in formalin fixed and paraffin embedded tissues. The results demonstrated that formalin fixed tissue preserved all stages of viral replication, while paraffin embedded tissues were useful for viral diagnostics, not all replication stages could be visualized.

An interesting wildlife case in which TEM aided in a diagnosis was the epizootic mortality of dolphins that occurred along the New Jersey coast in late summer 2013. Mortalities were related to dolphin morbillivirus, although one dolphin had histological lesions in the adrenal gland suggestive of a herpesvirus infection. TEM of the associated adrenal lesion reprocessed from paraffin confirmed the presence of a herpesvirus. Additionally cells within the lesion contained viral replication stages within the nucleus suggestive of a novel virus or an unusual replication stage. Further molecular studies are being conducted to identify the viruses associated with these lesions.

15c. Use of matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) for the speciation of pathogenic *Vibrio* in fish

Claire B. Miller[1,2], Seth Nydam[3], G. Kenitra Hammac[4], Timberly Maddox[1], Thomas Besser[1,2], Dubraska Diaz[1,2], Kevin Snekvik[1,2]*

[1]Washington Animal Disease Diagnostic Laboratory, Washington State University, Pullman, WA, USA, timberlymaddox@vetmed.wsu.edu
[2]Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA ksnek@vetmed.wsu.edu, cmiller@vetmed.wsu.edu, dubra@vetmed.wsu.edu, tbesser@vetmed.wsu.edu
[3]Paul G. Allen School for Global Animal Health, Washington State University, Pullman, WA, USA, snydam@vetmed.wsu.edu
[4]Indiana Animal Disease Diagnostic Laboratory, Purdue University, West Lafayette, IN, USA khammac@purdue.edu

Multiple *Vibrio* spp. cause significant morbidity and mortality in farmed marine fish leading to economic loss to fish producers. Unfortunately, speciation of these isolates is time consuming, costly, and often unsuccessful due to the diverse nature of the genus and lack of consistency in traditional culture identification methods. Therefore a rational and affordable, approach to speciation is needed to aid in correct identification and to allow accurate case interpretation. MALDI-TOF MS has been shown to be a rapid and cost effective method for speciation bacteria. However, no reports are currently available as to the accuracy of this method for *Vibrio* spp. Fifty-five presumptive *Vibrio* spp. isolates were tested by MALDI-TOF MS and compared to speciation using traditional biochemical testing and speciation by sequencing of three conserved genes. Thirty-four isolates (~62%) had scores higher than two to allow speciation. All five ATCC strains including *Listonella (Vibrio) anguillarum*, *Vibrio alginolyticus*, *Vibrio vulnificus*, *Vibrio ordalii*, and *Photobacterium damselae* subspecies *damselae* were correctly identified using MALDI-TOF MS using this criterion. An additional five isolates (~9%) were identified to the genus level with scores between 1.8 and 2. Two isolates (~4%) produced detectable scores less than 1.8 and did not allow for confident identification at the genus or species level. Fourteen isolates (25%) produced an output of no identification. Biochemical speciation of the *Vibrio* isolates was also performed for comparison with MALDI-TOF MS using the following conditions, with or without the addition of 2% NaCl solution to media, growth at 15°C and 20°C; incubation for 24 and 48 hours. Speciation using traditional biochemical methods was highly inaccurate, and varied with salt concentration, temperature during growth, and time of incubation. ATCC strains were identified using 2% enriched salt at 15°C which was consistent with MALDI-TOF, but only under these specific conditions. Only one non-ATCC isolate, *Photobacterium damselae* subspecies *damselae*, was concordant between MALDI-TOF MS and biochemical testing. DNA sequencing of multiple gene targets was presumed to be the most accurate and speciation by this method was completed on all 55 isolates. There was modest to good agreement between the sequencing data and MALDI-TOF MS. These findings indicate the MALDI-TOF MS database is moderately accurate but further training is needed for confident use in *Vibrio* speciation of fish-origin.

15d. POCKIT™ system: A field-deployable tool for rapid, specific, and sensitive on-site diagnosis of aquaculture animal diseases

Chen Su*, Li-Juan Ma, Pin-Hsing Chou, Yu-Chun Lin, Shih-Han Weng, Yun-Long Tsai, Pei-Yu Lee, Hsiao-Fen Grace Chang

GeneReach Biotechnology Corporation, Central Taichung Science Park, Taichung City, 407, Taiwan peiyu329@genereachbiotech.com

Offering high sensitivity and specificity in detecting pathogens, polymerase chain reaction (PCR) assays have been included into bio-security measures and proven to improve overall shrimp production at large-scale facilities. Timely and cost-effective point-of-need diagnosis should provide the same benefits to various aquaculture facilities. POCKIT™ Nucleic Acid Analyzer (POCKIT™, GeneReach) is a field-deployable tool to aid rapid on-site detection of target pathogens. POCKIT™ provides optimized conditions for fluorescent probe-based insulated isothermal PCR (iiPCR) and automatically generates simple readouts within one hour from nucleic acid samples. Based on iiPCR technology, IQ Plus assays have been developed to work on POCKIT™ for easy and fast pathogen detection. The reagent works in a lyophilized format, allowing shipping and storage without refrigeration. Assays are available for various important aquaculture pathogens, including acute hepatopancreatic necrosis disease (AHPND)/early mortality syndrome (EMS) and white spot syndrome virus. Here we show the results of IQ Plus AHPND/EMS Kit. Causing large-scale shrimp die-offs, AHPND/EMS has led to huge economic losses to the shrimp farming industry. Targeting the virulence-associated plasmid of *Vibrio parahaemolyticus* in shrimp or farming environment, IQ Plus AHPND/EMS Kit could be used to monitor any potential threats of EMS by screening for the presence of the virulence-associated plasmid in shrimp, water, or other sources. We compared the performance of IQ Plus Kit with a qPCR assay. Testing extracted nucleic acid of a virulent *V. parahaemolyticus* strain, the detection limit of real-time PCR was at 10^{-6} dilution. The sensitivity of IQ Plus AHPND/EMS Kit was as good as qPCR. In addition, IQ Plus EMS/AHPND Kit generated no signals from host genomic DNA and non-virulent strains of *Vibrio* species, indicating excellent specificity in detecting the virulence-associated plasmid. Working in a lyophilized format, these sensitive and specific IQ Plus assays are useful tools for on-site diagnosis of aquaculture diseases.

16) General Session: Parasites

16a. Persistence of ichthyophoniasis external signs in Pacific herring *Clupea pallasii*

Lucas M. Hart[1*], Carla Conway[2], Diane Elliott[2], Paul K. Hershberger[1]

[1]Marrowstone Marine Field Station, U.S. Geological Survey, Nordland, WA 98358, USA, lhart@usgs.gov
[2]Western Fisheries Research Center, U.S. Geological Survey, Seattle, WA 98115, USA

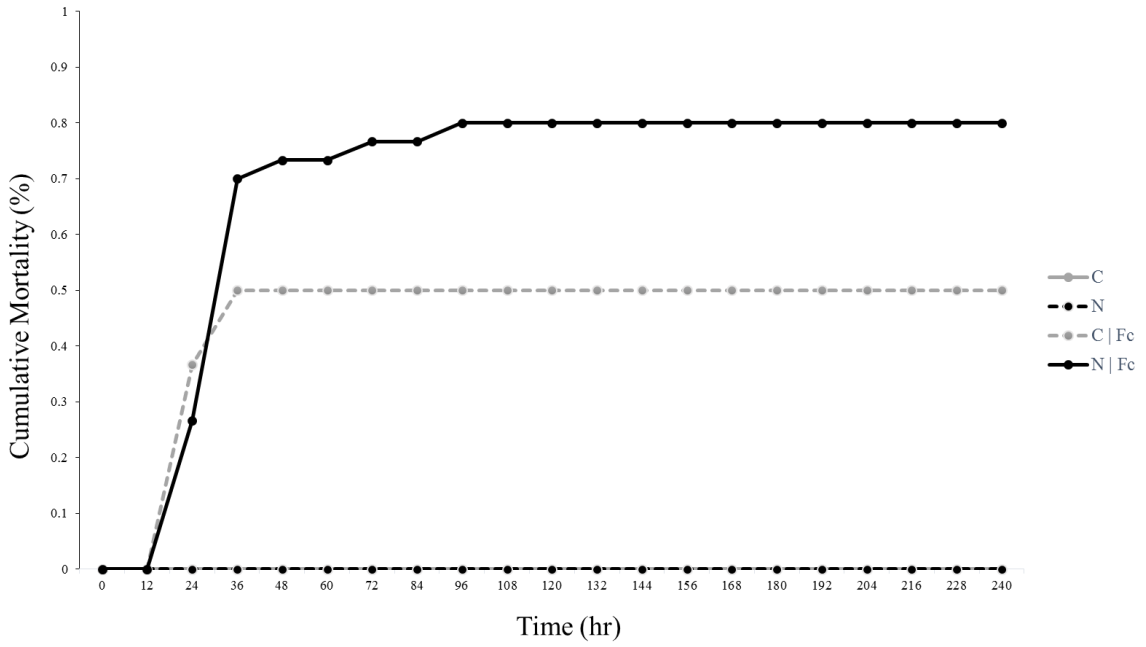
The progression of ichthyophoniasis related external signs in Pacific herring was highly variable and asynchronous between individuals after injection with pure parasite isolates. Observed signs included 'sandpaper skin', open lesions, pigmented ulcers and / or bleeding ulcers. External sign prevalence plateaued 35d post-exposure and persisted in 73-79% of exposed individuals until the study was terminated at 147d post-exposure. Among a second group of infected herring, external signs were completely resolved in only 10% of the fish after 429d. Mortality onset preceded external sign appearance and correlations were not observed between these metrics. Histological examination of infected somatic tissues indicated an apparent affinity of the parasite for host red muscle, and an absence of the parasite in epidermal tissues. Host responses consisted primarily of inflammation, fibrosis, and necrosis in the somatic muscle and other tissues. The persistence and asynchrony of external signs and host response indicated that they were neither precursors to host mortality nor did they provide reliable metrics for hindcasting the date of exposure. However, the long-term persistence of clinical signs in Pacific herring can be useful at ascertaining ichthyophoniasis related mortality in regularly observed populations.

16b. Co-infection dynamics of *Nanophyetus salmincola* and two bacterial diseases in Chinook salmon

Sean R. Roon*, Michelle Jakaitis, Julie D. Alexander, Jerri L. Bartholomew

Oregon State University, Department of Microbiology, Nash 220, Corvallis, OR 97331
roons@onid.orst.edu, jakaitim@onid.orst.edu, alexanju@science.oregonstate.edu, bartholj@science.oregonstate.edu

Our survey data suggest a high prevalence of the *Nanophyetus salmincola* in wild juvenile Chinook (*Oncorhynchus tshawytscha*) salmon rearing in upper Willamette tributaries in Oregon, USA. This trematode is not typically associated with salmon mortality; however, sublethal effects of this parasite are a physiological burden that is thought to have a negative impact on salmon fitness and survival. We conducted a series of challenges evaluating if *N. salmincola* infections decrease resistance to two common freshwater pathogens, *Flavobacterium columnare* and *Aeromonas salmonicida*. These bacteria are of concern for hatcheries rearing juvenile salmonids in the Upper Willamette tributary, and therefore, may also be potential concern for wild salmonids in proximity to a facility experiencing an epizootic. Results of these two challenges exhibit increased mortality for co-infection groups compared to single infection groups. Tanks of juvenile Chinook salmon in the *F. columnare* and *N. salmincola* co-infection group experienced significantly higher mortality compared to tanks exposed only to *F. columnare.* Tanks challenged with *A. salmonicida* also resulted in a trend of increased co-infection mortality. Our results support the hypothesis that sublethal infection of *N. salmincola* can increase susceptibility to disease in salmon. We provide evidence that encysted metacercariae of *N. salmincola* may be of concern in a freshwater system. Increased *N. salmincola* density may suppress immune function, and therefore, lead to the synergistic mortality observed in co-infection groups.



16c. *Mikrocytos*: An extremely divergent eukaryotic genus of microcell parasites and the changing landscape of current research introduced by modern molecular techniques.

Geoff J. Lowe[1,2]*, Gary R. Meyer [1,3], Cathryn L. Abbott [1,4]

[1]Fisheries and Oceans Canada, Pacific Biological Station, 3190 Hammond Bay Road, Nanaimo, BC, V9T 6N7, Canada
[2]Aquatic Animal Health Section, Molecular Genetics, Geoff.Lowe@dfo-mpo.gc.ca
[3]Aquatic Animal Health Section, Molecular Genetics, Gary.Meyer@dfo-mpo.gc.ca
[4]Aquatic Animal Health Section, Shellfish Health, Cathryn.Abbott@dfo-mpo.gc.ca

The genus *Mikrocytos* is traditionally known for its only named species, *Mikrocytos mackini*, the microcell parasite which occurs in oysters from the Pacific Northwest of North America. To date, multiple factors have conspired to create difficulty for scientific research on *Mikrocytos* parasites. These include their tiny cell size, infections that are often of light intensity, lack of suitable cell lines and techniques for *in vitro* culture, and the seasonal nature of the disease (at least in the case of *M. mackini*). Until recently, high genetic divergence has complicated the phylogenetic placement of *Mikrocytos* and a lack of informative taxonomic characters has precluded new species descriptions. Further, molecular detection of *M. mackini* has been hampered by a lack of DNA sequence data and cross-reactivity with newly-discovered *Mikrocytos* spp. Even though these challenges presented major obstacles to the acquisition of new scientific knowledge on these parasites for some time, new molecular data for this genus, generated by Next Generation Sequencing (NGS) techniques has begun paving the way forward and redressing problems associated with its unknown evolutionary origins, problematic molecular detection, and poorly-characterized diversity. Here we review the findings of two recent phylogenetic studies clarifying the taxonomic placement of *Mikrocytos*. With the increasing availability of NGS technology to compliment traditional DNA-based methods there are now sufficient molecular techniques to detect novel *Mikrocytos* parasites and to characterize its related diversity.

16d. Re-evaluation of the myxosporean family Ortholineidae

Mark A. Freeman[1], Thitiporn Laoprasert[2], Paul Brown[3], Phaik Eem Lim[1,4]

[1]Institute of Ocean and Earth Sciences, University of Malaya, Kuala Lumpur, 50603 Malaysia
[2]Aquatic Animal Health Research Institute, Department of Fisheries, Jatuchak, Bangkok, 10900 Thailand.
[3]Marine and Freshwater Fisheries Research Institute, Snobs Creek, Private Bag 20, Alexandra, Victoria 3714 Australia
[4]Institute of Biological Sciences, University of Malaya, Kuala Lumpur, 50603 Malaysia

The myxosporean family Ortholineidae Lom et Noble, 1984 (suborder Variisporina) currently contains genera of a mixed origin with respect to the site of infection in fish. *Ortholinea* Shulamn, 1962, *Neomyxobolus* Chen et Hsieh, 1960 and *Kentmoseria* Lom et Dyková, 1995 are all reported as being ceolozoic in the renal systems of marine and freshwater fishes. Whereas, *Cardimyxobolus* Ma, Dong et Wang, 1982 and *Triangula* Chen et Hsieh, 1984 are histozoic, forming large polysporous plasmodia in gills and other tissues. However, all genera have myxospores that are sub-spherical to irregularly ellipsoidal, with bilateral symmetry along an often prominent but straight sutural line, and are hence included in the family. Tissue tropism is known to be a robust feature for grouping myxosporeans together and is supported in multiple molecular phylogenetic studies. Currently there is a lack of molecular data for this family with sequences only available for *Ortholinea* and *Cardimyxobolus*.

In this study we generate SSU rDNA sequence data for two more species of *Ortholinea* from peninsular Malaysia, one *Neomyxobolus* sp. from Thailand, and *Triangula percae* from Australia. *Ortholinea fluviatilis* and *Ortholinea* sp. were sampled from the urinary bladders of the puffer fish, *Tetraodon fluviatilis*, and the scat, *Scatophagus argus*, from Kuala Selangor, Malaysia. A histozoic myxosporean with a *Neomyxobolus* morphotype (*Neomyxobolus* sp.) was discovered infecting the somatic muscle of the kuhlii loach *Pangio kuhlii* from Chanthaburi Province in Eastern Thailand. *Triangula percae* was sampled from the brains of infected introduced European redfin perch *Perca fluviatilis* from Victoria, Australia.

Phylogenetic analyses showed that the new and all previously reported SSU rDNA sequences for the Ortholineidae grouped within the Platysporina and not the Variisporina. *Triangula percae* grouped with *Cardimyxobolus* sp. and members of the Myxobolidae. *Neomyxobolus* sp. occupied a solitary branch in all phylogenetic trees but was well supported as a member of the Myxobolidae. The new sequences for *Ortholinea* spp. robustly grouped with other myxosporeans infecting the urinary system, including *O. orientalis*, and formed a sister clade to *Myxobolus* spp. infecting salmonid nerve tissues.

We present these finding and discuss the possible importance of other features that could be useful in systematic studies of the Platysporina, such as the presence of iodinophilous vacuoles in mature spores. Our findings suggest that the Ortholineidae should be transferred from the Variisporina to the Platysporina.

16e. Development of monoclonal antibodies for polar filaments and valves of *Myxobolus honghuensis* (Myxosporea: Bivalvulida)

Luo Jia[1,2,3]*, Dan Li[1,2,3], Yanhua Zhai[1,2,3], Junfa Yuan[1,2,3], Zemao Gu[1,2,3]

[1]Department of Aquatic Animal Medicine, College of Fisheries, Huazhong Agricultural University, Wuhan, 430070, People's Republic of China jialuo@webmail.hzau.edu.cn
[2]Freshwater Aquaculture Collaborative Innovation Center of Hubei Province, Wuhan, 430070, People's Republic of China guzemao@mail.hzau.edu.cn
[3]Key Lab of Freshwater Animal Breeding, Ministry of Agriculture, Wuhan, 430070, People's Republic of China jfyuan@mail.hzau.edu.cn

Myxobolus honghuensis Liu et Gu, 2012, which infects the pharynx of allogynogenetic gibel carp *Carassius auratus gibelio* (Bloch) are often results in high mortality. To facilitate the immunological research of polar filaments and spore valves, which are important during the invasion of host tissues, two monoclonal antibodies (MAbs) were prepared from mice immunized with soluble protein from sonicated *Myxobolus honghuensis* spores. The immunofluorescence analysis revealed that MAb 1C7 reacted specifically and strongly with the polar filaments of spores, MAb 3B7 localized almost wholly on spore valves. The ELISA titer of MAb 1C7 and MAb 3B7 is 1: 124000 and 1:248000. The isotype of MAb 1C7 and MAb 3B7 is IgM and IgG1 respectively. From western blotting analysis, MAb 1C7 recognized two prominent protein bands of 130Kda and 180Kda; MAb 3B7 recognized a band of 28Kda. Moreover, proteins reacted with MAb 1C7 and MAb 3B7 were BCLF1 and ANGI_AOTTR identified by mass spectrometry. These two proteins are supposed to associated with invading and envading the host. This study demonstrated that MAb 1C7 can be used in the further biological study of polar filament function, including parsing the protein composition. Also, MAb 3B7 can be employed in the further analysis of surface antigens on spores, including characterization of proteic and carbohydrate epitopes. Thus these MAbs could be important for understanding host-parasite interactions.

17) General Session: Viruses III

17a. Phylogenetic analysis of IPNV isolates from Atlantic Canada

Dante R. Mateo[1]*, Spencer J. Greenwood[2], David B. Groman[3], Carmencita Yason[1,4]

[1]Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, C1A 4P3 Canada dmateo@upei.ca
[2]Department of Biomedical Sciences, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, C1A 4P3 Canada sgreenwood@upei.ca
[3]Aquatic Diagnostic Services, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, C1A 4P3 Canada groman@upei.ca
[4]Regional Diagnostic Virology Services, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, C1A 4P3 Canada yason@upei.ca

Infectious pancreatic necrosis virus is one of the most important viruses of fin-fish, in particular for salmonids. Since the 1990's, this virus primarily affected farm reared Atlantic salmon (*Salmo salar*), brook trout (*Salvelinus fontinalis*) and rainbow trout (*Oncorhynchus mykiss*) in Atlantic Canada. In order to learn about the phylogenetic relationship amongst isolates recovered from infected fish and strain representatives of existing phenogroups, a phylogenetic analysis was conducted. For this analysis, an 851 nucleotide segment that corresponded to the VP2 gene coding region was used to construct phylogenetic trees. The analysis revealed that the isolates from Atlantic Canada belong to 3 genogroups: I, III, and IV. This is not surprising considering that these genogroups contain Canadian representative strains, i.e. Jasper and Jasper ATCC are included in genogroup I, Canada 1 and ASV are included in genogroup III, and Canada 2 and 3 are included in genogroup IV. Interestingly most of the representative Canadian strains were, however, originally isolated from rainbow trout, a finding that suggests some horizontal transfer of the virus within Canada. Among the isolates from Atlantic Canada, the sequence similarity among pairs ranged from 100% to 72.5%, and when comparing them with the representative strains it ranged from 99.2% to 65.7%. Overall, these results suggest that there is not a single origin of IPNV in fish from Atlantic Canada and that the virus has disseminated among salmonids, likely due to stocking enhancement programs as well as the expansion of aquaculture since the 1980's.

17b. Development and diagnostic validation of a reverse transcription quantitative PCR (RT-qPCR) assay for detection of infectious pancreatic necrosis virus (IPNV)

Sharon Clouthier[1]*, Tamara Schroeder[1], Carol McClure[2], Melissa Lindsay[1], Sunita Khatkar[1], Crystal Collette-Belliveau[3], Lisa Gaudet[3], Eric Johnsen[4], Jason Allen[1], Adrian Zetner[1], Eric Anderson[5]

[1] Fisheries & Oceans Canada, Freshwater Institute, 501 University Crescent, Winnipeg, MB, R3T 2N6 sharon.clouthier@dfo-mpo.gc.ca; tamara.schroeder@dfo-mpo.gc.ca; melissa.lindsay@dfo-mpo.gc.ca; sunita.khatkar@dfo-mpo.gc.ca; jason.allen@dfo-mpo.gc.ca; adrian.zetner@dfo-mpo.gc.ca

[2] AquaEpi Research, RR3, 300 Heartz Road, Charlottetown, PEI, C1C 0H8 cmcclure@upei.ca

[3] Fisheries & Oceans Canada, Gulf Fisheries Center, 343 University Ave, Moncton, NB, E1C 9B6 crystal.collette-belliveau@dfo-mpo.gc.ca; lisa.gaudet@dfo-mpo.gc.ca

[4] New Brunswick Research and Productivity Council, 921 College Hill Road, Fredericton, NB, E3B 6Z9 eric.johnsen@rpc.ca

[5] Box 28, Group 30, RR2, Ste Anne, MB, R5H1R2, Canada mainebiotek@hotmail.com

An IPNV RT-qPCR assay was developed and then validated to assess its fitness as a diagnostic test method according to guidelines outlined by the World Organization of Animal Health. Performance characteristics of the new RT-qPCR test were evaluated using samples from three different salmonid fish species naturally infected with IPNV. Four laboratories participated in the study. The RT-qPCR assay was capable of detecting isolates from all seven genogroups of IPNV except possibly genogroup II. The limit of detection was defined as 20 copies of plasmid encoding target sequence from genogroup V. Diagnostic validation revealed that the RT-qPCR assay performance was dependent on the prevalence and virus load within a population. Diagnostic sensitivity (DSe) and specificity (DSp) estimates of ≥86% and ≥88%, respectively, obtained for populations with low pathogen levels increased to ≥97.1% and 100%, respectively, for populations with high pathogen levels. For comparison, the DSe of a conventional RT-PCR assay was reduced to 35% and virus isolation by cell culture to 26% using samples from the low virus load population. The DSp for these two tests remained high regardless of virus load in the fish population. Repeatability and reproducibility of the RT-qPCR assay for all populations was high with 85% agreement among all test results within and between three laboratories. Estimates of test precision were in perfect agreement (kappa = 1.0) among samples from the high virus load population but were only in moderate to substantial agreement (kappa = 0.43 to 0.66) among samples from the low virus load population. A similar but stronger pattern was observed with the conventional RT-PCR (kappa = 0.27 to 0.6) and virus isolation (kappa = 0.02 to 0.51) assays. The performance characteristics of the IPNV RT-qPCR test indicate that it is suitable for use as diagnostic assay.

17c. Risk assessment of piscine reovirus (PRV) infection in Pacific salmon and trout

Maureen K Purcell[1]*, Kyle A. Garver[2], James R. Winton[1]

[1]US Geological Survey – Western Fisheries Research Center, 6505 NE 65th Street, Seattle, WA 98115 USA, mpurcell@usgs.gov, jwinton@usgs.gov

[2]Pacific Biological Station, Fisheries and Oceans Canada, Aquatic Animal Health, 3190 Hammond Bay Road Nanaimo, BC V9T 6N7, Canada, Kyle.Garver@dfo-mpo.gc.ca

Piscine reovirus (PRV) is a newly described virus that has been linked to heart skeletal muscle inflammation (HSMI) in farmed Atlantic salmon (*Salmo salar*) in Norway. Surveys have revealed that PRV is ubiquitous in asymptomatic wild and farmed Atlantic salmon in both the marine and freshwater environments in Norway. The high prevalence of PRV in asymptomatic fish has raised questions about the exact relationship between PRV and the disease HSMI. PRV genetic material has also been detected in farmed Atlantic salmon in Chile, Scotland and Ireland, as well as in trout and salmon from British Columbia (BC), Canada. However, there have been no confirmed reports of HSMI disease in Ireland, Chile or BC. The BC detections of PRV raise concerns about the risk the virus may pose to native Pacific salmon and trout species. Here, we report on controlled laboratory challenge studies to assess the risk of PRV to Chinook salmon. Juvenile fish were injected with 0.2 µM filtered tissue homogenates containing PRV genetic material. Fish were monitored daily for mortality and sampled periodically over a 3 month period for hematological and histopathological evaluation. Blood was sampled from infected individuals to assess hematocrit values, investigate incidence of intraerythrocytic inclusion body formation, and to determine PRV RNA copy number by quantitative reverse-transcriptase PCR. Early results indicate that PRV replicates in Chinook salmon and produces inclusion bodies in the cytoplasm of red blood cells. However, we observed no mortality and no significant reduction in blood hematocrit values in the PRV infected group suggesting that PRV is of low virulence in Chinook salmon.

17d. Isolation and genomic identification of a novel Aquareovirus detected in the endangered fountain darter, *Etheostoma fonticola*

Luke R. Iwanowicz[1]*, [1]Deborah D. Iwanowicz[1], Teresa D. Lewis[2], Tom Brandt[3]

[1]USGS, Leetown Science Center, Fish Health Branch, 11649 Leetown Road, Kearneysville, WV 25430 USA liwanowicz@usgs.gov; diwwanowicz@usgs.gov
[2]U.S. Fish and Wildlife Service, Southwestern Native Aquatic Resources and Recovery Center, P. O. Box 219, Dexter NM 88230 USA Teresa_Lewis@fws.gov
[3]U.S. Fish and Wildlife Service, San Marcos Aquatic Resource Center, 500 East McCarty Lane, San Marcos, TX 78666 USA tom_brandt@fws.gov

In 2003, a putative reovirus was isolated from wild fountain darters (FODs; *Etheostoma fonticola*) inhabiting the San Marcos River (SMR) during routine fish health monitoring. The fountain darter is endemic to the SMR and Comal River (CR) in Texas, and is a federally listed species for which active genetic management and restoration plans are being developed. The virus has never been detected in FODs from the CR and little is known about its pathogenicity or transmission potential. A panel of commonly used fish cell lines was tested for virus permissibility at 15ºC and 25ºC. The EPC cell line is nonpermissive to the virus, while the CHSE-214 cell line is permissive at both temperatures and the best choice for diagnostic culture of this isolate. We have conclusively identified this virus as an Aquareovirus, and approximately 99% of the genome has been sequenced via next generation and Sanger sequencing methods assuming a genome size of ~23.8 kb. To date 6 of the 11 segments of this dsRNA virus are completely sequenced. This virus shares greatest sequence identity (Segment 2; ~80% homology) to the Turbot Aquareovirus. Diagnostic PCR primers that target genome segments 3, 5, 6, 7 and 10 as well as a quantitative PCR assay have been developed for molecular confirmation of field isolates. We are currently validating these primer sets against a number of phylogenetically similar isolates. It is unknown if this isolate is a pathogen as it has never been associated with a disease outbreak. Pathogenicity and interspecies transmission studies are in progress.

17e. Development and analytical validation of quantitative PCR assays for detection of sturgeon nucleocytoplasmic large DNA viruses (NCLDVs)

Sharon C Clouthier[1]*, Elissa JD VanWalleghem[1,2], Eric D Anderson[3]

[1] Fisheries & Oceans Canada, Freshwater Institute, 501 University Crescent, Winnipeg, Manitoba R3T 2N6, Canada sharon.clouthier@dfo-mpo.gc.ca
[2] University of Manitoba, Department of Biological Sciences, Winnipeg, MB, R3T 2N2, Canada elissa.vanwalleghem@dfo-mpo.gc.ca
[3] Box 28, Group 30, RR2, Ste Anne, MB, R5H1R2, Canada mainebiotek@hotmail.com

Several mimivirus-like NCLDVs of sturgeon (*Acipenseridae*) previously recognized as unclassified members of the family *Iridoviridae* pose a potential disease risk for strategies designed to aid the recovery of threatened sturgeon populations in North America. These include the white sturgeon iridovirus (WSIV) found in white sturgeon *Acipenser transmontanus*, the Missouri River sturgeon iridovirus (MRSIV) isolated from pallid sturgeon *Scaphirhynchus albus* and shovelnose sturgeon *S. platorynchus*, the shortnose sturgeon *A. brevirostrum* irido-like virus and Namao virus (NV) detected in lake sturgeon *A. fulvescens*. We describe the development and analytical validation of two quantitative PCR (qPCR) assays. The tests designated as Q1 and Q2 were designed to target separate regions of the sequence encoding the major capsid protein (mcp) gene of Namao virus or all sturgeon NCLDVs, respectively. The analytical performance characteristics of sensitivity (ASe) and specificity (ASp) as well as repeatability of both assays were established. Each test reliably detected plasmid DNA ranging from $5x10^7$ to 5 copies per reaction and displayed a limit of detection of at least 50 plasmid copies. The Q2 assay demonstrated an expected inclusivity of all of the sturgeon mimiviruses tested whereas the Q1 assay detected all but WSIV. The two qPCR assays did not yield detectable amplification with synthetic target sequences from a panel of NCLDV isolates representing the families *Iridoviridae*, *Mimiviridae*, *Phycodnaviridae*. Repeatability estimates using plasmid DNA serially diluted over 7 orders of magnitude were high with coefficient of variation values ranging from 0.59 to 3.74 within a run and 0.68 to 3.79 between runs. The Q1 assay detected Namao virus more often than histology in samples collected over a four year period from wild populations of Manitoba lake sturgeon broodstock and their progeny. These molecular assays should improve or supplement the throughput, precision and accuracy of diagnosis for these viruses.

18) Special Session: Aquatic Diagnostic Laboratory Quality Assurance

18a. Inter-laboratory proficiency tests on detection of notifiable fish diseases

Niels .J. Olesen*, Niccoló Vendramin, Anemone Ojala, Susie S. Mikkelsen

National Veterinary Institute, Technical University of Denmark, Copenhagen, Denmark
njol@vet.dtu.dk

Part of the functions and duties of the European Union Reference Laboratory for Fish Diseases (EURL) is to organize periodic comparative tests of diagnostic procedures at Community level as described in Council Directive 2006/88/EC. The primary aim of the tests is to standardize and harmonize diagnostic methods in between laboratories in order to ensure safe and trustworthy trade of fish.

A qualitative and quantitative proficiency test has been carried out annually since 1996. The last 5 years the test was divided into proficiency test 1 (PT1) and proficiency test 2 (PT2). PT1 was designed to primarily assess the identification of the fish viruses: viral hemorrhagic septicemia virus (VHSV), infectious hematopoietic necrosis virus (IHNV), epizootic hematopoietic necrosis virus (EHNV), spring viremia of carp virus (SVCV), and infectious pancreatic necrosis virus (IPNV) by cell culture based methods. PT2 was structured with the aim of assessing the ability of participating laboratories to identify the fish pathogens: infectious salmon anaemia virus (ISAV), Cyprinid herpesvirus 3 (CyHV-3) (otherwise known as *koi herpes virus* - KHV) and *Aphanomyces invadans* the causative agent of epizootic ulcerative syndrome (EUS) by biomolecular methods (PCR- based). The number of National Reference Laboratories (NRLs) participating in PT1 and PT2 was in 2013 43 with laboratories from all over the world.
The proficiency tests are accredited by the Danish Accreditation and Metrology Fund –DANAK under registration number 515 for proficiency testing according to the quality assurance standard EN ISO/IEC 17043. Thereby being indispensable requisites for obtaining and maintaining accreditation in the participating laboratories.

Participants are asked to identify and quantify the contents within a deadline of 8 weeks. The EURL collate the answers and processed them statistically and graphically to provide the individual laboratory with a unique picture of its performance in relation to the other participants. Each participant is assigned a code number to ensure discretion.

Occasionally the tests are designed with additional purposes, e.g. by including other common fish viruses for differential diagnosis, for testing cell line susceptibility within and between laboratories, for testing the ability of laboratories to detect double infections and to differentiate between genotypes. The laboratories are also encouraged to sequence and characterize the viral genes, giving a unique opportunity to compare detailed sequencing results conducted in various laboratories on exactly the same virus isolates. Surprisingly high variations are observed, indicating that sequences retrieved from large repositories, with often poor proof reading, should be used with caution. This paper describes the different approaches in designing the test over a 17-year period and comments on the observations made and overall performance of participants during that period. Reports from the various proficiency tests can be downloaded on the following link http://www.eurl-fish.eu/Activities/proficiency_tests

18b. Veterinary laboratory association quality assurance program (VLAQAP) virtual microscopy module – A model for aquatic histopathology proficiency testing

David B. Groman

Aquatic Diagnostic Services, Atlantic Veterinary College, University of Prince Edward Island, 550 University Ave., Charlottetown, Prince Edward Island, C1A4P3, Canada groman@upei.ca

The availability of internationally acceptable proficiency testing programs for veterinary diagnostic laboratory are limited. This is due, in part, to a lack universally acceptable criteria, protocols and standardized testing reagents. In addition, for viable agents such as viruses, fungi, bacteria and parasites, as well as body fluids, international import regulations present barriers to the easy distribution of these test analytes. There are, however, less stringent regulations for the distribution of fixed / non-viable test materials such as genomic sequences, preserved parasites, fixed cytology and histopathology slides. The VLAQAP, currently provided through the Atlantic Veterinary College, offers proficiency testing to over 300 commercial, governmental and university veterinary diagnostic labs worldwide. The VLAQAP has recently developed a web based delivery format (see http://www.vlaqap.org/), which is ideal for instantaneous distribution of digitally scanned testing material such as parasite whole mounts and histology slides. Digital images of the test materials are scanned using an Olympus VS120-S5 imaging system, loaded onto a computer server at the University of Prince Edward Island and distributed to client via the VLAQAP website. Clients are provided with case history information and asked to examine the images over the web and submit via the website a diagnosis. Diagnoses are compared to previously determined benchmarks and compared with those supplied by other participating laboratories. The advantage of using a virtual microscopy system for proficiency testing of diagnostic histopathology and cytology services include, uniform specimen preparation and distribution as well as ease of use for the diagnostic pathologist. This presentation will provide a live demonstration of the Aquatic Virtual Microscopy Module currently available to Aquatic Diagnostic Laboratories worldwide.

18c. Quality Assurance according to ISO 17025 at fish diagnostic laboratories in Europe: Practice and status

Olga LM Haenen[1]*, Niccoló Vendramin[2], Anemone Ajola[2], Niels J. Olesen[2]

[1]National Reference Laboratory for Fish, Shellfish and Crustacean Diseases, Central Veterinary Institute, part of Wageningen UR, P.O. Box 65, 8200 AB Lelystad, The Netherlands olga.haenen@wur.nl
[2]DTU-National Veterinary Institute, Section for Virology, Fish Diseases Unit (EURL), Bülowsvej 27, 1870 Frederiksberg C, Denmark njol@vet.dtu.dk

Quality Assurance (QA) accreditation according to ISO/IEC 17025 is a must for veterinary diagnostic laboratories, including those for fish diagnosis. Since the late nineties, the European Union Reference Laboratory for Fish Diseases encouraged its National Reference Laboratories (NRL) for Fish Diseases to acquire accreditation. Additionally, at the EAFP Conference in 1999, a worldwide attended QA workshop was held (Haenen et al., 1999). The result was a steep increase in ISO 17025 accredited fish diagnostic laboratories since the start of this century, both in Europe and in many other areas like Chile, the US, and Canada.

ISO/IEC 17025 is the global recognized standard that was developed specifically for testing and calibration laboratories that intended to be accredited, often done by a national accreditation body. An important difference from ISO 9001 is the fact, that in ISO 17025 the scientific quality of the test is accredited, and therefore, validations and proficiency tests are integrated and obligatory to conduct. In the EU the NRLs for Fish Diseases must be accredited according to ISO 17025, primary for surveillance and diagnosis of the principal notifiable fish diseases. EU lists the following fish diseases as non-exotic: Viral Hemorrhagic Septicemia (VHS), Infectious Hematopoietic Necrosis (IHN), Infectious Salmon Anemia (ISA), and Koi herpesvirus disease (KHVD), while Epizootic Hematopoietic Necrosis (EHN) is listed as exotic for the EU.

Getting accreditation is a time-consuming and expensive process, and asks for continuous updating and refinement. In case of new techniques, like real-time PCRs the process may be speeded up to a few months by using a so called *Flexible Scope* on QA: it is possible to obtain accreditation by notifying the accreditation board after conducting a standardized validation procedure. Once this scope is accredited additional tests using the same methodology can be added to the scope without an additional audit.

In this lecture, an overview of daily practice of using ISO 17025, and of validation, including that of modern tests is presented. Additionally, the increase of QA status since 2003 of the fish NRLs of the EU for specific tests is shown and discussed. It is concluded, that ISO 17025 is a valuable tool for standardization and harmonization of tests for diagnosis and surveillance of fish diseases, and that more and more European laboratories obtain accreditation The high costs of obtaining and maintaining accreditation is however a risk for future development.

- EURL, 2014. http://www.eurl-fish.eu/Activities/annual-meetings (accessed 23 May 2014)
- Haenen, O.L.M., E.-M. Bernoth and D. Groman, 1999. Quality assurance in fish disease diagnosis. Bull. Eur. Ass. Fish Pathol. 19(6): 302-309.
- ISO/IEC International Standard 17025 (2005). General requirements for the competence of testing and calibration laboratories. International Organisation for Standardisation (ISO)/International Electrotechnical Commission (ISO/IEC), ISO Central Secretariat, 1 rue de Varembé, Case Postale 56, CH - 1211, Geneva 20, Switzerland.
- OIE Quality Standard and Guidelines for Veterinary Laboratories. http://web.oie.int/boutique/ accessed 23 May 2014.

18d. Improvements are needed in reporting of accuracy studies for diagnostic tests used for detection of finfish pathogens

Ian A. Gardner[1]*, Tim Burnley[2], Charles Caraguel[3]

[1]Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, Prince Edward Island C1A 4P3, Canada, iagardner@upei.ca

[2]Eastern Epidemiological Services, Cornwall, Prince Edward Island C0A 1H8, Canada, burnley.tim@gmail.com

[3]School of Animal & Veterinary Sciences, Roseworthy Campus, University of Adelaide, Roseworthy, South Australia 5371, Australia, charles.caraguel@adelaide.edu.au

Accurate diagnostic tests are central to the detection, control and prevention of pathogen spread in aquaculture. The World Organisation for Animal Health (OIE) specifies that test accuracy in aquatic species should be estimated to assess a test's "fitness for the intended purpose(s)". Regardless of the intended purpose of the test, complete and transparent reporting of design elements and results of a test accuracy study is essential so that readers can appraise the validity of the study, including the potential for bias in diagnostic sensitivity and specificity estimates.

The Standards for Reporting of Diagnostic Accuracy (STARD) statement (www.stard-statement.org) was published to encourage improved reporting of key elements of test accuracy studies in human medicine. The STARD does not prescribe directly how to design a test accuracy study. Rather, it focuses on clear and transparent reporting of its key elements, namely the 25 items in its checklist. Although STARD principles apply broadly to test accuracy studies in all species, the guidelines do not account for unique considerations in animal production such as use of experimental challenge studies, varied testing purposes, different epidemiological units and sampling designs, and use of latent class analysis methods to account for imperfect reference

We evaluated the reporting quality of studies evaluating test accuracy for finfish diseases using the 25 items in the STARD checklist. Based on a database search, 11 studies that included estimates of diagnostic accuracy were identified for independent evaluation by all authors. Ten of 11 studies were for viral infections (including 3 listed by OIE: infectious salmon anaemia, viral haemorrhagic septicaemia, and infectious hematopoietic necrosis virus) and one was for *Parvicapsula minibicornis*. Eight studies used field samples only, 2 used experimental samples, and 1 used both. All studies evaluated samples collected post-mortem. For each study, STARD checklist items were scored as 'yes', 'no', or 'not applicable'. For most items, classifications were similar among reviewers. Only 10 of 25 items were consistently reported in most (≥ 80%) papers and reporting of the other items was highly variable (mostly between 30-60%). Three items ('number, training and expertise of readers and testers'; 'time interval between index tests and reference standard'; and 'handling of indeterminate results, missing data and outliers of the index tests') were reported in <10% of papers. Two items ('time interval between index tests and reference standard', and 'adverse effects from testing') were considered less relevant to the fish health context because test samples usually are collected post-mortem. Modification of STARD to increase relevance to aquatic food animals should facilitate use by authors and thereby improve the overall reporting quality regardless of how the study was designed. An international initiative is underway to develop an aquatic version of STARD which will be broadly applicable to finfish, molluscs and crustaceans.

18e. Influences of government regulators on aquatic laboratory quality assurance: A global perspective

Kim C. Klotins[1], David B. Groman[2]*

[1]National Veterinary Program Specialist, Domestic Disease Control Program, National Aquatic Animal Health Program, Canadian Food Inspection Agency, Ottawa, Ontario, Canada kim.klotins@inspection.gc.ca

[2]Aquatic Diagnostic Services, Atlantic Veterinary College, University of Prince Edward Island, 550 University Ave., Charlottetown, Prince Edward Island C1A4P3, Canada groman@upei.ca

Fish and seafood trade is an important activity for many countries and occurs at an international and domestic scale. Since aquatic animal diseases were listed by the World Organization for Animal Health (OIE), there have been increasing requirements to certify the disease status of aquatic animals and aquatic animal products to manage risks of disease introduction and spread. Part of that certification may require diagnostic testing of animals or product. To facilitate trade between member countries, the OIE provides guidelines and standards, including the establishment of a Competent Authority within the country that would oversee the certification process. That authority must reside within the government of the country. It is expected that the competent authority would implement their program in a manner that is consistent with the guidelines and standards provided by the OIE. The OIE recommends that all laboratories that provide services to the aquatic animal health program operate under a quality assurance program. There are several ways a Competent Authority can follow to meet this recommendation including the use of legislation, policy and national standards or criteria. This presentation will describe the process followed by several countries, including Canada, and identify benefits and challenges.

19) Special Session: Myxozoan Origins and Diversity Outreach

19a. Myxozoans as cnidarians

Beth Okamura*

Department of Life Sciences, Natural History Museum, London, United Kingdom

There is now strong evidence for the phylogenetic placement of Myxozoa within the Cnidaria and it is therefore timely to explore their evolutionary history in this context. In particular, what cnidarian features may have facilitated the transition to an endoparasitic lifestyle? This talk will explore how we may understand the transition to endoparasitism by myxozoans in view of their cnidarian origins. I will summarize the collective evidence for placement within the Cnidaria and consider how certain traits, such as polar capsules and tetraradial musculature, may help to identify the cnidarian sister group. I will then compare and contrast myxozoan and cnidarian life histories and examine the difficulties of identifying common stages between myxozoans and their free-living relatives. Further discussion will explore whether cnidarian traits, such as a propensity to produce novel propagative stages and extensive epithelial surfaces, predisposed a myxozoan precusor to evolve an endoparasitic lifestyle, and how various animals may have been incorporated initially as hosts resulting in the complex life cycles observed today. These issues will appear in chapters contributed to ‘Myxozoan Evolution, Ecology and Development’, a book reviewing current knowledge of this topical group to be published in the near future by Springer.

19b. *Bipteria* sp. – old parasite in an old host: tracing the origin of myxosporean parasitism in the vertebrates

Alena Kodádková[1,2], Pavla Bartošová-Sojková[1], Ivan Fiala[1]*

[1]Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic alena.kodadkova@gmail.com, bartosova@paru.cas.cz, fiala@paru.cas.cz

[2]Faculty of Science, University of South Bohemia in České Budějovice, Branišovská 31, 370 05 České Budějovice, Czech Republic

Myxozoan parasites alternate between their vertebrate (mostly fish) and invertebrate (mostly annelid) hosts. It is assumed that Myxozoa evolved within cnidarians as a sister lineage to the Medusozoa (Cnidaria). Based on the molecular and fossil dating of jellyfish, medusozoans evolved during Cambrian radiation (540 - 510 Ma), which suggests an evolutionary old origin of myxozoans. We discovered a new myxosporean *Bipteria* sp. from the gall bladder of rabbit fish, *Chimaera monstrosa* (Holocephala), from the North Atlantic. Morphology of *Bipteria* sp. myxospore is characterised by crescent shape with wing-like appendages similar to the *Ceratomyxa* morphotype that is supposed to be evolutionary primitive. Based on the SSU rDNA phylogeny, *Bipteria* sp. represents the most basal single-species branch of the marine myxosporean lineage suggesting *Bipteria* is an evolutionary old parasite. We made an ultrametric tree of representatives of all myxosporean clades. The tree was provided with timescale calibrated at the presumed origin of the Myxozoa. The node representing the origin of *Bipteria* sp. fits to the time of origin of its vertebrate host, *C. monstrosa*, in the Silurian era. The first branching (marine) chloromyxids of the freshwater lineage diversified with the origin and diversification of their elasmobranch hosts. The rapid diversification of the main clades of both marine and freshwater lineages happened when the bony fish appeared on the Earth. Furthermore, the supposed switch from the marine to the freshwater environment within the freshwater myxosporean lineage coincides with the origin of oligochaetes from their polychaete ancestors. Based on the performed analysis, we hypothesize multiple origin of parasitism in the vertebrate host during the myxozoan evolution.

19c. Environmental DNA reveals novel myxosporean diversity

Hanna Hartikainen[1,2]*, David Bass[1], Beth Okamura[1]

[1] Department of Life Sciences, The Natural History Museum, Cromwell Road, London, SW7 5BD, UK d.bass@nhm.ac.uk b.okamura@nhm.ac.uk

[2] ETH Zürich & Eawag, Institute for Integrative Biology, Ueberlandstrasse 133, 8600 Dübendorf, Switzerland Hanna.Hartikainen@eawag.ch

The increasing use of environmental DNA (eDNA) and massively parallel deep sequencing has revolutionized the detection of novel microbial diversity and characterization of community structure and function. Such approaches are increasingly used to discover novel parasite diversity, to monitor parasite occurrence and to characterize distribution patterns. Yet, myxozoans are conspicuously absent from most amplicon and metagenomic eDNA surveys from filtered water, soils and sediment. We hypothesized that this is due to the inability of regularly employed general primers to amplify myxozoans and their relative scarcity in small volume samples. We developed a targeted, nested PCR primer approach to detect all known myxosporeans in eDNA samples and screened 115 marine water, 35 terrestrial soil, 24 faecal and 270 freshwater eDNA extractions. PCR products from positive samples were pooled according habitat type, cloned and sequenced. The newly developed specific primers detected myxoporeans across the known phylogenetic diversity, including both marine and freshwater lineages. Representatives of *Myxobolus* clade were most commonly encountered. Large volume water samples and kicking up sediment significantly improved detection likelihood, suggesting that myxozoan spores are relatively rare in the water column. We detected *Myxobolus articus* and other two previously unsequenced myxoboliid species from otter spraints. *Myxobilatus gasterostei* was found frequently in freshwaters, along with *Chloromyxum truttae* and two undescribed SSU rDNA types. Samples from South Africa yielded three SSU rDNA types for which no close matches were found in Genbank. Phylogenetically these sequences clustered in the *Myxobolus* clade. Our results show that with specific primers, eDNA can be a valuable gateway for detecting novel myxozoan diversity, can be used as a monitoring tool and can provide insight into life-cycle studies of myxozoans.

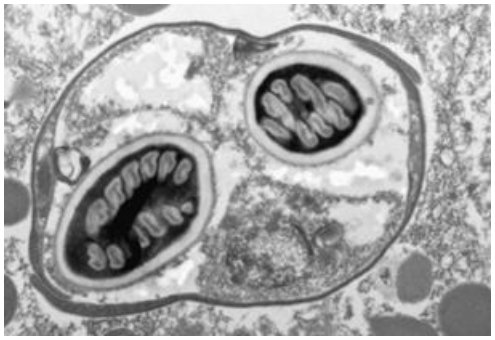
19d. Is there a future for the order Multivalvulida in myxosporean systematics?

Mark A. Freeman[1], Ivan Fiala[2], Alena Kodádková[2,3], Árni Kristmundsson[4]

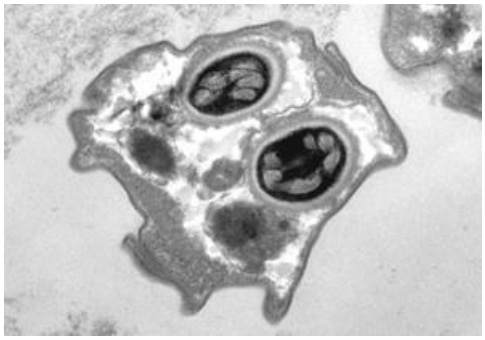
[1]Institute of Ocean and Earth Sciences, University of Malaya, Kuala Lumpur, Malaysia
[2]Institute of Parasitology, Academy of Sciences of the Czech Republic, Branišovská 31, České Budějovice, Czech Republic
[3]Faculty of Science, University of South Bohemia in České Budějovice, Branišovská 31, České Budějovice, Czech Republic
[4]Institute of Experimental Pathology, at Keldur, University of Iceland, Iceland

Myxosporean taxonomists have traditionally classified myxosporeans into two orders, the Bivalvulida and the Multivalvulida, based on the number of valves contained in the myxospore shell. The Bivalvulida contain two valves and the Multivalvulida three or more. The Multivalvulida contains two families, the Trilosporida with three shell valves and the Kudoidae with four or more. The Kudoidae contained genera named according to the shell valve number, *Pentacapsula* (5 valves), *Hexacapsula* (6 valves) etc. Whipps et al. (2004) proposed that all genera with four or more valves be assigned to the genus *Kudoa*, leaving it as the sole genus in the family. The main rationale for this was the limited genetic distance observed between members of the *Kudoa*, irrespective of shell valve number, compared to other genera, such as *Myxobolus* in the Bivalvulida. However, since that revaluation of the family Kudoidae, a sub-clade of myxosporeans have been robustly placed within the Kudoidae, in phylogenetic analyses, that only have two shell valves and superficially resemble sphaerosporids.

We present morphological and molecular data on two-valved (sphaerosporid) members of the Kudoidae and conclude that they are simply two-valved members of the genus *Kudoa*. This confirms that spore valve number is not a valuable character in myxosporean systematics, and that priority should be given to other features such as tissue tropism, DNA analyses and other morphological and ultrastructural features. In addition, we suggest a new genus, *Gasteromyxum*, for basal kudoids, which reasserts an origin from within the Variisporina for the Multivalvulida. The value of retaining the taxonomic orders Bivalvulida and Multivalvulida in myxosporean systematics is discussed.



Gasteromyxum elopsi n. sp.



Two-valved kudoid (sphaerosporid)

Whipps CM, Grossel G, Adlard RD, Yokoyama H, Bryant MS, Munday BL, Kent ML (2004). Phylogeny of the Multivalvulida (Myxozoa: Myxosporea) based on comparative ribosomal DNA sequence analysis. J. Parasitol. 90: 618-622.

19e. Freshwater myxozoans in China: epidemiology, taxonomy and prospects

Zemao Gu*, Yang Liu, Luo Jia, Mingjun Huang, Qingxiang Guo

Lab of Freshwater Animal Breeding, Ministry of Agriculture, College of Fisheries, Huazhong Agricultural University, Wuhan, 430070, People's Republic of China guzemao@mail.hzau.edu.cn

Myxozoans are ubiquitous metazoan parasites mainly found in fish and invertebrate host. In China, merely in freshwater fish, more than 600 species were reported and they are widely distributed all over the country except for some places with orphan status in myxozoan research, like Tibet. Almost all fish species in China are threatened by myxosporidiosis to a different extent and the epidemiology pattern varies from province to province depending on different cultured species, climate pattern and breeding habit. For instance, in central China and eastern China, *Myxobolus honghuensis* Liu & Gu, 2012, causing nearly 100% host mortality, *Myxobolus turpisrotundus* Zhang, 2009, parasitizing on the head, fin, gills and reducing the commercial value sharply, *Thelohanellus wuhanensis* Xiao & Chen , 1993, which parasitizes on the skin and causes a high mortality more than 90%, are common and keep increasing along the Yangzi River Basin, especially in Hubei Province, Jiangsu Province and north of Zhejiang Province where the susceptible fish host, allogynogenetic gibel carp *Carassius auratus gibelio* (Bloch) is widely cultured. In northern China, the *Thelohanelus kitauei* Egusa, 1981, which parasitizes in the gut of common carp (*Cyprinus carpio*), is a major problem and causes huge loss. Myxosporidiosis involving freshwater fish species other than carp, like channel catfish (*Ictalurus punctatus*), yellow catfish (*Pelteobagrus fulvidraco*), snakehead (*Channa argus*), could be found in southern China but not dominate. The current taxonomy research mainly focus on identification of new species and revealing cryptic species, solving synonyms and homonyms, and other controversial taxonmic topic on species level and genus level, e.g. investigating the validity of the genus *Henneguya* with morphological and molecular evidence. Besides, the ongoing project on setting up DNA barcodes database based on SSU rDNA, with host-specificity, tissue-specificity and available SSU rDNA informations included, can serve as a useful tool for identification and has broad applicability for diagnosis. Future research will focus on expansion and testing maturity of the DNA barcodes database, seeking for more suitable and effective molecular markers, establishing life history models and starting transcriptome and genome study to solve controversial taxonmic topics.

19f. *Ceratonova shasta*: Evolution of (how we perceive) a parasite

Jerri L. Bartholomew*, Stephen D. Atkinson

Department of Microbiology, Nash Hall 220, Oregon State University, Corvallis, OR 97330

Ceratomyxa shasta is a long-standing taxonomic outlier to all other *Ceratomyxa* spp. It is histozoic (rather than coelozoic), has a freshwater life cycle (as opposed to marine) and is phylogenetically distant. The recent description of a new myxozoan species from freshwater sticklebacks led us to propose erecting a new genus, *Ceratonova*, to contain the two species: *C. shasta* n. comb. and *C. gasterostea* n. sp. This redescription provided the opportunity to look back at the changes in our understanding of this parasite and forward to what we are learning from new approaches. While our knowledge of some aspects of *C. shasta*, such as the life cycle, have changed dramatically, the geographic and host ranges have not. However, new information about the invertebrate host provides an explanation for the unusually static and restricted geographic range of *C. shasta*. Similarly, a better understanding of parasite genetics is informing us about host-parasite co-evolution. Our knowledge is expanding rapidly, with molecular diagnostic methods providing tools for predicting disease impacts on wild populations and epidemiological approaches providing critical insights about alternatives for disrupting disease dynamics. Sequencing of the genome and transcriptome is providing insights into differences between the host-specific parasite genotypes as well as into the basic biology of the parasite.

20) General Session: Diseases of Invertebrates

20a. Microparasites causing reduced commercial value of northern shrimp, *Pandalus borealis*

Árni Kristmundsson[1]*, Mark A. Freeman[2]

[1]Institute for Experimental Pathology at Keldur, University of Iceland, Reykjavik, Iceland, arnik@hi.is
[2]Intitute of Oceanic and Earth Sciences, University of Malaya, Malaysia, Kuala Lumpur, Malaysia, mark@um.edu.my

Northern shrimp, *Pandalus borealis*, also called pink shrimp or deepwater prawn, is a commercially valuable species distributed in the northern parts of the Atlantic and Pacific Oceans. During processing, in early winter 2013, black spots of unknown aetiology were noticed in a portion of the shrimp catch, originating from three different locations; shallow infjord and deeper outfjord waters off the NW coast of Iceland and off the Labrador coast in Canadian waters. Samples of affected shrimps from all sites were sent to the Fish disease Laboratory at the Institute for Experimental Pathology at Keldur, to check whether infectious agents caused this phenomenon.
Clinical signs were similar in shrimps from all sites, i.e. black spots of different sizes apparently starting in the chitinous outer shell with a subsequent extension into the muscular tissues. Histopathological examination revealed severe muscular necrosis in all shrimps from all sites.

PCR and DNA sequencing revealed that there was a microsporidian infection present, but only in the deeper outfjord waters off the NW coast of Iceland. This microsporidian was identified as *Thelohania butleri* (although the generic assignment is in doubt), a known parasite of the smooth pink shrimp *Pandalus jordani* from the Pacific coast of Canada. Three different and novel alveolate sequences were also sampled, one from each site. The sequence from the infjord waters off Iceland was most similar to dinoflagellate sequences from the order Syndiniales, which are known to be parasitic in crustacean and other marine organisms. The sequence from the outfjord waters, which occurred as a co-infection with *T. butleri*, was most similar to a basal apicomplexan. Whilst the Canadian samples had yet another alveolate sequence more related to the ciliates, and similar to apostome ciliate *Pseudocollinia* spp. found as parasites of the Arctic krill *Thysanoessa raschii*.
We present these finding and demonstrate the histopathological changes from shrimp at each site. We also consider the possibility of co-infections of parasites not yet detected by PCR and whether some of the DNA sequences obtained could be from non-pathogenic alveolates, such as ectocommensal ciliates.

20b. Targeting essential genes utilizing RNA interference to elucidate shrimp-WSSV interaction

Mary Beth B. Maningas[1,2,3], David Angelo V. Guanzon[1]*, Jassy Mary S. Lazarte[3], Rod Russel R. Alenton[3], Maria Violeta R. Tare[3], Hidehiro Kondo[4], Ikuo Hirono[4]

[1]Department of Biological Sciences, College of Science, University of SantoTomas
[2]Molecular Biology and Biotechnology Laboratory, Research Center for Natural and Applied Sciences, University of Santo Tomas,
3Graduate School, University of Santo Tomas, España,1015, Manila, Philippines
marybethmaningas@yahoo.com/mbmaningas@mnl.ust.edu.ph
[4]Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology, Minato-ku, Tokyo, 108-8477, Japan

White Spot Syndrome Virus (WSSV) remains the most widespread and devastating infectious agent that hit the shrimp aquaculture industry worldwide. To date, there are no known effective strategies yet to combat WSSV infection. This study aimed to elucidate host-pathogen interaction through the functional study of both a viral and a host gene. Utilizing RNA Interference, we elucidated the function of VP9 gene of WSSV, contig23 (c23) and protein kinase (*Mr*PK) found in the shrimp genome identified to have high homology with WSSVORF-325 and WSSV-PK. Four set-ups using *Macrobrachium rosenbergii* shrimps were prepared for treatment of VP9-dsRNA, c23-dsRNA, *Mr*PK-dsRNA, GFP-dsRNA, and PBS. Each shrimp treatment group was challenged with WSSV and survival rate was recorded. VP9-, c23-, and GFP-dsRNA injected shrimps showed a significant survival rate of 80%, 100% and 100%, respectively, in contrast to 20% of the PBS injected shrimps at 10 days post-infection (dpi). These results are corroborated with the re-infection survival assay and the RT-PCR analysis. For the PK gene, open reading frames (ORFs) that encode 424-aa polypeptide was found in *Marsupenaeus japonicus* with sequences for *Penaeus monodon* and *Macrobrachium rosenbergii* also obtained. Phylogenetic analysis revealed that the three ORFs have 30% homology to WSSV-PK. Interestingly, there was no effect in expression when shrimps were infected with WSSV but PK was downregulated when infected with *Vibrio* sp. suggesting that it might also have a role in bacterial infection. Challenge test data based on three species of shrimp strongly indicate that VP9 can be a good target gene for RNAi therapeutics.

20c. Functional elucidation of *Mr*C20 by RNA interference

David Angelo V. Guanzon[1]*, Francisco G. Bolinao IV[1], Alfred Jerald I. Salvador[1], Joseph Carlo V. Vergel[1], Hidehiro Kondo[2], Ikuo Hirono[2], Mary Beth B. Maningas[1,3]

[1]Department of Biological Sciences, College of Science, University of Santo Tomas, España, Manila, 1015 Philippines

[2]Laboratory of Genome Science, Tokyo University of Marine Science and Technology, Konan 4-5-7, Minato, Tokyo 108-8477, Japan

[3]Research Center for Natural and Applied Sciences. University of Santo Tomas, España, Manila, 1015 Philippines

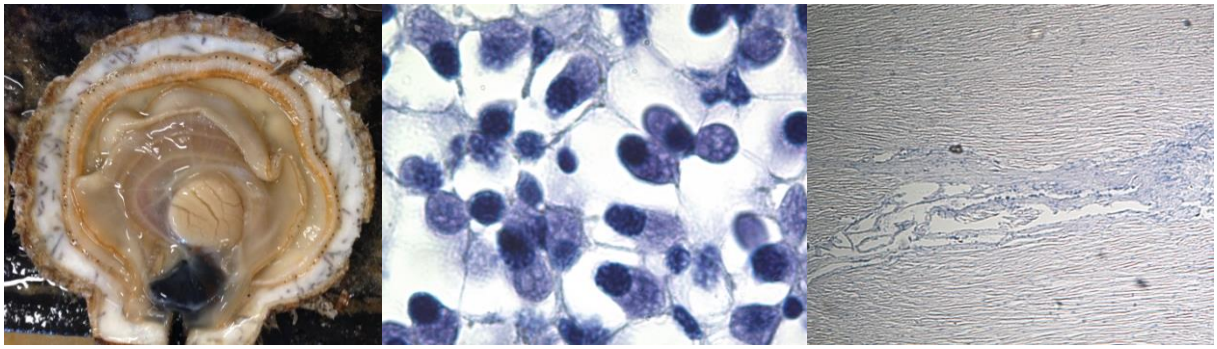
Shrimp and prawn aquaculture is a major source of income in the intertropical countries of the Southeast Asian region. However, the country experiences a great decline in production due to a major viral pathogen, the White Spot Syndrome Virus (WSSV) which continues to prevail despite many preventive measures applied to deter the virus. RNA Interference (RNAi) technology has been employed to reveal functions of genes both in the virus and its host with the aim of controlling WSSV by elucidating complex host-virus interactions. This study determined the involvement of *Mr*C20, a DNA fragment that is part of the genome of *M. japonicus* and was shown to be present in *M. rosenbergii*. Rapid cloning of 5' and 3'-cDNA ends PCR (RACE-PCR) was employed to determine the unknown 5' and 3'-cDNA termini of *Mr*C20. The RACE-PCR products of *M. rosenbergii* was sequenced, assembled, and a majority consensus tree was constructed. Phylogenetic analysis revealed that *Mr*C20 is highly homologous to WSSV VP466, a viral nucleocapsid protein that functions in viral penetration by binding WSSV virions to host cells. The clustering of WSSV VP466 with both *Mr*C20 and *Mj*C20 suggests viral mimicry. *Mr*C20's high homology to WSSV VP466 made it a prime candidate for RNAi, which if silenced provides protective capacities and further reveals its complex host-virus interaction. Moreover, *Mr*C20 is ubiquitously expressed in vital organs suggesting that it is essential to metabolic functions of the prawn. *Mr*C20 may also play a role in the shrimp immune system as highlighted in its expression in the gills and hemocyte. One Way Analysis of Variance (ANOVA) of the mortality assay indicates that gene silencing of *Mr*C20 has a significant protective effect in terms of the survival of the prawn. Further statistical analysis by Scheffe's test determined that *Mr*C20-dsRNA treated prawns are provided protective effects against WSSV compared to GFP-dsRNA and PBS treated prawns.

20d. Apicomplexan infection of the Atlantic sea scallop *Placopecten magellanicus*

Árni Kristmundsson*[1], Susan Inglis[2], Kevin Stokesbury[2], Mark A. Freeman[3]

[1]Institute for Experimental Pathology at Keldur, University of Iceland, Reykjavik, Iceland, arnik@hi.is
[2]University of Massachusetts-Dartmouth, SMAST, Fairhaven, MA USA, singlis@umassd.edu, kstokesbury@umassd.edu
[3]Institute of Oceanic and Earth Sciences, University of Malaya, Malaysia, Kuala Lumpur, Malaysia, mark@um.edu.my

Atlantic sea scallop, *Placopecten magellanicus*, meats are normally firm and creamy white. However scallops with small, darkened (gray) meat have been observed in temporally and spatially specific locations along the Eastern Sea Board since 1949. These scallops have been associated with reduced harvestable biomass and mass mortality events. Large numbers of these scallops are observed in the scallop rotational management areas on Georges Bank following periods of fishing closures. In 2013, the Closed Area I access fishing area was closed early due to the number of "gray meat" scallops and reduced harvestable biomass. A similar phenomenon has been experienced in the wild stock of Iceland scallop, *Chlamys islandica*, in Icelandic waters causing a total collapse in the stock with a subsequent fishing ban imposed.
Samples from affected scallops (gray meat), caught in Georges Bank in winter 2013-2014, were examined for the presence of pathogens by histopathological and molecular methods.



Muscular tissues of all scallops examined were infected with an apicomplexan parasite. Infections were found both intracellular inside muscle fibres, and also in the intracellular spaces. Various developmental stages of the apicomplexan parasite were identified, including developing trophozoites, merozoites and sporozoites. Severe histopathology was observed in relation to the infections, which was characterized by varying degrees of focal or disseminated muscular necrosis. PCR and DNA sequencing confirmed the presence of an apicomplexan parasite that is closely related to the one identified from the Iceland scallop, which is believed responsible to the recent collapse in that stock.
Based on the prevalence, infection intensity and the histopathological changes observed and the similarity to a known pathogen from scallops from Icelandic waters, it seems highly probable that this apicomplexan is responsible for the grey meat phenomenon in *Placopecten magellanicus* from Georges Bank in US waters.

21) General Session: Outreach and Physiology

21a. US Food and Drug Administration's Phish-Pharm Database - A searchable database of pharmacokinetics data in fish – 2014 update

NR Hasbrouck*, TC Crosby, R Reimschuessel

US Food and Drug Administration, Center for Veterinary Medicine, Office of Research, 8401 Muirkirk Road, Laurel, MD 20708 USA nicholas.hasbrouck@fda.hhs.gov, tina.crosby@fda.hhs.gov, renate.reimschuessel@fda.hhs.gov

Due to the increased demand for seafood as a food product, the need for veterinary care and safe and effective therapeutic drugs for the aquaculture industry has emerged. As part of the US Food and Drug Administration/Center for Veterinary Medicine's commitment to streamlining the drug approval process for minor species, the Phish-Pharm database was updated to Version 5 in 2014. The literature database now consists of almost 600 articles that detail drug metabolism, depuration, and pharmacokinetics in multiple fish species.

Phish-Pharm allows users to search for information using eight fields: drug/chemical, drug class, common name, genus and species, route of administration, sample analyzed, author's names, and water type. Additional data fields include metabolites identified, depletion time, half-life (t ½), water temperature, average animal weight, dosage, protein binding, clearance, volume of distribution in a central compartment (Vc) or volume of distribution at steady state (Vd), a comments section, and additional fields listing the citation, authors, title, method of analysis, and internet links.

As part of an interactive demonstration, we will highlight features of the Phish-Pharm database, showing how the database can display trends that may otherwise be difficult to recognize and identify data gaps for future research in aquatic animal medicine, especially for food species.

21b. Aquatic animal health courses: converting to online and the infusion of one health and interdisciplinary views

Iskande L.V. Larkin*, Heather T.D. Maness

College of Veterinary Medicine, University of Florida, PO Box 100136, Gainesville, FL 32610 USA ivlarkin@ufl.edu; htdaniel@ufl.edu

The majority of Chief Academic Officers consider distance learning fundamental in long-term strategic planning.[1] However, significant concern exists about educational quality, integrity, and effectiveness in online classrooms.[1] This has initiated a nationwide (and global) conversation that is likely to improve all classroom settings by emphasizing instructional design based upon pedagogical theory.[2] With this in mind, the Aquatic Animal Health Program at the University of Florida cautiously entered into online education. In our courses we utilized technology (recorded lectures, online videoconferencing/discussion, and survey tools) to: 1) increase participation by expert lecturers (biologists, veterinarians, and government employees to provide content), 2) diversify students in the course enhancing student-student interactions (undergraduate, veterinary, and graduate students benefitting from professional perspectives and each other's), and 3) evaluate student satisfaction.

Students enrolled in our online courses provided feedback on each format/tool (responses to individual questions ranged from 77 to 158 students). Students responded favorably to the technology with the majority noting a similar level of learning as a classroom-based course for the recorded lectures (54%) and online discussion sessions (59%). Furthermore, a strong proportion responded that they learned more or significantly more with recorded lectures (31%). Overall, the technologies used are viewed as successful methodologies for learning. The vast majority of students (74%) felt the technology allowed them to understand the material better and half felt it increased their interest in the subject matter. Thus, program resources will continue to be allocated for further development of distance education curricula and additional technologies will be explored for continued improvements in education within this specialized field.

Acknowledgements
This work was conducted under UF IRB Protocol #: U-1113-2011. Support is provided by UF AAHP including the Self-funded Online Education Program, UF CVM, UF Whitney Laboratory for Marine Bioscience, and Florida Fish and Wildlife Conservation Commission.

Literature Cited

1. Allen IE, Seaman J. 2011. Going the Distance: Online education in the United States 2011. Boston (MA): Babson Survey Research Group and Quahog Research Group, LLC. Available from: http://www.babson.edu/Academics/centers/blank-center/global-research/Documents/going-the-distance.pdf
2. Summers JJ, Waigandt A, Whittaker TA. 2005. A Comparison of Student Achievement and Satisfaction in an Online Versus a Traditional Face-to-Face Statistics Class. Innovative High Educ 29:233-250.

21c. An examination of job market and valued knowledge and skills for veterinarians in aquatic animal health

Heather T.D. Maness[1,2]*, Sebastian Galindo-Gonzalez[3], Iskande L.V. Larkin[1], T. Grady Roberts[3], Ruth Francis-Floyd[1,2]

[1]College of Veterinary Medicine, University of Florida, PO Box 100136, Gainesville, FL 32610 USA htdaniel@ufl.edu ivlarkin@ufl.edu rffloyd@ufl.edu
[2]College of Agriculture and Life Sciences, University of Florida, Gainesville, FL USA
[3]College of Agriculture and Life Sciences, University of Florida, PO Box 112060, Gainesville, FL 32611 USA sgalindo@ufl.edu groberts@ufl.edu

There has been some research directed at the veterinarian job market and/or technical and non-technical competencies of veterinarians but analysis in specific veterinary fields such as aquatic animal health is lacking. In December 2013, a 43 item survey was conducted of 99 targeted aquatic animal health professionals and had a response rate of 70%. Respondents, predominately veterinarians (83%), represented government, universities, private practice, corporations and non-profits (including zoos and aquariums), as well as other areas (such as independent contractor). Participants have been employed in the field (partially or primarily) for a mean of 19.5 years.

The majority responded that the average recent veterinary graduate does not have the knowledge or experience needed in any of the aquatic taxon categories (mammals, fish, birds, invertebrates, reptiles, and amphibians) to meet their organization's needs. The largest deficiency is in fish medicine (both fresh and saltwater species). The average desired experience is 42% fish, 32% mammal, 11% reptile/amphibian, 11% bird, and 4% invertebrate. Effective use of educational resources, including critically evaluating scientific literature, was viewed as more valuable than an additional year of work experience in small animal medicine. For non-technical skills, honesty/integrity was most valued and commonly possessed by veterinary employees and colleagues. Teamwork/interpersonal skills was valued second most yet listed first for commonly needing improvement. Respondent's perspective of the job market included negative perceptions of the rehabilitation field (static or slight decrease) and more positive perceptions of aquaculture and one health (static to slight increase).

Acknowledgements
This work was conducted under UF IRB Protocol #: 2013-U-1219. The authors would like to thank all of the respondents as well as Drs. Martine de Wit, Glen Israel, Hugh Mitchell, Jena Questen, David Scarfe, Thomas Waltzek, and Roy Yanong for their support and assistance.

21d. Exogenous insulin modulated the cellular accumulation in the exudate of tilapia during infectious aerocistite

Marco A. A. Belo[1,2*], Ed J.R. Prado[2], Alessandra C. Moraes[2], Elizabeth P. Foz[1], Roberto Barbuio[2], Vanessa P. Faria[2]

[1]Laboratory of Animal Pharmacology and Toxicology, Camilo Castelo Branco University - UNICASTELO, 950 Hilário da Silva Passos Ave., Descalvado/SP. maabelo@hotmail.com; betinafoz@hotmail.com.

[2]Dep. of Veterinary Pathology – São Paulo State University/UNESP–Jaboticabal/SP. ed_johnny@hotmail.com; alecris_moraes@hotmail.com; betovet04@yahoo.com.br; vanessapavesifaria@hotmail.com

Nile tilapia, *Oreochromis niloticus*, GIFT (±532g) , masculinized , were divided into two groups of 21 animals each treated or not with insulin (Lantus ®), subcutaneously route a single dose of 10 IU-kg^{-1} (both inoculated with *Aeromonas hydrophila* in the swim bladder). Seven animals from each treatment were sampled at 6, 24 and 48 hours post-inoculation (HPI) for exudate collection. After deep anesthesia with benzocaine, fish were necropsied to collect the exudate. Total cells were counted in neubauer chamber and differential cell counts in smear.

Tilapia treated with insulin showed significant (P<0.05) increase in the number of cells in the exudate, influenced principally by granulocytes (6 and 24 HPI), as well as significant (P<0,05) increase in lymphocytes and macrophages (6 HPI), when compared to infected control. Low counts of thrombocytes were observed 48 HPI in fish treated with insulin. On the other hand, tilapia only infected (Control) presented increase in the cell counts and granulocytes during the evolution of acute infectious aerocistite, as long as fish treated with insulin showed the highest counts of lymphocytes (6 HPI) and total cells and granulocytes (24 HPI). It was not observed macrophages 6HPI in control fish.

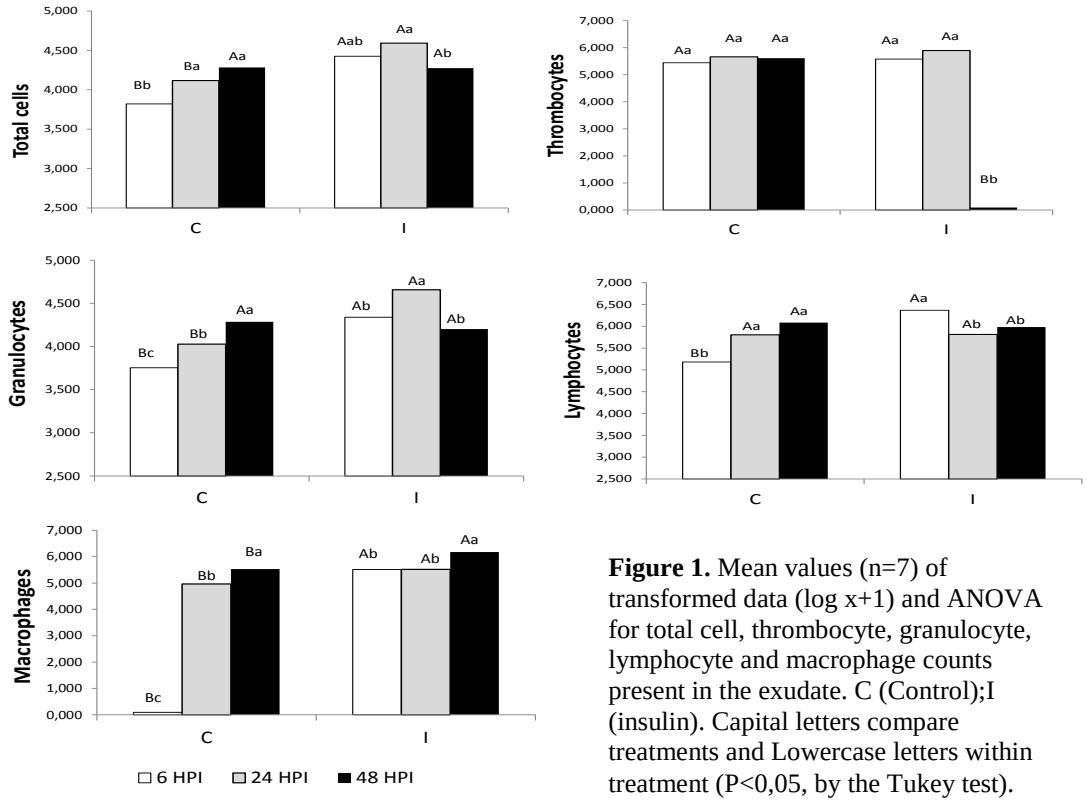


Figure 1. Mean values (n=7) of transformed data (log x+1) and ANOVA for total cell, thrombocyte, granulocyte, lymphocyte and macrophage counts present in the exudate. C (Control);I (insulin). Capital letters compare treatments and Lowercase letters within treatment (P<0,05, by the Tukey test).

21e. Pharmacokinetics and effects on thromboxane production by cownose ray (*Rhinoptera bonasus*) whole blood cells following single intramuscular injection of carprofen

Cara L. Field[1*], Heather K. Kynch[2]

[1]Georgia Aquarium, 225 Baker Street, Atlanta, GA 30313 USA cfield@georgiaaquarium.org
[2]School of Veterinary Medicine, University of California Davis, Davis, CA 95616 USA hkknych@ucdavis.edu

Elasmobranchs are subject to inflammation secondary to a variety of causes, and possess COX-1 and COX-2, analogous to mammalian COX enzymes. Cyclooxygenase (COX) converts precursor molecules into pro-inflammatory prostaglandins and leukotrienes such as thromboxane A2 (TXA2). Non-steroidal anti-inflammatory drugs (NSAIDS) such as the COX-2 selective carprofen are commonly used to reduce inflammation in many species. TXA2 production by Atlantic stingray cells *in vitro* was partly reduced by indomethacin, but *in vivo* COX inhibitor effects have not been studied. We compared TXB2 production by blood cells from captive cownose rays (*Rhinoptera bonasus*) treated with a single intramuscular dose of carprofen (4 mg/kg; n=10) with untreated cownose ray cells (n=6). We also collected blood samples from carprofen-treated rays at 1, 3, 6, 12, 24, 36, 48, 72 and 96 hours post administration to establish pharmacokinetic properties of this drug. Blood collected 6 and 24 hour post-administration was assessed for TXB2 production. Maximal TXB2 production was elicited by stimulation of blood cells with the calcium ionophore A23187. Control ray blood was treated with carprofen (4mg/L) *in vitro* for comparison with *in vivo* results. Indomethacin *in vitro* greatly inhibited TXB2 production from controls. Control cells treated with carprofen *in vitro* produced reduced amounts of TXB2 indicating a response by these cells to this COX-2 inhibitor. However TXB2 production by carprofen-treated cells was maximal in response to A23187 at both 6 and 24 hours, suggesting that this single dose does not reduce TXB2 production *in vivo*.

22) Special Session: Myxozoan Epidemiology & Infection Dynamics

22a. Invertebrate hosts and the epidemiology of proliferative kidney disease

Inês Fontes[1*], Hanna Hartikainen[2], Nick Taylor[3], Beth Okamura[1]

[1]Natural History Museum, London, U.K. i.fontes@nhm.ac.uk, b.okamura@nhm.ac.uk
[2]EAWAG, Dübendorf, Switzerland, hanna.hartikainen@eawag.ch
[3]Cefas, Weymouth, U.K. nick.taylor@cefas.co.uk

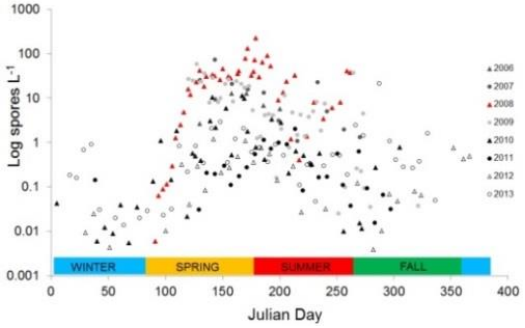
Proliferative kidney disease (PKD) is an emerging disease in wild salmonids. The myxozoan causative agent, *Tetracapsuloides bryosalmonae* (*Tb*), uses freshwater bryozoans as primary hosts. A major aim of our research is to model the epidemiology of PKD which requires characterizing the population dynamics, development, prevalence and burden of *Tb* in bryozoans and transmission to fish. Bryozoans can reproduce asexually via dormant stages (statoblasts) and colony fragmentation. Our previous research has demonstrated host condition-dependent development of *Tb* within *Fredericella sultana* (*Fs*). Thus, *Tb* cycles between virulent, spore-producing overt infections and benign covert infections. Extensive sampling demonstrates that covert infections persist throughout the year in *Fs* populations (ranging from 23-92% in 3 rivers systems in southern England). In addition, PCR and sequencing demonstrate substantial vertical transmission with a high proportion of statoblasts (30-39%) and colony fragments (26-74%) carrying *Tb* infections. These results contribute to parameterisation of our epidemiological model and provide insights into how bryozoans can act as persistent disease reservoirs, thus promoting annual disease outbreaks on fish farms.

22b. Long-term surveillance of a fish pathogen by molecular quantification of waterborne stages in river samples

Sascha L. Hallett*, Gerri R. Buckles, Charlene N. Hurst, R. Adam Ray, Jerri L. Bartholomew

Department of Microbiology, 226 Nash Hall, Oregon State University, Corvallis, Oregon, USA
halletts@science.oregonstate.edu

Ceratonova shasta causes enteronecrosis in juvenile salmon and trout in the Pacific Northwest of North America and is limiting their recovery in the Klamath River. Transmission of this freshwater myxozoan parasite occurs through waterborne stages: actinospores, released from polychaete worms, develop into myxospores in salmonid fishes. In response to the high prevalence and severity of *C. shasta* infection in Klamath River salmonids, we developed a parasite monitoring program that included river water sampling and molecular quantification of waterborne stages in these samples. In 2006, we established 5 mainstem index sites, which spanned 212 river kilometers, and 4 sites in tributaries. Weekly, automatic samplers collected and pooled 1L of river water every 2h for 24h. Replicate 1L samples from the pool were filtered through a 5µm membrane and total DNA extracted. Total *C. shasta* was quantified using a TaqMan qPCR, which targeted the ssrRNA gene. Over 8 years (2006–2013), we assayed ~5000 samples. From a subset of these, we determined relative abundance of host-specific ITS-1 *C. shasta* genotypes using a SYTO9 qPCR and sequencing. The river water samples yielded spatial and temporal data of parasite density and genetic diversity across high-impact and low-impact years. Parasite abundance differed significantly among sites, seasons and years. The mainstem was the primary source of parasite, tributaries contributed little. The parasite was most abundant in spring, when salmonids out-migrate. ITS-1 genotype I dominated, which reflects the relatively high numbers of its specific host, Chinook salmon. We are now exploring relationships among parasite occurrence, invertebrate and vertebrate host life histories, and water temperature and flow. Direct measurement of waterborne parasite stages was a pragmatic alternative to direct host sampling and facilitated semi-real-time reporting via weekly, online updates. These data inform epidemiological and predictive model development and host management strategies.



Abundance of *Ceratonova shasta* across 8 years at one Klamath River index site. Each data point represents average density (spores/L) of the parasite in 3 x 1L river water samples.

This research was funded by the Bureau of Reclamation, U.S. Department of the Interior.

22c. Seasonal fluctuation of myxozoa infection in rohu, *Labeo rohita,* in Myanmar

Kay Lwin Tun[1,2]*, Hnin Hnin Htay[2], July Maung Maung[2], Hiroshi Yokoyama[1], Tomoyoshi Yoshinaga[1]

[1]Laboratory of Fish Diseases, Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan atyoshi@mail.ecc.u-tokyo.ac.jp

[2]Laboratory of Aquatic Bioscience, Department of Zoology, University of Yangon, University Avenue, Myanmar kaylwintun@gmail.com

Myanmar government has undertaken ongoing series of democratic reforms in political, economic and administrative sectors that attracts an enormous amount of foreign investment to Myanmar. Four of the top five most attractive areas for foreign investment are related to fishery. Meanwhile, aquaculture in Myanmar has developed in recent decades with the annual production of 800,000 tons. However, relatively little work has been done to study the parasites and diseases of cultured fish. The present study was conducted to investigate the seasonal occurrence of myxozoan parasites in culture fish Rohu, *Labe rohita* (Cypriniformes: Cyprinidae) in Myanmar. A total of 50 individuals of Rohu were monthly sampled from culture farm located in Yangon environs from June 2012 to November 2013. Skin, gills and internal organs, viz., eyes, brain, gills, heart, swim bladder, liver, gallbladder, muscles, intestine and kidneys were examined for infection. Three species of myxozoa, *Zschokkella* sp., *Myxobolus* sp. and *Thelohanellus* sp. were observed. *Zschokkella* sp. infected the gallbladder while *Myxobolus* sp. and *Thelohanellus* sp. were found in the skin and gills of fish. Parasites were detected in July 2012, when fish were 2 to 3 months old, and declined from June 2013 when the fish were 11 months old (Fig). Although environmental factors such as water temperature and rain fall were recorded in study area, prevalence of infections are more related to age of fish than to environmental factors. Ongoing studies are being conducted on histopathology of infected fish as well as small subunit ribosomal RNA gene analysis of parasites.

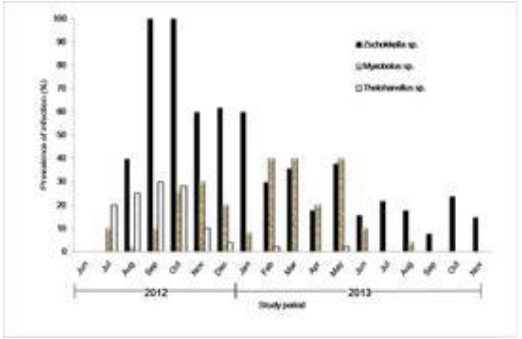


Fig. Prevalence of myxozoa infection in *Labeo rohita* cultured in Yangon environs, Myanmar

22d. Blebbing around: motility of *Ceratonova shasta* (Myxozoa) in rainbow trout

Gema Alama-Bermejo[1-3], Astrid S. Holzer[2], Juan A. Raga[3] and Jerri Bartholomew[1]

[1]Department of Microbiology, Oregon State University, Corvallis, OR 97331, USA bartholj@science.oregonstate.edu

[2]Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, České Budějovice 37005, Czech Republic astrid.holzer@paru.cas.cz

[3]Marine Zoology Unit, Cavanilles Institute of Biodiversity and Evolutionary Biology, Science Park, University of Valencia, 46980 Paterna, Spain toni.raga@uv.es, gema.alama@gmail.com

Amoeboid motility of eukaryotic cells relies on membrane protrusion with the production of 3-dimensional projections or pseudopodia (*i.e.* lamellipodia, filopodia and blebs). Myxozoans are microscopic metazoan parasites. In their non-spore stage, these parasites are motile, as most animals. This motility, although little studied is relevant to understanding how myxozoans migrate and proliferate during infection. *Ceratonova shasta* (formerly *Ceratomyxa shasta*) is a virulent parasite affecting wild and cultured salmonids in the rivers of the Pacific Coast of North America. *C. shasta* type II is a highly virulent genotype in rainbow trout that results in a systemic infection with the production of ascites during the last steps of infection. Pre-sporogonic and sporogonic stages collected from intestine, bile and ascites showed active motility and membrane protrusion. To characterize their motility, these stages were studied in detail using confocal laser scanning microscopy, light and electron microscopy, as well as transcriptome data for identification of motility promotors to characterize their motility.
Abundant blebs were observed on pre-sporogonic and spore forming stages. Each bleb increased in size and displaced the membrane to the sides. Sometimes, blebs were produced in only one side and appeared to be pushing the parasite toward the opposite direction. Switching between blebs and filopodia\lamellipodia was observed in some stages. Static parasite stages showed abundant filopodia (filamentous-actin positive) all around the surface. Bleb-based motility of *C. shasta* is compared with the filopodia-based motility, recently described for similar developmental stages from the myxozoan *Ceratomyxa puntazzi*. Functionality in relation to habitat is discussed. Motility genes are potential virulence factors candidates for drug targeting in parasites.

22e. Emerging numbers of motile myxozoan blood stages in common carp – A closer look at *Sphaerospora molnari,* a parasite on the rise

Astrid S. Holzer[1]*, Ashlie Hartigan[1], Sneha Patra[1,2], Edit Eszterbauer[3]

[1] Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, Branišovská 31, 37005 České Budějovice, Czech Republic astrid.holzer@paru.cas.cz, ashlie.hartigan@paru.cas.cz, snehapatra@gmail.com

[2] Faculty of Science, University of South Bohemia, Branišovská 31, 37005 České Budějovice, Czech Republic snehapatra@gmail.com

[3] Institute for Veterinary Medical Research, Centre for Agricultural Research, Budapest, Hungary eedit@vmri.hu

In recent years, we detected emerging numbers of myxozoan proliferative blood stages, in the literature also called UBOs (unidentified blood objects) or C-stages, at different carp culture sites in Central Europe. Using molecular diagnostics, we were able to demonstrate that most of these stages represent *S. molnari*, a parasite known to form spores in the epithelia of the gills and the skin. While spore-forming stages cause marked dystrophic changes and necrosis in the infected epithelia, the etiology of proliferative blood stages of *S. molnari*, which are able to reach any organ or tissue via the blood stream, is unknown to date. In the present study we aimed at determining *S. molnari*'s dispersion in the blood system and its extravascular location in the fish host, thus better understanding the intrapiscine development and potential relation to pathology. Using *in situ* hybridisation, we were able to show that *S. molnari* is able to invade different organs from the vascular system, thereby contributing to pathology at various sites. Most importantly, we were able to demonstrate that *S. molnari* serves as an important co-factor or precondition for the development of Swim Bladder Inflammation (SBI) in common carp, a disease which had previously been related to the kidney parasite *S. dykovae*, formerly known as *S. renicola*. Furthermore, PCR of monthly carp blood samples demonstrated that proliferative blood stages of *S. molnari* are present year-round while spore formation in the gills is restricted to the spring season. We detected extraordinary high densities of blood parasites during the summer months, and we determined that the proliferation of *S. molnari* in the blood is temperature dependent. This prompts us to predict emerging numbers of *S. molnari* as water temperatures are on the rise in Central European carp ponds. Due to the parasite's importance, present studies in our laboratory focus its extrapiscine life cycle and transmission pathways as well as on the discovery of parasite genes and proteins of particular importance for host exploitation, with the aim to develop antiparasitic strategies based on these molecules.

22f. Preliminary attempts to reveal the life cycle of *Sphaerospora molnari* (Myxozoa)

Sneha Patra[1, 2]*, Ashlie Hartigan[1], Astrid S Holzer[1]

[1]Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, Branišovská 31, 37005 České Budějovice, Czech Republic snehampatra@gmail.com; ashlie.hartigan@paru.cas.cz; astrid.holzer@paru.cas.cz

[2]Faculty of Science, University of South Bohemia, Branišovská 31, 37005 České Budějovice, Czech Republic

Life cycles of myxozoans alternate between invertebrates (oligochaetes, polychaetes and bryozoans) and vertebrates (mostly fish). The intermediate hosts of the members of the *Sphaerospora* sensu stricto clade are either fish or amphibians. However, little is known about their definitive hosts since the life cycle of *Sphaerospora dykovae* is the only one that has been described to date. But infection experiments were not confirmed by DNA sequencing.

In order to determine the invertebrate host of *S. molnari*, in this present study, we performed the following experiments using infected donor fish and laboratory-reared SPF carp to determine how newly stocked carp fry can become infected in common carp aquaculture in traditional ponds:

1. Artificial fish-to-fish transmission of proliferative blood stages by IP injection
2. Natural fish-to-fish transmission of blood stages using blood-sucking leeches (*Hemiclepsis marginata*).
3. Oral transmission by feeding of fish tissues infected with blood stages
4. Indirect transmission in cohabitation aquaria, using different types of sediments from *S. molnari*-enzooic ponds and containing a variety of potential invertebrate hosts.

All experiments were evaluated by microscopic examinations as well as by PCR, using *S. molnari*-specific primers. With regard to natural infection in carp ponds, we determined that transmission via an invertebrate host is likely the most common infection pathway. In targeted cohabitation studies, we were able to determine the preferred habitat of the definitive host, thus limiting the number of potential invertebrate hosts to only a dozen.

While aiming to identify the definitive host of *S. molnari* including the morphological and developmental study of its actinosporean stage, we believe that elucidation of the life cycles of members of *Sphaerospora* sensu stricto clade could contribute significant information on the evolution of the Myxozoa.

23) Special Session: Shellfish Diseases

23a. Evaluation of the role of a novel plasma iron binding protein in eastern oyster host defense against the protozoan parasite *Perkinsus marinus*

Jerome La Peyre[1*], Sandra Casas[1], Jaren Lee[1], Julie Gauthier[2], Jean-Philippe Beguel[1]

[1]Department of Veterinary Science, School of Animal Sciences, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, USA jlapeyre@agctr.lsu.edu
[2]Loyola University, New Orleans, LA 70803, USA

Mammalian iron-binding proteins transferrin and lactoferrin have been found to be effective in reducing *P. marinus* proliferation and an increase in infection in *P. marinus* intensity with the addition of iron to oysters maintained in aquaria has been previously reported. Depriving invading microbes of iron essential for their growth is a well-known innate defense mechanism of vertebrates but very little is known about possible iron withholding system in invertebrates including oysters. Segon is a recently characterized metal binding protein that is secreted by oyster hemocytes and make up about 20% of plasma protein. Its high association with iron suggests a potential oyster host defense role. Oysters (*Crassostrea virginica*) were therefore exposed to iron carbonyl (5 or 20 mg/L daily) and challenged with *P. marinus* and then sampled to measure segon expression along with whole organism responses (mortality, condition), tissue responses (histopathology alterations), cellular responses (hemocyte density, viability, phagocytic capacity, reactive oxygen species production) and subcellular responses (dominin expression, lysosomal stability, superoxide dismutase activity, glutathione and HSP70 protein concentrations, lipid peroxidation) in relation to iron concentrations and *P. marinus* infection intensities. Results obtained so far indicate no change in segon transcription levels but significant differences in other host responses after iron exposure. Surprisingly although mortality was greater in oysters receiving iron, *P. marinus* infection intensity was highest in oysters not exposed to iron in contrast to an earlier report.

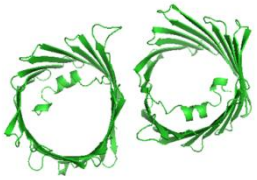
23b. Metabolic changes during the herpesvirus OsHV-1 µVar infection in the oyster

Charlotte Corporeau[1]*, David Tamayo[1], Fabrice Pernet[1], Claudie Quéré [1], Stéphanie Madec[2]

[1]Ifremer, Laboratoire des sciences de l'Environnement Marin (UMR 6539, LEMAR), 29280 Plouzané, FRANCE. charlotte.corporeau@ifremer.fr

[2]Laboratoire Universitaire de Biodiversité et Ecologie Microbienne (EA3882), IFR148 ScInBioS, Université Européenne de Bretagne, ESMISAB, Technopôle de Brest Iroise, 29280 Plouzané, FRANCE. Stephanie.madec@univ-brest.fr.

Since 2008, mass mortalities of juvenile *Crassostrea gigas* have notably affected all rearing sites along coasts of France when seawater temperature exceeds 16°C (EFSA, 2010). Recent mortality events are also reported in UK, Australia and New Zealand and results of diagnostic tests show that they are associated with the detection of a particular genotype of the ostreid herpesvirus 1 (OsHV-1) named µVar (Segarra et al., 2010). Our aim is to better understand the pathogenesis of OsHV-1 µVar and to determine which metabolic pathways might be affected during infection in oyster. Eight month-old *Crassostrea gigas* were anesthetized and injected in the muscle with high load (challenged oyster) or low load (control oyster) inoculum of OsHV-1 µVar. At 2 days post-injection, quantity of OsHV-1 DNA in challenged oysters was 10.000 times higher than in that of control oysters. Then, for the first time, we used a two-dimensional (2-D) proteomic approach to identify metabolic signatures of OsHV-1 µVar infection between challenged *versus* control oysters. We analyzed 10 proteomes (5 challenged *versus* 5 control oysters, N=2 technical replicates for each analysis) obtained from individuals that exhibited contrasted infection status, as measured by their individual OsHV-1 DNA level. Our results identified 25 abundant protein spots that showed a marked change in accumulated levels. Interestingly, challenged oysters exhibited an increased glycolysis and an accumulation of a specific protein, the porin VDAC, which reflects a "Warburg effect" (Warburg, 1956). The Warburg effect is an atypical metabolism that favour OsHV-1 µVar by providing cellular energy and building blocks during viral genome replication in oyster, and was initially reported in cancer cells and more recently in shrimp infected with virus (Chen et al., 2011). Further researches are currently on-going to investigate how environmental factors and farming practices could control the Warburg effect in *Crassostrea gigas* to delay or protect from disease mortality risk.



The VDAC porin protein.

23c. Skulking behind an MSX smokescreen: SSO prevalence in Maine and Massachusetts

Cem Giray[1]*, Diane Murphy[2], Marcy L. Nelson[3]

[1]Kennebec River Biosciences Inc., 41 Main Street, Richmond, Maine 04357 USA cgiray@kennebecbio.com

[2]Cape Cod Coop Ext & Woods Hole Sea Grant, Box 367, Barnstable, Massachusetts 02630 USA dmurphy@barnstablecounty.org

[3]Marcy L. Nelson , Maine Department of Marine Resources, PO Box 8, 194 McKown Point Road, West Boothbay Harbor, Maine 04575 USA marcy.nelson@maine.gov

Haplosporidium costale (SSO) has been reported from the US as far north as Maine and since the 1980s, but in part due to the absence of any significant level of mortalities related to its detection, this organism appears to have been largely ignored by regulatory testing requirements in New England for the past 20+ years. Due to the potential for high level of associated mortalities, regulatory testing instead has targeted another Haplosporidian, *H. nelsoni* (MSX). Testing conducted in Maine and Massachusetts since 2010 indicates varying prevalence of SSO in wild and farmed populations of the Eastern oyster (*Crassostreae virginica*). In some cases SSO detection was associated with disease and mortalities, while in other cases not; in some cases SSO was present solo, while in others in co-occurrence with MSX and/or *Perkinsus marinus*. SSO prevalence, co-occurrence with MSX, detection via PCR versus histology, relationship with any mortalities, and shifts in presence of MSX versus SSO in farmed and wild *C. virginica* and potential relationship with the use of MSX-resistant *C. virginica* in aquaculture are investigated.

23d. Development of a TaqMan real-time PCR assay for the detection of *Perkinsus olseni* in Australian abalone

David M. Cummins[1*], Brian J. Jones[2], Mark St. J. Crane[1], Nicholas Gudkovs[1]

[1] CSIRO Australian Animal Health Laboratory (AAHL) Fish Diseases Laboratory, Geelong, VIC 3220, Australia. [*] david.cummins@csiro.au

[2] Ministry for Primary Industries, Investigation and Diagnostic Centres and Response, Wallaceville, Upper Hutt 5018, New Zealand

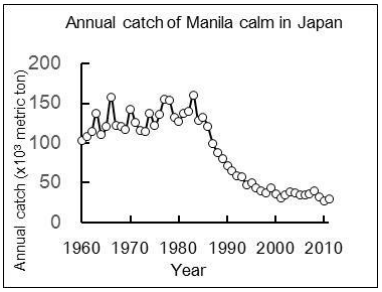
Perkinsus olseni is a protozoan parasite of molluscs and was first described in association with disease in Australian abalone in the early 1980's (Lester and Davies, 1981). Since this time, outbreaks of Perkinsosis in Australia have been responsible for significant economic loss in the wild abalone fishery through the depletion of stocks in coastal waters of both South Australia (SA) and New South Wales (NSW). With increasing investment in aquaculture and the need to better manage wild stocks, the development of rapid molecular assays, such as real-time PCR (qPCR) for the detection and diagnosis of disease has become a priority. Here we describe the development of a TaqMan real-time PCR assay to detect *P.olseni* in abalone. We demonstrate the assay's specificity and sensitivity using a range of plasmid and genomic samples. The assay was further validated by testing wild abalone for the presence of *P.olseni* from known infected and *Perkinsus*-free areas alongside *OIE Manual of Diagnostic Tests for Aquatic Animals* methods such as histology, Ray's Fluid Thioglycollate Medium (RFTM) and conventional PCR. To date, the test appears to be specific and sensitive in detecting *P.olseni* in abalone.

23e. Impact of the protozoan *Perkinsus olseni* on wild Manila clam populations in Japan

Tsukasa Waki, Tomoyoshi Yoshinaga*, Miki Takahashi, Tatsuya Eki, Jun Shimokawa

Laboratory of Fish Diseases, Department of Aquatic Bioscience Graduate School of Agricultural and Life Sciences, University of Tokyo atyoshi@mail.ecc.u-tokyo.ac.jp

Manila clam *Ruditapes phillipinarum* is one of major shellfish resources in Japan. However, the catch of Manila clam has clearly declined since the 1980s due to the depletion of the resources (Figure). The infection with the protozoan parasite *Perkinsus olseni*, which is an OIE-listed disease, was reported in Manila clams in Japan in the late 1990s for the first time, when the infection had prevailed nation-widely. Although the infection with the parasite was suspected as a cause of the depletion of Manila clam resources, most researchers working for recovery of Manila clam fisheries were still suspicious of the involvement of the parasite to the resource depletion, mainly due to the lack of information on the virulence of the parasite. Thus, we evaluated the virulence of the parasite by challenge experiments and estimated the impact of the parasite on wild Manila clam populations by field studies in three tidal flats. In the challenge experiments, clams showed significantly higher mortalities than control clams, mortalities occurred when mean infection intensities reached 10^6 cells/g wet tissue weight (WWT), and the infection intensities and mortality rates increased more rapidly in juveniles than in adults and at higher temperatures. In other challenge experiments, growth, condition index, filtration activity, and burrowing activity significantly decreased in challenged groups. The field studies revealed that, in the sampling station in Ariake Bay where the Manila clam catch had much declined, the density of each year class of clams began to decrease when their infection intensities reached up to ~10^6 cells/g WTW, which was close to the lethal intensity suggested by the challenge experiments. In the other two study areas, where clam resources were relatively abundant, the infection intensities in the populations were much lower than those in Ariake Bay. The results of the challenge experiments and field studies indicate that *P. olseni* infection has negative impact on the survival of wild Manila clams and is one of major causes of the depletion of Manila clam resources in Japan.

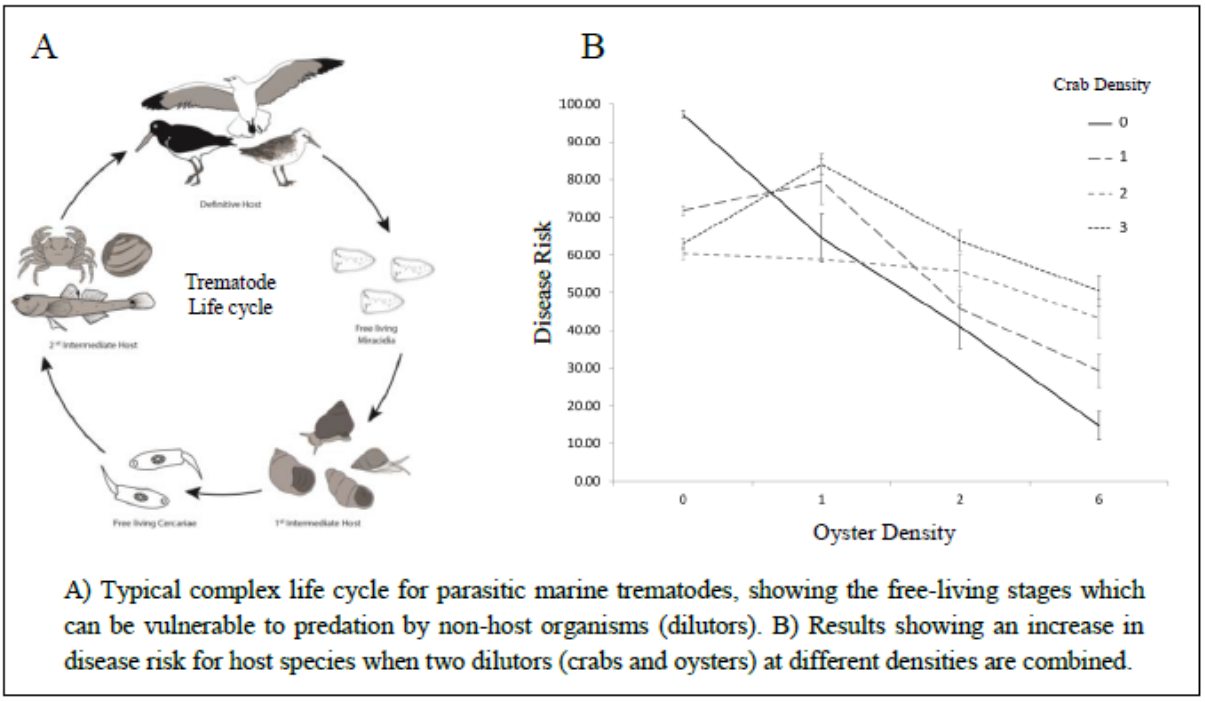


23f. Biodiversity reduces disease risk in aquatic systems: If only it were so simple!

Jennifer E Welsh[1]*, Corina Brussaard[2], Jaap van der Meer[1], David W Thieltges[1]

[1]Department of Marine Ecology, NIOZ Royal Netherlands Institute for Sea Research, Postbus 59, 1790 AB, Den Burg (Texel), Netherlands Jennifer.welsh@nioz.nl, Jaap.van.der.Meer@nioz.nl, David.Thieltges@nioz.nl
[2]Department of Biological Oceanography, NIOZ Royal Netherlands Institute for Sea Research, Postbus 59, 1790 AB, Den Burg (Texel), Netherlands Corina.Brussaard@nioz.nl

Infections in aquatic organisms are problematic worldwide. However, ecological research has suggested that an increase in biodiversity results in a decrease in disease risk in hosts via the so called dilution effect, a hypothesis which is now being tested in aquaculture systems. This dilution effect has primarily looked at the abundance and competency of host species and microparasite diseases. Here, we extend this idea that biodiversity can reduce disease risk in host species to include non-host organisms (dilutors) and macroparasites. Using a marine trematode parasite which is known to infect commercially valuable shellfish and non-host organisms known to reduce its parasitic free-living stages we designed mesocosm experiments that manipulated both the density and the diversity of the dilutors. Our initial results show that in simplistic systems non-host organisms can indeed reduce free-living parasites and therefore, reduce disease risk in aquatic host species. However, when diversity is increased the results indicate that the effects of biodiversity on disease risk are actually complex and not only depend on the identity of the dilutor but also the density. In such multifaceted systems the results showed complex interactions between dilutors that can both reduce or enhance the disease risk for a target hosts. Hence, the relationship between diversity and disease risk seems much more idiosyncratic than the 'biodiversity reduces disease risk' hypothesis suggests.



A) Typical complex life cycle for parasitic marine trematodes, showing the free-living stages which can be vulnerable to predation by non-host organisms (dilutors). B) Results showing an increase in disease risk for host species when two dilutors (crabs and oysters) at different densities are combined.

24) General Session: Immunostimulants I

24a. Immunostimulant effects of recombinant cytokine administration in the Japanese pufferfish, *Takifugu rubripes*

Ryusuke Nagamine[1]*, Gouranga Biswas[1], Jun-ichi Hikima[2], Masahiro Sakai[2], Tomoya Kono[2]

[1]Interdisciplinary Graduate School of Agriculture and Engineering, University of Miyazaki, Miyazaki 889-2192, Japan nb14001@student.miyazaki-u.ac.jp
[2]Department of Biochemistry and Applied Biosciences, Faculty of Agriculture, University of Miyazaki, Miyazaki 889-2192, Japan jhikima@cc.miyazaki-u.ac.jp, m.sakai@cc.miyazaki-u.ac.jp, tkono@cc.miyazaki-u.ac.jp

Prevention of diseases in fish through immunostimulation is a preferred method to the use of vaccines and antibiotics in aquaculture. Cytokines play an important role for regulation of immune response. Therefore, immunostimulation using recombinant cytokines may be an efficient technique for disease prevention in fish. The cytokine cDNAs [*i.e.*, interferon (IFN) -□, IFN-□rel, interleukin (IL)-4/13A, IL-4/13B, IL-6, IL-17A/F-1, IL-17A/F-2 and IL-17A/F-3] of Japanese pufferfish (*Takifugu rubripes*) without signal peptide sequence were amplified by PCR and inserted to pCold-His expression vector for recombinant protein synthesis. Constructed plasmid was transformed into competent *Escherichia coli* E15 cells. The transformed cells were grown in Luria-Bertani (LB) medium at 37°C for overnight, and then the cultures were shifted to 15°C to induce protein expression. Recombinant cytokines were purified using Ni-NTA column, and endotoxin was removed in polymyxin B column. The purified recombinant cytokines in PBS [5μg (in 100μL PBS) were intramuscularly injected to healthy Japanese pufferfish (10 g body weight) and control fish received a 100 μL PBS injection. Head kidney was collected at 1, 3 and 5 days post injection (dpi), and lysozyme activity, superoxide anion production and phagocytic activity were measured to examine the effect of immunostimulation by the recombinant cytokines. Lysozyme showed high activity at 1 dpi in rIFN-□ and rIL-17A/F-3 injected fish, at 5 dpi in rIL-4/13A injected fish and all through the time course in IL-4/13B injected fish. Superoxide anion production increased at 1 dpi in all types of cytokine injected pufferfish except in rIL-4/13B and rIL-17A/F-1 injected fish. Fish received rIFN-□ injection had an elevated phagocytic activity at 1 and 3 dpi, whereas rIFN-□rel and rIL-4/13A treated fish showed an increased phagocytic activity only at 1 and 3 dpi, respectively. The results suggested the potentiality of recombinant cytokine proteins as effective immunostimulants to the Japanese pufferfish.

24b. Oral immunoprophylaxis of finfish using alginate microencapsulation

Bikramjit Ghosh*[1], Kenneth D. Cain[2], Barbara F. Nowak[1], Andrew R. Bridle[1]

[1]NCMCRS, AMC, University of Tasmania, Launceston, Tasmania, Australia
[2]Department of Fish and Wildlife Resources and The Aquaculture Research Institute, University of Idaho, Moscow, ID, USA

Oral delivery is a potential solution to constraints associated with the immunoprophylaxis methods most prevalent in aquaculture: injection and immersion. However, oral immunogen delivery has produced inconsistent outcomes in fish. This is primarily attributed to antigen degradation, solutions to which are typically complex and expensive. Here, we developed and validated a method for oral fish immunoprophylaxis using alginate microcapsules (aMCs). An emulsion/internal-gelation protocol was adapted to minimize impact on the material being encapsulated. Microcapsules were characterized *in vitro* using lysozyme and bovine serum albumin (BSA). Post-encapsulation change in bioactivity of lysozyme was used to determine protocol impacts on the encapsulated substance. aMC release dynamics were tested at different pH levels and temperatures using BSA. Uptake and systemic distribution was verified using FITC-labeled BSA-aMCs *ex vivo* in intestinal explants, and combined in feed for *in vivo* administration to *Salmo salar* fry. *Oncorhynchus mykiss* fry were immunized against *Flavobacterium psychrophilum* infection with microencapsulated live attenuated oral vaccine. Microencapsulation did not significantly reduce lysozyme bioactivity. BSA release from aMCs was pH- and temperature-responsive. Uptake and translocation of aMCs was visible *ex vivo* and *in vivo*. Oral immunization significantly increased survival against bacterial challenge ($F=11.4$; $p=0.01$), and was comparable to IP immunization. Our findings indicate this method could be a convenient, effective alternative to prevalent finfish immunoprophylaxis strategies.

24c. Haematological, histopathological changes and antimicrobial residue in sub-adult *Clarias gariepinus* (Burchell, 1822) infected with multidrug resistant *Pseudomonas aeruginosa* exposed to some selected medicinal plant extracts

Oghenebrorhie M Amrevuawho[1], Adeolu A Akinyemi[1], Onyenoro GN Ezeri[1]*, Olufunmilayo M Bankole[2], Micheal Agbaje[3], Paul A Akinduti[3]

[1] Department of Aquaculture and Fisheries Management, Federal University of Agriculture Abeokuta, Ogun State, Nigeria. PMB 2240 call4mavis@yahoo.com, adeoluakinyemi@yahoo.com , ezeri2000@yahoo.com

[2] Department of Microbiology, Federal University of Agriculture Abeokuta, Ogun State, Nigeria. PMB 2240 aadmokole@yahoo.co.uk

[3] Department of Microbiology and Parasitology, Coll. of Vet. Medicine, Federal University of Agriculture Abeokuta, Ogun State, Nigeria. PMB 2240 mikeabgaje@yahoo.com, niyiakinduti@gmail.com

This study aims to evaluate the haematological, histopathological changes and antimicrobial residue in *Clarias gariepinus* sub-adults artificially infected with *Pseudomonas aeruginosa* and exposed to some selected medicinal plant extracts and oxytetracycline. Type culture of *P. aeruginosa*; ATCC 27853 was exposed to aqueous extract of *Moringa oleifera, Allium sativum, Allium cepa, Zingiber officinale* and oxytetracycline at concentration of 100%, 75% and 50% to evaluate their antibacterial sensitivity using agar well diffusion method. Three experimental fish from each treatment were tested and checked for their cellular immune response and histopathological changes, respectively. Antimicrobial residue in the fish muscle was determined using PREMI 25 test kit. While the parked cell volume(PCV), haemoglobin (HB) concentration, red blood cell(RBC) and lymphocyte of the infected fish were relatively lower, the white blood cell(WBC) and neutrophil was significantly ($p>0.05$) higher than infected fish treated. Histopathological changes revealed degeneration of gill lamellae and gastric glands in stomach cells of infected fish, however, organs of infected treated fish showed slight regeneration. Antimicrobial residues were found in all fish muscles except for infected fish treated with ginger. Conclusively, though all treatment inhibited the growth of *P. aeruginosa*, only *Z. officinale* inhibited pathogen without residue in the fish muscles.

Table 1: Mean values of blood parameters of *P. aeruginosa* infected *C. gariepinus* sub-adult exposed to oxytetracycline and plant extracts

Blood Parameters	Moringa	Onion	Garlic	Ginger	Oxytetracycline	Infected	Uninfected
PCV (%)	34.33 ± 2.1^{b}	24.33 ± 2.0^{a}	25.33 ± 0.9^{ab}	30.67 ± 3.7^{ab}	29.33 ± 4.2^{ab}	22.33 ± 0.3^{a}	34.67 ± 5.2^{a}
HB (g/dl)	9.67 ± 0.5^{c}	7.70 ± 0.5^{ab}	8.07 ± 0.2^{abc}	9.20 ± 0.8^{bc}	9.57 ± 1.2^{bc}	6.97 ± 0.2^{a}	9.77 ± 0.2^{a}
RBC ($x10^{12}$/L)	1.90 ± 0.3^{ab}	1.40 ± 0.1^{ab}	$1.60\pm0,1^{ab}$	1.90 ± 0.3^{ab}	1.93 ± 0.2^{b}	1.27 ± 0.1^{a}	2.23 ± 0.3^{a}
WBC ($x10^{9}$/L)	14.03 ± 1.8^{abc}	10.77 ± 0.7^{a}	11.97 ± 0.9^{ab}	14.63 ± 1.9^{abc}	14.93 ± 1.7^{bc}	17.13 ± 0.5^{c}	10.80 ± 0.3^{a}
NEU (%)	33.67 ± 7.6^{a}	38.00 ± 2.6^{ab}	37.33 ± 3.5^{ab}	34.33 ± 4.4^{ab}	35.00 ± 4.0^{ab}	47.33 ± 1.2^{b}	30.33 ± 2.9^{a}
LYM (%)	66.67 ± 8.4^{b}	61.67 ± 2.7^{ab}	61.33 ± 2.7^{ab}	65.33 ± 4.7^{ab}	64.00 ± 4.0^{ab}	52.33 ± 0.9^{a}	69.00 ± 2.3^{a}

24d. Effect of *Tetracera potatoria* and *Psidium guajava* on growth and haematology of cultured *Clarias gariepinus*.

BO Oyebanji[1]*, OU Eyenre[2], OL Olatunji[3]

Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria
[1]oyebanji.bukola44@gmail.com
[2]eyenreokeoghene@gmail.com
[3]olatunjiolayinka012@gmail.com

The use of natural products seems to be the most promising method of preventing fish diseases and improving growth offering an alternative to the drugs, chemicals and antibiotics currently used in fish culture. A 56 day study was conducted to evaluate the effect of dietary inclusion of *Tetracera potatoria* root extract (TP) and *Psidium guajava* leaf extract (PG) on growth and haematological parameters of African catfish, *Clarias gariepinus.* One hundred and twenty juvenile of initial weight of 30 ± 0.3g were randomly distributed into plastic tanks (45 L) at 10 fish / tank and replicated twice. Experimental animals were fed with commercial fish feed (42% crude protein) at 5% body weight twice a day. Mean water quality parameters ranged from: temperature 26^{o}C-28^{o}C, Dissolved oxygen 5.13 mg/L – 6.25mg/L, pH 6.81-6.95. Group 1 animals served as control without inclusion of extract, groups 2 and 3 had 250mg/kg and 500mg/kg inclusion of TP, groups 4 and 5 had 250 and 500mg of PG respectively, group 6 animals had 600mg/kg inclusion of vitamin C. The weight of the animals were recorded weekly for the period of the experiment while PCV, Hb, RBC WBC, differential WBC count, and weight of viscerals were determined at the conclusion of the experiment. MCH, MCHC, MCV, viscerosomatic and hepatosomatic indices were afterwards calculated from these parameters. The mean weight gain (54.5g/fish), final standard length and the viscerotropic index were significantly different ($p<0.05$) across the group and highest values recorded in fish supplemented with 250mg/kg TP. The highest percentage survival of 100% was recorded in groups 2, 3 and 6 animals. There was no significant change in the blood parameters of fish across the group. It can be concluded that the natural extracts had no toxic effects on the fish but they can serve as natural growth promoters in aquaculture production hence lead to increased productivity.

24e. Functional genomics studies of the impact of diets containing camelina oil and/or camelina meal on Atlantic cod and Atlantic salmon immune responses

Marije Booman, Qingheng Xu, Matthew L Rise*

Department of Ocean Sciences, Memorial University of Newfoundland, St. John's, NL A1C5S7 Canada mrise@mun.ca

Aquaculture feeds for carnivorous fish such as cod and salmon currently contain fish oil (FO) and fish meal (FM), non-sustainable ingredients largely harvested from wild fish stocks. The replacement of FO and FM with plant products in fish feeds can improve the sustainability of aquaculture. *Camelina sativa*, an oilseed with high lipid content (40%), is an alternative source of oil and protein for aquafeeds. However, research is needed to ensure that camelina product-containing diets do not have a negative effect on fish health. Our studies used microarray and quantitative reverse transcription – polymerase chain reaction (qPCR) analyses to investigate the effects of replacing dietary FO with camelina oil (CO), with or without inclusion of camelina meal (CM), on fish anti-viral and anti-bacterial immune responses. Juvenile Atlantic cod and Atlantic salmon smolts were fed FO-based control diets, or one of a group of experimental diets containing different amounts of CO or a combination of CO and CM. At the end of each feeding trial, fish were administered intraperitoneal injection of saline (control for both species), the viral mimic poly(I:C) (Atlantic cod), or formalin-killed *Aeromonas salmonicida* (Atlantic salmon). Functional genomics analyses (20K microarray with qPCR for cod; qPCR for anti-bacterial biomarker genes for salmon) with spleen templates were used to investigate the effects of the diets on basal immunity (pre-injection) and on the response to the immune stimulation. All fish showed strong gene expression responses to the immune stimulation, but differences between diets were modest and limited to a few immune-related genes. In summary, Atlantic cod and Atlantic salmon fed on camelina product-containing diets can mount strong anti-viral and anti-bacterial immune responses, comparable to those of fish fed a diet based on marine ingredients.

25) Special Session: Myxozoan Pathogenicity, Genomics, and Transcriptomics

25a. Development of *Henneguya ictaluri* in the channel catfish, blue catfish, and their hybrid cross

Thomas G Rosser[1]*, Matt J Griffin[1,2], Lester H Khoo[1,2], Linda M Pote[1], Terrence E Greenway[2], David J Wise[2]

[1]Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, 240 Wise Center Drive, Mississippi State, MS 39762 USA tgr49@msstate.edu, griffin@cvm.msstate.edu, khoo@cvm.msstate.edu, lpote@cvm.msstate.edu
[2]Thad Cochran National Warmwater Aquaculture Center, Mississippi State University, PO Box 197, Stoneville, MS 38776 USA dwise@drec.msstate.edu, greenway@drec.msstate.edu

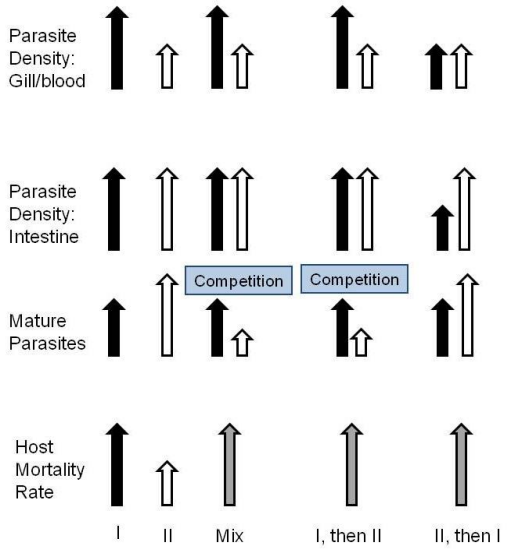
Henneguya ictaluri, the myxozoan parasite responsible for Proliferative Gill Disease (PGD) in cultured channel (*Ictalurus punctatus*) and blue (*I. furcatus*) x channel hybrid catfish, has persisted in catfish aquaculture since it was first identified in the early 1980s. The life cycle of the parasite consists of a myxospore stage in channel catfish and an actinospore stage released by the benthic oligochaete *Dero digitata.* However, it is unknown if the channel catfish × blue catfish hybrid is a true intermediate host for the parasite. Previous work has demonstrated blue catfish are refractive to the disease. Conversely, during acute stages of infection hybrids display clinical signs of disease comparable with channel catfish, although is to a lesser extent histologically and with lower parasite burdens as determined by quantitative PCR. Our studies focused on the development of the parasite in blue catfish, channel catfish and their hybrid cross through a series of experimental challenges exposing fish to pond water containing *H. ictaluri* actinospores. Fish were sampled weekly for 12 weeks, and gill, brain, heart, anterior kidney, posterior kidney, spleen, liver, and stomach tissues were taken for histological processing and qPCR analysis. Parasite stages were present throughout multiple organs in channel and hybrid catfish at various time-points, with channel catfish tissues having higher levels of parasite DNA than hybrids. Consistent with previous work, *H. ictaluri* was not detected histologically or molecularly in any blue catfish at any time point. Histologically the mature myxospores were only observed in the channel catfish, beginning at 8 weeks post-infection. The results support previous work demonstrating blue catfish are refractory to infection by *H. ictaluri* and also suggest that *H. ictaluri* may not complete its life cycle through the hybrid catfish.

25b. Within-host parasite competition in a myxozoan-fish system

Charlene N. Hurst[1,2*], J. L. Bartholomew[1]

[1]Department of Microbiology, College of Science, Oregon State University, Corvallis, OR, USA, bartholj@science.oregonstate.edu
[2]NOAA National Marine Fisheries Service, West Coast Regional Office, 1201 NE Lloyd Blvd., Portland, OR, USA, charlene.n.hurst@noaa.gov

Concomitant pathogen infections are common in wildlife, creating the potential for pathogen interactions, which can effect infection and disease dynamics. We hypothesized that competition would occur between two genotypes of *Ceratonova shasta* with differing levels of virulence in Chinook salmon (*Oncorhynchus tshawytscha).* This competition would cause an increase in overall virulence and a decrease in within-host replication and mature parasite production in fish following mixed infections. When exposure to each genotype occurred sequentially, we hypothesized that the first infecting genotype would have a competitive advantage evidenced by relatively higher replication and mature parasite production. Our results demonstrated that infections with both genotypes simultaneously resulted in competition leading to a reduction in the number of mature parasites compared to single genotype infections. In sequential infections, the first infecting genotype produced the majority of mature parasites, suggesting an advantage for the first infecting parasite genotype. Regardless of the order or timing of genotype exposure used to achieve a mixed infection, both genotypes persisted in the host for long enough to produce mature parasites for transmission, demonstrating the ability of both genotypes to coexist despite competitive pressure. Over time, competitive suppression may lead to selection for increased genotype virulence to optimize their competitive advantage. In the natural environment, where other selective pressures exist, such as host heterogeneity, the outcome of a competitive interaction is less predictable, but may lead to exclusion of the less virulent genotype in resistant fish.



25c. The effect of inbreeding on the susceptibility of brown trout (*Salmo trutta* m. *fario*) to the whirling disease parasite *Myxobolus cerebralis*

Edit Eszterbauer*, Barbara Forró, Csaba F. Guti, Dennis M. Kallert

Institute for Veterinary Medical Research, Centre for Agricultural Research, Budapest, Hungary
eszterbauer.edit@agrar.mta.hu; forro.barbara@agrar.mta.hu; guti.csaba@agrar.mta.hu; d.kallert@gmx.de

Myxobolus cerebralis, the myxozoan parasite causing whirling disease in salmonids, is responsible for heavy economical and ecological losses worldwide. The species has a wide host range, but significant differences are observable among the susceptibility of various fish species and strains. Although the original host, brown trout (*Salmo trutta* m. *fario*), is susceptible, the symptoms are milder and the mortality of infected fish is significantly lower than in other highly susceptible species, such as rainbow trout (*Oncorhynchus mykiss*). Brown trout, however, due to sublethal infections with often subclinical symptoms, may greatly contribute to the distribution of the parasite and has etiological relevance.
The aim of our study was to perform *in vivo* infection trials with *M. cerebralis* to explore whether the genetic homogeneity of brown trout broodstock influences the susceptibility of offspring to the parasitic disease. Using genetic markers, the individual inbreeding coefficient and the pairwise relatedness were estimated for the Atlantic lineage broodstock in the Aufsess trout farm, Germany. With targeted fertilization, three offspring groups (inbred, non-inbred and closely related ones) were created, which considerably differed in the level of inbreeding. Furthermore, offspring of Atlantic-Danubian hybrid broodstock and Steelhead rainbow trout (as positive control) from the Lillafüred trout hatchery, Hungary, were used in the infection trials. Our findings indicate that the inbreeding status of broodstock has significant influence on the intensity of parasite infection in the subsequent generations. Therefore, the preventive measure against the parasite should also be completed with the regular genetic freshening of broodstock under controlled conditions.

Financial support: Fachberatung für Fischerei des Bezirks Oberfranken, Alexander von Humboldt Foundation and János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

25d. Pathogenicity studies of *Myxobolus honghuensis* (Myxosporea: Bivalvulida) using suckling mice model

Qingxiang Guo*, Zemao Gu, Luo Jia, Jianhua Qin, Hong Li

Lab of Freshwater Animal Breeding, Ministry of Agriculture, College of Fisheries, Huazhong Agricultural University, Wuhan, 430070, People's Republic of China
guzemao@mail.hzau.edu.cn

Myxozoans are ubiquitous metazoan parasites and some species can cause severe fish diseases around the world. However, a recent research shows *Kudoa septempunctata* has stirred up food-borne illness in Japan and can cause diarrhea, elevated fluid accumulation ratio in suckling mice with the possible mechanism related to the sporoplasm invasion. That is the first certain report of human pathogenicity myxozoan spores, even though some species, i.e. *Kudoa* spp. *Myxobolus* spp. Henneguya spp. , had long been involved with some unsolved human pathogenic cases. In this study, in order to evaluate the interaction of *Myxobolus honghuensis*, another myxozoan which parasitizes in the pharynx of important food fish allogynogenetic gibel carp *Carassius auratus gibelio* (Bloch), with mice and extrapolate the results to human, BALB/c suckling mice test combined with hematological and histological methods were used. Suckling mice test showed that there was no statistically difference of fluid accumulation ratio between mice inoculated with 1.65×10^6 spores and negative control after 8-h incubation. Spores did not disrupt intestinal histology and no abnormal bowel movements were observed within 20 h post inoculation. No anomalous hematology parameters but a slight leukopenia only consisting of monocytes were recorded in mice inoculated with 1.65×10^6 spores at 6 h post inoculation. These results suggest that *Myxobolus honghuensis* spores could not cause diarrhea and elevated fluid accumulation ratio in BALB/c suckling mice and the ability for myxozoan spores causing gastrointestinal symptoms may differ in *Kudoa* species and *Myxobolus* species.

25e. Insights into the genome of *Ceratonova shasta*, a myxozoan parasite of salmonids

Stephen D Atkinson, Shawn T O'Neil, Eli Meyer, Sascha L Hallett, Jerri L Bartholomew

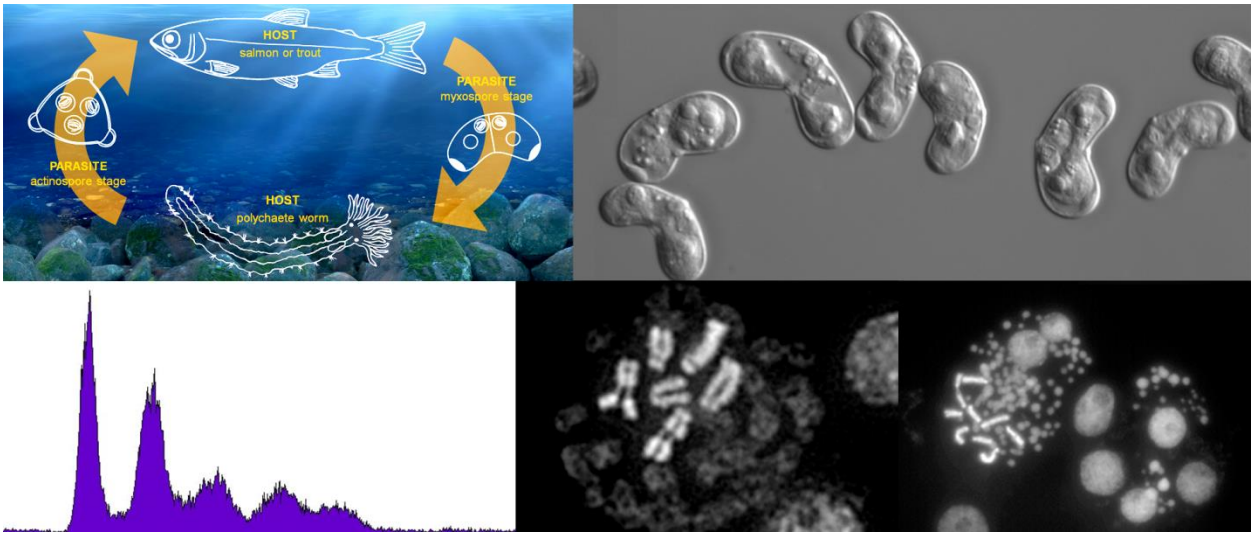
Oregon State University, Corvallis, Oregon, USA

Myxozoans are a common, speciose, yet persistently enigmatic group of parasitic Cnidaria. We are using a multi-disciplinary approach to characterize both the genome and transcriptome of *Ceratonova shasta* (syn. *Ceratomyxa shasta*), an economically and ecologically important intestinal parasite of salmon and trout in North America.

Genome properties: We determined the chromosome number by examination of DAPI-stained mitotic myxospore sporogenic stages from fish, using fluorescence microscopy. The *C. shasta* nuclear genome is organized into 3 chromosomes, with diploid and possibly triploid nuclear division observed in cell spreads. We estimated its genome size to be 105-160Mb, using flow cytometry, fluorescence microscopy and genome sequencing. We determined the relative copy numbers of rRNA genes to single-copy nuclear genes to be between 340 and 810X, using SYTO9 real-time PCR assays.

Genome sequencing: Total DNA was extracted from purified myxospores, an Illumina library prepared and sequenced on a single lane of an Illumina HiSeq2000. Raw reads comprised ~400Gb with ~300X coverage of the genome. Reads were assembled using Velvet, SOAP denovo and CLC Genomics Workbench: Velvet produced our best draft genome assembly, which comprised 15,423 scaffolds (total assembly size 105Mb; N_{50}=46.9Kb).

Transcriptome sequencing: RNA was sampled from different *C. shasta* genotypes, which infected different salmonid hosts. Non-normalized libraries were prepared, barcoded and sequenced in a paired-end 100nt run on a single lane of Illumina HiSeq2000. Reads were filtered of adaptor and primer sequences, assembled using Trinity and annotated against the NCBI database. Gene ontogeny labels and taxonomy labels were applied also.



25f. Transcriptome analysis of *Sphaerospora molnari* (Myxozoa: Myxosporea) and putative peptidase characterization.

Ashlie Hartigan[1]*, Martin Kašny[2,3], Astrid S. Holzer[1]

[1]Institute of Parasitology, Biology Centre of ASCR, České Budějovice, Czech Republic
ashlie.hartigan@paru.cas.cz, astrid.holzer@paru.cas.cz
[2]Department of Parasitology, Faculty of Science, Charles University in Prague, Viničná 7, Prague 128 44, Czech Republic, kasa@post.cz

Myxozoan parasites are an emerging group of pathogens being investigated around the world however the majority of this research is focused on taxonomy or their role in important diseases of wild and cultured fish. There is very little known about the "omics" (genomes, transcriptomes or proteomes) behind the functional molecular biology of these parasites. Peptidases play an important role in host parasite interactions, such as host cell invasion, parasite nutrient uptake and reproduction. Here we present the results of transcriptomic analysis of *Sphaerospora molnari*, with special focus on peptidases. This species has been implicated as a causative agent in gill sphaerosporosis as well as Swim Bladder Inflammation of cyprinids with large scale economic and animal health impacts. *Sphaerospora molnari* invades its host *Cyprinus carpio*, circulates and proliferates in the blood as motile, multicellular plasmodia which cause inflammation of different tissues before producing myxospores in the gills and skin surface. We used the total RNA from proliferative blood stages of *S. molnari* to obtain a transcriptome. By using Illumina HiSeq 52 046 864 reads of both parasite and its host (1:4 ratio) were generated. Assembly was completed in Trinity giving 89 241 contigs (N50=1540), which were screened against a number of datasets including the genomes and transcriptomes of the only other available Myxozoan NGS data from *Myxobolus cerebralis* and the host species *Cyprinus carpio* or other reference model fish like *Danio rerio* (Zebra fish) to remove host transcript contamination. The filtered transcriptome was then screened for putative proteins, and in particular peptidases, these were predicted and classified based on homology, motif searches, gene ontology and biological pathways. Among the predicted peptidases, e.g., disintegrin-like peptidase (metallo-peptidases), and cathepsins (cysteine peptidases) were revealed, which have been previously shown to be important virulence factors in other parasite groups. We used transcriptomic data to explore the important functional proteins of *S. molnari* in order to further the molecular/biochemical characterization of selected peptidases and their biological roles.

26) General Session: Aquaculture / Hatchery Issues

26a. Land-based aquatic practices in New Zealand – Building our awareness to improve biosecurity response preparedness

Jeannine Fischer*

Ministry for Primary Industries, Pastoral House, 25 The Terrace, PO Box 2526, Wellington 6140, New Zealand Jeannine.fischer@mpi.govt.nz

New Zealand has a broad range of land-based aquatic practices, including production of goods for sale or personal use, research and education, and for enhancement of wild stocks. New Zealand's Ministry for Primary Industries (MPI) currently maintains a registry of these licensed fish farming operations. Details of current on-farm biosecurity procedures and farm/ health management of unlicensed aquatic practices and the risks they present to New Zealand's biosecurity system remain largely unknown. These practices may pose a biosecurity risk by introducing or spreading pests and diseases into New Zealand's natural waterways. The unlicensed aquatic practices include, but are not limited to, research facilities, commercial ornamental fish breeders, trust hatcheries that carry out enhancement programmes, fish-out ponds.

The "Land-based aquatic practices" research project involves identifying the range of unlicensed aquatic practices and developing a database of key information about their operations. Additionally, a survey and assessment of these operators will be undertaken to ascertain operator understanding of biosecurity and the biosecurity management practices they have in place. The aim of this research is to improve MPI's knowledge of the land-based aquatic sector and will assist in building relationships with these practitioners. It will also increase MPI's understanding of the range of pest and disease management awareness and on -farm practices, perceptions and needs by the sector to grow. This research will also contribute to planning for greater resilience to biological threats affecting New Zealand's primary industries.

26b. Risks of pathogen entry and amplification at three hatcheries in the Willamette River Basin, Oregon, U.S.A.

Michelle Jakaitis[1]*, Sean R. Roon[1], Sascha L. Hallett[1], Richard A. Holt[1], Antonio Amandi[2], Jerri L. Bartholomew[1]

[1]Oregon State University, Department of Microbiology, Nash 226, Corvallis, OR 97331
jakaitim@onid.oregonstate.edu, roons@onid.oregonstate.edu, halletts@onid.oregonstate.edu, holtr@onid.orst.edu, bartholj@science.oregonstate.edu
[2]Oregon Department of Fish and Wildlife, Nash 524, Corvallis, OR 97331
amandia@onid.orst.edu

Hatcheries are often perceived as a source of pathogen amplification, potentially increasing disease risk to wild populations during epizootics; at the same time, wild fish may introduce pathogens into hatcheries. Incoming and exiting hatchery water is not treated, creating a potential pathogen entry and exit portal, but not much is known about this dynamic. In 2011-2013 we conducted sentinel fish exposures to examine the potential for pathogen transmission into and out of three Oregon hatcheries. In 2011, juvenile rainbow trout and Chinook salmon were held in hatchery influents and effluents for week-long exposures in June, July, and August. In 2012, summer monitoring was extended through early October, to see if sentinel fish became infected in the hatchery influent or effluent, indicating an incoming pathogen source or potential hatchery outbreak. Sentinel fish were also exposed during hatchery outbreaks of *Flavobacterium psychrophilum* (2011, 2012), *F. columnare* (2012), and *Aeromonas salmonicida* (2011, 2012) and at one hatchery that had low *Flavobacterium columnare* infection prevalence (2013). Sentinel fish were placed at additional sites downstream of hatchery effluents during hatchery outbreaks. All fish were transferred to the Salmon Disease Lab, Oregon State University, for monitoring. Results indicated that pathogen transmission occurred from the hatchery to sentinel fish during outbreaks of *A. salmonicida* and *F. columnare* in late summer, and during low levels of *F. columnare* infection in 2013. In these cases there was increased mortality in fish held at multiple downstream sites below the hatchery. No target pathogens were detected in sentinels held upstream of the hatcheries. Low levels of non-target bacteria were detected in sentinels during non-outbreak exposures, and minimal sentinel mortality occurred at this time. Our results suggest that pathogen transmission can occur from a hatchery to sentinel fish held immediately downstream of hatcheries undergoing disease outbreaks, but this may be a limited effect dependent on distance, dilution, and pathogen.

26c. First nationwide survey documenting and analysing causes of loss of fish and its predisposing factors in Norwegian salmonid aquaculture

Hogne Bleie[1]*; Aud Skrudland[2], Marit Stormoen[3], Randi I. Krontveit[3]

[1]MSD Animal Health Norge, Thormölensgt. 55, 5008 Bergen, Norway, hogne.bleie@merck.com;
[2]Mattilsynet (Norwegian Food Safety Authority), PO Box 383, 2381 Brumunddal, Norway, aud.skrudland@mattilsynet.no,
[3]Centre for Epidemiology and Biostatistics, Faculty of Veterinary Medicine and Bioscience, Norwegian University of Life Sciences, marit.stormoen@nmbu.no and randi.krontveit@nmbu.no

A nationwide survey covering the generations of Atlantic salmon *(Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss*) transferred to sea in the autumn of 2010, spring of 2011 and autumn of 2011 was carries out retrospectively after the stocks were harvested and end point biological data were available. The production data were retrieved by questionnaires being filled in by site managers and returned to the project manager. One questionnaire was filled in for each group of fish, which was defined as uniform smolt from one smolt plant transferred to sea to a specific site during a short time interval. A total of 1.066 groups were covered, representing 318 sea water sites, 59 proprietors, stocks from 139 smolt plants and a total of 307 million individual fish. Data documenting causes of loss of fish were separated in three consecutive intervals; from stocking and through the third month at sea, from the fourth month to the end of the 10^{th} month, and from the 11^{th} month to harvest. The absolute numbers of fish registered lost were given and further divided in to categories and specific causes of loss. All data were retrieved from the database of biomass recordings maintained by all fish farmers in Norway. Data were plotted anonymously into an Excel spreadsheet and transferred to STATA 12 ©for epidemiological analysis. A whole range of overt loss factors, geographical differences, predisposing management regimes and biological strategies were disclosed and will be presented. The project was led by Mattilsynet, financed by The Norwegian Seafood Research Fund- FHF and the statistical analysis carried out by the Norwegian University of Life Sciences.

26d. A farm-level view of furunculosis in salmonids and challenges faced for natural resource management of wild trout in New Jersey

J Lovy[1]*, SE Friend[1], S Crouse[2], D DiCarlo-Emery[3]

[1]Office of Fish & Wildlife Health & Forensics, N.J. Division of Fish & Wildlife, NJ 07863, USA
Jan.Lovy@dep.nj.gov, Sarah.Friend@dep.nj.gov
[2]Lebanon Fisheries Laboratory, N.J. Division of Fish & Wildlife, NJ 08833, USA
Shawn.crouse@earthlink.net
[3]Animal Health Diagnostic Laboratory, N.J. Department of Agriculture, NJ 08628, USA
Denise.dicarlo-emery@ag.state.nj.us

Furunculosis, caused by the bacterium *Aeromonas salmonicida salmonicida*, occurred in New Jersey's state fish hatcheries for the first time in thirty years. The farmed species most affected by the disease during these epizootics included brook and brown trout and freshwater-raised Atlantic salmon. Despite available antibiotics for treating the disease, the presence of carrier fish within populations remained problematic in fish destined for release into the environment. Since its first isolation from the Pequest trout hatchery in September 2013 the bacterium caused chronic disease in trout populations, characterized grossly by "boils" within the musculature of the fish. More recently, the bacterium became established in Atlantic salmon, which showed little external disease signs, but internally had a severe hemorrhagic septicemia. The presence of furunculosis in the hatcheries led to the humane euthanasia of about 230,000 salmonid fish based on a policy developed for the long-term protection of the state's trout waters. In the wake of these hatchery outbreaks, several field studies were conducted to monitor for the presence of *A. salmonicida salmonicida* in wild and previously stocked fish. Bacterial strains were compared with biochemical tests and antibiotic sensitivities to determine if strain variations exist within the state. This presentation will discuss the furunculosis epizootics and farm level management, actions taken to limit the spread of the bacterium to wild fish, and the results of field surveys in the wild.

26e. Naturally infected catfish concurrently transmit *Ichthyophthirius multifiliis* and *Edwardsiella ictaluri* to naive catfish

Xu, De-Hai[1]*, Craig Shoemaker1, Qi-Zhong Zhang[2]

[1]U.S. Department of Agriculture, Agricultural Research Service, Aquatic Animal Health Research Unit, 990 Wire Road, Auburn, AL 36832, USA. dehai.xu@ars.usda.gov
[2]Hydrobiology Institute, Jinan University, Guangzhou, Guangdong 510632, P. R. China

Bacterium *Edwardsiella ictaluri* and parasite *Ichthyophthirius multifiliis* (Ich) are two common pathogens of channel catfish (*Ictalurus punctatus*) which cause major losses to catfish aquaculture. There is limited information available whether fish naturally coinfected with Ich and *E. ictaluri* can concurrently transmit both pathogens to naive fish. The objective of this study was to expose naive catfish to naturally infected fish that carried Ich and *E. ictaluri* to provide clinical evidence for transmission of both pathogens. Three tanks of fish were exposed to naturally coinfected fish and two tanks were utilized as mock-infected controls in each of the two trials. In trial I, 34 out of 60 fish (56.7%) exposed to two infected fish per tank died at day one. All remaining fish (100%) died two days post exposure. Of the dead fish, all showed heavy Ich infection and *E. ictaluri* was isolated from the kidney of 50% of the dead catfish. In trial II, the cumulative mortality in fish exposed to 2 coinfected fish per tank was less than 20% during days 1-7 post exposure. Most of the fish died from 8 to 14 days post exposure to the coinfected fish. Ninety-six % of the fish were positive for both Ich and *E. ictaluri* in trial II. The results demonstrated that fish naturally coinfected with Ich and *E. ictaluri* could concomitantly transmit both pathogens to naive fish. In aquaculture management, precaution is needed to thoroughly examine fish prior to shipment or purchase to prevent the spread of aquatic animal pathogens.

26f. Summary and treatment effectiveness of cases examined at the Kentucky State University Fish Disease Diagnostic Laboratory from 2009 through June 2014

R. Durborow*, J. Kelso, J. Ma, C. Frederick, T. Ogunsanya, A. Redden, K. Campbell, W. Kahill

Aquaculture Research Center, 103 Athletic Road, Kentucky State University, Frankfort, KY 40601 USA robert.durborow@kysu.edu

The Fish Disease Diagnostic Laboratory at the Kentucky State University Aquaculture Research Center diagnosed 268 fish disease cases between 2009 and June 2014. Cases included a massive die-off of 500,000 silver carp in the Cumberland River in Kentucky just below Lake Barkley dam (Figure 1) and a case of heavy gill flukes and *Trichodina* sp. on the gills of tilapia (Figure 2). A summary of disease cases over this five and a half year period will be presented and will include species of fish examined, parasites and bacteria responsible for causing morbidity and mortality, and other causes of disease including water quality degradation and poor nutrition. Fish species examined included largemouth bass, channel catfish, rainbow trout, brown trout, bluegill sunfish, yellow perch, paddlefish, koi, goldfish, channel catfish x blue catfish hybrids, and freshwater prawns. The most common diseases included *Aeromonas hydrophila* and *Flavobacterium columnare* bacteria, *Ichthyophthirius multifiliis* and *Trichodina*. KSU FDDL examined cases submitted from Kentucky and surrounding states including Ohio, West Virginia, Tennessee and Texas. Treatment effectiveness and cost were evaluated.

Figure 1. Silver carp killed below Lake Barkley dam, Kentucky in April 2014.

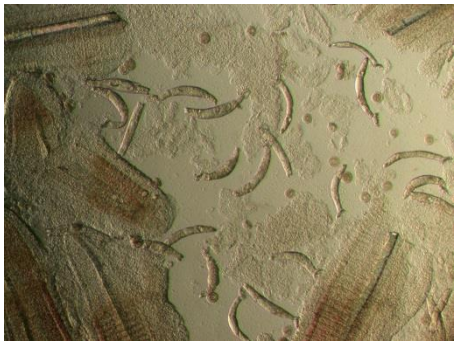


Figure 2. Heavy Trichodina and gill fluke infestation on the gills of tilapia.

27) General Session: Immunostimulants II

27a. Dietary effects on immunity, stress, and efficacy of a live attenuated *Flavobacterium Psychrophilum* vaccine

Kenneth D Cain*, Sudheesh Ponnerassery

Department of Fish and Wildlife Sciences and the Aquaculture Research Institute, University of Idaho, 875 Perimeter Dr MS1136. Moscow, ID 83844-1136

Rainbow trout were fed a basal commercial salmonid diet with or without added nutrient and immunostimulatory properties. The effect of diet was tested following immersion vaccination of fish using two formulations of a live attenuated coldwater disease vaccine (B.17 or ILM-B.17). The basal diet and the health promoting diet were fed to fish for 2 weeks prior to vaccination with B.17, ILM-B.17, or a TYES media control. Following primary vaccination, fish were fed each diet for 2 more weeks, booster immunized, and then fed the respective diets for one additional week at which time all groups were switched to the basal diet until the end of the experiment. At 7 weeks post vaccination all groups were injection challenged with *Flavobacterium psychrophilum* and monitored until approximately 60% cumulative mortality was observed in negative control groups. Just prior to disease challenge, a chlorine spike occurred resulting in substantial mortality among treatment groups. Interestingly, the fish fed the health promoting diet had significantly higher survival than fish fed the basal diet. Mortality ranged from 38-86% in groups of fish fed the basal diet and 4-18% in those fed the health promoting diet. Anti-*F. psychrophilum* antibody titers developed in fish from all vaccinated groups and were significantly higher than controls. Those fish vaccinated with the ILM-B.17 vaccine and fed the health promoting diet showed the highest elevated responses. Following challenge, RPS values ranged from 37.6% – 70%; however, chlorine induced mortality just prior to challenge did not allow for effective statistical comparisons to be made in this trial. Currently, the study is being repeated and thus far antibody responses appear to parallel initial observations. Results will be presented, and to date suggest that health based diets may promote improved immunity, stress response, and possibly enhance aquaculture vaccine efficacy.

27b. Antimicrobial activity of *Origanum vulgare* L. on protection against *Lactococcus garvieae* and *Vibrio anguillarum* in rainbow trout (*Oncorhynchus mykiss*, Walbaum)

Oznur Diler*, Oznur Gormez, Abdullah Diler

Suleyman Demirel University, Faculty of Egirdir Fisheries, oznurdiler@sdu.edu.tr

The wide and frequent use of antibiotics in the past has resulted the resistance development in pathogens in recent years. Plant extracts such as essential oils have increased attention as potential alternatives to growth promoters for animal production. In this study, the effect of *Origanum vulgare* L. on antibacterial activity was investigated in *Oncorhynchus mykiss*. Experimental diets supplemented with four concentrations (0.125, 1.5, 2.5, 3.0 ml kg-1) *Origanum vulgare* L. for twelve weeks. Dietary application of *O. vulgare* L. as protection for Lactococcosis and Vibriosis were studied. Feeding with *O. vulgare* L. oil significantly reduced mortality following injection with *Lactococcus garvieae* and *Vibrio anguillarum*. Dietary administration of 2.5 and 1.5 ml kg-1 of *O. vulgare* L. significantly reduced mortality. The highest survival rate was observed in the group fed with the 3.0 ml kg-1. These results indicated that dietary administration of *O. vulgare* L. improved disease resistance of rainbow trout (*O. mykiss*).

27c. The external administrated fish cytokine will increase the survival in bacterial and virus challenge

John Han-You Lin[1,2,3]*, Chin-Chou Lin[1], Shih-Jie Lin[1],Wan-Ching Kuo[1,4,] Yi-Fan Foung[1,4], Han-Tso Lin[4]

[1]Institute of Biotechnology, College of Bioscience and Biotechnology, National Cheng Kung University, Taiwan
[2]Research Center of Agricultural Biotechnology, National Cheng Kung University, Taiwan
[3]Center of Biosciences, National Cheng Kung University, Taiwan hanyou@mail.ncku.edu.tw
[4]Department of Biotechnology, Ming Chuan University, Taiwan

The aquaculture is the growing industry in the worldwide. However, the disease outbreak cause a huge economic lose. Utilized the antibiotics has the public health concern, and it could not treat or prevent virus infection. Development a novel and effectively method to control disease outbreak is an urgent issue. Immunoprophlaxys method may be the best way to control the disease. The host immune reaction was regulated by cytokines, a group of peptides or small proteins. These cytokines trigger on various the innate or adaptive immunity. Our hypothesis is try to increase the survival via external administrated fish cytokine. In this study, we utilized a multifunction teleost cytokine, grouper IL-6, as a model. The recombinant grouper, gIL-6, expressed via the *Escherichia coli* BL21 (DE3) protein expression system. The biology function of recombinant IL-6 was confirmed by measure the mRNA of immune related genes expression in the IL-6 stimulated fish by quantitative real-time PCR. Then, a bacterial pathogen (*Vibrio compbelli*) and a virus pathogen (nodavirus, NNV) was challenge the fish pretreat or treat recombinant IL-6. The results shown the fish pretreat or treatment with recombine IL-6 showing the higher survival rate than the control group; the bacteria and virus load in the organs of IL-6 treatment fish was also lower than the control group. These results indicated fish increase the survival by administrated external cytokine. The cytokine will trigger on innate immune responses and help fish eliminated the pathogen in the body. That may be an alternative way to prevent or treatment of disease by using recombinant teleost cytokine.

27d. A probiotic provides significant protection against *Flavobacterium psychophilum* in rainbow trout after injection by two different routes

S. LaPatra[1]*, T. Feringer[2], K. Cain[2]

[1]Clear Springs Foods, Inc., Research Division, Buhl, ID USA scott.lapatra@clearsprings.com
[2]Department of Fisheries and Wildlife, University of Idaho, Moscow, ID USA
kcain@uidaho.edu

To determine the potential for protection against *Flavobacterium psychrophilum* infection, a study was conducted where a probiotic, *Enterobacter* sp. strain C6-6, was delivered to rainbow trout, *Oncorhynchus mykiss*, via injection. Two separate studies were conducted. In a preliminary study groups of rainbow trout (mean weight, 2 g) were either left unhandled or intramuscularly (IM) injected with a standardized concentration of either a 48 or 72 h culture of C6-6 and subsequently challenged with two different doses of *F. psychrophilum* 7 , 28 and 56 days post-injection (PI). The relative survival ranged from 66 to 87%, 42 to 53% and 0 to 18% at 7, 28 and 56 days PI, respectively. In the second study groups of rainbow trout (mean weight, 1.3 g) received either an intraperitoneal injection (IP) of phosphate buffered saline (PBS; negative control), supernatant from a C6-6 culture, formalin killed C6-6, or live C6-6 and were subsequently challenged with *F. psychrophilum* 7 or 28 days PI. Log rank survival analysis showed a significant ($p<0.05$) reduction in mortality for fish receiving the treatments at both 7 and 28 days PI. Additionally, at 28 days PI, fish receiving the formalin killed or live C6-6 had significantly increased antibody titers against *F. psychrophilum*. This was not expected and suggests that protection observed at 28 days could be in part due to a cross protective adaptive immune response. Antibody titers were not detected at 7 days PI but significant protection was observed and indicates that innate immunity was most likely responsible for this. Taken together, results from this study indicate that protection against *F. psychrophilum* after either IM or IP injection of this naturally occurring bacterium, either alive or dead, is at least in part dependent on the enhanced immune function(s) in the treated fish. This may shed light on protection mechanisms associated with the use of the *Enterobacter* sp. C6-6 strain, and may be useful as a potential alternate strategy for reducing the impacts from *F. psychrophilum* infection through non-specific immune-enhancement during times of increased fish stress or as a possible adjuvant.

27e. Optimizing the efficacy of a live attenuated *Flavobacterium psychrophilum* vaccine for coldwater disease

Sudheesh Ponnerassery*, Kenneth D Cain

Department of Fish and Wildlife Sciences and the Aquaculture Research Institute, University of Idaho, 875 Perimeter Dr MS1136. Moscow, ID 83844-1136 USA

Vaccine optimization studies were carried to improve the efficacy of a live attenuated strain of *Flavobacterium psychrophilum* (CSF259-93B.17) as vaccine against coldwater disease. The growth of the vaccine strain under iron limited conditions was optimized by growing the bacteria in iron limited medium (ILM) (tryptone yeast extract salts medium, TYES containing 0-60 µM concentrations of a known iron chelator, 2, 2, bipyridine) at 15^0C. Bacteria grown in higher doses of 2, 2, bipyridine (50 and 60 µM) grew slowly compared to the bacteria grown in normal TYES medium up to 72 h of incubation. However, when the cultures reached 96 h, the bipyridine treated bacteria recovered from the inhibition and grew to higher counts similar to untreated bacteria. The viable count of bacteria grown in ILM reached 1.08×10^{11} cfu/ml at 96 h of incubation compared to a count of 1.28×10^9 cfu/ml at 72 h. Further optimization of the ILM vaccine is being carried out by vaccinating different size groups of rainbow trout (0.5 g, 1.0 g and 2.4 g) and for different vaccination delivery durations (1.5, 3, 6, and 30 min). The ILM vaccine was diluted 1:10 using the rearing water and 150 fish per treatment were immersion vaccinated. Similarly, the control groups of 150 fish in each size group were immersed in TYES media diluted 1:10. The fish were fed a standard trout fry diet (BioOregon Fry, Skretting) at 2% body weight. Fish were booster immunized using a similar dose of the vaccine 2 weeks post initial immunization. Five fish from each treatment group were bled and sera collected every two weeks for measuring specific immune response using a standard ELISA method. Vaccinated fish developed significantly higher specific antibody titers than the control fish at 2, 4 and 6 weeks post initial vaccination. The experiments are ongoing and the fish will be challenged with a virulent strain of *F. psychrophilum* at 8 weeks post initial vaccination. In addition, field evaluation to determine safety and efficacy of the ILM vaccine is underway.

28) Special Session: Environmental Contaminants and Fish Health I

28a. Evaluation of potential disease causing agents in young of the year smallmouth bass in the Chesapeake Bay watershed

Megan V Kepler[1,2]*, Vicki Blazer[3], Tyler Wagner[4], Heather Walsh[5], Geoff Smith[6]

[1] Intercollege Graduate Degree Program in Ecology, Pennsylvania State University, University Park, Pa USA
[2] Pennsylvania Cooperative Fish and Wildlife Research Unit, 413 Forest Resources Building, Pennsylvania State University, University Park, PA 16841 USA mvk10@psu.edu
[3] U.S. Geological Survey, Fish Health Branch, Leetown Science Center, 11649 Leetown Road Kearneysville, WV 25430 USA vblazer@usgs.gov
[4] U.S. Geological Survey, Pennsylvania Cooperative Fish and Wildlife Research Unit, Pennsylvania State University, 402 Forest Resources Building, University Park, PA 16802 USA txw19@psu.edu
[5] West Virginia University Research Corporation, 866 Chestnut Ridge Rd, Morgantown, WV 26506 USA hwalsh@usgs.gov
[6] Pennsylvania Fish and Boat Commission, Division of Fisheries Management, 1601 Elmerton Ave., Box 67000, Harrisburg, PA 17106 USA geofsmith@pa.gov

Smallmouth bass and other species within the Chesapeake drainage have shown varying signs of disease for a number of years. In the Potomac drainage, skin lesions and periodic fish kills of adults in the spring have occurred. Since 2005, smallmouth bass in the Susquehanna drainage have been displaying characteristics of disease in both adults and young of the year (YOY) with only mortality occurring in the YOY. Bacterial pathogens (*Flavobacterium* and *Aeromonas*), largemouth bass virus, trematode and myxozoan parasites have all been identified, suggesting environmental stressors and potential immunosuppression. The presence of YOY disease between drainages was evaluated through examining microscopic pathology, the presence of parasites, and concentrations of contaminants (e.g., PCBs, DDE, pesticides, and fungicides). A random sample of YOY smallmouth bass were collected at a total of 20 sites (16 sites in PA, 2 sites in MD, and 2 sites in WV) for histological evaluation of parasites, inflammation and general tissue abnormalities. Aquatic macroinvertebrates, specifically Oligochaetes, were collected in an attempt to identify the intermediate hosts for myxozoan parasites. One parasite of interest is *Myxobolus inornatus*, previously identified in YOY smallmouth bass in Pennsylvania. Sediment samples were also collected from 6 sites in PA, 2 sites in MD, and 2 sites in WV to investigate potential variability in contaminants concentrations and to identify contaminants of concern. Myxozoan parasites were identified in fish sampled throughout Pennsylvania and at one site in Maryland. Molecular identification of parasites is currently ongoing. A regional evaluation of disease and contaminants in YOY smallmouth bass is important to the development of predictive frameworks for future outbreaks in addition to understanding various risk factors associated with disease.

28b. Potential risk factors for skin and liver tumors of white sucker and brown bullhead

Vicki S. Blazer[1*], Heather L. Walsh[2], Cassidy M. Hahn[2], Ryan P. Braham[2], Luke R. Iwanowicz[1]

[1]U.S. Geological Survey, Leetown Science Center, Kearneysville, WV 25430 USA
vblazer@usgs.gov, liwanowicz@usgs,gov
[2]West Virginia University, Division of Forestry and Natural Resources, Morgantown, WV 26506 USA hwalsh@usgs.gov, Cassidy.hahn@gmail, ryan.braham@hotmail.com

Fish tumors, particularly orocutaneous and liver tumors, have been used extensively as indicators of ecosystem health and the adverse effects of anthropogenic contaminants. Many studies have shown an increased prevalence of neoplasms at contaminated sites versus less-contaminated sites. Brown bullhead (*Ameiurus nebulosus*) and white sucker (*Catostomus commersonii*) are indicator species at Great Lakes Areas of Concern (AOC) and elsewhere. Tumors of the lips, barbels and body surface include papilloma, squamous cell carcinoma, melanoma and osteoma. Liver tumors include hepatic cell adenoma, hepatic cell carcinoma, cholangioma and cholangiocarcinoma. The Great Lakes Restoration Initiative began in 2010 and one major objective was to address beneficial use impairments (BUIs) at AOC, one of which is the "fish tumors or other deformities" BUI. While resources have been directed at assessing the prevalence of tumors at various AOC, there has been less emphasis on determining the associated risk factors. Historically, polycyclic aromatic hydrocarbons (PAHs) have been the contaminants most associated with liver tumors in fishes. Less evidence exists for the role of specific contaminants in observed orocutaneous tumors. However, at some AOC, despite remediation of contaminated sediments, directed at removal of PAHs and PCBs (polychlorinated biphenyls), the prevalence of both liver and body surface neoplasms remains above that of less impacted sites. Measuring chemical concentrations in specific tissue as well as molecular analyses are providing evidence for the role of other factors in carcinogenesis. Other chemicals including arsenic and chemicals of emerging concern such as estrogens as initiators and/or promoters of carcinogenicity is considered. Additionally, the role chronic inflammation, and proliferative responses related to pathogens (viral) and parasites (myxozoans) as risk factors will be discussed.

28c. An evaluation of biological markers as indicators of exposure to genotoxic and mutagenic compounds in the Great Lakes Basin, United States

Ryan P. Braham[1]*, Vicki S. Blazer[2], Heather L. Walsh[1], Cassidy M. Hahn[1], Patricia M. Mazik[3]

[1]West Virginia University, Morgantown, WV 26506, USA, rbraham@usgs.gov, hwalsh@usgs.gov, cmhahn@usgs.gov
[2]USGS, Leetown Science Center, 11649 Leetown Road, Kearneysville, WV 25430, USA, vblazer@usgs.gov
[3]USGS, WV Cooperative Fish and Wildlife Research Unit, Morgantown, WV 26506, USA, pmazik@wvu.edu

Biological endpoints sensitive to genotoxic and mutagenic contamination are widely used as indicators of anthropogenic contamination. In the Great Lakes basin, the "fish tumors or other deformities" is a biological use impairment (BUI) at Areas of Concern (AOC) that uses fish metrics to quantify the relative system health. While legacy chemicals have been associated with liver tumors, less is known about the risk factors associated with skin tumors. The micronucleus (MN) assay was incorporated to assess four species of fishes collected from eight AOC's and one non-AOC site during the spring, 2011. Skin and liver tumors were also quantified at these sites. The four species evaluated were brown bullhead (*Ameiurus nebulosus*), largemouth bass (*Micropterus salmoides*), smallmouth bass (*Micropterus dolomieu*), and white sucker (*Catostomus commersoni*). Micronuclei and/or other nuclear abnormalities (NA) were observed at all sites; however NA generally occurred at a higher rate and severity than MN. Micronuclei and other NA were observed at differing occurrence and severity rates by species. Bass species generally expressed MN and NA at a higher occurrence rate and severity than bullhead or suckers collected at the same site. Interestingly, while skin and liver tumors were observed in bullhead and white suckers, they were not observed in bass. This apparent site and species affect should be considered when evaluating biological endpoints sensitive to genotoxic and mutagenic compounds. The correlation of existing sediment and water quality data to these biological endpoints will be discussed.

28d. Biological effects of environmental contaminants on gene expression endpoints in largemouth bass (*Micropterus salmoides*) and smallmouth bass (*Micropterus dolomieu*) from Great Lakes areas of concern

Cassidy M. Hahn[1*], Luke R. Iwanowicz[2], Vicki S. Blazer[2], Heather L. Walsh[1], Ryan P. Braham[1], Patricia M. Mazik[3]

[1]West Virginia University, Division of Forestry and Natural Resources, Morgantown, WV 26506, USA Cassidy.Hahn@gmail.com, Ryan.Braham@hotmail.com, hwalsh@usgs.gov
[2]USGS, Leetown Science Center, Fish Health Branch, 11649 Leetown Road, Kearneysville, WV 25430, USA liwanowicz@usgs.gov, vblazer@usgs.gov
[3]USGS, West Virginia Cooperative Fish & Wildlife Research Unit, West Virginia University, Morgantown, WV 26506, USA pmazik@wvu.edu

A recent shift in environmental monitoring of the Great Lakes watershed includes the evaluation of a new group of compounds collectively referred to as contaminants of emerging concern (CECs). At Great Lakes Areas of Concern, both CECs and legacy contaminants are often present resulting in the exposure of aquatic organisms to complex chemical mixtures. In order to assess the cumulative impact of these chemical mixtures a suite of biological indicators from the molecular to the organismal level was evaluated in resident pelagic largemouth bass and smallmouth bass. Next generation sequencing technologies were used to identify biomarkers genes in these non-model species and gene expression analyses were conducted using the nCounter assay from Nanostring Technologies. Gene expression analyses were designed to complement histological assessments, plasma analysis of vitellogenin, estradiol and 11-keto-testosterone and water and sediment contaminants at each sampling location. Correlations between these biomarkers as well as seasonal and species variation of gene expression endpoints will be discussed.

28e. Contaminant-associated health effects in fishes from the Ottawa and Ashtabula Rivers, Ohio

Luke R. Iwanowicz[1]*, Vicki S. Blazer[1], Heather Walsh[2], Cassidy Hahn[2], David S. DeVault[3], Jo Ann Banda[3]

[1]USGS, Leetown Science Center, Fish Health Branch, 11649 Leetown Road, Kearneysville, WV 25430 USA liwanowicz@usgs.gov; vblazer@usgs.gov

[2] West Virginia University, Division of Forestry and Natural Resources, Morgantown, WV 26506, USA, hwalsh@usgs.gov; cmhahn@usgs.gov

[c]US Fish and Wildlife Service, Ecological Services, 1 Federal Drive, Fort Snelling, MN 55111 USA devault.consulting@gmail.com; joann_banda@fws.gov

Resident fish populations serve as ecologically relevant sentinels of aquatic ecosystem health that allow health metrics from individuals to be used as a proxy of environmental health. Here we comparatively assessed the health of the Ottawa River and Ashtabula River using histologic, immunologic, and endocrine biomarkers in both brown bullheads (BB; *Ameiurus nebulosus*) and largemouth bass (LMB; *Micropterus salmoides*). Biomarker metrics were compared to fish collected from a reference site (Conneaut Creek). Both BB and LMB were collected from 3 locations in the Ottawa River across an ~8 mile gradient during the Spring of 2009. The following year BB and LMB were collected from the Ashtabula River and Conneaut Creek. Whole bodies were analyzed for approximately 30 legacy contaminants. Differences in immunological biomarkers were noted between species with significant reductions in bactericidal activity in BB at downriver sites in the Ottawa River. In general immune function appeared to be most greatly impaired in the Ottawa River compared to the Ashtabula or Conneaut. Total PCBs were significantly negatively correlated with bactericidal activity and other immunological endpoints across sites. Contaminant body burdens were lowest at the reference site and highest at the downriver site in the Ottawa River. Total PCB concentrations ranged from 0.19 – 22.21 mg/kg in BB and 0.06 – 56.9 mg/kg in LMB. In general fish from the Ottawa River appeared to be more greatly impacted than those from the Ashtabula River and Conneaut Creek. While PCBs may partially explain these differences, there is evidence that others chemicals and land-uses contribute to this observation.

28f. The pollution sentinel *Fundulus heteroclitus*: Application to sediment remediation efforts

Wolfgang K. Vogelbein[1]*, Michael Unger[1], Joe Rieger[2]

[1]Dept. of Environmental and Aquatic Animal Health, Virginia Institute of Marine Science, College of William and Mary, Rt. 1208, Gloucester Point, VA 23062 USA wolf@vims.edu
[2]Elizabeth River Project, 475 Water Street, Portsmouth, VA 23704 USA jrieger@elizabetriver.org

The mummichog, *Fundulus heteroclitus*, a small cyprinodontid teleost common to eastern USA estuaries, has a geographic distribution ranging from Nova Scotia, Canada to northern Florida. However, local sub-populations are thought to be non-migratory, with a very restricted summer home range of ~50 m. We therefore consider this small, highly abundant estuarine fish to be an effective bioindicator of local environmental quality. Histopathological surveys of mummichogs from variously degraded habitats along the eastern USA have indicated a strong positive association between high prevalences of hepatic and extra-hepatic neoplasms and sediment polycyclic aromatic hydrocarbon (PAH) concentrations. We have observed a broad spectrum of liver lesions including degenerative, inflammatory and compensatory proliferative changes, pre-neoplasms and hepatic, biliary, exocrine pancreatic and vascular neoplasms in mummichogs inhabiting a number of estuarine habitats in Virginia and Maryland. In the Elizabeth River, Virginia, these lesions clearly track a sediment-PAH gradient, with highest lesion prevalences occurring in fish from several creosote-contaminated sites in the highly industrialized portions of the river. In contrast, liver lesions attributable to toxigenicity essentially are at background levels in the more residential stretches of the river. The mummichog was recently adopted by the Virginia Dept. of Environmental Quality, the Elizabeth River Project and The Living River Trust as a sentinel to monitor environmental quality and, given the considerable baseline information that we have amassed over the years on toxicopathic liver lesion prevalences throughout the system, to track environmental recovery following sediment remediation projects currently underway within the river. The rationale for this approach is based on the assumption that if remediation projects are effective in reducing bioavailability of sediment PAHs and other chemical contaminants, then the expected outcome of mummichog histopathology monitoring is that liver lesion prevalence and severity should decrease over time. Preliminary findings suggest that using mummichog liver histopathology in conjunction with sediment chemical analyses will provide a critical tool for setting mitigation goals and assessing sediment remediation success in the future.

29) General Session: Immunology

29a. Transcriptome response associated with protective immunity in T and B cell deficient zebrafish

Aparna Krishnavajhala[1], Alan Zhao[2], Xiu-Feng Wan[3], Larry Hanson[4]*, Lora Petrie-Hanson[5]

[1]Department of Biochemistry and Molecular Biology, College of Arts and Sciences, Mississippi State University, ak265@msstate.edu
[2]Department of Basic Sciences, College of Veterinary Medicine, PO Box 6100, Mississippi State University, 39762-6100 nz65@.msstate.edu
[3]wan@cvm.msstate.edu
[4]hanson@cvm.msstate.edu
[5]lora@cvm.msstate.edu

Rag1$^{-/-}$ mutant zebrafish lack T and B lymphocytes. However, when re-exposed to homologous bacteria, these fish mount a response that provides specific protection. To further define this response, we utilized microarray analyses on kidney tissue to determine the mechanisms underlying innate immune system memory in zebrafish. We also analyzed interferon (IFN) gamma by RT-qPCR on kidney tissues and sorted kidney leukocytes. IFN gamma is produced by activated NK cells and could indicate if this cell mediates the protective response seen in lymphocyte deficient zebrafish. Pathological studies and *in situ* hybridizations were performed to observe tissue changes and location of the cells that produced IFN gamma. Following bacterial re-exposure, zebrafish transcripts in cell receptor activation, cell proliferation and cytotoxic function categories were differentially expressed. We found high expression of IFN gamma in the lymphocyte like cell population after bacterial exposure and this was induced to a higher level in fish that had been vaccinated. The phagocytic cell population showed no induction of IFN gamma. The pathological changes were less severe in the vaccinated fish. Our microarray and pathological findings indicate that the primary immune response of mutant zebrafish is not impaired, and they demonstrate an enhanced innate immune response following secondary bacteria exposure. Following homologous secondary exposure, mutant zebrafish kidney cell population is undergoing upregulated cell receptor activation, cell cytotoxic functions and cell proliferation. This cell population expresses IFN gamma. In mice activated T cells, NK-T cells and NK cells have been shown to be the primary cells that express IFN gamma. Since rag 1 mutant zebrafish do not have T or NK-T cells, the IFN responding cell population is most likely NK cells.

29b. Effect of temperature on innate immune response of Japanese flounder (*Paralichthys olivaceus*)

Norie Kaneshige*, Hidehiro Kondo, Ikuo Hirono

Laboratory of Genome Science, Tokyo University of Marine Science and Technology, Konan 4-5-7 Minato-ku, Tokyo 108-8477, Japan hirono@kaiyodai.ac.jp

Fish are poikilotherms, and their immune system depends on temperature. Although antibody responses in fish are strongly influenced by temperature, it is still uncertain how the immune responses are regulated at different temperatures. To understand the mechanism, we evaluated the gene expression profile of immune related genes at different temperatures by quantitative PCR (qPCR) and microarray. First, Japanese flounder (ABW=4.2 g) were immunized with 2.3×10^8 CFU/fish of formalin-killed *Edwardsiella tarda* (*E. tarda* FKC) by intra-peritoneal injection and maintained at 15˚C and 22˚C. Spleen was collected at 3, 6 and 12 hours post-injection (hpi) and 1, 3, 7 and 14 days post-injection (dpi). mRNA levels of immune related genes were calculated by qPCR. Another group of Japanese flounder (ABW=12.9 g) were also immunized with 3.4×10^8 CFU/fish of *E. tarda* FKC by intra-peritoneal injection and maintained at 15˚C and 22 ˚C. Spleen was collected at 6 hpi and 3, 14 and 28 dpi. The gene expression profiling was conducted by microarray. Challenge test was also conducted. Fish were first immunized with *E. tarda* FKC and kept at 15˚C and 22˚C for 28 days. Subsequently, the water temperatures were shifted to 24 ˚C with 7 days. Fish were infected with 3.7×10^4 CFU/fish of *E. tarda* after 35 days post-immunization, and mortality was monitored for 14 days. qPCR results showed that IL-1β mRNA levels were strongly upregulated at both temperatures at 3 hpi. On the other hand, IFN-γ mRNA levels strongly increased at 15˚C at 6 hpi and at 22˚C at 3 hpi. The microarray analysis showed that *E. tarda* FKC injection upregulated IL-1β expression until 28 dpi. Challenge test revealed that mortality was delayed and the fish survived longer in the *E. tarda* FKC injected group compared to the control group.

29c. Differential mortality of wild-type and T and B lymphocyte deficient zebrafish infected with a novirhabdovirus suggest that lymphocytes mediate age and temperature associated resistance.

Du Ngoc Nguyen*[1,2], Lorelei Ford[1,3], Lora Petrie-Hanson[1,4], Larry Hanson[1,5]

[1]Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, Starkville, MS.
[2]dnn24@cvm.msstate.edu
[3]ford@cvm.msstate.edu
[4]lora@cvm.msstate.edu
[5]hanson@cvm.msstate.edu

In order to study the contribution of innate defenses and lymphocyte based immunity to protection against a primary viral infection in fish, wild-type (WT) and rag *1*$^{-/-}$ zebrafish (T and B lymphocyte deficient) were infected with the novirhabdovirus, Snakehead Rhabdovirus (SHRV), by intraperitoneal (IP) injection. Both strains of fish were highly susceptible to SHRV. Diseased fish demonstrated exophthalmia, hemorrhaged fin bases and protruding scales. The susceptibilities of the two strains were significantly different and the difference increased with the age of fish (p-value < 0.0004) and the water temperature (p-value < 0.0041). All mortality in the WT fish occurred between day 3 and 10 post infection whereas the rag *1*$^{-/-}$ fish continued to die for at least one more week. The difference in losses were more pronounced at 28°C as the WT fish became substantially more resistant to the virus but the lymphocyte deficient fish remained highly susceptible. In cell culture SHRV had similar growth kinetics at 24 and 28°C suggesting that reduced virus replication was not a factor in the lower mortality.

Initial evaluation of gene expression of interferon gamma (IFNγ) and Myxovirus-resistance A (MxA) by quantitative reverse transcriptase PCR (qRT PCR) demonstrated that expression of these genes was low in un-exposed fish but increased substantially in both strains within 48 hours of exposure to SHRV. The strong IFNγ response in lymphocyte deficient fish suggests Natural Killer cell activation. These data suggest that innate immunity is important controlling virus infection early during the infection, but then the acquired immune system clears the infection. Furthermore, age and the environmental temperature have a strong effect on this acquired immune response. These results may help explain the temperature and age associated resistance observed in other Novirhabdovirus diseases in fish.

29d. Cytosolic sensor, DDX41 activates antiviral and inflammatory immunity in response to stimulation with dsDNA in Japanese flounder, *Paralichthys olivaceus*

Jun-ichi Hikima[1]*, Nhu Truong Quynh[2], Fernand F. Fagutao[2], Masahiro Sakai[1], Tae Sung Jung[2], Takashi Aoki[3]

[1]Department of Biochemistry and Applied Biosciences, Faculty of Agriculture, University of Miyazaki, Miyazaki 889-2192, Japan jhikima@cc.miyazaki-u.ac.jp, m.sakai@cc.miyazaki-u.ac.jp
[2]Lab of Aquatic Animal Diseases, College of Veterinary Medicine, Gyeongsang National University, Jinju, 660-701, Korea tqnhu@ctu.edu.vn, ffagutao@gmail.com, jungts@gsnu.ac.kr
[3]Consolidated Research Institute for Advanced Science and Medical Care, Waseda University, Tokyo 162-0041, Japan aokitaka@aoni.waseda.jp

In mammals, DDX41, one of the receptors belonging to the DExD family, has been known as a cytosolic DNA sensor and mediates antiviral response in host cells. However, its mechanism and structure in lower vertebrates such as fish remain poorly understood. Here, we cloned the full-length cDNA of DDX41 from Japanese flounder (JfDDX41) and investigated its role in immune response to viral DNA. JfDDX41 consists of 2,269 bp encoding 614 amino acids residues. The JfDDX41 gene was subsequently determined, revealed to be 5,812 bp in length containing 17 exons and 16 introns. At the amino acid sequence level, the full-length JfDDX41 showed high homology to the DDX41s of other vertebrates: 94% with medaka and 76% with human. JfDDX41 mRNA is located in all tissues and is distinctly expressed in fish naturally infected with LCDV (lymphocystis disease virus). High expression levels were examined in heart, liver, kidney and stomach. The JfDDX41 mRNA level increased significantly in the adherent (monocyte-like) cells stimulated with DNA virus. Reporter assay result showed that transcriptional activity of type-I IFN promoter was enhanced in the JfDDX41-overexpressed HINAE cells treated with C-di-GMP (dinucleotides). The overexpressing JfDDX41 also induced the antiviral and inflammatory cytokine gene expression by cytoplasmic C-di-GMP treatment. The results suggest that JfDDX41 plays an important role in the induction of antiviral activity and inflammatory response as the first-reported cytosolic DNA sensor in fish.

29e. Discovery of the nasopharynx-associated lymphoid tissue of rainbow trout

Irene Salinas[1*], Luca Tacchi[1], Rami Musharrafieh[1], Erin T. Larragoite[1], Scott LaPatra[2]

[1]Center for Evolutionary and Theoretical Immunology. Department of Biology, University of New Mexico, Albuquerque, New Mexico, USA. isalinas@unm.edu
[2]Clear Springs Foods Inc Research Division, Buhl, Idaho, USA. scott.lapatra@clearsprings.com

The nasopharynx-associated lymphoid tissue (NALT) is thought to be the first line of defense against airborne pathogens. NALT has only been identified thus far in birds and mammals. However, the olfactory organ of aquatic vertebrates such as teleost fish is also bombarded by waterborne antigens. We hypothesize for the first time that NALT is an ancient and conserved arm of the mucosal immune system in all vertebrates, terrestrial and aquatic. Using rainbow trout as a model, we reveal the main features of teleost NALT at the cellular and molecular level. Trout NALT follows the main canonical features present in other fish mucosa-associated lymphoid tissues including diffuse lymphoid cell populations, a preponderant number of IgT B cells and IgT levels in mucus as well as a diverse associated bacterial community (microbiota). Nasal delivery of live attenuated infectious hematopoietic virus vaccine (IHNV) was used a model to identify the main innate and adaptive immune molecules that play a role in trout nasal immune responses using microarray. Moreover, nasal vaccination using IHNV or ERM vaccines induces high levels of protection against systemic and mucosal challenges both at 7 and 28 days post-vaccination. Our results reveal for the first time that NALT is an important arm of the mucosal immune system of fish and open up important avenues for the development of nasal vaccines for use in aquaculture.

29f. Nasal vaccines for use in aquaculture

Irene Salinas[1]*, Scott LaPatra[2]

[1]Center for Evolutionary and Theoretical Immunology. Department of Biology, University of New Mexico, Albuquerque, New Mexico, USA. isalinas@unm.edu
[2]Clear Springs Foods Inc Research Division, Buhl, Idaho, USA. scott.lapatra@clearsprings.com

Nasal vaccines are commonly used in domestic animals, farmed terrestrial animals and humans. Nasal vaccines require small amounts of antigen and stimulate both local and systemic immune responses. Based on our findings that have revealed the presence of a mucosal immune system present in the olfactory organ of teleosts, we aimed to evaluate the effectiveness of two previously developed vaccines in rainbow trout: a live attenuated infectious hematopoietic necrosis virus vaccine (IHNV) and a killed enteric red mouth (ERM) bacterin. The nasal route was compared to the immersion and injection routes in a series of different experiments. Moreover, delivery of both vaccines into a single nare or each vaccine into separate nares was also tested. Challenge to live pathogens at 7 and 28 days post-vaccination revealed that delivery of IHNV into the left nare and ERM into the right nare is the most effective of all the vaccination regimes tested, including injection of IHNV and immersion with ERM vaccines. Our results demonstrate the nasal vaccines are a novel a promising way to effectively control aquatic infectious diseases.

30) Continuing Education I: Existing & Emerging Programs, Procedures, and Issues Involving Aquatic Animal Health & Welfare for the Practicing Aquatic Veterinarian

30a. Weissellosis – An important emerging disease in farmed rainbow trout

Timothy J. Welch[1], David P. Marancik[1], Christopher M. Good[2]*

[1]National Center for Cool and Coldwater Aquaculture, 11876 Leetown Road, Leetown, WV USA Tim.Welch@ars.usda.gov

[2]The Conservation Fund's Freshwater Institute, 1098 Turner Road, Shepherdstown, WV 25443 USA c.good@freshwaterinstitute.org

Since 2007, disease outbreaks associated with *Weissella* sp. bacteria in cultured rainbow trout have been reported on farms in China and Brazil. In the summer and fall of 2011, the authors visited two trout farms in North Carolina to investigate reports of severe, prolonged mortalities in larger fish approaching market size (0.5–1.0 kg). *Weissella* sp. were isolated from sampled moribund fish, and gene sequence analysis revealed 99% homology to isolates collected from the Chinese and Brazilian outbreaks. Laboratory-based challenge experiments replicated both the disease signs and induction of mortality in exposed healthy rainbow trout, and the pathogen was readily re-isolated from experimentally infected fish showing signs of infection. Weissellosis reoccurred in 2012 on one of the North Carolina farms, demonstrating environmental persistence of the agent through the winter months, and suggesting that, without intervention, the pathogen has the potential to become a lasting and endemic disease problem in USA farmed rainbow trout. In this presentation, we describe the ongoing disease situation in North Carolina, as well as the development of an experimental vaccine to control weissellosis.

30b. Contact zoonotic risks for aquaculture professionals in warm water aquaculture

Olga LM Haenen[1]*, Joyce Evans[2], Franck Berthe[3]

[1]National Reference Laboratory for Fish, Shellfish and Crustacean Diseases, Central Veterinary Institute, part of Wageningen UR, P.O. Box 65, 8200 AB Lelystad, The Netherlands olga.haenen@wur.nl

[2]United States Department of Agriculture, Agricultural Research Service, Stoneville, MS, 38776 USA Joyce.Evans@ARS.USDA.GOV

[3]European Food Safety Authority (EFSA), Animal Health and Welfare Panel, Largo N. Palli 5/A, Parma, I-43100, Italy Franck.BERTHE@efsa.europa.eu

Aquaculture production and consumption of aquacultural products increases. This growth enhances an increase in zoonotic infection from either handling or ingestion of these products. The principal pathogens acquired topically from fish or shellfish through spine/pincer puncture or open wounds are *Aeromonas hydrophilia*, *Edwardsiella tarda*, *Mycobacterium marinum*, *Streptococcus iniae*, *Vibrio vulnificus* and *V. damsela*. All of these indigenous pathogens have also been associated with disease outbreaks in food fish. Outbreaks are often related to management factors such as quality and quantity of nutrients in the water and stocking density, which increase bacterial loads on the external surface of the fish. As a result, diseased fish are more likely to transmit infection to aquaculture professionals, including fish processors. This paper will provide an account of worldwide topically acquired human cases of zoonosis from the principal fish and shellfish zoonotic pathogens and discuss risks, and required prevention.

Reference

- Haenen, O.L.M., J.J. Evans, and F. Berthe, 2013. Bacterial infections from aquatic species: Potential for and prevention of contact zoonoses. Rev. sci. tech.Off.int Epiz. 32 (2): 497-507.

30c. Histopathology for aquatic cases in practice: When should I use it, what samples do I take and what's it going to tell me.

Kevin R Snekvik[1,2]*

[1]Washington Animal Disease Diagnostic Laboratory, College of Veterinary Medicine, Washington State University, Pullman WA 99164-7034 USA ksnek@vetmed.wsu.edu
[2]Department of Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, WA 99164-7040 USA

Histopathology is one of many tools at a clinician's disposal for determining an accurate diagnosis and in turn deciding upon an appropriate treatment protocol. In aquatics, histopathology can be used for diagnosing disease in individual animals, but it is also effective at both diagnosing disease and evaluating underlying causal factors that are affecting groups of animals within a collection or production system. This presentation will focus on situations in which histopathology can be a valuable tool and times when it is less likely to be helpful. Sampling will be covered, including when is a sample going to be non-diagnostic, how to submit small fish for histopathology and which tissue samples to collect from larger fish and what is their diagnostic relevance. Lastly, a few representative cases will be presented showing how histopathology can provide insight into treatment failures or support the modification of treatment for remaining fish within a population.

30d. Florfenicol: Correlation of pharmacokinetics in channel catfish (*I. punctatus*) with minimal inhibitory concentration values against *Aeromonas hydrophila* and the control of associated mortalities in INAD field studies

Patricia S. Gaunt[1]*, Cory Langston[1], Christopher Wrzesinski[2], Bonnie Johnson[3], Louis Crouch[1], Dana Gao[1], Paul Adams[2], Fangshi Sun[2], Richard Endris[2]

[1]Mississippi State University, College of Veterinary Medicine, 240 Wise Center Road, MS State, MS 39762 USA gaunt@cvm.msstate.edu
[2]Merck Animal Health, 556 Morris Avenue, Summit, NJ 07901 USA richard.endris@merck.com
[3] US Fish and Wildlife Service, 4050 Bridger Canyon Road, Bozeman, MT 59715 USA bonnie_johnson@fws.gov

Florfenicol (FFC) is currently approved to control mortality associated with enteric septicemia of catfish (ESC) and columnaris disease in catfish (*Ictalurus punctatus*) at a dose rate of 10 mg/kg one time a day for 10 days. Efforts are underway to gain its approval for control of mortality associated with *Aeromonas hydrophila* in catfish. The objective of this report is to demonstrate with a multidose pharmacokinetic study that the minimal steady state concentration (Css (min)) of FFC in plasma after administration one time a day for 10 days exceeds the FFC minimal inhibitory concentration 90 (MIC_{90}) values for *A. hydrophila* and therefore indicates susceptibility of these bacteria to FFC. These values were then correlated with the clinical performance of FFC in controlling mortality associated with *A. hydrophila* in catfish in Investigational New Animal Drug (INAD) field trials.

A multidose oral pharmacokinetic study was conducted at a dose rate of 10 mg/kg one time a day for 10 days to assess the plasma concentration of FFC in channel catfish. At various time points ranging from predose to 120 hours after the final administration, blood was sampled from individual fish (10 fish per time point). The plasma was assayed for FFC using an LC-MS/MS method. The pharmacokinetic modeling of the results was performed using the computer program WinSAAM.

Catfish in commercial ponds experiencing field outbreaks of *A. hydrophila* were treated under INAD permits with FFC-medicated feed at a dose rate of 10 mg/kg body weight. The MIC values of FFC were determined for *A. hydrophila* isolated in these studies by the broth microdilution assay using commercially-prepared plates.

Results of the pharmacokinetic studies demonstrated that after administration of FFC to catfish, the mean terminal half-life (t1/2), maximum concentration at steady-state (Css(max)), minimal concentration at steady-state(Css(min)), time of Css(max) Tmax, and Vc/F were 9.0 h, 9.72 µg/mL, 2.53 µg/mL,8 h, and 0.653 L/kg, respectively. In the INAD field studies, catfish ate the medicated feed, and mortality diminished after initiation of treatment. The MIC_{90} of FFC against *A. hydrophila* from the field outbreaks was 0.5µg/mL.

The FFC concentration vs. time curve demonstrated that for the 10 day treatment period the Css (min) was above the MIC_{90} against *A. hydrophila*. These values along with the clinical performance in the INAD field studies support a FFC label claim for control of mortality associated with *A. hydrophila* in catfish.

.

31) Special Session: Environmental Contaminants and Fish Health II

31a. 17α-Ethinylestradiol dysregulates both mRNAs and miRNAs leading to impaired immune responses in zebrafish

Siba R Das[1,2], James C Woodson[1], Rachel Bessire[1], Donald E Tillitt[3], James Winton[1], John D Hansen[1]*

[1]Western Fisheries Research Center, U.S. Geological Survey, 6505 NE 65th St, Seattle, WA jwoodson@usgs.gov, rbessire@usgs.gov, jwinton@usgs.gov, jhansen@usgs.gov
[2]Department of Epidemiology, University of Washington, Seattle, WA dassiba@uw.edu
[3]Columbia Environmental Research Center, U.S. Geological Survey, Columbia, MO dtillitt@usgs.gov

17α-ethinylestradiol (EE2) is an endocrine disrupting compound found in a variety of aquatic ecosystems at ng L^{-1} concentrations and studies indicate that EE2 can alter development and reproduction for fish species. A relatively unexplored area though, is the impact of EE2 on immunity in fish. We assessed the impact of EE2 on the innate immune transcriptome in zebrafish by exposing fish to EE2 followed by a immune stimulation using peptidoglycan and poly(I:C) to mimic microbial infection. This microarray study identified gene pathways involved in immunity that were down-regulated upon exposure to EE2 implying that EE2 likely suppresses innate immunity. To further test this hypothesis, fish were exposed to EE2 and challenged with a LD30 of *F. noatunensis* using our established zebrafish/*Fno* challenge model. In addition, the microarray analysis suggested the involvement of miRNAs for regulating gene pathways impacted by EE2. Therefore we extended our study to identify miRNAs that may perturb innate immune pathways in fish. Individual miRNA libraries (n=36) from the EE2 experiment were sequenced using Illumina technology. Reads were filtered, trimmed and aligned to the current miRBase 20 database for zebrafish. Reads were normalized and differentially expressed miRNAs were calculated. Twenty-four and 66 miRNAs were significantly dysregulated upon exposure to 1 and 10ng L^{-1} levels of EE2, respectively. Nine miRNAs (e.g. dre-miR-725) were specific to the low dose exposure of EE2, 42 miRNAs (e.g. dre-miR-375) were restricted to the high dose exposure with 11 miRNAs (e.g. dre-miR-137) being shared between the environmentally relevant doses of EE2 exposure. We also applied this methodology to the PGN and poly(I:C) samples. Putative miRNA gene targets were identified from the miRBase target database and correlated with the previous microarray results. Overall, our analysis implies that miRNAs participate in regulating innate immunity in fish and that exposure to EDCs perturbs this process.

31b. Pathology working group review of histopathologic specimens from three laboratory studies of Diclofenac in trout

Jeffrey C. Wolf[1]*, Christine Ruehl-Fehlert[2], Helmut E. Segner[3], Klaus Weber[4], Jerry F. Hardisty[5]

[1]Experimental Pathology Laboratories, Inc., Sterling, Virginia, USA

While the pathology peer review / pathology working group (PWG) model has long been used in mammalian toxicologic pathology to ensure the accuracy, consistency, and objectivity of histopathology data, application of this paradigm to ecotoxicological studies has thus far been limited. In the current project, the PWG approach was used to evaluate histopathologic sections of gills, liver, kidney, and/or intestines from three previously published studies of diclofenac in trout, among which there was substantial variation in the reported histopathologic findings. The main objectives of this review process were to investigate and potentially reconcile these interstudy differences, and based on the results, to establish an appropriate no observed effect concentration (NOEC). Following a complete examination of all histologic sections and original diagnoses by a single experienced fish pathologist (pathology peer review), a two-day PWG session was conducted to allow members of a four-person expert panel to determine the extent of treatment-related findings in each of the three trout studies. The PWG was performed according to the United States Environmental Protection Agency (US EPA) Pesticide Regulation (PR) 94-5 (EPA, August 24, 1994). In accordance with standard procedures, the PWG review was conducted by the non-voting chairperson in a manner intended to minimize bias, and thus during the evaluation, the four voting panelists were unaware of the treatment group status of individual fish and the original diagnoses associated with the histologic sections. Based on the results of this review, findings related to diclofenac exposure included minimal to slightly increased thickening of the gill filament tips in fish exposed to the highest concentration tested (1000 □g/L), plus a previously undiagnosed finding, decreased hepatic glycogen, which also occurred at the 1000 □g/L dose level. The panel found little evidence to support other reported effects of diclofenac in trout, and thus the overall NOEC was determined to be > 320 □g/L. By consensus, the PWG panel was able to identify diagnostic inconsistencies among and within the three prior studies; therefore this exercise demonstrated the value of the pathology peer review / PWG approach for assessing the reliability of histopathology results that may be used by regulatory agencies for risk assessment.

31c. Analytical toxicology of seafood and communications post-DWH Oil Spill: Bridging environmental and public health concerns

Andrew S. Kane[1,2]*, Makyba K.S. Charles[1], Ross M. Brooks[1], Babette Brumback[1], Angela B. Lindsey[3], Traci Irani[3], Anne Mathews[4], Leah D. Stuchal[1,5], Steven M. Roberts[1,5], J. Glenn Morris[1,2]

[1]Departments of Environmental and Global Health; and Biostatistics, College of Public Health & Health Professions, University of Florida, Gainesville, Florida USA kane@ufl.edu mkscharles@gmail.com, brooks02@ufl.edu, brumback@ufl.edu
[2]Aquatic Pathobiology Laboratories; and Emerging Pathogens Institute, University of Florida, Gainesville, Florida USA jgmorris@epi.ufl.edu
[3]Center for Public Issues Education in Agriculture and Natural Resources, University of Florida, Gainesville, Florida USA ablindsey@ufl.edu irani@ufl.edu
[4]Department of Food Sciences and Human Nutrition, Institute for Food and Agricultural Sciences, University of Florida, Gainesville, Florida USA anne.mathews@ufl.edu
[5]Center for Environmental and Human Toxicology, College of Veterinary Medicine, University of Florida, Gainesville, Florida USA lstuchal@ufl.edu, smr@ufl.edu

The Deepwater Horizon oil spill caused concern for aquatic animal health in the Gulf of Mexico, as well as for public health and seafood safety. Human risk-based limits for polynuclear aromatic hydrocarbons (PAHs) in seafood have been established by the US FDA, and are based on national fish consumption and body weight estimates. Other studies have suggested that coastal Gulf communities consume more seafood than the national average. The goal of this NIEHS-sponsored project was to conduct analytical toxicology on edible portions of inshore-caught Gulf seafood, and to obtain seafood consumption rates for Gulf coast communities using an in-person food frequency questionnaire (FFQ). The FFQ determined consumption rates for finfish, shrimp, oyster and blue crab using a validated seafood portion guide. Yearly consumption estimates were used to calculate average daily consumption rates. Initial FFQ data indicated that seafood consumption in Gulf coast communities is higher than the national average with Gulf coast residents consuming 33-492% more seafood (oyster, fish, crab and shrimp, but not blue crab) than the national average. Further, seafood consumption distributions varied substantially among communities. Toxicology data on parent PAHs and respective alkylated homologues indicated that contaminant levels in edible portions of seafood are very low - near limits of detection, and orders of magnitude lower than FDA's Levels of Concern. Risk communication and outreach efforts associated with this project addressed gaps by BP and federal agencies that failed to effectively address community concerns, and to demystify processes that were used to regulate fishery closures and re-openings. This study shows the importance of using regional seafood consumption patterns when developing risk-based levels of concern for contaminants in seafood. The multidisciplinary approach and expertise applied in this effort underscores the complex nature of addressing contaminant burdens in seafood species amidst varying community and regional perspectives, and limitations in analytical oil spill chemistry and risk assessment methodology.

31d. Toxicology of sodium cyanide in four aquacultured fish species of importance in Colombia

Jaime F. González [1,2*], Diana M. Ochoa[2], Javier F. Borbón[2], Victoria Rodríguez[2], Angie L. León[2], Shirley Marroquín[2], Pablo D. Jiménez[2], Rene A. Díaz[2], Marlon Muñoz[2], María L. Correal[2]

[1]Laboratory of Aquatic Toxicology, School of Veterinary Medicine and Animal Science, Universidad Nacional de Colombia, Bogotá. jfgonzalezma@unal.edu.co.
[2]AQUÁTICA: Research Group in Aquatic & Environmental Toxicology, School of Veterinary Medicine and Animal Science, Universidad Nacional de Colombia, Bogotá.

Colombia´s government is fostering the mining industry as a cornerstone for economic growth. Sodium cyanide (NaCN) is used in the gold extraction process, which brings up concerns due to likely water pollution and toxic effects on fish. Six experiments were performed with juveniles (n=6-8 fish/treatment) of red tilapia (*Oreochromis sp*.), Nile tilapia (*Oreochromis niloticus*), white cachama (*Piaractus brachypomus*) and yamú (*Brycon amazonicus*) exposed during 24 h to waterborne NaCN. All the fish species displayed acute signs of poisoning within the first 15 minutes of exposure to 1.0 and 2.5 ppm NaCN, whereas the 0.25 ppm-exposed remained asymptomatic. Clinical signs were either respiratory distress (tilapia) or central nervous system signs: increased hyperactivity, spiral swimming, tremors and seizures (all the species). Hepatic catalase activity (tilapia) decreased in cyanide-exposed fish. Blood glucose (all the species), plasma lactate (red tilapia, yamú) (Table 1) and hematocrit (tilapia, cachama) were significantly higher in cyanide-exposed fish, whereas plasma proteins remained unaltered. Hepatic rhodanese decreased its activity as cyanide concentration exposure was higher (yamú). Plasma cholinesterase had a higher activity in short time exposure whereas it was inhibited at longer exposure times. The most notorious biochemical changes after cyanide exposure happened in blood glucose, plasma lactate, cholinesterase activity and rhodanese activity. The present work showed the risks fish species may confront when exposed to cyanide as a water contaminant.

Table 1. Blood glucose and plasma lactate (mean ± s.e.) in experimental fish exposed to cyanide. Different letters between controls and exposed of each species, $p < 0.05$, T-test.

Treatment	**Blood glucose** (mg/dL)			
	red tilapia	Nile tilapia	cachama	yamú
Controls	59 ± 25 [a]	53 ± 6 [a]	105 ± 30 [a]	114 ± 12 [a]
CN-exposed	153 ± 29 [b]	209 ± 23 [b]	358 ± 70 [b]	182 ± 9 [b]

Treatment	**Plasma lactate** (mg/dL)	
	red tilapia	yamú
Controls	11 ± 3 [a]	34 ± 4 [a]
CN-exposed	71 ± 16 [b]	74 ± 3 [b]

32) General Session: Bacteria IV

32a. Non-lethal samples for detection of *Renibacterium salmoninarum* in juvenile Chinook salmon (*Oncorhynchus tshawytscha*)—an update

Diane G Elliott*, Constance L McKibben, Carla M Conway, Dorothy M Chase, Maureen K Purcell, LynnMarie J Applegate

U.S. Geological Survey, Western Fisheries Research Center, 6505 NE 65th Street, Seattle, WA 98115 USA dgelliott@usgs.gov, cmckibben@usgs.gov, cmconway@usgs.gov, dchase@usgs.gov, mpurcell@usgs.gov, capplegate@usgs.gov

Non-lethal sampling methods for pathogen testing can reduce the need to sacrifice large numbers of fish to determine population infection prevalence, and combination of non-lethal sampling with tagging procedures allows monitoring of performance and survival of fish after testing. Molecular pathogen detection assays such as polymerase chain reaction (PCR) enable testing of small tissue samples, and real-time PCR (qPCR) allows rapid quantification of pathogens in samples. In previous experiments fin clips, gill snips, and surface mucus scrapings were determined to be suitable samples for non-lethal testing of Chinook salmon (*Oncorhynchus tshawytscha*) between 2.8 and 15.2 g in weight. We tested these sampling methods for detection of the bacterial kidney disease (BKD) agent *Renibacterium salmoninarum* in juvenile Chinook salmon following an immersion challenge with the bacterium, and compared nested PCR (nPCR) and qPCR results from non-lethal samples with analysis of kidney tissues by nPCR, qPCR, bacteriological culture, enzyme-linked immunosorbent assay (ELISA), fluorescent antibody test (FAT) and histopathology / immunohistochemistry. *Renibacterium salmoninarum* was detected by the PCRs in >50% of fin, gill, and mucus samples from challenged fish. Among the non-lethal assays, qPCR testing of mucus samples showed the best overall diagnostic performance characteristics for use as a proxy for lethal kidney sample testing to detect and quantify *R. salmoninarum* in juvenile fish. Mucus qPCR was the only non-lethal assay exhibiting both diagnostic sensitivity and specificity estimates >90% for distinguishing between *R. salmoninarum*-exposed and non-exposed fish in the tested population. In addition, the *R. salmoninarum* levels detected by mucus qPCR reflected changes in bacterial load estimated by kidney sample analyses, as evidenced by significant positive correlations ($P \leq 0.01$) observed between mucus qPCR *R. salmoninarum* DNA quantity estimates and kidney *R. salmoninarum* infection intensity scores at each sample time point from 3 to 21 weeks after immersion challenge, and in two different stocks of Chinook salmon.

32b. Bacterial kidney disease in a captive and endangered Chinook salmon broodstock: Did we manage it or did it manage us?

Mary Peters[1]*, Susan Gutenberger[1], Casey Risley[2], Peter Long[2], Jim Rockowski[3], Speros Doulos[3]

[1] US Fish and Wildlife Service, Lower Columbia River Fish Health Center, 201 Oklahoma Rd, Willard, WA 98605, mary_peters@fws.gov, susan_gutenberger@fws.gov
[2] US Fish and Wildlife Service, Columbia River Gorge National Fish Hatchery Complex, Little White Salmon National Fish Hatchery, 56961 SR 14, Cook, WA 98605, casey_risley@fws.gov, peter_long@fws.gov
[3] US Fish and Wildlife Service, Little White Salmon NFH, retired

From July 2008 to February 2014, Little White Salmon National Fish Hatchery raised five year classes (brood years) of an endangered run of spring Chinook salmon originating from the White River in the upper Columbia River watershed. As part of their recovery plan, adult captive broodstock were spawned as they matured, their progeny were raised at the hatchery and those progeny were then transferred back to the White River for acclimation and release. Captive broodstock were received at the hatchery as adults, post-smolts, juveniles or redd- pumped eggs and fry. Broodstock were reared on river water in outdoor raceways with low densities, predator control, and biosecurity measures.

These fish presented several challenges as a result of spending their entire lives in freshwater. There were four predominant causes of mortality during the captive broodstock lifecycle: smolting in freshwater, post-spawn male die-off, *Renibacterium salmoninarum* and *Salmincola californiensis*. During smolting (from 18 to 25 months old), losses were low and ranged from 0-3.4% (median = 0.2%) monthly mortality. The males were live-spawned and returned to a raceway to die naturally; there were no males which recovered to spawn a second year. However, intensive fish health management was required to control *Renibacterium salmoninarum* and *Salmincola californiensis* infections. We did not expect the *S. californiensis* infection, however, the copepod's presence on native trout in the watershed was apparently enough to infect the captive broodstock at 3 years of age. It was resistant to hydrogen peroxide treatments, however, SLICE® (emamectin benzoate) was very effective. We did anticipate the *R. salmoninarum* infection and implemented an aggressive control plan. A combination of azithromycin injections and oral treatments 2-4 times per year, in combination with biosecurity at the hatchery, was successful in controlling *R. salmoninarum* until the final year class.

32c. Phenotypic and genotypic heterogeneity amongst *Streptococcus iniae* isolates recovered from cultured and wild fish in North America, Central America and the Caribbean islands

Lucy Chou[1,2], Matt Griffin[3], Trellor Fraites[2], Cynthia Ware[3], Hugh Ferguson[4], Natalie Keirstead[2,5], John Brake[2], Judy Wiles[6], John P. Hawke[6], Rodman G. Getchell[7], Patricia Gaunt[3], Esteban Soto[1,2]*

[1] Center for Conservation Medicine and Ecosystem, School of Veterinary Medicine, Ross University, Main Island Road, West Farm, St. Kitts, West Indies LucyChou@students.rossu.edu; esoto@rossvet.edu.kn

[2] Department of Biomedical Sciences, School of Veterinary Medicine, Ross University, Main Island Road, West Farm, St. Kitts, West Indies TFraites@rossvet.edu.kn

[3] Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, PO Box 197, Stoneville, Mississippi, 38776, USA griffin@cvm.msstate.edu; cware@cvm.msstate.edu; Gaunt@cvm.msstate.edu

[4] School of Veterinary Medicine, St. George's University, PO BOX 7, St. George's, Grenada HFerguson@sgu.edu

[5] Drug Safety and Metabolism Unit, AstraZeneca R&D Boston, Waltham, Massachusetts 02451 Natalie.keirstead@astrazeneca.com

[6] Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Skip Bertman Drive, Baton Rouge, Louisiana 70803, USA jhawke1@lsu.edu; jwiles@vetmed.lsu.edu

[7] Aquatic Animal Health Program, Department of Microbiology and Immunology, College of Veterinary Medicine , Cornell University, Upper Tower Road , Ithaca , New York , 14853 , USA rgg4@cornell.edu

Members of the genus *Streptococcus* (specifically *S. iniae* and *S. agalactiae*) are important pathogens of cultured and wild fish species worldwide. Both are considered zoonotic agents that can cause severe diseases and mortality in humans and other mammals. During the last decade, outbreaks of streptococcosis have occurred in a wide range of cultured and wild fishes in the Americas and Caribbean islands. In this project, over thirty *S. iniae* isolates recovered from different fish species and locations were characterized phenotypically and genetically. Species identities were confirmed by biochemical identification, in addition to amplification and sequencing of the 16S rRNA gene. Repetitive element palindromic PCR (*rep-PCR*) fingerprinting, as well as biochemical and antimicrobial susceptibility profiles, suggest a single strain of *S. iniae* was responsible for two different disease outbreaks amongst reef fishes in the Caribbean, one in 1999 and another in 2008. Interestingly, a majority of the isolates recovered from cultured fish in the Americas were genetically distinct from the Caribbean isolates and presented a trend towards higher minimal inhibitory concentration (MIC) to several antibiotics and greater genetic variability. The biological significance of this genetic variability is unclear, but could have implications in future vaccine development and treatment.

32d. Phylogenomic analysis of *Weissella ceti* isolated from diseased rainbow trout (*Oncorhynchus mykiss*) and comparison with other *Weissella* species

Henrique C P Figueiredo, Siomar C Soares, Felipe L Pereira, Carlos A G Leal*

Aquacen – National Reference Laboratory for Aquatic Animal Diseases, Veterinary School, Federal University of Minas Gerais, MG, Brazil figueiredoh@yahoo.com

Weissella ceti is an emerging pathogen for cultivated rainbow trout (*Oncorhynchus mykiss*). The disease is characterized by acute cases of hemorrhagic septicemia. Outbreaks of *W. ceti* infections have been reported in rainbow trout farms at China, Brazil and USA and genetic relationships and diversity between isolates from those countries are unclear. The evolutionary and phylogenomic relationships between pathogenic and commensal species of *Weissella* are not well understood. Comparative analysis of whole genome sequence (WGS) data provides genetic information available for reconstructing the evolutionary history of pathogens. The aims of this study were to determine the phylogenomic relationships between *W. ceti* strains isolated from USA and Brazil, as well as, to evaluate evolutionary and pathogenomic relations between *Weissella* species. Brazilian strains of *W. ceti* WS08, WS74 and WS105 were sequenced with ION Torrent platform using fragment and mate-pair libraries; assembled with Mira and Newbler; annotated using RAST, Artemis, tRNAscan-SE, RNAmmer and the Interproscan database; and comparatively analyzed using Gegenees, SplitsTree4, PIPS, BRIG and BIGSdb. Interestingly, *W. ceti* clustered together with the non-pathogenic species *Weissella koreensis* in whole genome phylogenomic analysis; however, it appeared together with other pathogenic species, *Weissella cibaria* and *Weissella confusa*, in polymorphism-based, PAI and virulence factors phylogenetic analyses. *W. ceti* strains were shown to be very similar at the nucleotide level and genome synteny. Ten pathogenicity islands (PAIs) were found in this bacterium species. One of them (PAI 6a or 6b) was exclusively detected in strains WS74 and WS105, phage-like regions. PAI 2 is species-specific and two others present large deletions in other *Weissella* species. In conclusion, *W. ceti* seems to share PAIs and virulence factors with pathogenic *Weissella* species, has probably adapted to fish hosts by incorporating the variant PAIs, and presents different pathogenic lineages in Brazil resulting from the recent acquisitions of phage regions.

32e. Rainbow trout fed diets with varying content of marine and plant origin; how does that influence the outcome of experimental infections of the fry with *Flavobacterium psychrophilum* and *Yersinia ruckeri*?

Lone Madsen[1]*, Hans-Christian Ingerslev[2], Mette Boye[3], Inger Dalsgaard[4]

[1] National Veterinary Institute, Technical University of Denmark (DTU VET), Bülowsvej 27, 1870 Frederiksberg C, Denmark loma@vet.dtu.dk
[2] DTU VET hain@vet.dtu.dk
[3] DTU VET mboy@vet.dtu.dk
[4] DTU VET inda@vet.dtu.dk

Feed for rainbow trout aquaculture has traditionally been based on marine resources such as fish meal and fish oil. Because of a shortage of marine resources as well as the growing production of farmed fish, the feed industry has been forced to partially exchange fish meal protein with proteins derived from plants, like soy bean meal. This has been shown to affect the salmonid intestinal mucosa, and in addition, plant-based dietary proteins have been associated with changes in disease susceptibility in salmon and it has been suggested that these special diet types weakens the immune status of the fish.

One major cause for losses in Danish freshwater fish farms is the fry disease rainbow trout fry syndrome (RTFS), caused by the bacterium *Flavobacterium psychrophilum*, and experiences of the fish farmers suggest that the diet type is an important factor for disease development. Enteric redmouth disease caused by *Yersinia ruckeri* is also an economically important disease which causes problems in rainbow trout fry as well as larger fish.

Rainbow trout were fed from first-feeding with five different diets; diet A with marine fish oil (conventional fry diet), diet B (an organic version of A), diet C with rape seed oil (like B but with rape seed oil exchanging marine fish oil), diet C with pea protein (like B but added pea protein) and diet E with rape seed oil and pea protein. When the fry had reached sizes 1.5 g and 4 g, groups of fish from the five diet groups were infected with *Flavobacterium psychrophilum* and *Yersinia ruckeri*, respectively. An intraperitoneal injection model was used for *F. psychrophilum*, whereas a bath challenge was used for *Y. ruckeri*. Before and after infection, samples were taken from internal organs including the intestine for traditional bacteriology and only intestinal samples for next generation sequencing. The cumulative mortalities among the diet groups did not differ between groups in either of the two infection trials, suggesting that the diets did not have different effects on the immune status of the fish, when it comes to survival after infection, meaning that plant content did not seem to weaken the immune status of the rainbow trout fry. Results of the trial as well as the bacteriological examinations and the next generation sequencing results will be presented.

32f. Identification of O-antigen biosynthetic genes specific to Serotype 01 *Yersinia ruckeri:* role in virulence and protective immunity

Timothy J. Welch[1]*, Scott E. LaPatra[2]

[1]National Center for Cool and Cold Water Aquaculture, ARS-USDA, Leetown, WV 25430 USA tim.welch@ars.usda.gov
[2]Research Division, Clear Springs Foods, Inc., Buhl, ID 83316 USA scott.lapatra@clearsprings.com

Yersinia ruckeri, the etiologic agent of enteric redmouth disease, causes a hemorrhagic septicemia that primarily affects farmed rainbow trout (*Oncorhynchus mykiss*, Walbaum). *Y. ruckeri* strains comprise several O-serotypes; however the majority of outbreaks are caused by serotype O1 isolates of this pathogen. Here, we used genome sequencing to identify a large cluster of O-antigen biosynthetic genes specific to serotype O1 *Y. ruckeri* strains. This cluster consisted of genes encoding proteins predicted to function in O-antigen and LPS biosynthesis including proteins predicted to function in the biosynthesis of Legionamic acid, a nonulosonic acid know to be part of the O-polysaccharide repeat of O1 *Y. ruckeri*. Targeted mutation of one of the identified nonulosonic acid biosynthesis genes (*nab2*) resulted in loss of LPS synthesis and cross reactivity with anti-O1 serotyping antisera. This loss of LPS biosynthesis was also shown to cause a dramatic reduction in serum resistance and a complete loss of virulence in a rainbow trout challenge model. Vaccination of rainbow trout with bacterin vaccines derived from the nab2 mutant and its wild type parent strain followed by IP injection challenge demonstrated that the LPS component of the bacterin vaccine is required to elicit a protective response. A similar protective response was observed when fish were vaccinated with purified LPS thus showing that LPS alone is sufficient to elicit a protective response. We also present a PCR-based method that can be used to specifically identify serotype O1 *Y. ruckeri* strains.

33) Continuing Education II: Existing & Emerging Programs, Procedures, and Issues Involving Aquatic Animal Health & Welfare for the Practicing Aquatic Veterinarian

33a. Business and Marketing 101 for the well run, profitable and enjoyable private aquatic animal practice

Jena Questen*

East West Veterinary Service, PO Box 5393, Greenwood Village, CO 80155 USA
drquesten@gmail.com

This presentation will discuss modern marketing for the veterinary practitioner, with an introduction to the most common social media outlets. We will simplify and discuss the roles of each of these to the veterinary practitioner, and demonstrate practical ways to utilize these tools to make an aquatic animal practice more efficient, productive, and profitable, while simultaneously saving time and improving management concerns.

33b. Global Initiatives to Advance Aquatic Veterinary Education

Palić[1]*, AD Scarfe[2], CI Walster[3], and L-D Urdes[4]

[1]Chair for Fish Diseases and Fisheries Biology, Faculty of Veterinary Medicine, Ludwig-Maximilians-Universität, Kaulbachstr. 37, München 80539, Germany. d.palic@lmu.de
[2]American Veterinary Medical Association, 1931 N. Meacham Rd., Schaumburg, IL, USA.
[3]The Island Veterinary Associates, 132 Lichfield Rd., Stafford, Staffordshire ST17 4LE, UK.
[4]University of Agriculture & Veterinary Medicine, Marasti Bvd. 59, Bucharest 71331, Romania

With the rapid growth of global aquaculture and an increase in disease outbreaks among aquatic animal species there is a pressing need to ensure an adequate workforce of well-trained and skilled of veterinarians and veterinary para-professionals to service the aquaculture industries. This aquatic veterinary workforce is equally important to providing a wide variety of services for aquaculture supporting industries, and governmental programmes targeted at the prevention, control and possibly eradication of aquatic animal diseases.

Several International, Regional or National initiatives to examine veterinary educational programmes to meet contemporary needs of society have begun. These include evaluations by the North American Veterinary Education Consortium, the European Association of Establishments for Veterinary Education and other organizations. In addition the World Organisation for Animal Health's (OIE) has provided tools for evaluating the infrastructure of country's veterinary workforce, and an OIE Ad hoc Group on Veterinary Education is examining the minimum veterinary educational needs to support a country's ability to respond to disease.

While many of these initiatives have yet to fully embrace aquatic veterinary education and training, preliminary information suggests a number of courses actually exit at many veterinary schools in Europe, America and Australia and New Zealand. In addition a number of continuing education and professional development (CEPD) programs focused on aquatic veterinary subject matter supplements traditional veterinary skills. In addition, the number training programs (sometimes assisted by OIE) that prepare veterinarians to provide services on behalf of government agencies to as part of a national regulatory workforce to produce 'Official Veterinarians' (in Europe) or 'Accredited Veterinarians' (in US, Australia, and elsewhere), are increasing. Furthermore, programmes to recognize private practitioners having 'day-one' or core competencies (knowledge skills and experience –KSEs– as generally required of any veterinary degree) obtained from are veterinary and non-veterinary curricula, CEPD or other sources. Similarly, advanced veterinary training and Board certification in aquatic veterinary medicine exist in N. America, Australia/New Zealand and a new initiative has now started in Europe.

Of interest to many veterinarians is a new Certified Aquatic Veterinarian Programme being coordinated by the World Aquatic Veterinary Medical Association which recognized veterinarians having core KSEs. The programme has is designed to allow veterinarians to obtain KSE from a variety of sources, including veterinary and non-veterinary academic programs, extra-curricular CEPD programs and self-study of eight core areas. These include both traditional veterinary subject matter found in veterinary curricula (e.g. pathobiology, epidemiology of important aquatic animal diseases, clinical diagnostics, therapeutic and biologic agents, public health, zoonotic diseases and seafood safety, etc.) and subject matter usually covered in pre-veterinary courses or non-veterinary curricula (e.g. Anatomy, physiology, life support systems, industry structure and), all with a focus on aquatic animals. It also requires KSE involving legislation and regulations that affect the practice of aquatic veterinary medicine.

33c. Aquatic veterinary education opportunities at AVMA-COE accredited schools

Jeffries, Jayme L[1,2*], Scarfe, A. David[3]

[1]College of Veterinary Medicine, University of Illinois, 2001 S. Lincoln Ave, Urbana, IL 61801
jjeffri2@gmail.com
[2]Headquarters Externship Program, American Veterinary Medical Association, 1931 N. Meacham Rd, Schaumburg, IL 60173
[3]American Veterinary Medical Association, 1931 N. Meacham Rd, Schaumburg, IL 60173
dscarfe@avma.org

The anticipated need for veterinary professionals and paraprofessionals capable of providing animal health support to the rapidly expanding farmed seafood industry has been established. In order to meet this need, adequate training opportunities which provide these individuals with the necessary knowledge and skills to serve successfully in these roles must be in place. Established offerings in aquatic medicine at veterinary schools have traditionally been diverse in content, variably offered, non-core in nature, and largely unadvertised outside of individual institutions. These characteristics have resulted in an unknown overall status of the profession's capacity to adequately prepare a veterinary workforce in this field. To address this, we developed a survey tool that was distributed to all AVMA-COE accredited veterinary schools which aimed to discover the opportunities available in aquatic medicine available at each institution. Results summarized in this report will enable the profession to evaluate the current state of training opportunities, in order to support measures to refine, expand or standardize the current system in a way that enables meeting future goals in workforce supply for aquatic veterinarians.

33d. National veterinary accreditation program (NVAP) – Aquatics modules

Lynn H. Creekmore[1]*, Christa L. Speekmann[2], Kathleen H. Hartman[3]

[1]USDA APHIS VS, 2150 Centre Ave, Bldg B, MS 3E13 Fort Collins, CO 80526 USA
Lynn.H.Creekmore@aphis.usda.gov
[2]USDA APHIS VS, 4700 River Road, Riverdale, MD 20737 USA
Christa.L.Speekmann@aphis.usda.gov
[3]USDA APHIS VS, 1408 24th Street, SE, Ruskin, FL 33570 USA
Kathleen.H.Hartman@aphis.usda.gov

In order to improve and expand regulatory veterinary support and services to U.S. aquaculture industries, USDA APHIS has developed three NVAP modules specifically for aquatic animal health topics and issues. The goal of the NVAP program and the aquatics modules are to focus on information that accredited veterinarians should possess in order to conduct regulatory activities in aquatic animals, such as reporting outbreaks of reportable aquatic animal diseases and issuing export health certificates. NVAP module 13 addresses "aquatic animal health regulations and health certification"; module 14 covers the "evaluation of aquatic animals for detection of reportable diseases and pathogens; and module 15 reviews "preventing disease introduction and spread in aquaculture". Currently, modules 13 and 15 are available online, at no cost, to APHIS accredited veterinarians and anyone interested in viewing the material. Each module takes about an hour to go through and contains self-graded knowledge assessments. Module 14 is expected to be posted later this year. Modules may be found at this website: http://www.aphis.usda.gov/wps/portal/aphis/home/?1dmy&urile=wcm%3apath%3a%2Faphis_content_library%2Fsa_our_focus%2Fsa_animal_health%2Fsa_vet_accreditation%2Fct_aast.

33e. Components of a model certificate of veterinary inspection useful for ensuring fish are not infected with priority diseases

A.D. Scarfe*

American Veterinary Medical Association, 1931 N. Meacham Rd., Schaumburg, IL, USA.
dscarfe@avma.org

Certificates of Veterinary Inspection (CVI), frequently referred to as "Health Certificates," are one of the most important documents needed to ensure animals are not infected with infectious and contagious disease-causing agents. They are useful for a number of purposes, but perhaps the most important being documenting that animals moved in commerce are free from specific pathogens of concern. Frequently they are a regulatory requirement imposed by a government agency that seeks to prevent the introduction of an important disease from entering areas under their jurisdiction. CVIs can also contribute to disease control and eradication at any level (from the farm to the nation), such as envisaged in the U.S. Disease Traceablity Program[a].

Numerous examples of CVI or "Health Certificates" have been in use with for terrestrial livestock for many decades. To increase their utility many are now becoming available electronically as e-CVIs using web-based systems. In the U.S., the Federal government is currently developing standards for the use of CVIs in interstate movement of animals[b]. However, those currently available for aquatic livestock (farmed aquatic animals) are often inconsistent in capturing all the necessary data needed, and are difficult to use.

When correctly designed a CVI that contains specific information about the source, destination, identity and conditions of the animals that are inspected, the results of clinical and diagnostic disease screening, and certifying statements by the person issuing the certificate[c] (and, if required, endorsed by a government agency) can accomplish these goals.

In building on CVIs used for terrestrial livestock and earlier concepts about the information needed in a CVI for finfish and other aquatic livestock[d], the AVMA's Aquatic Veterinary Medicine Committee (AqVMC) have been considering a model CVI that might accomplish these primary goals, and be simple and inexpensive to use. By illustrating how a model CVI may be structured to capture the information needed, the AqVMC hopes to lay the groundwork for others to develop suitable CVIs for their specific purposes and needs.

[a]See http://tinyurl.com/nw3sxmu for more details. Note – as currently outlined the U.S. Animal Traceability Program has yet to include aquatic livestock (farmed species used in aquaculture).
[b]United States Department of Agriculture, Animal & Plant Health Inspection Service (2012). Data Standards for Interstate Certificates of Veterinary Inspection (ICVI). Accessible at http://tinyurl.com/qdno3po.
[c]In the U.S., Australia, New Zealand and some other countries regulatory functions for issuing CVIs are usually given to "Accredited Veterinarians"; in the EU these veterinarians are called "Official Veterinarians."
[d]Starling, D. Palić & AD Scarfe (2007). Refinement and Use of Certificates of Veterinary Inspection (Health Certificates) for Optimal Assurance of Disease Freedom in Aquatic Animals. *In*: Dodet B & OIE Scientific & Technical Department (eds). The OIE Global Conference on Aquatic Animal Health. *Dev. Biol.* (Basel), 129: 103-113.

35) Continuing Education III: Existing & Emerging Programs, Procedures, and Issues Involving Aquatic Animal Health & Welfare for the Practicing Aquatic Veterinarian

35a. The trials and tribulations of wild-caught ornamental fish: A veterinarian's perspective on the collection, transport and sustainability of wild-caught ornamentals

Timothy J. Miller-Morgan[1,2*], Jerry R. Heidel [1,2,3]

[1]Oregon Sea Grant–Aquatic Animal Health, College of Veterinary Medicine, Oregon State University, tim.miller-morgan@oregonstate.edu
[2]Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Oregon State University
[3]Department of Biomedical Sciences, College of Veterinary Medicine, Oregon State University

Wild-caught ornamental fisheries exist in many countries including: Brazil, Columbia, India, Indonesia, Sri Lanka, the Philippines, and Malaysia. There has been much debate about whether or not many of these fisheries are sustainable. While a number of these fisheries are sustainable or have the potential to be sustainable there are always opportunities for improvement of general health management throughout the chain of custody.

The journey for a wild-caught ornamental fish may be prolonged and highly stressful. Morbidity and mortality among these fish throughout the chain of custody from the wild to the wholesale/retail facility increases collection pressure on wild populations and potentially results in a significant economic cost to the industry.

In other animal industries the involvement of veterinarians on the husbandry team to assist with animal health assessment, recommendation of clinically proven treatment and management methodologies, and participation in husbandry staff training regarding animal health management has been shown to have a positive impact on overall animal health.

Over the last 12 years the Aquatic Animal Health program has had the opportunity to work with a number of sectors within the ornamental fish industry dealing in wild-caught ornamental fish from freshwater and marine environments.

The focus of each project was to characterize the health status and health management procedures of wild-caught ornamental fish in the country of origin before export or as they arrived in the United States. Once these characterizations had been established staff would begin to work with key stakeholders to refine or develop health management methodologies based upon these findings. The ultimate goal of each project is to reduce overall morbidity and mortality resulting in reduced collection pressure on the wild environments, increased fish survival, and improved industry economics.

We will discuss some of our general findings as well as the practical aspects of this type of industry/veterinary partnership, some of the general health management techniques we developed for import facilities, the specific benefits to the partner companies and our observations of fish health and husbandry at facilities in the export countries.

35b. Principles of biosecurity for the ornamental fish industry - How you can help your clients improve fish quality and health

Timothy J. Miller-Morgan[1,2]*, Jerry R. Heidel [1,2,3]

[1]Oregon Sea Grant–Aquatic Animal Health, College of Veterinary Medicine, Oregon State University, tim.miller-morgan@oregonstate.edu
[2]Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Oregon State University
[3]Department of Biomedical Sciences, College of Veterinary Medicine, Oregon State University

Biosecurity consists of the practices and procedures used to prevent the introduction, emergence, spread, and persistence of infectious agents and disease within and around fish production and holding facilities. Furthermore, these practices help eliminate conditions that can enhance disease susceptibility among the fish. In short, biosecurity precautions are put in place to exclude and contain fish pathogens. Biosecurity practices are applicable to all levels of the ornamental fish industry: producers, wholesalers, retailers, and hobbyists. Proper use of biosecurity measures will help prevent introduction of infectious disease in a fish facility, and will also help minimize the risk of diseases being passed from producer to hobbyist. Such practices will lead to a more sustainable industry since decreased or reduced disease leads to: reduced wild collection to replace losses; decreased losses among broodstock and grow out fish lots; decreased financial output to treat or manage disease outbreaks; and improved overall quality of fish for the export or domestic market. As import-export regulations for ornamental fish become increasingly stringent on a global level, veterinarians may be called upon to assist ornamental fish facilities in the planning and implementation of biosecurity programs. We will present a brief overview of the major considerations that should be taken into account when developing a biosecurity program for an ornamental fish facility.

Designing and implementing biosecurity practices can be simplified if we consider some basic themes**: pathogen exclusion**, **pathogen containment**, and **basic best health practices**. We will consider the elements of each, and show how these elements will allow you to hinder access of pathogens into a facility, control the spread of pathogens that may emerge, and promote high health and disease resistance among the fish in the facility. The overlap of practices addressing these themes will become evident.

Basic biosecurity procedures are uniform across the industry, but the biosecurity plan will be tailored to meet the special needs of each business. As the scope, needs, and finances of the business change, the facility manager will modify and adjust biosecurity measures accordingly, yet maintain the basic tenets of good biosecurity practices.

35c. Developing & implementing practical aquatic veterinary biosecurity programs to meet international (OIE) standards & national regulations

D. Palić[1]*, A.D. Scarfe[2], C. Walster[3]

[1]Chair for Fish Diseases and Fisheries Biology, Faculty of Veterinary Medicine, Ludwig-Maximilians-Universität, Kaulbachstr. 37, München 80539, Germany. d.palic@lmu.de
[2]American Veterinary Medical Association, 1931 N. Meacham Rd., Schaumburg, IL, USA.
[3]The Island Veterinary Associates, 132 Lichfield Rd., Stafford, Staffordshire ST17 4LE United Kingdom

The concepts behind the components of ideal aquaculture biosecurity programs that are effective and practical for producers, and meet national and international objectives have been discussed for a number of years in different forums. To be truly effective and acceptable for aquaculture industries and regulatory agencies biosecurity programs need integration of numerous we-accepted processes and procedures, and should also:

- be practical and economic;
- focus only on infectious and contagious diseases;
- address the prevention, control and eradication of diseases in or on a definable epidemiological unit/s;
- be based on well-established, sound scientific-justifiable veterinary procedures that are incorporated in internationally accepted standards (OIE Code and Manual); and,
- involve public-private partnerships with collaboration between producers, aquatic veterinarians, paraveterinary professionals, and regulatory authorities.

At the core of a biosecurity program is defining an epidemiologic unit (a well-defined geographical population of animals – e.g. a farm) on which all biosecurity steps or processes will be implemented. A second important principle is that all procedures implemented for a selected epidemiological unit must be thought out ahead of time, and well documented. This requires both an *a priori* evaluation, and a written biosecurity plan that addresses all steps and processes, specific for the unit under consideration. Third, as these procedures are implemented, they must be fully documented. Along with periodic on-site evaluation of operations and animals, the written plan and the documentation of implemented procedures becomes the focus for auditing and certification.

To be effective and justifiable the processes and procedures need to involve several formal processes, including:

- hazard and risk analysis (hazard identification and prioritization, risk assessment/evaluation, risk management and/or mitigation, and communication);
- analysis and remediation of critical control points (including evaluation and mitigation plans for correcting practices where disease could enter or leave the epidemiological unit);
- epidemiological principles (including necessary diagnostics, surveillance, monitoring and determining the status or freedom of diseases in the epidemiological unit);
- emergency preparedness (contingency protocols for disease control and eradication);
- auditing and certification of procedures and records that provide assurance of disease freedom (the latter of which are useful as compliance incentives).

Abstracts for Poster Presentations

P-1. ISA virus of low and high virulence spread differently in Atlantic salmon after infection by immersion challenge

Maria Aamelfot[1]*, Alastair McBeath[2], Debes Christiansen[3], Iveta Matejusova[2], Knut Falk[1]

[1]Norwegian Veterinary Institute, Ullevålsveien 68, Pb 750 Sentrum, N-0106 Oslo, Norway, maria.aamelfot@vetinst.no
[2]Food and Veterinary Authority, Falkavegur 6, FO-100 Tórshavn, The Faroe Islands
[3]Marine Scotland Science, 375 Victoria Road, PO Box 101, Aberdeen, AB11 9DB, Scotland

Infection with infectious salmon anemia virus (ISA virus) causes disease of varying severity in Atlantic salmon farming. Observations from the field suggest that variations in the host environment and differences in virulence among the various ISA virus strains cause differences in mortality and disease progression in salmon.

In the study, the spread of ISA virus isolates with high and low virulence and pathology and mortality after infection was examined using an immersion challenge. Immersion infection is more similar to natural infection than infection with injection, thereby providing a more accurate picture of what happens with natural infection in the sea. The experiment was performed at VESO Vikan, in Norway. Spread of the virus was examined using real time PCR and immunohistochemistry in several organs, including blood.

The low virulent virus replicated faster and produced more protein in gills, heart and kidney at earlier time points after immersion than the highly virulent virus. This was particularly noticeable in the gills with real time PCR. The highly virulent virus reached a higher peak viral load in all the tested organs and blood. The highly virulent virus caused 100 % mortality 21 days after infection, while the low virulent caused 15 % mortality.

The results of the study indicate that the highly virulent and low virulent viruses are taken up and spread differently in the salmon. This may be one of the reasons for the large differences in mortality and pathological changes between the groups. The way the fish were infected (i.e. by immersion) may have been essential to see the differences between virus strains.

P-2. Expression and purification of recombinant outer membrane proteins and secreted proteins of *Aeromonas hydrophila* strain ML09-119

Hossam Abdelhamed*, Attila Karsi, Mark L. Lawrence

Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS. 39762-6100, USA ha240@msstate.edu, karsi@cvm.msstate.edu, lawrence@cvm.msstate.edu.

Aeromonas hydrophila is a reemerging pathogen of channel catfish, and outbreaks from 2009 to 2012 have caused the loss of more than 8 million pounds of catfish in Alabama and Mississippi. Genome sequencing revealed a clonal group of isolates with unique genetic and phenotypic features that is highly pathogenic in channel catfish. Comparison of the genome sequence of a representative catfish isolate (ML09-119) from this pathogenic clonal group with other *A. hydrophila* isolates revealed several outer membrane proteins and secreted proteins unique to strain ML09-119. The goal of this research was to determine how effective these proteins will be in stimulating specific protective immunity in catfish against *A. hydrophila* infection. Therefore, we amplified, expressed, and purified nine *A. hydrophila* proteins, including fimbrial proteins, a transferrin-binding protein, major outer membrane protein OmpA, and others. Immunogenic potential of these proteins will be determined in juvenile catfish by injection followed by experimental infection with *A. hydrophila* strain ML09-119.

P-3. Deletion of TolQ and TolR in *Edwardsiella ictaluri* and effects on virulence

Hossam Abdelhamed*, Jingjun Lu, Mark L. Lawrence, Attila Karsi

Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762-6100, USA 6100, USA ha240@msstate.edu, jlu@cvm.mssate.edu, Lawrence@cvm.msstate.edu, karsi@cvm.msstate.edu

Edwardsiella ictaluri is a Gram-negative facultative intracellular pathogen causing enteric septicemia of channel catfish (ESC). In Gram-negative bacteria, the Tol system consists of four envelope proteins, TolQ, TolR, TolA, and TolB, and it functions to maintain outer membrane integrity and in macromolecule transport. It also contributes to virulence of several pathogens. TolQ and TolR are the two most common proteins of the Tol-Pal system, sharing structural and functional similarity with ExbB/ExbD and MotA/MotB. To understand the role of TolQ and TolR in *E. ictaluri* pathogenesis, we developed *Ei*ΔTolQ, *Ei*ΔTolR, and *Ei*ΔTolQR mutants using an in-frame deletion technique. Our results indicated that deletion of TolQ and TolR did not affect growth kinetics of *E. ictaluri* in both iron-replete and iron-depleted media. Their attenuation and vaccine efficacy were determined in catfish fingerlings following experimental infection by bath immersion. The *Ei*ΔTolQ, *Ei*ΔTolR, and *Ei*ΔTolQR mutants showed 28.93%, 19.70%, and 39.82% mortalities compared to 46.91% mortalities caused by strain 93-146. Also, the three mutants were able to protect channel catfish against subsequent infection with wild type *E. ictaluri*.

P-4. Response of *Piscirickettisa salmonis* to iron limited conditions

O Alamarza[1], C Segovia[1,2], V Maracaja-Coutino[3], C Sanchez[3], J Santander[1,2,3,4]*

[1]Microbiology and Immunity Laboratory, Faculty of Sciences, Universidad Mayor, Huechuraba, Chile 8580745
[2]Integrative Genomics PhD program, Faculty of Sciences, Universidad Mayor, Huechuraba, Chile 8580745
[3]Center for Genomics and Bioinformatics, Faculty of Sciences, Universidad Mayor, Huechuraba, Chile 8580745
[4]School of Life and Sciences, Arizona State University, Tempe, Arizona 85287.
jasantanderm@asu.edu

The aquaculture industry is one of the most important sources of human food and it has the fastest growth-rate of all animal-producing food sectors. In Chile, as well as in the global aquaculture industry, infectious diseases are the most serious issue that treats substantiality production. The salmon-culture is the biggest fish aquaculture in the country and among of the top 3 producers in the world. The bacterial pathogen with major impact in the national salmon industry is *Piscirickettsia salmonis*, a fastidious Gram-negative, facultative intracellular pathogen, causative agent of the pathology Salmonid Rickettsial Septicemia (SRS) or piscirickettsiosis. Recent studies showed that different levels of susceptibility to *P. salmonis* infection between different salmonid fish populations, correlates with variations observed in the expression of iron withholding mechanism-related genes, like ferritin and transferrin, two proteins responsible for sheltering iron ions inside the host cell. These suggests that salmonid fish displays a defense mechanism aimed to avert the proliferation of the infecting bacteria by maintaining extremely low concentrations of free iron in the infected tissue. Bacteria have evolved different mechanisms to obtain iron directly from the natural reserves of the host, these strategies consider the use of soluble molecules like siderophores or surface attached proteins that bind and sequester iron directly from the host cell iron transport machinery. Here we determined the optimal conditions to grow *P. salmonis* under iron-limited conditions. Total mRNA was purified from *P. salmonis* grown under iron-rich and iron-limited. The genome-wide *P. salmonis* transcriptional response under iron replete and iron limited conditions was characterized using RNA-seq. We identified the major component of the iron acquisition system of *P. salmonis*, including iron regulated uptake proteins, hemolytic proteins and *fur* regulated genes, allowing understanding *P. salmonis* physiology and pathogenesis.

P-5. Evaluation of nonlethal sampling techniques for Infectious Hematopoietic Necrosis Virus in steelhead (*Oncorhynchus mykiss*)

David R. Burbank*, Luciano V. Chiaramonte, Tyson R. Fehringer, Phil M. Mamer

Eagle Fish Health Lab, IDFG, 1800 Trout Rd., Eagle, ID 83616 USA
david.burbank@idfg.idaho.gov

Prior to moving or liberating fish from a hatchery, a subset of the population is sampled for pathogens to identify risks to the receiving facility/body of water. Currently, standard protocols involve taking a lethal sample which can create problems when dealing with highly valued or endangered fish. Although nonlethal samples for salmonid viral pathogens of concern have been previously evaluated, many of these studies used more specific and generally more sensitive molecular assays instead of cell culture. However, the specificity of primers and the difficulty in detecting viral replicating agents versus genetic material make molecular methods undesirable when looking for multiple virus types. Furthermore, comparative studies between accepted lethal sampling techniques using kidney and spleen tissue and lesser evaluated nonlethal techniques are limited. Our lab evaluated three previously identified nonlethal sampling methods (mucus/skin scrape, pectoral fin tissue and gill tissue) for viral replicating agent detection using cell culture and compared these results to a standard lethal sample (pooled kidney and spleen tissue). Samples were collected from steelhead (*Oncorhynchus mykiss*) broodstock historically demonstrating natural infections with infectious hematopoietic necrosis virus (IHNV) at the Dworshak National Fish Hatchery over three separate spawning events. Of the three nonlethal samples collected, both mucus/skin 83% (33/40) and fin preparations 83% (33/40) appeared to be more sensitive than pooled kidney and spleen tissue 45% (18/40) at detecting IHNV, while detections in gill tissue were less frequent 20% (8/40). Although the ability to detect viral replicating agents from a nonlethal sample exposed to the environment may be less than ideal for determining an active infection for a specific fish, it may provide a picture of the overall health of a population. Accordingly, this information can be used in decision making prior to movement or liberation, while decreasing lethal sampling and increasing fish in the river.

P-6. A refined pulsed field gel electrophoresis method to characterize *Flavobacterium columnare*

Tina C Crosby*, Charles M Gieseker

Office of Research, Center for Veterinary Medicine, US Food and Drug Administration, 8401 Muirkirk Road, Laurel, MD 20708 USA tina.crosby@fda.hhs.gov, charles.gieseker@fda.hhs.gov

Flavobacterium columnare, a fish pathogen causing Columnaris disease, leads to extensive mortality in many commercially important aquacultured fish species worldwide. Soto et al. (2007) developed a pulsed field gel electrophoresis (PFGE) method to genotype *F. columnare* and to correlate the PFGE grouping to virulence. The PFGE method developed, however, did not work as well for some *F. columnare* strains that have adherent cells. These cells form clumps that yield poor band resolution due to incomplete cell lysis and enzymatic cuts. In addition, the enzyme used, MluI, generates many bands that lie close together, making analysis difficult (Figure 1). We refined the method to include a de-clumping step to allow better cell lysis and improved enzymatic digestion. We also identified a different enzyme, PasI, which provides fewer and better differentiated bands (Figure 2). The development of a PFGE method for *F. columnare* that works for both adherent and non-adherent strains is critical because the new method may be used to differentiate among bacterial strains; identify possible correlations between subgroups; and determine epidemiological relatedness.

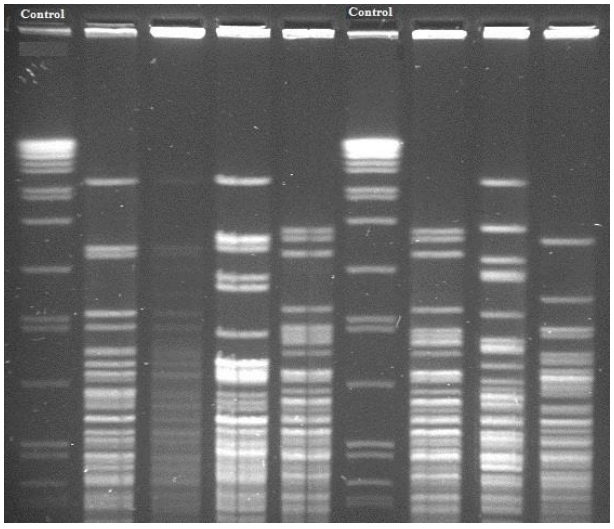


Figure 1 – MluI Enzyme

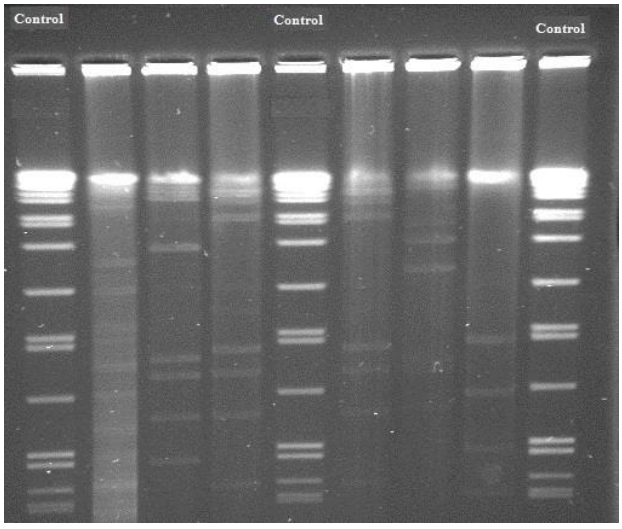


Figure 2 – PasI Enzyme

P-7. Bacteria from Chilean salmonid farms exhibit growth inhibition of fish pathogens and quorum sensing blocking

Mery de la Fuente[1,2], Claudio D Miranda[3,4]*, Paz Jopia[2], Nicolás Guiliani[5]

[1]Centro Regional de Estudios Ambientales, 2766 Cristobal Colón Avenue, Talcahuano, Chile merydelafuente@gmail.com
[2]Laboratorio de Biopelículas y Microbiología Ambiental, Centro de Biotecnología, Universidad de Concepción, Concepción, Chile pjopia@udec.cl
[3]Aquatic Pathobiology Lab, Department of Aquaculture, Universidad Católica del Norte, 1281 Larrondo, Coquimbo, Chile cdmirand@ucn.cl
[4]Centro de Estudios Avanzados en Zonas Áridas (CEAZA), 1281 Larrondo, Coquimbo, Chile
[5]Laboratorio de Comunicación Bacteriana, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile nguilian@uchile.cl

The main aim of this study was to screen in 80 Gram negative strains isolated from various sources of freshwater Chilean salmonid farms the presence of bacterial inhibitory activities including growth inhibition of the fish pathogens *Aeromonas hydrophila*, *Vibrio anguillarum* and *Flavobacterium psychrophilum* and quorum sensing blocking. Only 10 strains belonging to the *Pseudomonas* genus inhibited at least one of the assayed fish pathogens and from these, 2 strains were able to inhibit all assayed pathogenic species. Siderophore production by the inhibitory strains was determined using the chrome azurol S assay, observing that 9 strains produced siderophores. Apparently, siderophores produced by the inhibitory strains are mainly attached to the cell membranes and are not shed into the supernatant because the activity of these strains was more efficient in the coculture assay compared to the activity of the cell-free supernatant. When the 80 strains were examined for QS blocking activity using acyl homoserine lactone (AHL) monitor strains *Chromobacterium violaceum* CV026 and *Agrobacterium tumefaciens* NT1 (pZLR4), only strains *Pseudomonas putida* FF16 and *Raoultella* sp. R5B1 exhibited a quorum sensing blocking activity. Using thin-layer chromatography developed with the *A. tumefaciens* NTL4 (pZLR4) reporter strain was determined that both QS blocking strains produced C6-HSL and C8-HSL type molecules. Strain R5B1 did not show growth inhibition properties, whereas strain FF16 produced siderophores and also inhibited growth of *A. hydrophila* and *F. psychrophilum*, becoming a good probiotic candidate to be used in fish farming.

P-8. Quantification of *F. columnare* in tissues using real-time polymerase chain reaction

G. Derek Gibbs[1], Michael J. Mauel[2]*, Matthew J. Griffin[3], Mark L. Lawrence[1]

[1]Mississippi State University, College of Veterinary Medicine, 240 Wise Center Drive, P.O. Box 6100, Mississippi State, MS 39762 gdg7@msstate.edu, lawrence@cvm.msstate.edu
[2]Mississippi State University, College of Veterinary Medicine, Veterinary Research and Diagnostic Laboratory, 3137 Highway 468 West, P.O. Box 97813 Pearl, MS 39208 mmauel@mvrdl.msstate.edu
[3]Mississippi State University, College of Veterinary Medicine, Thad Cochran National Warmwater Aquaculture Center, 127 Experiment Station Road, P.O. Box 197, Stoneville, MS 38776 griffin@cvm.msstate.edu

Flavobacterium columnare, the causative agent of Columnaris disease, infects a wide variety of freshwater and brackish water fishes. Columnaris disease has been responsible for significant losses to farm-raised channel catfish *Ictalurus punctatus,* which makes it of particular concern to commercial producers in the southeastern United States. Using modifications to previously established protocols, specifically targeting a 203 bp nucleotide region of the chondroitin AC lyase gene. The assay was tested to determine its ability to detect isolates from separate *F. columnare* genomovars. There were no significant differences between the isolates, suggesting limited variation in copy numbers of the target gene. The linear dynamic range for the isolates covered 6 orders of magnitude ranging from 10 to 1.0E6 copies of target DNA. Tissues from gill, liver, spleen, and kidney experimentally inoculated with live bacteria did not adversely affect the sensitivity of the assay. The specificity of the assay was tested against 5 taxonomically or ecologically relevant isolates; *Flavobacterium johnsoniae, Pseudomonas aeruginosa, Aeromonas salmonicida, Edwardsiella tarda,* and *Edwardsiella ictaluri*. There was no amplification of the target sequence for any of these organisms. Following the completion of assay development, channel and hybrid catfish fingerlings were challenged with a *F. columnare* isolate from each genomovar, one a highly virulent isolate and an isolate of low virulence. The real-time PCR was used to determine changes in the concentration of bacteria within catfish tissues occur over time. The results presented here give insight into the pathogenesis of *F. columnare* and a possible protocol for early detection of columnaris infections. Early detection will give the producer time to prevent an extensive outbreak.

P-9. Pox virus related gill disease in Atlantic salmon

Mona Gjessing*, Ole Bendik Dale, Torstein Tengs

For almost 20 years, a special kind of gill disease has been described in some disease outbreaks of salmon fry. The farms typically experience acute, high mortality. It is often a recurrent problem in some farms and reports on substantial losses are common. Histopathological changes are typically apoptosis of epithelial cells in the gills. In such gills, we have detected pox-like virus particles by electron microscopy and molecular results strongly suggest that this is a previously undescribed pox virus.

Results related to pathological changes and viral molecular characteristics will be presented.

P-10. The first report of a Hepadnavirus isolated from fishes: evidence of Hepatitis B Virus infection in white sucker (*Catostomus commersoni*) from the Great Lakes region

Cassidy M. Hahn[1]*, Luke R. Iwanowicz[2], Vicki S. Blazer[2], Robert S. Cornman[3]

[1]West Virginia University, Division of Forestry and Natural Resources, Morgantown, WV 26506, USA Cassidy.Hahn@gmail.com
[2]USGS, Leetown Science Center, Fish Health Branch, 11649 Leetown Road, Kearneysville, WV 25430, USA liwanowicz@usgs.gov, vblazer@usgs.gov, rcornman@usgs.gov
[3]USGS, Leetown Science Center, Ecosystems Mission Area, 11649 Leetown Road, Kearneysville, WV 25430, USA rcornman@usgs.gov

A novel hepadnavirus has been identified in liver and skin samples from white sucker collected in the Great Lakes Region. Raised skin lesions of different morphological presentations and liver samples were collected from white sucker (*Catostomus commersoni*) to establish a transcriptome from which biomarkers of physiological status could be identified. Total RNA was extracted from these sample types and enriched for non-ribosomal RNA. Barcoded libraries were prepared for an RNA-seq workflow and sequenced using an Illumina HiSeq 2000. Contigs were generated bioinformatically and BlastX searches against the NCBI viral database indicated the presence of a Hepatitis B-like virus. Numerous lines of evidence support that this is a legitimate Hepatitis B virus present in replicative stages in both the liver and skin of white suckers. Both viral DNA and RNA transcripts have been amplified and sequenced. Re-sequencing of the virus via Sanger sequencing has confirmed that the genome is circular and conforms to the prototypical codon organization utilizing three overlapping open reading frames. The genome is approximately 3543 base pairs and most similar to Duck Hepatitis B Virus (3027 bp). All seven viral proteins have been identified by similarity searches and percent amino acid identities range from 29 to 43%. Screening of liver and skin samples (n=20) for viral DNA was positive in 20% of individuals indicating that the virus is not present in all fish and, therefore, is not simply an endogenous viral relic. To date, Hepatitis B viruses have not been isolated from fishes. While it is unknown whether this virus is associated with pathology in the white sucker, in birds and mammals these viruses are typically associated with liver pathology including inflammation, necrosis, hemorrhage and hepatocellular carcinoma.

P-11. Development of an immunochromatographic test kit for rapid detection of fish iridovirus

Sue-Min Huang*, Tzu-Ming Huang, Chen Tu, Hsiang-Jung Tsai

Division of Biology, Animal Health Research Institute, Taipei, 25158 Taiwan
smhuang@mail.nvri.gov.tw

An immunochromatographic test was developed for rapid diagnosis of fish iridovirus infections by monoclonal antibodies against the major capsid protein (MCP). The kit detected specifically the MCP of fish Ranavirus and Megalocytivirus, and the test results of one strain of Lymphocystivius and 20 strains of nervous necrosis virus were negative. The detection limit was 10^5 $TCID_{50}$/ml for viral culture. Totally 159 cases of fish iridovirus infection confirmed by polymerase chain reaction detection in Taiwan during 2001 to 2007 were detected by the kit and the sensitivity was 86.8% (138/159). Among the tissues of infected orange spotted grouper (*Epinephelus coioides*), including gill, heart, spleen and kidney, the signal of spleen specimen was highest and the signal of gill specimen was low. For experimentally infected fish, the pooled tissues of high-dose inoculation (10^7 $TCID_{50}$) showed positive at 3day postinfection (DPI), and the pooled tissues of low-dose inoculation (10^2 $TCID_{50}$) showed positive at 5 DPI and remained positive to 12 DPI. The test kit should be useful for rapid clinical diagnosis of fish iridovirus infection.

P-12. Determination of *in vitro* antibacterial activity of some plant essential oils on fish pathogenic bacteria

Aysegul Kubilay[1]*, Pinar Yıldırım[1], Huseyin Fakir[2], Gulsen Ulukoy[3]

[1]Department of Aquaculture, Faculty of Fisheries, Suleyman Demirel University, 32260 Isparta, Turkey aykub@yahoo.com
[2]Department of Forest Botany, Faculty of Forestry, Suleyman Demirel University, 32260 Isparta, Turkey huseyinfakir@sdu.edu.tr
[3]Department of Aquaculture, Faculty of Fisheries, Mugla Sıtkı Kocman University, 48000 Mugla, Turkey gulukoy@mu.edu.tr

In this study, essential oil extracts of *Phlomis armeniaca, Juniperus excelsa subsp. excelsa, Myrtus communis subsp. communis, Eucalyptus camaldulensis, Calamintha nepeta subsp. nepeta and Calamintha nepeta subsp. glandulosa* plants were investigated for their antibacterial activity against six fish bacteria which were *Aeromonas hydrophila, Pseudomonas sp., Yersinia ruckeri, Vibrio anguillarum* and *Lactococcus garvieae, Staphylococcus epidermidis* by agar well diffusion. The essential oil extracts of *C. nepeta subsp. nepeta, C. nepeta subsp. glandulosa and E. camaldulensis* showed antibacterial properties against *Staphylococcus epidermidis* and *Yersinia ruckeri* pathogens. Plant essential oil extracts, *P. armeniaca, J. excelsa subsp. excelsa, M. communis subsp. communis,* did not show antibacterial activity against bacterial fish pathogens. The immunostimulant feature and antibacterial activity of used plant essential oils have already been known to be therapeutic for human health against many bacterial diseases, also they have found to be effective against some bacterial fish pathogens. The results from the agar well diffusion method showed that 3 plants essential oils could inhibit the growth of bacterial fish pathogens. The essential oil extracts of *C.nepeta subsp. nepeta, C.nepeta subsp. glandulosa* and *E. camaldulensis* plants were determined to be potentially effective on against causes of staphylococcosis and yersiniosis, bacterial fish diseases.

P-13. Whole genome sequencing and diversity analysis of *Francisella noatunensis* subsp. *orientalis* isolated from Nile Tilapia

Carlos A G Leal[1]*, Lucas A Gonçalves[2], Siomar C Soares[1], Felipe L Pereira[1], Vasco A C Azevedo[2], Henrique C P Figueiredo[1]

[1]Aquacen – National Reference Laboratory for Aquatic Animal Diseases, Veterinary School, Federal University of Minas Gerais, MG, Brazil carlosleal@vet.ufmg.br
[2]Laboratory of Celular and Molecular Genetics, Institute of Biological Sciences, Federal University of Minas Gerais, MG, Brazil

The *Francisella* species are intracellular facultative bacteria that cause a variety of infections in terrestrial and aquatic animals. In this genus, *F. noatunensis* subsp. *orientalis* (FNO) has a remarked importance as an emergent fish pathogen, associated with outbreaks in Nile tilapia (*Oreochromis niloticus*) farms. The disease causes high mortality rates during outbreaks and has been reported in several countries. However, the phylogenomic relationships between isolates from different countries, as well as, evolutionary history of this pathogen are poorly characterized. Here, we have striven to determine the evolutionary history and genetic diversity of FNO worldwide using genomic data. Six Brazilian strains of FNO isolated from diseased Nile Tilapia were sequenced. Briefly, the genome sequencing was performed with ION Torrent and Ilumina platforms using fragment libraries; assembled with Mira and Newbler; annotated using RAST, Artemis, tRNAscan-SE, RNAmmer, and the Interproscan database; and comparatively analysed using Gegenees, SplitsTree4, PIPS, BRIG, and BIGSdb. *Francisella* species accommodated in two previously described phylogenetic clusters of species: *Philomiragia* group, composed by FNO, *F. noatunensis subsp noatunensis*, *F. philomiragia* and *Francisella sp.*; and "*Tularensis* group", with all *F. tularensis* species and subspecies. Brazilian FNO strains were shown to be highly clonal and only presented genomic rearrangements compared to isolates from other countries. However, despite the clonality, the number of pseudogenes varied from 252 and 317 in the strains from Indonesia and Costa Rica to ~364 in all Brazilian strains. Nine pathogenicity islands were found in FNO, including the previously described FPI inside PAI 7. PAIs 2, 6 and 9 are absent from all other *Francisella* species (species-specific PAIs), whereas PAIs 1 and 8 are only present in "*Philomiragia* group". These data suggest that FNO has first adapted to fish hosts by PAI acquisition and it is now passing through variable rates of genome decay in different countries.

P-14. Novel Chinook salmon Bafinivirus isolations from Ontario fish health monitoring

Stephen D. Lord*, Melinda J. Raymond, Peter J. Krell, Andrew M. Kropinski, Roselynn M.W. Stevenson

[1]Department of Molecular & Cellular Biology, College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1
slord@uoguelph.ca, mjoyce@uoguelph.ca, pkrell@uoguelph.ca, kropinsk@queensu.ca, rstevens@uoguelph.ca

To monitor the health of fish populations, the Fish Health Laboratory uses standard tissue culture procedures to detect the presence of filterable agents (viruses) capable of producing cytopathic effects (CPE) on fish cell lines. A new fish virus was detected in the fall of 2008 in pooled tissue samples from terminal spawning Chinook salmon in the Credit River, a tributary to Lake Ontario (case ONT 5769). The fish were sampled as part of a routine egg collection and, in the resulting fry, no virus was isolated and no unusual mortalities were observed.
The virus was not found in spawning Chinook in 2009 or 2010, but was again isolated in the fall 2011 and 2012 populations.
The virus was first isolated from organ and reproductive samples tested on RTG-2 cells; the same fish samples showed no CPE on CHSE-214 or EPC cell lines. Blind transfers from the positive RTG-2 assays produced CPE on RTG-2 cells incubated at 15^{o}C, 20^{o}C and 25^{o}C and also on EPC cells at 25^{o}C and 30^{o}C. Virus suspensions were concentrated by centrifugation and stained with uranyl acetate for electron microscopy. Virus particles were 45 nm wide and 120-130 nm long, with a bacilliform shape, rounded at both ends.

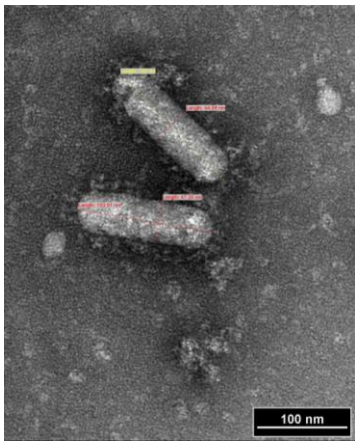


Figure 1: Electron micrograph of the 2008 Chinook virus.

Lack of growth following chloroform treatment suggested an enveloped virus, while growth in the presence of 5-iododeoxyuridine and treatment with RNase indicated the nucleic acid was RNA. PCR diagnostic tests using primers for the fish viruses IHNV, VHSV, KHV, LMBV and IPNV were negative. Other bacilliform viruses from fish have been reported, notably white bream virus (WBV) and fathead minnow virus (FHMV), but diagnostic primers for those viruses did not give positive PCR products.

An amplicon library prepared from RNA of purified cultured virus was sequenced by NGS and the primary sequence data, representing 80-fold coverage of the viral genome, was *de novo* assembled. While at the nucleotide level there was very little homology to the closest relatives, WBV and FHMV, the translated sequence showed a characteristic torovirus gene organization. However, a key conserved S-D-D signature motif of the replicase protein was missing in the Chinook isolate sequence. Based on the similarity of the size (27 kb) and the order and structure of the translated nucleotide sequence to those of WBV and FHM, the Chinook salmon virus appears to be a related but distinct isolate of the *Bafinivirus* genus (Salmon Bafinivirus). The significance of this virus in fish health is unknown but in routine health monitoring it can be a source of CPE in tissue culture.

Research support from the Ontario Ministry of Natural Resources, Fish Culture Section.

P-15. The relationship of infectious dose and lethal dose for two strains of the salmonid rhabdovirus IHNV that differ in virulence

Douglas G McKenney[1]*, Gael Kurath[2], Andrew R Wargo[3]

[1] Western Fisheries Research Center, USGS, 6505 NE 65th St, Seattle, WA 98122
dmckenney@usgs.gov
[2] Western Fisheries Research Center, USGS, 6505 NE 65th St, Seattle, WA 98122
gkurath@usgs.gov
[3] Virginia Institute of Marine Science, PO Box 1346, Gloucester Point, VA 23062
arwargo@vims.edu

Infection depends on the successful colonization of a host by a pathogen. For a virus to infect its host, it must come into contact with the host, gain entry into target cells, and replicate. There are a variety of factors that could influence the likelihood of a host becoming infected. It is typically assumed that the number of hosts infected will increase linearly as the concentration of virions in the environment increases. However, the relationship between viral concentration and infection could exhibit various forms, such as linear, logarithmic, or exponential. Furthermore, viral genetic differences could cause a genotype to be more effective at initiating infections at a given concentration. In this study we examined the relationship between dose size and the proportion of hosts infected by the fish rhabdovirus *Infectious hematopoietic necrosis virus* (IHNV), and whether this relationship differed between virus genotypes. We compared the dosage needed to infect 50% of hosts (ID_{50}) with a high (HV) or low (LV) virulence strain of the virus to determine how infectivity is associated with virulence. We exposed groups of fish to virus doses ranging from 10 to $2x10^5$ pfu/mL. Additionally, we ran experiments to calculate the dosage needed to kill 50% of fish (LD_{50}) and compared those values to the ID_{50} of each genotype. Both the ID_{50} and LD_{50} of the HV strain were lower than that of LV, by approximately 1 and 2.5 orders of magnitude, respectively. This indicates that the HV isolate was indeed more virulent and had an advantage over the LV isolate in initiating infections at viral concentrations found during epidemics.

P-16. Pathogenicity of *Vibrio splendidus* associated with massive mortalities of reared larvae of scallop *Argopecten purpuratus* (Lamarck, 1819)

Claudio D Miranda[1,2]*, Rodrigo Rojas[1], Rafael Opazo[3], Jaime Romero[3]

[1] Aquatic Pathobiology Lab, Department of Aquaculture, Universidad Católica del Norte, 1281 Larrondo street, Coquimbo, Chile cdmirand@ucn.cl

[2] Center for the Advanced Studies in Arid Zones (CEAZA), Web site: http://www.ceaza.cl, 1281 Larrondo Street, Coquimbo, Chile

[3] Biotechnology Lab, Institute of Nutrition and Food Technology, Universidad de Chile, 5524 El Líbano, Santiago, Chile ropazo2@yahoo.com ; jromero@inta.cl

Three strains (VPAP16, VPAP18 and VPAP23 strains) were isolated as the most predominant organisms from 3 different episodes of massive mortalities of larval cultures of the Chilean scallop *Argopecten purpuratus* occurred in different commercial hatcheries located in northern Chile. The main aims of this study were to identify the pathogenic strains and investigate their pathogenic activity. Based on selected phenotypic features and sequence identity of the 16S rRNA gene and the housekeeping gene, RNA polymerase α-chain *rpoA*, all pathogenic strains were identified as *V. splendidus*. Healthy 10–day–old scallop larvae cultures exhibited mortality percentages of 69.61 ± 3.35, 79.78 ± 6.11 and 61.73 ± 3.71% after 48 h when were inoculated with 1×10^6 CFU (colony forming units) mL^{-1} of VPAP16, VPAP18 and VPAP23 strains, respectively, and evidenced that concentrations $\geq10^4$ CFU mL^{-1} would probably be detrimental for the larval culture. The main clinical signs observed in challenged larvae for 24 h were bacterial swarms on the margins of the larvae, extension and disruption of the velum, detachment of velum cilia cells and digestive tissue necrosis. Otherwise, challenge assays using pathogenic strains stained with 5-([4,6-dichlorotriazin-2-yl]amino)fluorescein hydrochloride (5-DTAF) evidenced that after 1 h stained bacteria were detected in high density in the digestive gland and the margin of the shell. When larval cultures were inoculated with cell-free extracellular products (ECP) of *V. splendidus* strains, exhibited larval mortalities higher than 70% (VPAP16), 80% (VPAP18) and 50% (VPAP23) after 24 h, even when ECP were treated with proteinase K or heat, indicating that extracellular pathogenic activity is mainly mediated by non-proteic thermostable compounds. This study demonstrated for the first time the pathogenic activity of *V. splendidus* strains on reared-larvae of scallop *A. purpuratus* and prompts the necessity to maintain this species at concentrations lower than 10^4 CFU mL^{-1} to avoid episodes of mass mortalities in scallop hatcheries.

P-17. Inhibition of *Flavobacterium psychrophilum* adhesion *in vitro*

Anna Papadopoulou[1]*, Amy Howell[2], Tom Wiklund[1]

[1]Laboratory of Aquatic Pathobiology, Environmental and Marine Biology, Department of Biosciences, Åbo Akademi University, Tykistökatu 6, FIN-20520, Turku, Finland
apapadop@abo.fi

[2]Marucci Center for Blueberry Cranberry Research, Rutgers University, 125A Lake Oswego Rd. Chatsworth, NJ 08215, USA

Bacteria in aquatic environment have generally a strong tendency to attach and colonize any given surface, a survival mechanism which often represents a decisive step in the development of infectious disease. The fish pathogen *Flavobacterium psychrophilum* which occurs in two different phenotypes, rough and smooth, has recently been shown to have strong adhesive properties. The aim of this study was to screen the effect of certain anti-adhesive agents on the initial attachment of *F. psychrophilum* phenotypes *in vitro*. The adhesion experiments were done in 96-well *microtiter* plates *using* low nutrient conditions (natural lake water). The results showed that the cells of the rough phenotype showed poor adhesion in contrast to the cells of the smooth which exhibited a considerable attachment to polystyrene surface. This tight attachment of the smooth cells is suggested to be due to cell-cell adhesion. Carbohydrates, D- and L-amino acids, proteinase K, phytochemicals, fucoidan and an ion chelating agent significantly inhibited the adherence of cells of mainly the smooth phenotype. The results suggest that the anti-adhesive compounds blocked the cell to cell and not the cell to surface binding. We propose that these selected compounds inhibit cell adhesion by presumably inhibiting cell adhesion molecules-binding domain, suggesting a possible adhesion mechanism of *F. psychrophilum*. Based on these data, further studies are underway to determine whether these anti-adhesive agents are able to abrogate the bacterial cell contact with host target cells.

P-18. Antibiotic resistance testing of a genetic sublineage of *Renibacterium salmoninarum*

Linda D. Rhodes[1]*, A. Michelle Wargo Rub[2]

[1]Environmental & Fisheries Sciences Division, Northwest Fisheries Science Center, 2725 Montlake Boulevard East, Seattle, WA USA; linda.rhodes@noaa.gov
[2]Fish Ecology Division, Northwest Fisheries Science Center, Point Adams Research Station, 520 Heceta Place, Hammond, OR USA; michelle.rub@noaa.gov

Renibacterium salmoninarum is the etiologic agent for bacterial kidney disease (BKD) of salmon and trout, an endemic disease among free-ranging Pacific salmon and a persistent debilitating problem in hatcheries. In spite of decades of research, an effective vaccine against BKD for Pacific salmon has not been identified, and antibiotic treatments remain the principal pharmaceutical tool for managing infections. Because no antibiotics have been approved in the US for treating BKD, fundamental information such as minimum inhibitory concentrations (MIC) is often lacking. The most commonly used antibiotics have been macrolides (erythromycin and tulathromycin), although historically oxytetracycline was widely applied. Approval of an amphenicol, florfenicol, for a range of bacterial diseases in fish makes it a drug of interest for BKD. Furthermore, antibiotic combinations can significantly improve efficacy over single antibiotics, and underexplored for BKD. *R. salmoninarum* strains selected for testing are a genetic sublineage identified in a genome-wide analysis of 68 isolates from the UK, Norway, and North America. Two of the four sublineage strains were isolated from salmon receiving chemotherapeutic management, and one strain was previously shown to exhibit reduced susceptibility to macrolides. This study determines the susceptibility of the sublineage strains, compared to type strain ATCC 33209, to four relevant antibiotics (oxytetracycline, erythromycin, tulathromycin, florfenicol) and selected combinations of these antibiotics. This study permits comparisons between genome sequence variations and variations in antibiotic phenotype, as well as provides basic susceptibility data for future drug investigations.

P-19. Transcriptomics of temperature inducible chromosomal recombination in *Aeromonas salmonicida*

Cristopher Segovia[1,2], Manuel Ayala[1], Katherine Valderrama[1,3], Mario Moreno[4], Marcela Astete[4], Carolina Sanchez[4], Javier Santander[1,2]*

[1]Microbial Pathogenesis and Vaccinology Laboratory, Nucleus for Microbiology and Immunity, Faculty of Sciences, Universidad Mayor, Huechuraba, Chile
[2]Ph.D. Program in Integrative Genomics, Faculty of Sciences, Universidad Mayor, Huechuraba, Chile 8580745
[3]Ph.D Program in Aquaculture, Univerisidad Catolica del Norte, Coquimbo, Chile
[4]Sequencing Unit, Center for Genomics and Bioinformatics, Faculty of Sciences, Universidad Mayor, Huechuraba, Chile. javier.santander@umayor.cl

First described in the 19th century, *A. salmonicida* sp. *salmonicida* (here after *A. salmonicida*) is one of the oldest known fish pathogens. *A. salmonicida* is an important pathogen due to its nearly worldwide distribution, broad host range and potential devastating impacts on wild and farm fish. *A. salmonicida* is the causative agent of the so-called "typical" furunculosis, which causes economic losses in cultivated salmonids in fresh and marine waters. It also affects a variety of non-salmonid fish. *A. salmonicida* can be cultivated at temperatures as high as 34.5°C. At temperatures over 22°C its chromosome and virulence plasmid undergo recombination. Actually, the recommended temperature for the culture of *Aeromonads* has been as high as 28°C, it is likely that several *A. salmonicida* isolates, have significant differences with the original virulent isolated strain. In fact, American Type Culture Collection (ATCC) used to recommend temperatures of 26- 28°C for *A. salmonicida* since original stocking. The effects of this recombination are a faster growth and loss of virulence, both due to genetic recombination of the chromosome and virulence plasmid. As results the recombinant strains lack virulence. Here we determined that molecular effects of temperature induced recombination in A. *salmonicida*. It is known that *vapA*, an important virulence factor responsible for the synthesis of the A-layer, is lost due to insertion sequence (*IS*) *IS*AS1 and *IS*AS2 endogenous recombination. *A. salmonicida* A-layer is a VapA protein array that covers most of the LPS O-antigens and it is necessary for complement resistance and congo red A^+ phenotype. Using this phenotype, we determined the frequency of recombination at 28°C of several virulent isolates. Using transcriptmics, we determined that *rrn* mRNAs lost molecular weight and that the mRNA G+C composition shift after recombination. Also we determine the up- and down-regulated genes during recombination that might be related to the recombination machinery. The results support the idea that *A. salmonicida* is under accelerated evolution.

P-20. Phenotypic and molecular study of *Edwardsiella piscicida* isolated from farmed whitefish *(Coregonus lavaretus)* in Finland

Shafigh Shafiei[1,2], Krister Sundell[1], Sirpa Heinikainen[3], Takele Abayneh[4,5], Satu Viljamaa-Dirks[3], Tom Wiklund[1]*

[1]Laboratory of Aquatic Pathobiology, Environmental & Marine Biology, Department of Biosciences, Abo Akademi University, Turku, Finland twiklund@abo.fi ksundell@abo.fi
[2]Department of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran shafiei_sh@ut.ac.ir
[3]Finnish Food Safety Authority Evira, Kuopio, Finland satu.viljamaa-dirks@evira.fi sirpa.heinikainen@evira.fi
[4]Department of Food Safety and Infection Biology, Section for Microbiology and Immunology, Norwegian School of Veterinary Science, Oslo, Norway
[5]College of Veterinary Medicine & Agriculture, Addis Ababa University, Bishoftu/Debre-zeit, Ethiopia. takeletefera99@gmail.com

Edwardsiellosis is a systemic bacterial disease in fish, resulting in significant losses in the aquaculture industry all over the world. In recent (2000, 2002 and 2013) disease outbreaks of farmed whitefish (*Coregonus lavaretus*) in Finland, bacteria preliminary identified as *Edwardsiella* sp. were isolated. The bacterial isolates were phenotypically characterized and analyzed by PCR, ERIC-PCR, and Multilocus Sequence Analysis (MLSA). For comparative analysis, type strains of *Edwardsiella piscicida* (ET883^T) and *Edwardsiella tarda* (ATCC 15947^T) were also included in the study. All whitefish isolates and the type strains were Gram negative, motile short rods, catalase positive, oxidase negative, capable of growth at 40 and 42 °C, H_2S producing, with facultative anaerobic metabolism of glucose being able to ferment D- ribose, D-galactose, D-glucose, D-fructose, D-mannose, N-acetyl-glucosamine, D-maltose, L-fucose and potassium gluconate. All isolates from whitefish were identified as *E. piscicida* by PCR. ERIC-PCR and MLSA analysis showed that the isolates from whitefish and ET883^T clustered separately from ATCC 15947^T, indicating genetic differences. The findings of this study indicate that *E. piscicida* may be an emerging pathogen in farmed salmonids including whitefish in temperate geographical regions.

P-21. Culture and Characterization of *Flavobacterium branchiophilum* from Bacterial Gill Disease in Ontario Fish

Iwona G. Skulska[1]*, Melinda J. Raymond[1], Stephen D. Lord[1], Roselynn M.W. Stevenson[1]

Department of Molecular and Cellular Biology, College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1 iskulska@uoguelph.ca, mjoyce@uoguelph.ca, slord@uoguelph.ca, rstevens@uoguelph.ca

Routine diagnosis of bacterial gill disease (BGD) in cultured freshwater salmonid fish relies on microscopic examination of gill samples for the presence of copious long rods, which affect the respiratory function of the gills. Culture of the disease agent, *Flavobacterium branchiophilum,* is difficult, as it is quickly over-grown by faster-growing organisms from the aquatic environment. Denaturing Gradient Gel Electrophoresis (DGGE) indicated *F. branchiophilum* was the dominant organism on gills from Ontario BGD cases, with 76% of gills tested having a single DGGE band corresponding to that of authentic *F. branchiophilum.* However, after gills were cultured on plates of cytophaga agar (CA) many different DGGE bands were apparent and evidence of *F. branchiophilum* was rarely apparent (3% of cases). We improved isolation success by modifying Anacker and Ordal's cytophaga agar by additional salts (K^+, Mg^{+2}, Ca^{+2}), gelatin, and charcoal powder. Small pin-point colonies (*Figure 1*) were confirmed as *F. branchiophium* by PCR and 16S rRNA sequencing. Between July 2010 and May 2012, we isolated 48 cultures from eleven BGD outbreaks in four Ontario fish culture stations. Six outbreaks were in Atlantic salmon, three in rainbow trout, and two in brook trout, *Salvelinus fontinalis*. These isolates all retained the distinctive long-rod morphology (14-20 µM) observed on infected gills. In contrast, the type strain ATCC 35035, which grows more readily on CA, was consistently a shorter rod (5-11µM long). Electron microscopy showed all isolates to have surface structures resembling pili and extensive membrane vesicles and tubules. Comparisons of isolates from different outbreaks and fish hosts by molecular approaches could be useful in identifying potential sources of recurring BGD and identifying reservoirs of the pathogen. A set of primers that specifically amplifies all isolates of *F. branchiophilum* was developed for use in qPCR. In screening for useful primers, nucleotide we found that the gene encoding the DNA gyrase large subunit B (*gyrB*) differed in sequence in all isolates from brook trout examined in case ONT 6199. In addition, primers designed to amplify gyrase A, a hypothetical protein (YP_004843271.1) and succinate dehydrogenase (*sdhA*) failed to amplify in strain FL106, a representative isolate from case ONT 6199.

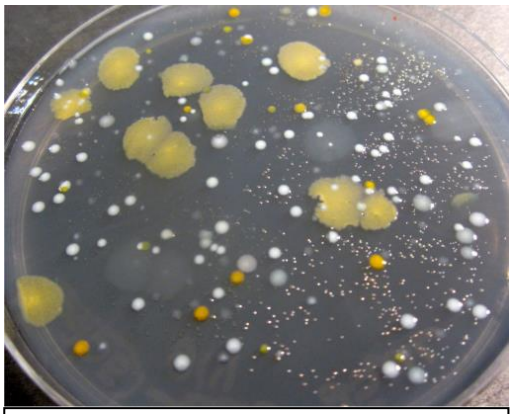


Figure 1: Charcoal mCA streaked with BDG infected gill tissue. Pinpoint colonies to right side of plate are F. branchiophilum.

Research supported by the Ontario Ministry of Natural Resources, Fish Culture Section

P-22. Assessing genetic markers for discerning serovars and strains of *Yersinia ruckeri*

Olivier Tremblay, Melinda J. Raymond, Christopher Ostrowski, Stephen D. Lord, Iwona G. Skulska, Roselynn M.W. Stevenson*

Department of Molecular and Cellular Biology, College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1 *rstevens@uoguelph.ca

Isolates of *Yersinia ruckeri*, the causative agent of Enteric Redmouth Disease or "yersiniosis" of salmonid fish, are described as genetically homogeneous despite recognizable phenotypic and serological differences. In routine monitoring of wild fish and provincial government hatchery stocks in Ontario between 1980 and 2012, *Y. ruckeri* was isolated from fish about twice a year (range: 0 to 7 isolations). All are authentic *Y. ruckeri* on the basis of their 16S rRNA sequences. Ontario isolates vary in sorbitol fermentation and reactions with diagnostic antisera. Serological varieties (serovars) of new isolates are tested using conventional serology or Western immunoblots of lipopolysaccharide (LPS) gel patterns but this approach is labour-intensive and the antisera used can vary between batches, rabbits and laboratories. Our objective has been to identify genetic sequences that would allow new isolates to be assigned to specific sero-groups based on rapid and convenient PCR-amplification techniques. Our previous studies with a panel of isolates representing major serovars failed to identify distinctive genetic markers for sub-groups within PCR-amplified target sequences for 16S rRNA, *gln*A and the 16S-23S ITS region. Bothe Enterobacterial Intergenic Repeat Consensus (ERIC) sequences random sets of ten-base-pair RAPD primers allowed some discrimination between strains but differences did not correlate with serological groups. In signature-tag mutagenesis studies, we had identified an O-antigen polymerase homolog in a serovar 1 strain of *Y. ruckeri*, RS 1154. Using that sequence and the published genome sequence for *Y. ruckeri*, we designed primers to amplify sequences for *wzy* (~750 bp), the O-antigen polymerase and to *wzx* (~1100 bp), the O-antigen flippase. In PCR amplifications, products were obtained from serovar 1 strains, including O:1 variants, but not from other serovars with different LPS gel patterns, so it is possible to confirm serovar 1 strains by PCR. To find a genetic marker for a second major serovar, represented by the sorbitol-fermenting Big Creek 74 (BC74) strain, we used suppression subtractive hybridization (SSH). The technique allows selective amplification of gene sequences that are present in one genome (tester DNA), but not the other (driver DNA). Two sequences seeming specific for BC74 were a 461 bp region of a *mal* T transcriptional regulator (OT4) and an 881 bp sequence (OT5) without a GenBank match. When tested against 15 strains of *Y. ruckeri*, the OT4 primers did react with some serovar 1 strains, so were discarded as a serovar marker. Primers for OT 5, the 881 bp sequence, did not react with serovar 1 strains and clearly differentiated BC74 and three other Pacific Coast isolates from nine other sorbitol-fermenting strains, which were isolates from Ontario and Norway. We compared the serological responses and silver-stained LPS gel patterns for reacting and non-reacting strains to determine whether they form coherent groups. The OT5 primers do not necessarily amplify sequences related to antigenic components and could be discriminating strains on the basis of their geographic sources. Group markers relating to bacterial antigens are relevant for understanding fish immune responses and for vaccine targeting, but it is equally useful to have molecular markers which provide convenient, consistent tools for differentiating isolates and sources of *Y. ruckeri*.

Research support from Ontario Ministry of Natural Resources and NSERC Canada.

P-23. *Aeromonas salmonicida* cyclic adenosine 3',5'-Monophosphate receptor protein (Crp) mutants in fish host.

Katherine Valderrama[1,2], Oscar Almarza[1], Javier Santander[1,3]*

Microbiology and Immunity Laboratory, Faculty of Sciences, University Mayor, Huechuraba, Chile, 8580745; PhD Program in Aquaculture, Catholic University of the North, Coquimbo, Chile; School of Life Sciences, Arizona State University, Tempe AZ 85287.
jasantanderm@asu.edu

Aeromonas salmonicida is a Vibrionaceae family member that causes a lethal disease called furunculosis in marine and freshwater fish. Being a mucosal facultative intracellular pathogen, this bacterium is an excellent candidate to develop immersion-oral live attenuated vaccines for the salmon-trout aquaculture industry. Deletion of the cyclic 3',5'-adenosine monophosphate (cAMP) receptor protein (*crp*) gene has been utilized in live attenuated vaccines for mammals and birds. Here we characterize the *crp* gene and report the effect of a *crp* deletion in *A. salmonicida*. The *A. salmonicida crp* gene and encoded protein are similar to other Enterobacteriaceae and Vibrionaceae family members, complementing *Salmonella enterica* and *Edwardsiella* Δ*crp* mutants in a cAMP-dependent fashion. The *A. salmonicida* Δ*crp-12* in frame deletion mutant demonstrated slight growth defects, loss of maltose utilization among other sugars, and lack of brown pigment synthesis. We found that the *A. salmonicida* Δ*crp-12* mutant was attenuated and conferred immune protection against *A. salmonicida* infection to the fish. We propose that deletion of the *crp* gene in *A. salmonicida* is an effective strategy to develop immersion live attenuated antibiotic-sensitive vaccines for the aquaculture industry.

P-24. Mixed mycobacterial infections in farmed sturgeons

Defeng Zhang[1, 2], Cheng Ji[3], Xujie Zhang[1], Tongtong, Li[1], Aihua Li[1]*, Xiaoning, Gong[1]

[1]State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, PR China liaihua@ihb.ac.cn
[2]Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou 510380, PR China zhangdefeng08@126.com
[3] Center for Circadian Clocks, School of Basic Medicine & Biological Sciences, Soochow University, Suzhou 215123, PR China jichenggreen@163.com

Mycobacteriosis of fish is a chronic progressive disease spreading all over the world (Novotny, Dvorska, Lorencova, Beran & Pavlik 2004). Based on microbiological and histopathological examinations and DNA sequencing, several outbreaks of mycobacteriosis in the reared sturgeons, including Chinese sturgeon (*Acipenser sinensis* Gray) and Amur sturgeon (*Acipenser schrencki*), were identified during 2009 to 2010. Forty nine isolates of nontuberculous mycobacteria（NTM）were isolated from 19 diseased sturgeons. In total, seven species of *Mycobacterium* were identified, namely, *Mycobacterium chelonae*, *Mycobacterium marinum*, *Mycobacterium gordonae*, *Mycobacterium fortuitum*, *Mycobacterium szulgai*, *Mycobacterium arupense*, and *Mycobacterium porcinum*. Among them, *M. marinum* was found to be more prevalent (89.5%) compared with the other mycobacterial species. When two molecular biological methods, PCR-DGGE (denaturing gradient gel electrophoresis) analysis and rpoB gene library sequencing, were used to analyze the mycobacterial DNAs extracted from the diseased fish tissues, mixed infections of two or three mycobacterial species were found being the predominant infection form (94.7%) in sturgeon mycobacteriosis. *M. marinum* was the only one species that caused sturgeon mycobacteriosis alone. Virulence assay showed that *M. marinum* possessed stronger pathogenicity to zebrafish killing 100% of fish in 28d at 10^3 cfu/fish than the other species. These results suggested that *M. marinum* is the major pathogenic bacteria in sturgeon mycobacteriosis, and the other mycobacterial species play minor roles in the co-infection. In addition, challenge experiments showed that equally mixed culture of three or four mycobacterial species possessed higher virulence to zebrafish than any individual species, suggesting a possible synergistic pathogenicity and probably explaining the popularity of mixed mycobacterial infections. To the best of our knowledge, this study is the first report on mycobacteriosis in farmed Chinese and Amur sturgeons as well as the first isolation of *M. porcinum* and *M. arupense* from diseased fish.

P-25. Systematic infection of *Ethynnus affinis* by cestode of the order Callitetrahynchus in Omani waters: pathological aspects.

Sarah Al Jufaili*, Um Kulthoum Al Kindi, Vladimir Machkevskyi, Nashwa Al Mazrooei

Fishery Quality Control Center Ministry of Fisheries Wealth, P.O. Box 427, P.C. 100, Muscat, Sultanate of Oman

A number of parasites are mainly known as aesthetic problem when infecting their fish hosts flesh, causing major economic problems in the fishery industry, cestode parasites belonging to the order *Trypanorhyncha* are among some of the these parasites. The infections on the fish musculature can reduce the market value of the affected fish. In year 2013 several incidences of massive parasitic infections have been reported by Fishery Biology Research team at the Marine Science Center. During their Tuna Biology project, several species of Tuna caught off Omani waters were found to inhabit numerous cysts in their internal organs. The incidence was reported to the Laboratory of Aquatic Parasitology, which upon examination of infected fish confirmed that the Tuna species were infected with a type of *Trypanorhyncha* Cestode belonging to the genus *Callitetrahynchus*. This genus is known to accommodate two species so far, both of them have been reported in the musculature of their hosts and were never reported from *E. affinis* before. The current *Callitetrahynchus* species is the only one so far with an infection site that is limited to the internal of their hosts with all organs being infected on various levels of intensities. The highest intensity level reaching to 112 parasites per fish and the mesenteries being the highest infected site followed by the liver. It was also noted that in some cases the parasites were found penetrating these vital organs which might imply some patholigcal reaction form the hosts side. Thus, the aim of this paper is to investigate the occurrence of these cestode on Omani Kawakawak fishes, describe the parasites to species level, discusses the parasitological indices of the infection and study the pathological impact of this infection on the host.

P-26. Investigations into the possible impacts of the digenetic trematode *Drepanocephalus spathans* on catfish aquaculture in the southeastern United States

Neely R Alberson[1*], Matt J Griffin[2], Lester H Khoo[2], Linda M Pote[1], Sylvie M Quiniou[3], Terrence E Greenway[4], Mary M O'Hear[1]

[1]Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS USA nrw2@msstate.edu, maryohear@gmail.com, lpote@cvm.msstate.edu,

[2]Thad Cochran National Warmwater Aquaculture Center, College of Veterinary Medicine, Mississippi State University, Stoneville, MS USA griffin@cvm.msstate.edu, khoo@cvm.msstate.edu

[3]Thad Cochran National Warmwater Aquaculture Center, Warmwater Aquaculture Research Unit, Agricultural Research Service, United States Department of Agriculture, Stoneville, MS USA sylvie.quiniou@ars.usda.gov

[4]Thad Cochran National Warmwater Aquaculture Center, Mississippi Agriculture and Forestry Experiment Station, Mississippi State University, Stoneville, MS USA greenway@drec.msstate.edu

Farm-raised catfish is an important industry in the southeastern United States. Over 80,000 water surface acres are devoted to catfish production and annual sales exceed $300 million. Disease related losses are a major impediment to catfish production, and digenetic trematodes play a significant role in production losses. The trematode *Bolbophorus damnificus* is associated with heavy losses in production due to mortality and a significant decrease in appetite, occurring even in mild infections. The rams-horn snail (*Planorbella trivolvis*), commonly associated with catfish operations in Mississippi, is a known intermediate host for *B. damnificus*, along with several other unidentified digenetic trematodes. The rams-horn snail has also been identified as an intermediate host in the life cycle of *Drepanocephalus spathans*, a parasite of the double-crested cormorant, a piscivorous bird found inhabiting ponds used in catfish production. Recent cohabitation trials, involving channel catfish (*Ictalurus punctatus*) and snails actively shedding *D. spathans* cercariae, resulted in mortalities within 7 days post-challenge. Previously thought inconsequential to catfish health, histopathology revealed *D. spathans* metacercariae encysted in and around the head region, with a predilection to the gill arch base, occluding blood vessels and resulting in death of some fish. A survey of digenetic trematodes from double–crested cormorants in the Mississippi Delta region found a 91% prevalence rate for *D. spathans*. The presence of double-crested cormorants on commercial catfish operations, in conjunction with populations of rams-horn snails, could have negative impacts on catfish health. Previous work, in addition to future studies including longevity studies, infectivity trials, bird transmission studies and pathology trials in channel catfish, and the development of a species-specific PCR assay, will be discussed.

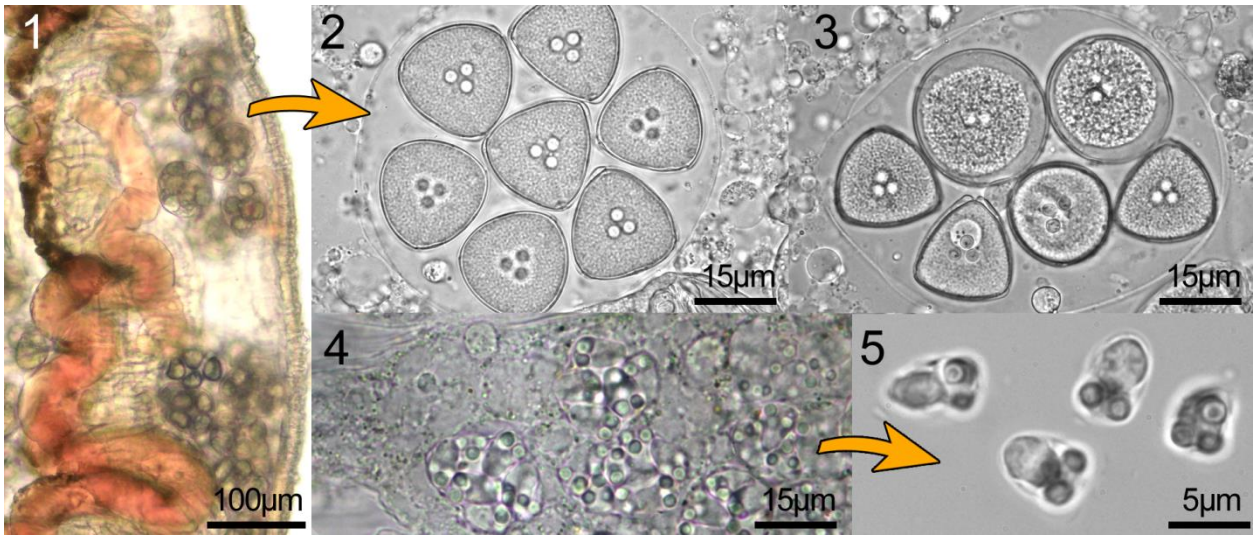
P-27. Prospecting for myxozoan infections in marine annelid worms to find the alternate host of *Kudoa inornata*, a pathogen of spotted seatrout

Stephen D Atkinson*[1], Isaure de Buron[2], D Diaz-Morales[1], Sascha L Hallett[1], Jerri L Bartholomew[1]

[1]Department of Microbiology, Oregon State University, Corvallis, Oregon, USA
[2]Grice Marine Laboratory, College of Charleston, Charleston, South Carolina, USA

Kudoa inornata is a myxosporean parasite (Cnidaria: Myxozoa) of spotted seatrout (*Cynoscion nebulosus*). Heavy infections result in post-mortem myoliquefaction and subsequent loss of value of fish fillets. No *Kudoa* life cycle is known. The few known myxosporean life cycles involve an obligate, alternate invertebrate host, typically an oligochaete or polychaete worm. We have demonstrated that *K. inornata* can infect seatrout at high prevalence and intensity in in-shore environments of Charleston Harbor, so we targeted those localities to find the parasite's invertebrate host. In May and July 2014, we collected intertidal invertebrates by hand, and by boat and grab from depths of 1-5 metres. Oligochaete and polychaete worms (1-100mm long) were separated from the substrate by hand, then examined fresh at 200-400x magnification. Overt myxosporean infections (cell-within-cell stages and mature 6-25µm actinospores) were photographed, and their ssrRNA genes sequenced using myxozoan primers.

We found myxosporeans in 27/170 (16%) oligochaetes but only 1/612 (0.2%) polychaetes. In oligochaetes, infections were in intestine or body cavity (Figs. 1-2). Some infections showed asynchronous development within pansporocysts (Fig. 3), a feature not observed previously. DNA sequencing revealed all oligochaete infections were most similar to *Myxobolus* or *Henneguya* spp. Sequences differed typically by 5-10%, suggesting at least 15 species are present in the oligochaetes, though a species accumulation curve suggests many myxozoan taxa remain to be found. In the polychaete, infection was in the tegument (Figs. 4-5) and was highly similar to *Kudoa* spp. sequences, but no exact match was found.



P-28. Two trematode species, one snail host: comparative patterns of cercarial maturation

Ana Born-Torrijos[1], Tsukushi Kamiya[2], Juan Antonio Raga[1], Astrid S. Holzer[3]*

[1]Cavanilles Institute for Biodiversity and Evolutionary Biology, Science Park, University of Valencia, PO Box 22 085, 46071 Valencia, Spain a.isabel.born@uv.es, toni.raga@uv.es
[2] Laboratoire MIVEGEC (UMR CNRS 5290, UR IRD 224, UM1, UM2), 911 Avenue Agropolis, BP 64501, 34394 Montpellier Cedex 5, France philophthalmus@gmail.com
[3] Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic astrid.holzer@paru.cas.cz

Digenean trematodes have complex life-cycles that include several intermediate hosts, thus requiring synchronization of parasite development with the host's availability. The two closely related species, *Cainocreadium labracis* and *Macvicaria obovata* (Opecoelidae), infect the same first intermediate host, the snail *Gibbula adansonii* in Els Alfacs lagoon (Ebro Delta, Spain). The cercariae of both species attach to the substrate immediately after emergence and utilize the sit-and-wait strategy of downstream host finding. In this context, the co-occurrence in time and space with their second intermediate hosts is of great importance.

Both species develop sporocysts that multiply asexually in the digestive gland and the gonads of their first intermediate host. This embryonic development has been recorded during three consecutive months (March to May), in 2011 and 2013. Six to 25 sporocysts from different snails have been analyzed for each species (*C. labracis* n=36, *M. obovata* n=15, double infected snails n=5) and the proportion of three different developmental stages has been recorded: mature stages (fully developed cercariae), immature cercariae (embryos) and germinal balls. Additionally, a Maturity Index for each sporocyst has been calculated based on the relative numbers of each stage.

Data have been explored with regression analyses in the software R, and the effect of snail size and infection status (single *versus* double infection) has been analyzed, showing differences between species. The maturity and development of *C. labracis* peaks in April, with significantly more mature and immature cercariae, thus coinciding with the higher incidence of small benthic fish (i.e. *Gobius niger*) that act as second intermediate host in the intertidal habitat. *Macvicaria obovata* shows a more protracted development, with immature cercariae increasing until late spring, so that the transmission window is extended, allowing the infection of the second intermediate snail host *Cyclope neritea,* over a prolonged period of time. *C. neritea* is abundant during the whole summer and early autumn.

P-29. The non-native monogenea *Thaparocleidus caecus* in India on its introduced host, *Pangasianodon hypophthalmus*: about two decades of unnoticed presence

Anshu Chaudhary[1,2], Hridaya Shanker Singh[1], Csaba Székely [2]*

[1]Molecular Taxonomy Laboratory, Department of Zoology, Ch. Charan Singh University, Meerut - 250 004, INDIA anshu8282@rediffmail.com

[2]Institute for Veterinary Medical Research, Centre for Agricultural Research, HAS, H-1581 Budapest, P.O. Box 18, HUNGARY szekely.csaba@agrar.mta.hu

In the context of biological invasion, scientists increasingly aware the impact of invasive species on native communities. The introduced species can act as vector of non-native parasites with devastating effects. Exotic species tremendously cause economic loss, modify ecosystem functions and can threaten the native ones. During a survey of non-native monogenean parasites in Meerut region, India, the freshwater iridescent shark of family Pangasiidae, *Pangasianodon hypophthalmus* (Previously *Pangasius sutchi*) was found infected with monogenean parasites. *Pangasianodon hypophthalmus*, a freshwater fish popular for used as food in India which is also abundantly available in Vietnam, Bangladesh, Indonesia and Thailand. It is a native of Mekong River in Vietnam has been introduced in several ecosystems worldwide, reproduced at a high rate, resulting in dense population of small specimens. P. hypophthalmus has been proven adaptable for intensive production in many countries and culturing this fish to boost up aquaculture.
Parasitological examination of *P. sutchi* revealed the presence of a non-native monogenean parasite of genus *Thaparocleidus*. The large number of parasites (~200) on a single fish, suggests their successful reproduction in the non-native Indian geographical region. Morphology and morphometrics of the parasite showed similarity with *Thaparocleidus caecus* (Mizelle and Kritsky, 1969) Gussev, 1978 of Southeast Asia. After morphological analysis we have examined 28S rDNA sequences of the parasites to substantiate the findings. This represents the first record of *T. caecus* in India and provides a clear avenue for human-assisted introduction of *P. hypophthalmus*.
The 28S rDNA sequence of *T. caecus* (627 bps) did not show a close relationship with any other *Thaparocleidus* represented in GenBank, except *Thaparocleidus* sp. BDY (EF100555) (98%). The 28S tree showed a better resolution within the clade for *T. caecus* (high bootstrap values of 100%). This similarity might be revised in the future as no 28S sequence for *T. caecus* species is available now. *T. ceaceus* is differentiated among *Thaparocleidus* species by 2% generic difference in their nucleotide sequence calculated by NCBI BLAST and also through analysis of MEGA software. This is the first and only 28S sequence of *T. caecus* (KF361477) available on Genbank database. Thus, it seemed possible that this non-native monogenea has remained unnoticed over the past decades.

Acknowledgements: Type D Scholarship provided by Balassi Institute, Hungarian Scholarship Board and UGC (University Grants Commission) India, under the Post Doctoral Fellowship to Anshu Chaudhary and by the Uttar Pradesh Government, Centre of Excellence, India, to HSS, project number (No.1486/70-4-2011-46(43)/2010).

P-30. The treatment of some protozoan and monogenean diseases using bath with high concentration of sodium chloride solution

Calin D. Cojocaru[1]*, Clementina L. Ardelean[2]

[1]State Sanitary Veterinary Laboratory-Aquatic Pathology Laboratory, 4 Surorile Martir Caceu, 300585, Timisoara, Romania, c_cojocaru_d@yahoo.com
[2]Banat University of Agricultural Sciences and Veterinary Medicine - King Mihai I of Romania Faculty of Animal Sciences and Biotechnologies - Section of Aquaculture, 119 Calea Aradului 300645 Timisoara, Romania, annalouissa@yahoo.com

3 fish species naturally infected with protozoans and monogeneans were treated by short time immersion (7-10 seconds) in a bath with 30% salt, sodium chloride. The remove of the parasites from the gills was completely, in all 3 fish: carp (*Cyprinus carpio*) and koi carp (*C. carpio koi*), from a pond, infected with *Trichodina sp., Dactylogurus solidus* and *Gyrodactylus sp.*, goldfish (*Carassius auratus auratus*) infected with *Gyrodactylus medius* and *Trichodina sp.*, collected from an aquaria and pike perch (*Sander lucioperca*), infected with *Ancyrocephalus paradoxus* and *Trichodina sp.*, caught from the Danube and reared in a recirculating system to induce artificial spawning. The success of the treatment is strongly dependent by the exposure time and faster placing in clean water after the treatment. The osmotic shock caused by sodium chloride, produce a coagulation and falling of the gills slime filled with parasites. According to the OIE recommendations, only few chemicals (including salt-sodium chloride-), are allowed to be used for treatment of aquacultured fish for human consumption purpose.

P-31. The prevalence and sanitary implications of *Eustrongylides excisus* (larvae) infection in the region of Iron Gates dam from Danube River, Romania

Calin-Decebal Cojocaru[1]*, Clementina-Luisa Ardelean[2]

[1] State Sanitary Veterinary Laboratory-Aquatic Pathology Laboratory, 4 Surorile Martir Caceu, 300585, Timisoara, Romania, c_cojocaru_d@yahoo.com

[2] Banat University of Agricultural Sciences and Veterinary Medicine - King Mihai I of Romania Faculty of Animal Sciences and Biotechnologies - Section of Aquaculture, 119 Calea Aradului 300645 Timisoara, Romania, annalouissa@yahoo.com

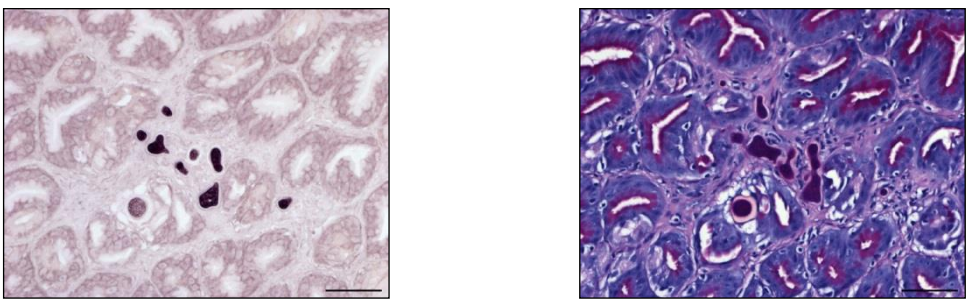
The prevalence of *Eustrongylides excisus* infection has sharply increased in the past 5 years. The intensity of infection is higher in pike perch (*Sander lucioperca*) – almost all of the specimens are infected in the Iron Gates dam, followed by European perch (*Perca fluviatilis*), Northern pike (*Esox lucius*), European catfish (*Silurus glanis*) and Asp (*Aspius aspius*). All these fish are well appreciated for culinary reasons and they are subject of commercial fishing. The health status of fish can be affected in higher infections with more than 20 larva per fish, especially when the liver is infected and ascita occurs. According to Europe Council Regulation 2074/2005, when the muscles are infected (very often in pike-perch, perch and pike), fish must be excluded from human consumption, even if the meat is frozen prior to cooking because the worms confer a disagreeable aspect. The zoonotic significance of *Eustrongylides excisus* is discussed, but the impact on food safety is major due to the economic losses caused by the impossibility of placing on the market of infected meat. The increasing of the prevalence is related to the global warming and eutrophication of Iron Gates Lake in the region of the dam, mainly because of Danube pollution.

P-32. Detection of *Ichthyophonus* by chromogenic in situ hybridization

Carla M Conway[1]*, Maureen K Purcell[1], Diane G Elliott[1], Paul K Hershberger[2]

[1]US Geological Survey – Western Fisheries Research Center, 6505 NE 65th Street, Seattle, WA 98115 USA, cmconway@usgs.gov, mpurcell@usgs.gov, dgelliott@usgs.gov
[2]US Geological Survey – Marrowstone Marine Field Station, 616 Marrowstone Point Road, Nordland, WA 98358, USA, phershberger@usgs.gov

Ichthyophonus hoferi is a protistan parasite in the class Mesomycetozoea infecting numerous marine and freshwater fish species over a broad geographic range. Ichthyophonus-like organisms have been reported in amphibians, reptiles, birds and invertebrates and may have been incorrectly classified under a single type species, *I. hoferi*. Although less sensitive than other detection techniques such as explant tissue culture, histopathological examination is effective for simultaneously evaluating host response and severity of *Ichthyophonus* infections. Histological sections showing positive periodic acid-Schiff (PAS) staining of multinucleate organisms 10-250 µm in diameter can be presumptive for *Ichthyophonus*, but lack of a definitive confirmatory test may lead to misdiagnosis, particularly when the organism is not cultured. We developed a chromogenic in situ hybridization (CISH) procedure that specifically detected *Ichthyophonus* ribosomal DNA in histological sections thereby complementing the histological diagnosis by providing highly specific molecular confirmation of the observed organism. A digoxigenin-labeled oligonucleotide probe was designed to target conserved portions of the 18S small subunit ribosomal gene of known *Ichthyophonus* species *I. hoferi* and *I. irregularis.* Formalin-fixed, paraffin-embedded tissues from naturally infected Chinook salmon, *Oncorhynchus tshawytscha*, and red-spotted newt, *Notophthalmus viridescens*, and experimentally infected Pacific herring, *Clupea pallasii*, rainbow trout, *O. mykiss*, and Pacific staghorn sculpin, *Leptocottus armatus*, were analyzed by CISH and PAS staining. In all fish species examined, probe hybridization was indicated by dark purple precipitates and correlated with the distribution and morphology of *Ichthyophonus* parasites observed in PAS-positive tissues and also identified its developmental stages in the presence of PAS-positive host cells. No hybridization occurred in PAS-positive, *Ichthyophonus*-like organisms in the red-spotted newt, supporting the hypothesis that the organism infecting amphibians is taxonomically distinct from fish-associated *Ichthyophonus*. The CISH procedure has utility for both diagnostic and research applications.



Ichthyophonus identified in the stomach tissue of rainbow trout by chromogenic in situ hybridization (left) and periodic acid-Schiff (right). Scale bars = 50 µm.

P-33. The influence of gender distribution on the reproduction and transmission dynamics of the parasitic salmon louse *Lepephtheirus salmonis* on wild salmon in British Columbia, Canada.

Ruth Cox[1]*, Maya Groner[2] George Gettinby[3], Crawford W. Revie[4]

[1]Centre for Veterinary Epidemiological Research, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, Canada. rucox@upei.ca
[2]Centre for Veterinary Epidemiological Research, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, Canada. mgroner@upei.ca
[3]Department of Mathematics and Statistics, University of Strathclyde, Scotland. g.gettinby@strath.ac.uk
[4]Centre for Veterinary Epidemiological Research, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, Canada. crevie@upei.ca

For any commercially or ecologically important parasite species, it is necessary to have a detailed understanding of the mating system in order to inform effective management and prevention strategies. This work focuses on the salmon louse, *Lepeophtheirus salmonis*, a parasitic copepodid which has a deleterious effect on wild and farmed salmon. As a dioecious species, it reproduces sexually on a host, with adult males forming mate guarding pairs with pre-adult females [1]. Little is known about the distribution of each gender on the salmon hosts nor about the gender ratio of the lice. Both of these factors will have marked consequences for successful reproduction of *L. salmonis* and therefore on parasite burdens.

Here we analysed a dataset consisting of more than 160000 wild fish that were sampled between 2003 and 2012 in British Columbia, Canada. Life stage and gender of all *L. salmonis* on each fish was recorded. We investigated the influence of the gender ratio and aggregation on the reproductive potential of the parasite population. Approximately 30% of fish were infested with at least one mobile louse. The gender ratio of lice on individual fish was male-biased in all years. Mobile lice of both genders were aggregated, with males tending to be more aggregated than females. As aggregation increased, the probability of mate pairing increased. The monthly mean proportion of hosts infested with at least one male and one female louse was 0.018 (±0.015 95%CI) and the monthly mean probability of a female louse being able to mate was 0.217 (±0.076 95%CI). Comparison with expected values calculated using models of dioecious parasites [3] showed that male and female lice tend to aggregate together on the host population. The models demonstrate that the level of aggregation of male and female lice has a considerable influence on the probability of reproduction. If males and females aggregate on the same host then the probability of reproduction can be many times higher than if the males and females tend to aggregate on different hosts (for the same value of mean lice abundance). The results have implications for a number of aspects of salmon health and management. These include the validity of lice population abundance estimates on wild or farmed fish when lice counts usually only record female adults, and the precision of salmon lice population dynamic models.

References

1. Ritchie G et al. (1996) J Exp Mar Biol Ecol 201: 285-298.

2. Bradley DJ & May RM (1978) Trans R Soc Trop Med Hyg 72: 262-273.

P-34. Development and validation of a molecular diagnostic assay for detection of the swimbladder nematode *Anguillicoloides crassus* in *Anguilla rostrata* glass eels

Spencer J Greenwood [1], David B. Groman[2]*, Scott R. Ault [3], Ron Heun [3], Ron Threader[4]

[1]Department of Biomedical Sciences, Atlantic Veterinary College, University of Prince Edward Island, 550 University Ave., Charlottetown, PE, Canada, C1A 4P3 sgreenwood@upei.ca
[2]Aquatic Diagnostic Services, Atlantic Veterinary College, University of Prince Edward Island, 550 University Ave., Charlottetown, PE, Canada, C1A 4P3 groman@upei.ca
[3]Kleinschmidt USA Ecological Services Group, 400 Historic Dr., PO Box 278, Strasburg, PA USA 17579. Scott.Ault@KleinschmidtGroup.com, Ron.Heun@KleinschmidtUSA.com
[4]266 McManus Road, White Lake, Ontario Canada K0A 3L0
Ron.Threader@KleinschmidtGroup.com

Anguillicolides crassus is a highly invasive exotic parasitic nematode of eels that has the potential to hinder energy reserves in adult eels thereby impairing both migration and spawning potential. Additionally, *A. crassus* poses a threat the lucrative glass eel market by the accidental introduction of infected glass eels to new watersheds. In Atlantic Canada, adult *A. crassus* have been found in yellow adult eels (*Anguilla rostrata*) from the Mira River and Sydney Harbour and from larval nematodes in elver eels from the St. Mary's River. Estimates of prevalence of infection are reported between 3-30% for eels sampled in New Brunswick and northern Nova Scotia but this likely represents an underestimate as these have relied solely based on gross observations. In order to prevent future introductions and to monitor for the presence of *A. crassus* in Canadian eels, diagnostic methods need to be developed that are rapid, sensitive, specific and reliable. An end-point analysis PCR was optimized and validated for the identification of *A. crassus* infections in glass eels based on an approximately 800 bp region of the large subunit ribosomal DNA (LSU rDNA) gene. The assay was validated using field samples of known *A. crassus* infected eels from an endemic area, Goose Creek, South Carolina. The assay was then used to investigate the infection status of glass eels from Atlantic Canadian sources (Nova Scotia and Newfoundland) where the prevalence in glass eels was unknown.

P-35. A case of systemic scuticociliatosis in a scorpionfish (Scorpaenidae)

Forgivemore Magunda[1,2]*, Christine M. Davitt [3], Kevin R. Snekvik [1,2]

[1]Department of Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, WA 991634 USA magundaf@vetmed.wsu.edu
[2]Washington Animal Disease Diagnostic Laboratory-Aquatic Health Services, Washington State University, Pullman, WA 99163 USA ksnek@vetmed.wsu.edu
[3]Franceschi Microscopy and Imaging Center, School of Biological Sciences, Washington State University, Pullman, WA 99164 USA davcmd@wsu.edu

Scuticociliatosis, a systemic disease caused by approximately 20 species of histophagous ciliated protozoa in the subclass Scuticociliatida of the phylum Ciliophora is an economically important, emerging disease of marine fish raised in aquaculture. Outbreaks of the disease have increased considerably in recent years resulting in massive deaths and significant economic losses. To increase recognition of this important emerging disease, we report a per-acutely lethal case of scuticociliatosis in a single adult, female, wild born Scorpionfish housed in an aquarium marine exhibit in the USA Pacific northwest. The case was submitted to the Washington Animal Disease Diagnostic Laboratory with a history of being found dead on day 23 of a 30 day standard quarantine protocol with no prior clinical signs. Gross findings included moderate skin edema with elevated scales, and hemorrhage and necrosis of the gonads, intestine, coelomic connective tissue, liver and gills. Histologically the infection was characterized by necrohemorrhagic oophoritis, enteritis, coelomitis, hepatitis and branchitis with high numbers of intralesional ciliated protozoa in the coelomic cavity. Fibrinonecrotic vasculitis with fibrinous thrombi associated with parasites disrupted branchial vessels. Protozoa from formalin-fixed tissues had histomorphology consistent with the phylum Ciliophora. The histological and ultrastructural morphology of the protozoa as determined by light microscopy and transmission electron microscopy are described. This case represents identification of an important emerging disease of fish in a previously unreported species and continuing identification of the syndrome in new locations.

P-36. Growth of the fish microsporidian parasite, *Loma salmonae*, within cell culture

Sarah H. McConnachie[1]*, Judy Sheppard[1], Glenda Wright[2], David J. Speare[1]

[1]Department of Pathology and Microbiology, Atlantic Veterinary College, Charlottetown, PEI
[2]Department of Biomedical Sciences, Atlantic Veterinary College, Charlottetown, PEI

Loma salmonae is the causative agent of Microsporidial Gill Disease of Salmon (MGDS), which causes chronic inflammatory branchitis and subsequent respiratory distress in affected salmonids. *L. salmonae* is an intracellular pathogen which targets cells within the gills, and cause the development of xenomas where spores develop and proliferate. Development of a rainbow trout (*Oncorhynchus mykiss*) – *L. salmonae* disease model has increased our understanding of the disease, and has helped answer questions relating to transmission, immunology, and the possibility of the use of deactivated spores as a vaccine. However, using an *in vivo* model is insufficient when trying to screen for effective treatments, or for growing spores at a production level for use as a vaccine. Human and insect microsporidians have been successfully cultured *in vitro* for many years, but the culture of fish microsporidians has not been as successful. Researchers believe that fish microsporidians are more reliant on an ideal host cell environment for xenoma development. Most successful spore production in culture has been with microsporidians that are non-xenoma forming. We have undertaken several studies that test the ability of *L. salmonae* to grow within three fish cell lines (RTgill-1, Rainbow Trout Macrophage, and CHSE-214), and one insect cell line (Aedes Albopictus). We present evidence of xenoma development within cell culture and will discuss ways to increase the productivity of the *in vitro* culture of *L. salmonae*.

P-37. Influence of temperature and fish stock on progression of *Ichthyophonus* infections in Chinook salmon (*Oncorhynchus tshawytscha*)

Constance L McKibben[1]*, Paul K Hershberger[2], Maureen K Purcell[1], Carla M Conway[1], Diane G Elliott[1]

[1] U.S. Geological Survey, Western Fisheries Research Center, 6505 NE 65th St., Seattle WA 09115 USA cmckibben@usgs.gov, mpurcell@usgs.gov, cmconway@usgs.gov, dgelliott@usgs.gov
[2] U.S. Geological Survey, Marrowstone Marine Field Station, 616 Marrowstone Point Road, Nordland WA 98358 USA phershberger@usgs.gov

The fish parasite *Ichthyophonus* sp. has been hypothesized as a driver of long-term declines in Yukon River Chinook salmon (*Oncorhynchus tshawytscha*) by reducing spawning effectiveness and contributing to pre-spawning mortality. However, most evidence supporting this hypothesis is based either on field survey data that show only correlations, or laboratory studies with surrogate host species that may exhibit different host responses to *Ichthyophonus.* One of our objectives was to examine the influence of water temperature on disease progression and mortality in juvenile Yukon River Chinook salmon. Better understanding of temperature effects on progression of ichthyophoniasis in these salmon would assist in development of models to predict prevalence and outcome of *Ichthyophonus* infections in the population under current temperature ranges and those that might be expected with continued climate change. Another objective was to investigate relative susceptibilities of Yukon River and Salish Sea Chinook salmon to *Ichthyophonus* disease, in an attempt to clarify the apparent paradox that ichthyophoniasis has not been a reported problem in Chinook salmon outside the Arctic-Yukon-Kuskokwim region, despite a high prevalence of *Ichthyophonus* infection in prey fishes in areas such as the Salish Sea. Juvenile Yukon River Chinook salmon and Kendall Creek (Salish Sea) Chinook salmon of the same age and size were challenged with *Ichthyophonus* by a 5-day feeding at 15°C with minced *Ichthyophonus*-infected Pacific herring (*Clupea pallasii*). Control fish from each stock were fed uninfected herring. After exposure, some Yukon River salmon and all the Kendall Creek salmon were retained at 15°C, while the remaining Yukon River fish were acclimated and held at either 10°C or 20°C. Triplicate tanks of test and control fish at each temperature were used for mortality assessment, and a fourth tank was used for periodic sampling. Infection prevalence was determined by culturing heart and liver tissue in MEM-5TA medium at 15°C for 21 days and examining for characteristic schizonts, and disease progression was evaluated by histopathology. Preliminary data indicated that some fish in each stock were successfully infected with *Ichthyophonus* after the feeding exposure. Mortality and disease progression results will be presented.

P-38. Methodologies for the isolation of free-living amoeba and identification of amoeba-resistant bacteria by co-culture with environmental amoeba

John McLean, LM Mutharia*

Department of Molecular and Cellular Biology, College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1. lmuthari@uoguelph.ca

Free-living amoebae are a diverse group of ubiquitous phagocytic protozoans that commonly inhabit natural and man-made terrestrial and aquatic environments. An important feature of amoeba is their ability to form highly resistant cysts during adverse conditions which may further protect internalized bacteria from disinfection regimens, antimicrobials and facilitate persistence in diverse environments and stressors. Amoeba can harbour bacterial pathogens which resist amoebal phagocytic killing mechanisms, survive and often multiply within the amoebal host and bacterial virulence factors required for intra-amoebal survival mirror those required for resistance and survival to animal phagocyte defense cells.

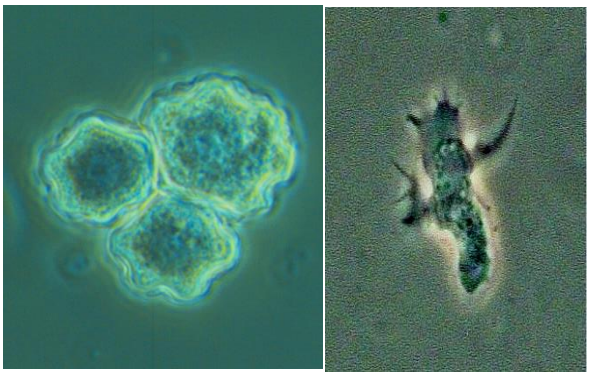


Figure 1. **Cysts and cells of *Acanthamoeba polyphaga***

The primary isolation of free-living amoeba can be extremely difficult and time consuming, the interactions between amoeba and bacterial pathogens is widely conducted *in vitro* using extensively laboratory-adapted amoebal species of *Acanthamoeba polyphaga* and *Acanthamoeba castellanii*. In this study, we present methodologies we developed for isolation of free-living amoeba from aquatic fish culture operations and terrestrial dairy farm operations. Persistent fungal contamination limits the efficacy of the amoeba walk-out protocol for direct isolation of amoeba from soils and sediment samples. Chemical and pH-based decontamination treatments with or without forced encystation of the resident amoeba were developed to clear environmental samples of fungi, leaving viable amoeba. Using these protocols, 10 species of amoeba have been isolated from 16 samples to date. A 77% successful amoeba isolation rate from environmental samples was achieved using our methodologies. Amoebae were isolated from 6/11 (54.5%) sediment samples from aquaculture operations.

Amoebae can be used as tools to discriminate between pathogenic and avirulent strains of bacterial pathogens in target environments. Unfortunately, studies investigating the relationship between resident environmental amoeba isolates and bacteria are lacking. We showed that only a few bacteria are truly resistant based on their ability to survive within cysts versus transient survival in trophozoites. Thus *Yersinia ruckeri* transiently survives predation but not in cysts.
Survival within amoeba was amoeba species-specific with some amoebal species killing all examined bacteria and others hosting some bacteria. Future studies will examine persistence of fish pathogens such as *Aeromonas salmonicida, Renibacterium salmoninarum* and *Y.ruckeri* in different amoeba species. This gap in knowledge limits water management and conservation options in aquaculture, including recirculation systems.

P-39. Molecular phylogeny and ultrastructure of *Myxobolus cuneus*, a parasite of patinga hybrid, and *Henneguya pseudoplatystoma*, a parasite of pintado hybrid

Tiago Milanin[1]*, Antônio A. M. Maia[2], Márcia R. M. Silva[2], Mateus M. Carriero[3], Edson A. Adriano[1,4]

[1]Department of Animal Biology, Universidade Estadual de Campinas, Campinas, SP, Brazil tiago_milanin@hotmail.com
[2]Department of Veterinary Medicine, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, SP, Brazil antomaia@usp.br
[3]Genetics and Evolution Department, Universidade Federal de São Carlos, São Carlos, SP, Brazil mmcarriero@gmail.com
[4]Department of Biological Sciences, Universidade Federal de São Paulo, Diadema, SP, Brazil edapadriano@gmail.com

Myxosporeans are metazoan parasites of vertebrates (primarily fish, but also amphibians, reptiles, birds and mammalians) and invertebrates (annelids). Among myxosporeans, species of the genus *Myxobolus* Bütschli, 1882 and *Henneguya* Thelohan, 1892, are most commonly found infecting fish. *Myxobolus cuneus* Adriano et al. 2006 and *Henneguya pseudoplatystoma* Naldoni et al. 2009 have been described, based on morphological, histopathological and ultrastructural analysis, to infect pacu (*Piaractus mesopotamicus*) and hybrid pintado (*Pseudoplatystoma corruscans x Pseudoplatystoma reticulatum*) from Brazilian fish farm, identifying the parasite as an important pathogen of these farmed fish species. In this study 18S rDNA gene sequencing of both *M.cuneus* and *H. pseudoplatystoma* was conducted. *M. cuneus* was found infecting the spleen of patinga, a hybrid fish resulting from the crossing of *P. mesopotamicus* and *Piaractus brachypomus*; *H. pseudoplatystoma* was found in the gill filaments of pintado hybrid fish. The fish utilized in this study were obtained from two fish farms from the state of São Paulo in Brazil. The study also provides previously unknown details of the host-parasite interface *M. cuneus*, which revealed that the plasmodial wall was composed of a single membrane connected to the ectoplasm of plasmodium through numerous pinocytic canals. The plasmodia revealed asynchronous development, and at different developmental stages displayed disporic pansporoblastos. Additionally, immature and mature spores were found at different depth levels of the plasmodium. Phylogenetic analysis by maximum likelihood showed that *M. cuneus* appears as a sister species of *Henneguya pellucida* Adriano et al. 2005, in a sub-clade composed mainly of myxosporean parasites of characiforms and *H. pseudoplatystoma* clustered in a sub-clade composed of *Henneguya/Myxobolus* spp. parasites of siluriform fishes.

P-40. Ultrastructure of two myxosporean species parasites of *Phractocephalus hemioliopterus* from the Amazon region

Juliana Naldoni[1], Antônio A M Maia[2], Edson A Adriano[1,2]*

[1] Department of Animal Biology, Universidade Estadual de Campinas, Campinas, SP, Brazil
jnaldoni@gmail.com
[2] Department of Veterinary Medicine, Universidade de São Paulo, Pirassununga, SP, Brazil
antomaia @usp.br
[3] Department of Biological Sciences, Universidade Federal de São Paulo, Diadema, SP, Brazil
edapadriano@gmail.com

The tropics of South America possess the greatest diversity of freshwater fish in the world, and this diversity is greater still in the Amazon region. Despite such diversity, few studies exist of host-parasite interaction of fish parasites from the Amazon. The present study describes the ultrastructural analysis of *Myxobolus* sp. and *Henneguya* sp. parasite of *Phractocephalus hemioliopterus* taken from the Rio Tapajos, in the state of Pará in Brazil. This siluriform fish of the pimelodidae family is a large Amazon catfish popularly known in Brazil as *pirarara. Myxobolus* sp. was found infecting the gill filaments of the fish, and ultrastructural analysis showed that plasmodia were surrounded by a capsule composed of connective tissue, with few layers of fibroblast-like cells. In the host-parasite interface, this capsule had a thin layer of loosely arranged collagen fibers occupying the space between the plasmodia and the capsule of fibroblast-like cells. The plasmodial wall of the parasite was composed of a single membrane, and had numerous projections and invaginations, with formations of vesicles in the ectoplasm of the plasmodia. Just below the thin ectoplasm zone there was a thin layer of numerous mitochondria. Adjacent to this mitochondria layer there was a layer composed of generative cells and sporoblasts at different stages of development. Mature spores were observed in the central zone. The polar capsules varied in size, with the smaller capsules having polar filaments arranged in three turns and the larger polar filaments arranged in six turns. The binucleate sporoplasm had numerous small dark sporoplasmosomes. *Henneguya* sp. was found infecting the gill lamellae and the plasmodia showed intralamellar type development. Ultrastructural analysis showed that *Henneguya* sp. was also surrounded by connective tissue, which was thinner than that observed in *Myxobolus* sp. There was a thin layer of electron-dense granular material, which prevented direct contact between the plasmodial wall and the connective tissue capsule. The plasmodial wall was composed of a single membrane, which was linked to the ectoplasm by numerous short thick pinocytic canals. Generative cells and sporoblasts in different developmental stages were found in all depth zones of the plasmodia, and mature spores were found from the peripherical zone to the deep zones of the plasmodia. This is the first study of myxozoans of *P. hemioliopterus.*

Study supported by FAPESP (Proc. nº 2013/21374-6).

P-41. Predicting the effects of climate change on myxozoan disease: a case study of *Ceratonova* (syn *Ceratomyxa*) *shasta*.

R. Adam Ray[1], Julie D. Alexander[2]*, Jerri L. Bartholomew[3]

[1]robert.ray@oregonstate.edu, Oregon State University Department of Fisheries and Wildlife, 104 Nash Hall, Corvallis, OR, 97330
[2]alexanju@science.oregonstate.edu, Oregon State University Department of Microbiology, 220 Nash Hall, Corvallis, OR, 97330
[3]bartholj@science.oregonstate.edu, Oregon State University Department of Microbiology, 220 Nash Hall, Corvallis, OR, 97330

Changes in the dynamics of infectious disease have been linked with climate change in aquatic systems. Climate related shifts in water temperatures and precipitation patterns will likely have important effects on the dynamics of myxozoan disease. However, predicting the magnitude and direction of specific responses is challenging. We present an overview of myxozoan disease dynamics in the context of climate change illustrated with data from a case study on disease (ceratomyxosis) dynamics in Klamath River salmonids. Using a model ensemble, we have predicted responses of *Ceratonova* (syn *Ceratomyxa*) *shasta* in three different types of water years (dry, median, wet) for three different future climate scenarios (hot/dry, moderate/median, and cold/wet) selected from 2020-2060, in the Klamath River CA, USA. The ensemble uses outputs from Global Climate Models (GCMs) as inputs for hydraulic and water temperature models, which are used as inputs for predictive statistical models. Outputs from the predictive models provide values for parameterizing the epidemiological model, which outputs an estimated basic reproductive number (R_0) for each climate scenario. The resultant R_0 values were scaled relative to empirical data collected in 2008 (high mortality in salmonids) and 2011, (low mortality) in the Klamath River. The majority of predicted future R_0 values were similar to the 2008 value, which provides compelling evidence that *C. shasta*-induced mortality will increase and remain high in the Klamath River.

P-42. Effects of coinfection of channel catfish (*Ictalurus punctatus*) with *Edwardsiella piscicida* and two digeneans, *Bolbophorus damnificus* and *Drepanocephalus spathans*

Stephen R. Reichley[1,2]*, Matt J. Griffin[1,2], Cynthia Ware[1], Terrence E. Greenway[1], Lester H. Khoo[1,2], Mark L. Lawrence[2], David J. Wise[1]

[1]Thad Cochran National Warmwater Aquaculture Center, P.O. Box 197, Stoneville, MS 38776 USA sreichley@cvm.msstate.edu, griffin@cvm.msstate.edu, cware@cvm.msstate.edu, greenway@drec.msstate.edu, khoo@cvm.msstate.edu, dwise@drec.msstate.edu

[2]College of Veterinary Medicine, Mississippi State University, P.O. Box 6100, Mississippi State, MS 39762 USA lawrence@cvm.msstate.edu

Bolbophorus damnificus (Digenea: Trematoda) is one of the most ruinous parasites affecting catfish production in the United States. Conversely, the impact of *Drepanocephalus spathans* (Digenea: Trematoda), a recently described parasite associated with farm-raised catfish, is presently unknown. Investigations of the genotypic variability of *Edwardsiella tarda* recently led to the adoption of a new species of *Edwardsiella*, *E. piscicida*. Research has suggested that *E. piscicida* is more prevalent in catfish aquaculture in Mississippi than *E. tarda*. The mortality associated with concurrent infections caused by *E. piscicida* and these trematode pathogens was investigated in this study. Six treatment groups were created: *E. piscicida* alone, *E. piscicida*/*B. damnificus*, *E. piscicida*/*D. spathans*, *B. damnificus* alone, *D. spathans* alone, and controls. The highest cumulative mortality was observed in the *E. piscicida*/*B. damnificus* group; followed by *E. piscicida* alone, *E. piscicida*/*D. spathans*, and *B. damnificus* alone. Mortality in fish challenged to *D. spathans* alone was similar to controls. This suggests coinfection of *B. damnificus* and *E. piscicida* can increase mortality in channel catfish. Future studies will be directed at investigating coinfection of *B. damnificus* and *D. spathans* with other *Edwardsiella* spp. in channel and channel x blue hybrid catfish.

P-43. A molecular and morphological survey of myxozoan actinospores isolated from *Dero digitata* in commercial channel catfish ponds in the Mississippi Delta

Thomas G Rosser[1]*, Matt J Griffin,[2] Lester H Khoo[2], David J Wise[2], Terrence E Greenway[2], Sylvie M A Quiniou[3], Linda M Pote[1]

[1]College of Veterinary Medicine, Mississippi State University, 240 Wise Center Drive, Mississippi State, MS 39762 tgr49@msstate.edu, griffin@cvm.msstate.edu, khoo@cvm.msstate.edu, lpote@cvm.msstate.edu

[2]Thad Cochran National Warmwater Aquaculture Center, Mississippi State University, PO Box 197, Stoneville, MS 38776 dwise@drec.msstate.edu, greenway@drec.msstate.edu

[3]Warmwater Aquaculture Research Unit, Agricultural Research Service, United States Department of Agriculture, PO Box 38, Stoneville, MS 38776 sylvie.quiniou@ars.usda.gov

The Myxozoa are an enigmatic group of metazoan parasites that primarily infect fish and aquatic annelids, but recently there have been reports of myxozoans identified from other vertebrate hosts (reptiles, amphibians, birds, and mammals). The typical myxozoan life cycle consists of myxospore stages that develop within the fish host following infection by an actinospore stage released by an aquatic annelid. To date more than 2,000 descriptions exist of myxospore stages from fish, while only 200 or so actinospores have been described. With approximately 50 life cycles confirmed, the use of small subunit ribosomal RNA gene sequence could assist in the elucidation of many unknown life cycles. In farm-raised catfish, the myxozoan *Henneguya ictaluri* is of significant importance. The etiologic agent of Proliferative Gill Disease (PGD), *H. ictaluri* is often associated with high fish mortalities and reduced production. The life cycle of *H. ictaluri* is known to consist of a myxospore stage in the gills of channel catfish and an actinospore stage shed by the benthic oligochaete *Dero digitata*. In addition, several other myxozoan species are also present in these ponds. In efforts to provide baseline data to elucidate these unknown life cycles, a survey of *D. digitata* from a commercial catfish pond was performed. Six different actinospore morphotypes were identified and characterized molecularly and morphologically. Two aurantiactinomyxons, two helioactinomyxons, one raabeia, and one triactinomyxon type were observed in this survey. Phylogenetic analyses of the SSU rRNA gene sequences demonstrated the raabeia and one of the helioactinomyxon types grouped with a group of ictalurid infecting myxozoans in North America, suggesting an ictalurid fish is a likely host to these parasites. Future work will focus on experimental completion of these life cycles and evaluate the pathology, if any, associated with these uncharacterized parasites.

P-44. A novel species of *Henneguya* from the gills of farm-raised channel catfish (*Ictalurus punctatus*)

Thomas G Rosser[1]*, Matt J Griffin[2], Sylvie M A Quiniou[3], Lester H Khoo[2], Linda M Pote[1]

[1]College of Veterinary Medicine, Mississippi State University, 240 Wise Center Drive, Mississippi State, MS 39762 tgr49@msstate.edu, griffin@cvm.msstate.edu, khoo@cvm.msstate.edu, lpote@cvm.msstate.edu

[2]Thad Cochran National Warmwater Aquaculture Center, Mississippi State University, PO Box 197, Stoneville, MS 38776

[3]Warmwater Aquaculture Research Unit, Agricultural Research Service, United States Department of Agriculture, PO Box 38, Stoneville, MS 38776 sylvie.quiniou@ars.usda.gov

The channel catfish *Ictalurus punctatus* is a host for at least eight different species of parasites belonging to the genus *Henneguya*, four of which have been molecularly characterized. Only two of these have known life cycles that have been experimentally confirmed to involve the benthic oligochaete *Dero digitata*. During a routine health screening of farm-raised channel catfish, several fish presented with deformed primary lamellae that harbored large, nodular, white cysts approximately 1.25 mm in diameter. Upon rupture, these cysts contained numerous *Henneguya* myxospores, with a typical lanceolate shaped spore body, measuring 17.1 ± 1.0 μm (mean ± SD; range = 15.0-19.3 μm) in length and 4.8 ± 0.4 μm (3.7-5.6 μm) in width. Pyriform shaped polar capsules were 5.8 ± 0.3 μm in length (5.1-6.4 μm) and 1.7 ± 0.1 μm (1.4-1.9 μm) in width. The two caudal processes were 40.0 ± 5.1 μm in length (29.5-50.0 μm) with a total spore length of 57.2 ± 4.7 (46.8-66.8 μm). The contiguous small subunit ribosomal RNA (SSU rRNA) gene sequence generated from myxospores of five excised cysts was not a match to any *Henneguya* sp. currently in GenBank. The greatest sequence homology (91% over 1900 bp) was with *Henneguya pellis*, a parasite associated with blister-like lesions on the skin of the blue catfish *Ictalurus furcatus*. Based on the unique combination of cyst and myxospore morphology, tissue predilection, host and SSU rRNA gene sequence we believe this isolate to be a previously undocumented species of the genus, *Henneguya bulbosus* sp. nov.

P-45. New primers for DNA barcoding of digeneans and cestodes (Platyhelminthes).

Niels Van Steenkiste[1], Sean A. Locke[2,3], Magalie Castelin[1], Geoff J. Lowe[1]*,
David J. Marcogliese[2], Cathryn L. Abbott[1]

[1]Aquatic Animal Health Section, Fisheries and Oceans Canada, Pacific Biological Station, 3190 Hammond Bay Road, Nanaimo, BC, Canada V9T 6N7, Niels.VanSteenkiste@dfo-mpo.gc.ca
[2]Fluvial Ecosystem Research Section, Aquatic Ecosystem Protection Research Division, Water Science and Technology Directorate, Science and Technology Branch, Environment Canada, St. Lawrence Centre, 105 McGill, 7th Floor, Montreal, QC, Canada H2Y 2E7
[3]Current address: Biodiversity Institute of Ontario, University of Guelph, 50 Stone Road East, Guelph, ON, Canada N1G 2W1

Digeneans (flukes) and cestodes (tapeworms) are among the most species-rich groups of parasitic metazoans and can seriously impact human health, fisheries, aqua- and agriculture, nature conservation and wildlife management. Identification of eggs, larval stages and adults to species, both in the environment and in their hosts, is crucial for preventive and remediating actions. Unfortunately, this is often hampered by their small size, hidden nature within hosts and lack of distinctive morphological features. DNA barcoding using the Folmer region of the mitochondrial cytochrome c oxidase subunit I (COI) gene can be a powerful tool for species detection and identification, but data are lacking in many digeneans and cestodes. Both commonly used 'universal' COI primers and those more recently developed to specifically target parasitic flatworms fail to amplify in several taxa. We generated COI alignments of digeneans, cestodes, monogeneans and turbellarians and found that high sequence variation at primer binding sites is the likely cause of problems amplifying across these taxa. Indeed, this high sequence divergence made it impossible to design primers that amplify across all parasitic flatworms; however, we developed new degenerate COI barcoding primers that amplify the Folmer region across digeneans and cestodes. Here we present PCR and sequencing methods for COI barcoding of these taxa, including alternative methods for overcoming initial failures that occurred in some taxa due to slipped-strand mispairings during PCR after a poly-T repeat, non-specific priming and suboptimal amplification. We successfully obtained COI barcodes from all 46 specimens tested, representing 23 families of digeneans and 6 orders of cestodes. This 100% success rate is a major improvement on existing methods for barcoding a broad taxonomic diversity of digeneans and cestodes. We expect methods presented here will lead to significant advances towards redressing the current paucity of sequence data for these taxa in public COI databases.

P-46. Parasitological investigations of the Asian seabass (*Lates calcarifer*) cultured in a fish farm in Setiu Lagoon, Malaysia

Csaba Székely[1]*, Muhammad Hafiz Borkhanuddin[1], Gábor Cech[1], Faizah Shaharom[2], Kartini Mohamed[2], Mohd Shukri Adam Embong[2], Kálmán Molnár[1]

[1] Institute for Veterinary Medical Research, Centre for Agricultural Research, HAS, H-1581 Budapest, P.O. Box 18, HUNGARY szekely.csaba@agrar.mta.hu
[2] Institute of Tropical Agriculture (AQUATROP), University Malaysia Terengganu, Kuala Terengganu, Malaysia

Asian seabass is a favourite food fish cultured mostly in the South Asian region and Australia in intensive and semi-intensive systems. In Malaysia the culture of this species started in 1976, when the Malaysian Government has embarked on the production using the wild and imported fry specimens in floating net cages in Setiu Lagoon on the east coast of Peninsular Malaysia (Norfatimah et al., 2009). Due to the high economic importance of Asian seabass culture, its diseases have been studied intensively (Rückert et al 2008). Here we report on the preliminary results of a parasitological investigation on Asian seabass individuals selected from net cages in Setiu Lagoon in February, 2013. The parasites detected were photographed and species belonging to the Myxozoa or Apicomplexa were fixed for histological, SEM and molecular processing. During the investigation period we found several specific and non-specific parasites. On the gills we found monogeneans of the genus *Laticola* specific for the Asian seabass. Most fish specimens were infected by the non-specific *Trichodina* spp. and *Cryptocarion irritans* damaging the gills and skin, respectively. Two *Henneguya* species were found on the gills (one of them located in the basal, the other in the apical region of the gill filaments), plasmodia harbouring spores of a third *Henneguya* species was found in the muscles. They seem to be species not described yet. The molecular processing of the *Henneguya* spp. is in progress. We found sporulated and unsporulated oöcysts of a *Goussia* species in the gut mucosa of two of the fifteen specimens investigated. The structure of oöcysts of this coccidium corresponded to that reported earlier by Gibson-Kueh et al. (2011). Sporulated oöcysts of the species bear the typical signs of the genus *Goussia*. Description of this species based on the morphology of fresh oöcysts is in progress.

Acknowledgements: OTKA K 100132, KTIA-AIK-12-1-2013-0017 and Malaysian Governmental Scholarship.

P-47. Taxonomic status of *Ichthyobodo* spp., ectoparasitic flagellates causing high mortality of Pacific salmon

Shigehiko Urawa[1*], Mark Freeman[2], Shinya Mizuno[3]

[1]Salmon Resources Division, Hokkaido National Fisheries Research Institute, Fisheries Research Agency, Sapporo 062-0922, Japan urawa@affrc.go.jp
[2]Institute of Ocean and Earth Sciences & Institute of Biological Sciences, University of Malaya, Kuala Lumpur 50603, Malaysia mark@um.edu.my
[3]Salmon and Freshwater Fisheries Research Institute, Hokkaido Research Organization, Eniwa, Hokkaido 061-1433, Japan mizuno-shinya@hro.or.jp

Parasitic flagellates belonging to the genus *Ichthyobodo* infect the skin and gills of various salmonid fishes. Heavy *Ichthyobodo* infections cause severe erosion of the skin epidermis of juvenile salmon, resulting in high mortality due to osmoregulatory failure when they migrate into the marine environment (Urawa 1993). In Japan, the parasites have been commonly recorded as *I. necator* from hatchery-reared juvenile chum and masu salmon. However, recent phylogenetic and morphological analyses have indicated the existence of two *Ichthyobodo* species infecting Atlantic salmon in Norway: a freshwater species, *I. necator sensu stricto* (s.s.) and an euryhaline species, *I. salmonis* (Todal et al. 2004, Isaksen et al. 2011). Their findings have encouraged us to re-examine the taxonomic positions of *Ichthyobodo* isolates from chum and masu salmon in Japan by molecular and morphological analyses as well as cross infection experiments. Our molecular analyses of 18S rDNA (1,950 bp) have indicated that Japanese isolates from chum and masu salmon have a 99% similarity with *I. salmonis* from Atlantic salmon in Norway, but only a 95% identity to *I. necator* s.s. from Atlantic salmon. Additional analyses of the ITS regions (960 bp) have clarified that isolates from chum salmon show a 99.4% similarity with *I. salmonis*, while isolates from masu salmon have a 97% and 96.8% similarity with *I. salmonis* and the chum isolate, respectively. Morphological observations using light- and electron microscopy have indicated that *Ichthyobodo* isolate from chum salmon is similar to *I. salmonis*, but significantly different in cell size, nucleus dimension, and the number of kinetoplasts from the masu isolate. Cross infection experiments have shown that the *Ichthyobodo* isolate from chum salmon easily infects the same host species but not masu salmon. The isolate from masu salmon infects masu but not chum salmon. The *Ichthyobodo* isolate from chum salmon is an euryhaline species reproducing both in fresh- and sea water (Urawa and Kusakari 1990). We have concluded that the isolate from chum salmon is identified as *I. salmonis*, while the *Ichthyobodo* isolate from masu salmon is a new species.

P-48. The effect of fluorescent dyes on the survival and infectivity of larval trematodes: Development of a methodology for determining the portals of entry of *Cardiocephaloides longicollis* into *Sparus aurata*

Gabrielle S. van Beest[1], Mar Villar-Torres[1], Astrid S. Holzer[2]*, Francisco E. Montero[1], Juan A. Raga[1], Ana Born-Torrijos[1]

[1]Cavanilles Institute for Biodiversity and Evolutionary Biology, Science Park, University of Valencia, PO Box 22 085, 46071 Valencia, Spain gavanbe@alumni.uv.es, marvito@alumni.uv.es, francisco.e.montero@uv.es, toni.raga@uv.es and A.Isabel.Born@uv.es

[2]Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic astrid.holzer@paru.cas.cz

The cercariae of the digenean trematode *Cardiocephaloides longicollis* penetrate the skin of gilthead seabream *Sparus aurata* (Sparidae) and migrate to the brain where they encyst as metacercariae of this fish, an important Mediterranean aquaculture species. The cysts could cause significant vision impairment leading to an increase in ‘‘conspicuous’’ behaviour favouring parasite transmission to the final host, as discussed in other sparids (Osset et al. 2005).

For the study of the penetration pattern of *C. longicollis* into its second intermediate host, cercariae of *C. longicollis* were used in different experimental assays. First, the effect of different *in vivo* fluorescent dyes on the survival and infectivity of labelled cercariae was tested. Thereafter, the selected dye helped us to determine the penetration points of *C. longicollis* into *S. aurata*.

Two different fluorescent dyes were tested: (1) 5(6)-Carboxyfluorescein N-hydroxysuccinimidyl ester (CFSE), which stains intracellular amines in life cells and concentrates in the acetabular gland of the cercariae, and (2) Hoechst 33342 (NucBlue), which specifically stains DNA, i.e. the nuclei of life or fixed cells. The effect of both dyes on survival and infectivity of the cercaria was tested with three different ascending concentrations: CFSE as 20 µM, 50 µM and 100 µM, and NucBlue as 1 drop/mL, 2 drops/mL and 3 drops/mL. The behaviour and the longevity of individual cercariae was recorded during 24 hours.

To evaluate post-staining infectivity of the trematodes, 50 stained cercariae were used to infect *S. aurata*, thereafter analysing the water where the fish were infected, to carry out the tails count, as cercariae lose them when they penetrate the host. Additionally, 20 days after the infection, fish brains were analysed and the number of encysted metacercariae was recorded.

P-49. Light microscopic study of *Myxobolus* sp. (Myxozoa) parasitic in kidney of *Leporinus friderici* Bloch, 1794 (Characiformes: Anostomidae) from Brazil.

Letícia G P Vidal[1]*, José L Luque[2]

[1]Universidade Federal Rural do Rio de Janeiro, Instituto de Veterinária, Programa de Pós-Graduação em Ciências Veterinárias, Seropédica, Rio de Janeiro, Brasil let_vidal@yahoo.com.br
[2]Universidade Federal Rural do Rio de Janeiro, Instituto de Veterinária, Departamento de Parasitologia Animal, Seropédica, Rio de Janeiro, Brasil luqueufrrj@gmail.com

The Neotropical region have the most diversified fish fauna of the world. Among fish pathogens, Myxozoa is an important group. Composed by highly specialized metazoan parasites, mainly of aquatic hosts with a wide host range. *Myxobolus* Bütschli, 1882 is the most common genus, with the greatest number of species infecting both marine and freshwater fishes. From South America, 30 species of *Myxobolus* have been reported and this number certainly will increase and some species can be found infecting the kidney and urinary system of their hosts. A light microscopy study of a myxosporean parasitizing the kidney of the freshwater fish *Leporinus friderici* Bloch, 1794 (Characiformes: Anostomidae) collected in Mogi Guaçú River (21°55'37" S, 47°22'03" W) in the State of São Paulo, Brazil, was carried out. This parasite produces spherical to ellipsoidal histozoic plasmodia, which were observed on 16 out of 24 (66.6%) *L. friderici* examined, and were 15 ± 2.7 mm long. Plasmodia were placed on glass slides, stained with Giemsa and examined using a light microscope. Measurements and photographs were taken with the use of the differential interference contrast microscope (DIC) Olympus BX 51 coupled with a digital camera Olympus UC 30 (Olympus, Center Valley, Pennsylvania). Mature spores were ovoid in frontal view with rounded extremities, and measured 12.9 ± 0.7 μm in total length and 9.6 ± 0.6 μm wide. Polar capsules were elongated 6.05 ± 0.5 μm long and 2.7 ± 0.2 μm wide. This is the first report of *Myxobolus* sp. in the kidney of *L. friderici* in São Paulo. More information about ultrastructure and genetics will be added to this study in order to confirm the possibility of a new taxon.

P-50. Host-parasite relationship and phylogeny of *Myxobolus* sp. parasite of *Brycon orthotaenia* from São Francisco River, Brazil

Suellen A Zatti[1], Juliana Naldoni[1], Kassia RH Capodifoglio[2], Tiago Milanin[1], Antônio AM Maia[1], Marcia RM Silva[2], Edson A Adriano[1,3]*

[1]Departamento de Biologia Animal, Universidade Estadual de Campinas, Campinas, SP, Brazil
[2]Departamento de Medicina Veterinária, Universidade de São Paulo, Pirassununga, SP, Brazil
[3]Departamento de Ciências Biológicas, Universidade Federal de São Paulo, Diadema, SP, Brazil
edapadriano@gmail.com

The genus *Myxobolus* Bütschli 1882 is the most speciose group within the myxozoans, with more than 850 species described. So far, 37 *Myxobolus* species have been described infecting fish species in South America and nine species have been reported for fish of the Bryconidae family. In this work is reported the occurrence of plasmodia of an unknown *Myxobolus* sp. infecting gill filaments of *Brycon orthotaenia* caught in São Francisco River, Minas Gerais state, Brazil. This characid fish of the family Bryconidae is popularly known in Brazil as *piraputanga*. The parasite was observed in three of the 39 *B. orthotaenia* specimens examined. The plasmodia were white and long, measuring 5 mm. Mature spores were oval shaped from the frontal view and biconvex from the lateral view, measuring 9.0 ± 0.3 µm in length and 6.2 ± 0.4 µm in width. The polar capsules were elongated and equal in size, measuring 4.7 ± 0.3 µm in length and 1.7 ± 0.1 µm in width. Histopathological analysis showed that the development of the parasite led to compression of the adjacent tissues and inflammatory infiltrate with granulocytic cells. Ultrastructure analysis revealed that the plasmodia were delimited by two membranes, which had numerous and extensive pinocytotic channels extending into the wide ectoplasm zone. The plasmodial wall exhibited abundant villi-like projections and a thin layer of granular material prevented direct contact between the plasmodial wall and the host tissue. Phylogenetic analysis, based on 18S rDNA, showed *Myxobolus* sp. clustering as a sister species of *Myxobolus oliveirai*, a parasite of other fish species of the genus *Brycon*.

Study supported by CNPq (Proc. nº 472747/2012-6).
Doctorate student supported by FAPESP scholarship (Proc. nº 2011/10738-1 - J. Naldoni; Proc. nº 2011/08549-6 - T. Milanin).

P-51. A new strategy to characterize different cell types from fish leukocytes using the bio-imaging technology of the confocal Raman microspectroscopy

Takashi Aoki[1]*, Masahiro Ando[1], Hiro-o Hamaguchi[2], Jun-ichi Hikima[3], Masahiro Sakai[3], Tadaaki Moritomo[4], Teruyuki Nakanishi[4], Haruko Takeyama[5]

[1]Institute for Nanoscience and Nanotechnology, Waseda University, Tokyo 162-8480, Japan, aokitaka@aoni.waseda.jp
[2]Department of Applied Chemistry and Institute of Molecular Science, National Chiao Tung University, Hsinchu 30010, Taiwan, hhama@nctu.edu.tw
[3]Department of Biochemistry and Applied Biosciences, Faculty of Agriculture, University of Miyazaki, Miyazaki 889-2192, Japan, jhikima@cc.miyazaki-u.ac.jp, m.sakai@cc.miyazaki-u.ac.jp
[4]College of Bioresource Sciences, Nihon University, Fujisawa 252-0880, Japan, moritomo.tadaaki@nihon-u.ac.jp, tnakanis@brs.nihon-u.ac.jp
[5]Department of Life Science and Medical Bioscience, Waseda University, Tokyo 162-8480, Japan, haruko-takeyama@waseda.jp

Isolation of a single type of cell from leukocytes needs a specific cell marker for confirming the identity. However, it is cumbersome to identify the specific cell marker and produce the specific antibody for fish because of evolutional differences from mammals. Currently, Raman spectroscopy is gaining attention as a powerful biochemical technique that allows for dynamic characterization and bio-imaging of living tissues and cells in absence of additional staining (*e.g.*, fluorescent stain). Different instrumentation strategies utilizing confocal detection optics, multispot, and line illumination have been developed to improve speed and sensitivity of the analysis of single cell type by Raman spectroscopy. In this study, the detection of CD4-positive cell (T-cell), an IgM-positive cell (B-cell), a thrombocyte and an erythrocyte isolated from ginbuna crucian carp (*Carassius auratus*) was conducted by non-invasive biochemical characterization of single cells using the confocal Raman microspectroscopy. The Raman spectra from the contents of ginbuna crucian carp cell (including the Raman spectra derived from proteins and nucleic acids) were detected and analyzed for visualization of a single cell. The spectra detected from ginbuna erythrocyte were easily divided from those of other cells. A couple of unique spectra from a $CD4^+$ cell were also detected when compared to the thrombocyte spectra. The results suggest possibility of cell separation by Raman spectroscopy technique for several cell-types of fish leukocytes. This strategy for a single cell analysis by Raman spectroscopy would have wide biomedical implications on biological live-detections in eukaryotic cells.

P-52. FISHPATHOGENS.NET – A richly visual fish pathogen database

Stephen D Atkinson[1]*, Sascha L Hallett[1], Casey P Dinsmore[2], Craig Banner[3], Jerri L Bartholomew[1]

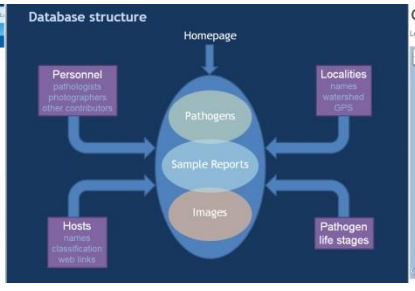
[1]Department of Microbiology, Oregon State University, Corvallis, Oregon, USA
[2]COSine, College of Science, Oregon State University, Corvallis, Oregon, USA
[3]Oregon Department of Fish & Wildlife, Corvallis, Oregon, USA

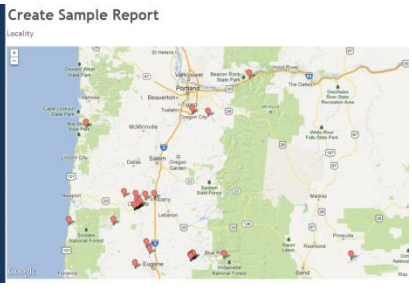
The popularity of recreational fishing in the Pacific Northwest of North America leads to the Oregon Department of Fish and Wildlife (ODFW) receiving many enquiries regarding abnormal appearing fish, e.g. “What is that large white cyst in my salmon fillet?”, “What are these worms in this bluegill?”, “Can I still eat it?”. ODFW supported us (OSU) to create a public database that is an easy-to-use, richly visual, web-based source of information for the pathogens of Oregon’s fishes: www.fishpathogens.net.

Our database cross-references three primary categories of information: >500 host and pathogen images from OSU and ODFW collections; >120 pathogen information summaries from published sources; and >600 fish examination reports. Data sub-categories include lists of >200 fish host species, and a Google Maps-based locality recording and searching tool. We are digitizing pathogen records from OSU surveys and ODFW health records of wild and hatchery fish. We aim to also incorporate significant but poorly-circulated data (reports, personal databases) from fisheries scientists.

Pathogens include viruses, bacteria, fungi, protozoans and metazoan parasites (acanthocephalans, myxozoans, crustaceans, platyhelminthes, nematodes). Non-specialists can identify common macroscopic pathogens and disease signs by stepping through a guided image search (“What’s That Spot?”, browse through galleries of host and pathogen images, or search based on particular hosts, pathogen types or localities. Pathogen information summaries can be printed out as “fact sheets”.

The database is hosted on a dedicated MySQL database server at OSU, using CentOS Linux, with Drupal 7 for webpage creation. We have designed both the output and input to be mobile-device-friendly, to promote accessibility by both the public and fish pathologists.





P-53. The effects of a sublethal dose of botulinum Serotype E on the swimming performance of channel catfish (*Ictalurus punctatus*) fingerlings

Rachel Beecham[1]*, T. Thomas[1], D. X. Gao[2], P. S. Gaunt[2]

[1]Mississippi Valley State University, Dept. of Natural Science, 14000 Hwy 82 W, Itta Bena, MS 38941, rvbeecham@yahoo.com

[2]Mississippi State University, College of Veterinary Medicine, P.O. Box 197, 127 Experiment Station Road, Stoneville, MS 38776

Visceral toxicosis of catfish (VTC) is a disease of cultured Channel Catfish *Ictalurus punctatus* in the MS Delta Region and surrounding states. The etiology of VTC is associated with botulinum serotype E (BoNT/E) which causes blockage of acetylcholine release at the neuromuscular junction leading to weakness and paralysis of skeletal muscles including those involved in swimming. This study attempted to determine if sublethal exposure to purified BoNT/E caused reductions in swimming performance and metabolism of channel catfish. Catfish swimming performance was assessed on stocker sized channel catfish (mean weight 62.35 ± 2.5 g) with 10 sham-injected fish and 10 fish injected with a sublethal dose of BoNT/E. A modified Blazka type swim chamber was used to assess swimming performance. We injected catfish with either 0.015% trypsin or 400 pg purified BoNT/E digested with 0.015% trypsin intracoelomically, then acclimated an individual catfish in the swim chamber for 17 hours prior to the swimming trial. Water temperature was maintained at ~28°C and dissolved oxygen was between 4 and 7 mg/L. A critical swimming speed (Ucrit) protocol was followed and dissolved oxygen and temperature were monitored every 2 minutes throughout the swim trial. Cost of transport was calculated from the oxygen consumption at each test speed (10-70 cm/s). There was a statistical difference between the Ucrits ($p = 0.0034$), but no differences were found between the cost of transports ($p=0.67$) of the sham injected and BoNT/E groups. There was a difference in the cost of transport as it relates to the speeds tested ($p<0.0001$) with cost of transports being highest at low speeds and decreasing as speed increased. These results indicate that botulinum E interferes with the swimming speed of the catfish which could contribute to the mortality from the disease of VTC and potentially make the fish more susceptible to predation.

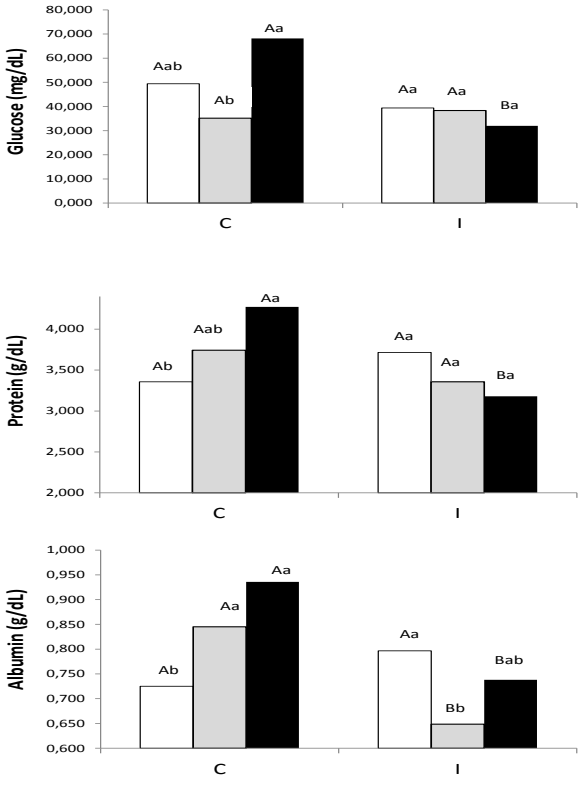
P-54. Insulin treatment affects carbohydrate, protein and fat metabolism in tilapia during acute inflammation

Marco A. A. Belo[1,2]*, Ed J.R. Prado[2], Alessandra C. Moraes[2], Elizabeth P. Foz[1], Roberto Barbuio[2], Vanessa P. Faria[2]

[1]Laboratory of Animal Pharmacology and Toxicology, Camilo Castelo Branco University - UNICASTELO, 950 Hilário da Silva Passos Ave., Descalvado/SP. maabelo@hotmail.com; betinafoz@hotmail.com.

[2]Dep. of Veterinary Pathology – São Paulo State University/UNESP–Jaboticabal/SP. ed_johnny@hotmail.com; alecris_moraes@hotmail.com; betovet04@yahoo.com.br; vanessapavesifaria@hotmail.com.

Nile tilapia , *Oreochromis niloticus*, GIFT (±532g) , masculinized , were divided into two groups of 21 animals each treated or not with insulin (Lantus ®), subcutaneously route a single dose of 10 IU-kg^{-1} (both inoculated with *Aeromonas hydrophila* in the swim bladder). Seven animals from each treatment were sampled at 6, 24 and 48 hours post-inoculation (HPI) for blood collection. Glucose, total protein, albumin, triglycerides and cholesterol were determined.



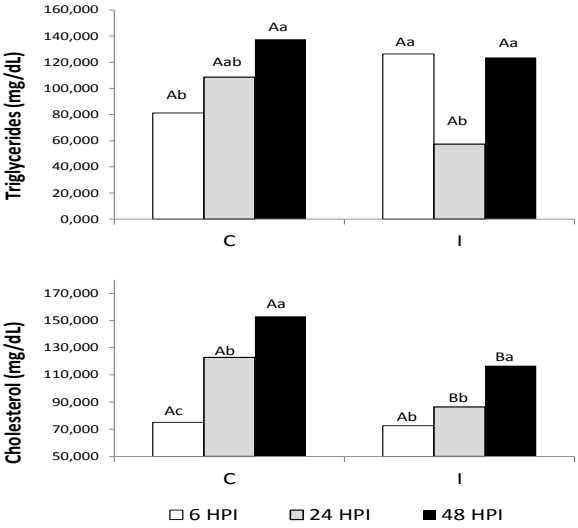


Figure 1. Mean values (n=7) and ANOVA for glucose, total protein, albumin, triglycerides and cholesterol. C (Control);I (insulin). Capital letters compare treatments and Lowercase letters within treatment (P<0,05, by the Tukey test).

Tilapia treated with insulin showed significant (P<0.05) decrease in the levels of glucose and total protein (48 HPI), as well as significant (P<0,05) decrease in albumin and cholesterol 24 and 48 HPI, when compared to infected control. On the other hand, tilapia only infected (Control) presented increase in the levels of total protein, albumin, cholesterol and triglycerides during the evolution of acute infectious aerocistite, as long as fish treated with insulin showed decreasing values of glucose and total protein, and significant (P<0,05) decrease of albumin and triglicerydes levels at 24 HPI (Figure 1).

P-55. Immunostimulant effects of the Mongolian dairy product derived lactic acid bacteria in the Japanese pufferfish (*Takifugu rubripes*)

Gouranga Biswas[1], Tomoya Kono[2], Jun-ichi Hikima[2], Masahiro Sakai[2]*

[1]Interdisciplinary Graduate School of Agriculture & Engineering, University of Miyazaki, Miyazaki 889-2192, Japan gbis47@gmail.com

[2]Department of Biochemistry & Applied Biosciences, Faculty of Agriculture, University of Miyazaki, Miyazaki 889-2192, Japan tkono@cc.miyazaki-u.ac.jp, jhikima@cc.miyazaki-u.ac.jp, m.sakai@cc.miyazaki-u.ac.jp

With increasing disease occurrence in intensive aquaculture, prevention of diseases by elevating innate immunity using biological products is a desirable option. Cytokines, small glycoproteins produced by immune-competent cells play an important role in host innate and adaptive immunity. Firstly, to compare immunostimulant efficacy of two lactic acid bacteria (LAB), *Lactobacillus paracasei* spp. *paracasei* (strain 06TCa22) (Lpp) and *L. plantarum* (strain 06CC2) isolated from the Mongolian dairy products, we assessed 16 cytokine gene expressions using multiplex RT-PCR analysis (GenomeLab Genetic Analysis System, GeXPS; Beckman Coulter Inc.) in the Japanese pufferfish (*Takifugu rubripes*) head kidney (HK) cells incubated with these two heat-killed LABs. Further, we used Lpp that exhibited better immunostimulant effects in the *in vitro* experiment, for oral delivery (1 mg/ g body weight/ day for 3 days) to pufferfish and investigated transcriptomic responses of 16 cytokines. At 24 h post treatment, fish were infected by an intramuscular injection of 0.1 mL *Vibrio harveyi* bacterial suspension (10^8 cfu/ mL). Additionally, we assessed superoxide anion production (SAP) and phagocytic activity (PA) of HK cells and resistance to *V. harveyi* infection in treated fish. Significant up-regulation of pro-inflammatory (IL-1β, IL-6, IL-17A/F-3, TNF-α and TNF-N), cell-mediated immunity inducing (IL-12p35, IL-12p40 and IL-18), antiviral/ intra-cellular pathogen killing (type I- IFN-1 and IFN-γ), anti-inflammatory (IL-10) and peripheral T cell expansion and survival controlling (IL-2, IL-7, IL-15, IL-21 and TGF-β1) cytokines was observed in the treated fish and also in *V. harveyi* infected fish. Furthermore, significantly increased SAP, PA and decreased load of pathogenic *V. harveyi* ($P < 0.01$; 0.05) were observed in the treated fish compared to untreated fish. Our results indicate the enhancement of cytokine-mediated immunity in the Japanese pufferfish by the use of the heat-killed Lpp as a potential immunostimulant to confer protection against *V. harveyi* infection.

P-56. Analysis of the kinome of the Pacific oyster *Crassostrea gigas* for the identification of signals in response to the environement.

Charlotte Corporeau[1]*, Yanouk Epelboin[1], Laure Quintric[2], Eric Guévélou[1], Vianney Pichereau[3]

[1]Laboratoire des sciences de l'Environnement Marin (UMR 6539, LEMAR), Ifremer, 29280 Plouzané, France. Charlotte.corporeau@ifremer.fr*
[2]Ifremer, Laboratoire Ressources Informatiques et Communications, 29280 Plouzané, France.
[3]Laboratoire des sciences de l'Environnement Marin (UMR 6539, LEMAR), UBO, 29280 Plouzané, France.

Phosphorylation generated by protein kinases is a central mechanism involved in a very large number of cellular processes: differentiation, division, proliferation, apoptosis, growth, survival, metabolism, and particularly in the signaling mechanisms. The study of the protein kinases of the Pacific oyster *Crassostrea gigas* is now possible thanks to the recently published genome (Zhang et al. 2012). The Pacific oyster is a sessile animal which undergoes all along his life environmental constraints and allows a large part of its energy to reproduction. Here we present the *in silico* analysis of the kinome in *C. gigas* to help understand how the oyster can control its physiological state for adaptation to sessile life and extensive set of responses to environmental stress. The predicted protein kinases in the *C. gigas* genome were identified, annotated and classified, according to both function and kinase domain taxonomy (Manning et al., 2002). Results show that the *C. gigas* kinome consists of a total number of 372 protein kinases, closer to the sea urchin kinome (353), an organism that also lives in the marine environment. Surprisingly, the diversity of *C. gigas* kinases diverges from sea urchin kinome since the number of tyrosine kinases (70) and tyrosine-kinase like (40) important for intracellular communication and cell-cell signaling resembles the human kinome (90 and 43 respectively). Moreover, *C. gigas* kinome lacks 15 atypical kinases and presents a particular abundance in Receptor guanyl-cyclase (RGC) (15 in more than sea urchin kinome, 18 in more than human kinome), mostly represented by a large amount of 17 natriuretic peptide receptors (NPR). Our work enabled the creation of the kinome of the Pacific oyster, provides a comparison with other species, in particular marine species, and highlights the link between composition of the kinome with the living environment of this organism.

P-57. Integrative analysis of the effects of microplastics in Pacific oyster *Crassostrea gigas*.

Charlotte Corporeau[1]*, Charlotte Laot[1], Rossana Sussarellu[1], Philippe Soudant[2], Christophe Lambert[2], Caroline Fabioux[2], Ika Paul-Pont[2], Nelly Le Goïc[2], Virgile Quillien[1], Hélène Hegaret[2], Anne-Laure Cassone[2], Marie Eve Julie Arsenault-Pernet[1], Myrina Boulais[1], Christian Mingant[1], Johan Robbens[3], Marc Suquet[1], Arnaud Huvet[1]

[1]Ifremer, Centre de Bretagne, LEMAR UMR 6539 UBO-CNRS-IRD-IFREMER, Plouzané, France
[2]CNRS, LEMAR UMR 6539 UBO-CNRS-IRD-IFREMER, Institut Européen de la Mer, Plouzané, France
[3]ILVO, Oostende, Belgium
Email: Charlotte.Corproreau@ifremer.fr

Plastics are persistent synthetic materials, which accumulate in the marine environment. Over time, biological, chemical and physical processes degrade large plastic items into microplastics (MP). As part of the MICRO European Interreg project (MICROPlastics – Is it a threat for the 2 seas Area?), MP biological effects were assessed through an integrative approach on the Pacific oyster *Crassostrea gigas*. The oyster *C. gigas* is a widely studied model organism that lives in the highly stressful intertidal zone. They are likely to be impacted by MP pollution, as they filter large volumes of water and can ingest microscopic particles while feeding.
Thanks to the *C. gigas* genome that was recently published by Zhang et al. (2012), we can now combine "-omics" data with ecophysiological parameters, in order to get information on mechanisms and biological processes that might be affected by MP contamination in oysters. In our project, adult oysters were exposed to MP during two-months under controlled conditions, using a mix of yellow-green fluorescent polystyrene MP (2 and 6 µm, 2000 particles mL^{-1}). The diet conditions were designed to induce gametogenesis in the laboratory. Throughout the experimental MP contamination, growth, survival, ingestion, assimilation, gametogenesis and immunological parameters were followed in adult oysters. The gametes produced by contaminated oysters were analysed in terms of number/size of oocytes and number/velocity of spermatozoa. The fertilization success was estimated for each female by the level of D-larval yield. Trans-generational effects were measured by studying larval development. Genomics and proteomics were then used to look for pathways disrupted by MP contamination and explain mechanisms linked with the phenotypical effects that were induced by MP in adult oyster and gametes.

P-58. Novel cytokine homologue gene, IL-17, in Kuruma shrimp *Marsupenaeus japonicas*

Mari Inada[1], Masahiro Sakai[2]*, Toshiaki Itami[2]

[1] Tokyo University of Marine Science and Technology, Konan 4-5-7, Minato-ku, Tokyo 108-8477, Japan minada0@yahoo.co.jp

[2] Faculty of Agriculture, University of Miyazaki, 1-1, Gakuen Kibanadai-nishi, Miyazaki, 889-2192 Japan itamit@cc.miyazaki-u.ac.jp

Cytokines are signaling protein molecules and have various physiological functions such as cell proliferation, cell differentiation and so on. In vertebrates, interleukin-17 (IL-17) is known as inflammatory cytokine and promotes the inflammatory response, cell migration and granulopoiesis. Regarding the study of IL-17, research is performed actively in vertebrates. On the other hand, reports are very few in invertebrates. In this study, we report the identification and characterization of genes of IL-17, from kuruma shrimp, *Marsupenaeus japonicus*. This is the first report in crustacean. The open reading frame of *Mj*IL-17 encodes a protein of 244 amino acids with an estimated molecular mass of 26.8 kDa. The *in silico* analyses such as domain, homology and phylogenetic analyses were performed. *Mj*IL-17 was conserved the IL-17 domain which is known as characteristic domain of IL-17 family. In genome analysis, *Mj*IL-17 had no intron. In *in vivo* experiment, gene expression analysis was performed. In various organs, *Mj*IL-17 didn't show the significant gene expression. Additionally, we obtained the similar data in pathogen infection experiment. These data suggested that *Mj*IL-17 gene hardly express or may be pseudo-gene in kuruma shrimp.

M. Inada is a recipient of the Japan Society for the Promotion of Science (JSPS). This study was supported, in part, by research grants from the JSPS and the JSPS Asian CORE Program.

P-59. Characterization of cytokine homologue genes, VEGF, MIF and Astakine, in Kuruma shrimp *Marsupenaeus japonicas*

Mari Inada[1]*, Toshifumi Yui[2], Masahiro Sakai[3], Toshiaki Itami[3]

[1]Tokyo University of Marine Science and Technology, Konan 4-5-7, Minato-ku, Tokyo 108-8477, Japan minada0@yahoo.co.jp
[2]Faculty of Engineering, University of Miyazaki, 1-1, Gakuen Kibanadai-nishi, Miyazaki, 889-2192 Japan tyui@cc.miyazaki-u.ac.jp
[3]Faculty of Agriculture, University of Miyazaki, 1-1, Gakuen Kibanadai-nishi, Miyazaki, 889-2192 Japan itamit@cc.miyazaki-u.ac.jp

Cytokines are known as signaling protein molecules for intercellular communication. In vertebrates, the vascular endothelial growth factor (VEGF) family is cystine-knot cytokines which promote angiogenesis, chemotaxis for macrophages and granulocytes, and lymphangiogenesis. The macrophage migration inhibitory factor (MIF) is an inflammatory multi-functional cytokine and involves in innate immunity. In invertebrate, Astakine is known as the invertebrate cytokine which has prokineticin domain and can induce the hematopoietic stem cell differentiation in freshwater crayfish, *Pacifastacus leniusculus*. In this study, we report the characterization of genes of VEGF, MIF and Astakine from kuruma shrimp, *Marsupenaeus japonicus*.

The full-length cDNA sequence of the *Mj*VEGF1, *Mj*MIF and *Mj*Astakine genes were 845 bp, 894 bp and 1,589 bp. The *in silico* analyses such as domain, homology and phylogenetic analyses were performed. In prediction of 3D structure, *Mj*VEGF1 formed dimer. On the other hand, MIF formed trimer. In *in vivo* experiment, gene expression analysis was performed. The gene expression of *Mj*VEGF1 and *Mj*Astakine increased after white spot syndrome virus (WSSV)-injection. These data suggested that *Mj*VEGF1 and *Mj*Astakine may be important in innate immunity in kuruma shrimp.

M. Inada is a recipient of the Japan Society for the Promotion of Science (JSPS). This study was supported, in part, by research grants from the JSPS and the JSPS Asian CORE Program.

P-60. Cytokine of TGF-β family, myostatin, in Kuruma shrimp *Marsupenaeus japonicas*

Mari Inada[1], Toshifumi Yui[2], Masahiro Sakai[3]*, Toshiaki Itami[3]

[1]Tokyo University of Marine Science and Technology, Konan 4-5-7, Minato-ku, Tokyo 108-8477, Japan minada0@yahoo.co.jp
[2]Faculty of Engineering, University of Miyazaki, 1-1, Gakuen Kibanadai-nishi, Miyazaki, 889-2192 Japan tyui@cc.miyazaki-u.ac.jp
[3]Faculty of Agriculture, University of Miyazaki, 1-1, Gakuen Kibanadai-nishi, Miyazaki, 889-2192 Japan itamit@cc.miyazaki-u.ac.jp

Cytokines are signaling protein molecules for intercellular communication and have various physiological functions. In vertebrates, Myostatin (MSTN) is included in the transforming growth factor beta (TGF-β) family. Additionally, MSTN is known as growth differentiation factor (GDF)-8 and shows more than 90% identity with GDF-11. Regarding physiological function, MSTN is involved in muscle growth. In this study, we report the Identification and characterization of genes of MSTN, from kuruma shrimp, *Marsupenaeus japonicus*.
The full-length cDNA sequence of the *M. japonicus* Mstn (*Mj*MSTN) gene comprises 1,391 bp. The open reading frame of *Mj*MSTN encodes a protein of 428 amino acids with an estimated molecular mass of 49.0 kDa. The *in silico* analyses such as domain, homology and phylogenetic analyses were performed. In prediction of 3D structure, *Mj*MSTN formed dimer. In *in vivo* experiment, gene expression analysis was performed. *Mj*MSTN gene showed high level expression in muscle and heart. In pathogen infection test, the gene expression of *Mj*MSTN decreased at 108 hours after white spot syndrome virus (WSSV)-injection. This data suggested that *Mj*MSTN may be involved in virus infection in kuruma shrimp.
M. Inada is a recipient of the Japan Society for the Promotion of Science (JSPS). This study was supported, in part, by research grants from the JSPS and the JSPS Asian CORE Program.

P-61. Molybdoflavo enzyme gene, Xanthine Dehydrogenase, in Kuruma shrimp *Marsupenaeus japonicas*

Mari Inada[1], Daisuke Shigeyoshi[2], Masahiro Sakai[2]*, Toshiaki Itami[2]

[1]Tokyo University of Marine Science and Technology, Konan 4-5-7, Minato-ku, Tokyo 108-8477, Japan minada0@yahoo.co.jp
[2]Faculty of Agriculture, University of Miyazaki, 1-1, Gakuen Kibanadai-nishi, Miyazaki, 889-2192 Japan itamit@cc.miyazaki-u.ac.jp

Free radicals such as reactive oxygen species (ROS) play a significant role in many physiological processes. In vertebrates, ROS such as the superoxide-derived hydrogen peroxide (H2O2) and superoxide (O2-) are involved in antibacterial and antiviral host defense mechanisms. In mammals, xanthine oxidoreductase (XOR) is members of the subfamily molybdo-flavoenzymes and is known to generate the ROS. The xanthine oxidase (XO) and Xanthine dehydrogenase (XDH) are known as XOR and are single-gene products. Conversion of XDH to xanthine oxidase (XO) is stimulated by inflammatory cytokines. In this study, we report the characterization of genes of XDH from kuruma shrimp, *Marsupenaeus japonicus*.
The open reading frame of *Mj*XDH gene were 4,086 bp and estimated mass was 150 kDa. In phylogenetic analysis, *Mj*XDH was closely related to the insect XDH and clustered with invertebrate XDH. Characteristic domain of XDH was well-conserved. The expression of *Mj*XDH gene decreased after the white spot syndrome virus (WSSV)-injection. This data suggested that *Mj*XDH may be involved in virus infection in kuruma shrimp.
M. Inada is a recipient of the Japan Society for the Promotion of Science (JSPS). This study was supported, in part, by research grants from the JSPS and the JSPS Asian CORE Program.

P-62. Oyster restoration research in Apalachicola Bay, Florida: engaging academic and community partnerships to address environmental and fishery challenge

Andrew S. Kane[1,2]*, Karl E. Havens[2], Shannon Hartsfield[3], Joe Taylor[4], Angela Lindsey[5], Tracy Irani[5], J. Glenn Morris[1]

[1]Department of Environmental & Global Health, College of Public Health and Health Professions; and University of Florida Emerging Pathogens Institute, Gainesville, Florida USA kane@ufl.edu jgmorris@epi.ufl.edu
[2]Florida Sea Grant College Program, Gainesville, Florida USA khavens@ufl.edu
[3] Franklin County Seafood Workers Association, Apalachicola, Florida USA shannonaber@yahoo.com
[4]Franklin's Promise Coalition, Apalachicola, Florida USA palme2blue@yahoo.com
[5]Center for Public Issues Education in Agriculture and Natural Resources, IFAS-University of Florida, Gainesville, Florida USA ablindsey@ufl.edu irani@ufl.edu

Apalachicola Bay, Florida, is renowned for production of high quality Eastern oyster (*Crassostrea virginica*). The oyster industry employs over 2,500 people, and supports one of Florida's few remaining heritage seafood fisheries. It contributes 90% of Florida's and more than 10% of the nation's oyster harvest. A severe decline in the Apalachicola Bay oyster fishery occurred in 2012, two years after the Deepwater Horizon oil spill, concomitant with reduced freshwater flow into the bay and severe drought throughout the watershed. Altered environmental conditions, particularly elevated salinity, gastropod oyster predators (*Stromonita, Melongena*), indwelling shell parasites (*Cliona, Polydora, Diplothyra;* Figure 1), and Dermo disease (*Perkinsis marinus*), represent multiple stressors that, along with uncoordinated resource management in this timeframe, made for the "perfect storm" to affect this fishery. Efforts supported through the NIEHS and the University of Florida Oyster Recovery Team engaged the regional seafood worker community to support needed management and monitoring plans, and demonstrated that oil spill-related contamination was not a cause of the fishery decline. These efforts are developing novel monitoring approaches, and have demonstrated the utility of community-based engagement to support of fishery-based science and risk communication.

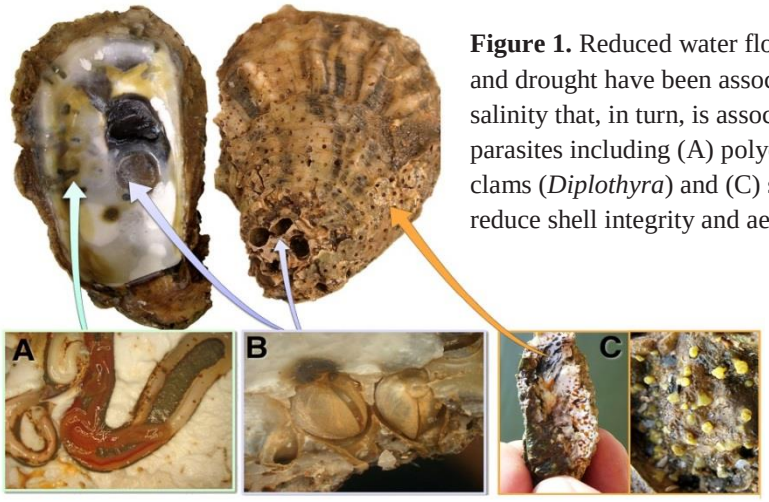


Figure 1. Reduced water flow into Apalachicola Bay and drought have been associated with elevated salinity that, in turn, is associated with shell-boring parasites including (A) polychaetes (*Polydora*), (B) clams (*Diplothyra*) and (C) sponge (*Cliona*), that all reduce shell integrity and aesthetics.

P-63. Splenic macrophage aggregates as potential biomarker of exposure in red Snapper sampled from the northern Gulf of Mexico post-DWH oil spill

Andrew S. Kane[1,2]*, Jaclyn Pine[1], Isabel C. Romero[3], David J. Hollander[3], William F. Patterson III[4]

[1]Department of Environmental and Global Health, College of Public Health and Health Professions, University of Florida, Gainesville, Florida USA
[2]Aquatic Pathobiology Laboratories, Emerging Pathogens Institute, University of Florida, Gainesville, Florida USA kane@ufl.edu
[3]College of Marine Sciences, University of South Florida, St. Petersburg, Florida USA isabelromero@mail.usf.edu davidh@marine.usf.edu
[4]Dauphin Island Sea Laboratory, University of South Alabama, Dauphin Island, Alabama USA wpatterson@disl.org

Macrophage aggregates were histologically observed and their severity ranked in the spleens of red snapper, *Lutjanus campechanus*, collected in 2011 and 2012 from the northern Gulf of Mexico. This study evaluated macrophage (MΦ) aggregate numbers, sizes and distribution based on fish condition index, location of capture, and hepatic PAH burden. Ranked MΦ severity observations (Figure 1) were validated and revealed positive correlations between MΦ ranks, and MΦ numbers and areas ($p<0.01$). A positive correlation between splenic macrophage severity rank and fish total length ($p<0.05$), but not fish weight ($p=0.15$), was observed. Metrics data further suggest that larger fish may have lower condition indices compared with literature values for red snapper from the northern Gulf in previous years. No differences in MΦ aggregate severity ranks were observed in red snapper sampled from multiple zones distributed East to West in the Gulf of Mexico ($F=0.668$, $p=0.57$). Splenic MΦ aggregate severity, however, appeared predictive of hepatic PAH burden ($F=4.71$, $p=0.004$), suggesting the utility of MΦ aggregate evaluation to discern levels of PAH exposure and uptake in red snapper. Results provide important precursor knowledge for longer term assessments of potential chronic effects of PAHs on fish communities in the Northern Gulf of Mexico.

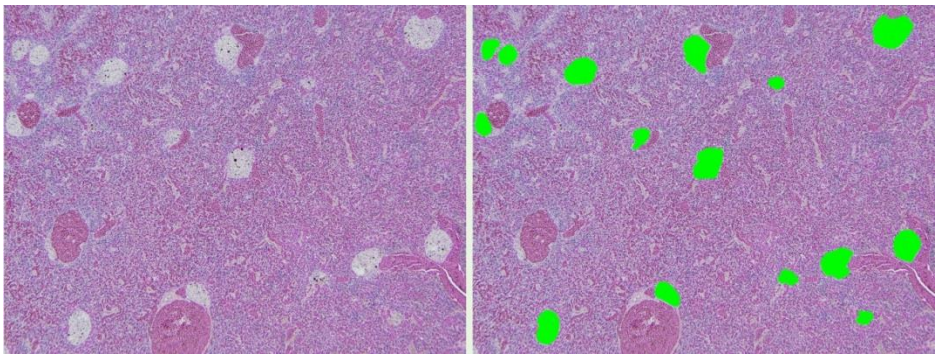


Figure 1. ***LEFT:*** Photomicrograph of red snapper spleen showing macrophage aggregates as pale, irregular cell clusters. ***RIGHT:*** Same photomicrograph with macrophage aggregates digitally masked to facilitate quantitative area and count analyses.

P-64. The relative proteome analysis of olive flounder cultured at different temperature

Jong-Oh Kim[1], Wi-Sik Kim[1], Joseph Kwon[2], Duwoon Kim[3], Myung-Joo Oh[1]*

[1]Department of Aqualife Medicine, College of Fisheries and Ocean Sciences, Chonnam National University, Yeosu, Republic of Korea jongoh.kim77@gmail.com (J-O Kim), wisky@jnu.ac.kr (W-S Kim), ohmj@jnu.ac.kr (M-J Oh)
[2]Korea Basic Science Institute, Daejeon, Republic of Korea joseph@kbsi.re.kr
[3]Department of Food Science and Technology, College of Agriculture and Life Sciences, Chonnam National University, Gwangju, Republic of Korea dwkim@jnu.ac.kr

Temperature is a primary physical factor affecting the life of fish. It is reported that temperature in the range of 15–25°C is optimal for the growth of the olive flounder. Viral hemorrhagic septicemia virus (VHSV) is the etiological agent of viral hemorrhagic septicemia (VHS), which is one of the most serious viral diseases affecting farmed olive flounder in Korea. Studies of VHSV infection defending on temperature were also reported. The olive flounder is susceptible to VHSV from 8°C to 15°C but not at 20°C. Therefore, we used a proteomics approach to find differences in spleen of olive flounder. The fish were reared at different temperature conditions (at 15°C and 20°C) for 2 weeks. The proteomic response was examined using label-free protein quantitation coupled with LC–MS^E tandem mass spectrometry and processed data were analyzed with ProteinLynx Global Server (PLGS). A total of 84 proteins from spleen were found to be differentially expressed between experimental groups based on the olive flounder protein database converted from expressed sequence tag (EST) database. A comparative protein analysis was performed to estimate the fold-change values indicated by the ratio of 20°C to 15°C. The top 10 of up-regulated proteins were SH3BGRL, PFN1, PPIF, PRDX1, YWHAE, ANXA5, YWHAG, LTA4H, YWHAB and PDXK, while down-regulated proteins were HBZ, KRT8, KRT19, KRT18, MSN, FN1, TUBB, TUBA3C/3D, TPM3 and TUBA1C. Most of all, hemoglobin subunit zeta (HBZ) expression level was dramatically decreased up to -3.448 fold. It explained olive flounder reared at the 15°C was induced to produce more hemoglobin. Ingenuity Pathway Analysis (IPA) was used to assign identified proteins into different molecular and cellular functional classes. A number of proteins were associated in specific biological function networks such as ‘Cell death and survival’, ‘Cellular function and maintenance’. Interestingly, the proteins related with ‘Cell death and survival’ were greatly up-regulated by rearing temperature. We expect that our study offer new insight into the systemic response of teleost fish.

P-65. Immune-related gene expression profiling in Pacific bluefin tuna (*Thunnus orientalis*) during larval stage

Keigo Kobayashi[1]*, Taiki Sakurai[1], Goshi Kato[1,2], Motoshige Yasuike[3], Yoji Nakamura[3], Atsushi Fujiwara[3], Kazunori Kumon[4], Hideki Nikaido[4], Hidehiro Kondo[1], Ikuo Hirono[1]

[1]Laboratory of Genome Science, Tokyo University of Marine Science and Technology, Konan 4-5-7, Minato, Tokyo 108-8477, Japan hirono@kaiyodai.ac.jp
[2]National Research Institute of Aquaculture, Fisheries Research Agency, 422-1 Nakatsuhamaura, Minami-ise, Mie 516-0193, Japan
[3]Aquatic Genomics Research Center, National Research Institute of Fisheries Science, Fisheries Research Agency, 2-12-4 Fukuura, Kanazawa, Yokohama, Kanagawa 236-8648, Japan
[4]Amami Station, Seikai National Fisheries Research Institute, Fisheries Research Agency, 955-5 Hyousakiyamahara, Setouchi, Oshima, Kagoshima 894-2414, Japan

Pacific bluefin tuna (*Thunnus orientalis*) is one of the most important fish species in terms of fishery in the Northern Pacific Ocean. The decrease of their resources is an important concern. Therefore, larviculture technologies have been developed, and cultivation technologies of Pacific bluefin tuna are established. However, high mortality caused by infection often occurs in larviculture. Understanding the development of immune system in larval Pacific bluefin tuna is important for protecting larvae from infection. We already analyzed global changes in gene expression in 1-10, 13, 15 and 25 days post hatch (dph) larvae using a 44K microarray. Result indicated that innate immune-related genes were already expressed at 1-15 dph larvae. On the other hand, adaptive immune-related genes were highly expressed at 25 dph larvae. To reveal the establishment of immune system of Pacific bluefin tuna during larval stage in more detail, we analyzed changes in mRNA levels of immune-related genes (C3, C3-2, LYSC, LYSG, RAG-1, RAG-2, TdT, TCRβ, CD3ε, CD4, CD8α, CD40, Igμ, MHCIα, MHCIIα and MHCIIγ) of 1, 3, 7 and 10-25 dph larvae using quantitative PCR. As a result, innate immunity is functioning in early larval stage and adaptive immunity is started to construct in 20 to 25 dph Pacific bluefin tuna.

P-66. Cytokine-induced CD4 positive T cell differentiation in the Japanese pufferfish, *Takifugu rubripes*

Tomoya Kono[1]*, Hiroki Korenaga[2], Ryusuke Nagamine[3], Gouranga Biswas[3], Jun-ichi Hikima[1], Masahiro Sakai[1]

[1]Department of Biochemistry and Applied Biosciences, Faculty of Agriculture, University of Miyazaki, Miyazaki 889-2192, Japan tkono@cc.miyazaki-u.ac.jp, jhikima@cc.miyazaki-u.ac.jp, m.sakai@cc.miyazaki-u.ac.jp
[2]Drug Discovery Platforms Cooperation Division, RIKEN Center for Sustainable Resource Science, Saitma 351-0198, Japan hiroki.korenaga@riken.jp
[3]Interdisciplinary Graduate School of Agriculture and Engineering, University of Miyazaki, Miyazaki 889-2192, Japan r-nagamine@cc.miyazaki-u.ac.jp, gbis47@gmail.com

CD4+ T cells are a central component of the adaptive immune response and classified into distinct sets based on their specific cytokine production patterns. Several reports have suggested that fish possess Th subset activity similar to that of mammals. Aims of the present study were to isolate and chracterize CD4+ T cell subsets from blood of the Japanese pufferfish (fugu), *Takifugu rubripes*based on their cytokine production profile. We produced specific antibodies against fugu CD4 and several cytokines [interferon (IFN)-γ, IFN-γrel, interleukin (IL)-4/13A and IL-4/13B], and their specificity was confirmed by Western blotting using peripheral blood leukocyte (PBL) lysate as template. Isolation of CD4+ T cells was performed with the magnetic activated cell sorting system. Sorted fugu CD4+ T cells were characterized by expression analysis of cell marker genes. Fugu CD4+ T cells expressed T-cell marker genes but not macrophage or B-cell marker genes. In addition, PBLs were stimulated with recombinant (r) fugu IFN-γ, IFN-γrel, IL-4/13A, IL-4/13B, lipopolysaccharide (LPS) and chitosan prior to sorting, and then intracellular cytokine staining was conducted using anti-cytokine antibodies. The rIFN-γ and rIFN-γrel stimulations especially upregulated the production of Th1 cytokines (IFN-γ and IFN-γrel) of CD4+ T cells. Moreover, the rIL-4/13A and rIL-4/13B stimulations especially enhanced the production of Th2 cytokines (IL-4/13A and IL-4/13B). The cytokine production of CD4+ T cells was also increased by LPS and chitosan stimulations. These results suggest that fish CD4+ T cells differentiate into effector cells by the cytokine or induction with immunostimulants as in mammals.

P-67. Validation of MALDI-TOF Mass Spectrometry for Rapid Identification of *Yersinia ruckeri*

Charlotte Ramey[1], Jeff Lewis[2], Jan Giles[1]*, Beatrice Despres[2], Anne Muckle[2], David Groman[1]

[1]Aquatic Diagnostic Services, University of Prince Edward Island, 550 University Ave., Charlottetown, Prince Edward Island, Canada, C1A4P3
[2] Department of Pathology and Microbiology, University of Prince Edward Island, 550 University Avenue Charlottetown, PEI, Canada, C1A 4P3

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) with BioTyper™ identification software was evaluated as a novel diagnostic tool for rapid identification of *Yersinia ruckeri*. A single *Y. ruckeri* Main SPectrum (MSP) protein profile was already present in the commercially available BioTyper™ database. Eleven field strains identified as *Y. ruckeri* using phenotypic testing, serology and 16s rDNA sequence analysis were cultured on Blood Agar and subsequently identified using the MALDI BioTyper™. Initially, nine of eleven field strains were correctly identified as *Y. ruckeri*(DSM 18506) with an average identification score of 2.132 ± 0.078 and two strains were incorrectly identified as *Y. enterocolitica.* Following the addition and updating of these 11 *Y. ruckeri* MSPs to the commercial BioTyper™ database, all 11 isolates were correctly identified on subsequent analysis. Preliminary statistical analysis (Composite Correlation Index and PCA cluster analysis) suggested that protein spectra from some strains were sufficiently unique to allow strain resolution using the updated BioTyper™ database. Re-examination of five strains revealed a distinct MSP preference common to all five. Early evidence suggests that the MSP creation method can influence strain self-identification. MALDI BioTyper™ can rapidly and reproducibly identify *Y. ruckeri* and should prove equally valuable for future identification of other aquatic pathogens.

P-68. Regulation pattern of IRF4 by STAT6 and c-Rel in zebrafish

Shun Li, Long-Feng Lu, Yong-An Zhang*

State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei 430072 China yzhang@ihb.ac.cn

Interferon regulatory factor 4 (IRF4) plays a pivotal role in both innate and adaptive immune responses in mammals. In adaptive immune system, the activation of IRF4 by STAT6 and c-Rel is crucial for B lymphocyte proliferation and differentiation. In fish, several B cell lineages have been characterized as in mammals, however, the signaling pathway of IRF4 and the relationship with STAT6 and c-Rel are still unknown. In this study, expression of zebrafish IRF4 (*Dr*IRF4) was monitored in several tissues at mRNA level. *Dr*IRF4 transcripts were upregulated in trunk kidney but downregulated in spleen by infection with spring viremia of carp virus (SVCV), and were immediately induced by treatment with poly I:C in zebrafish embryo fibroblast-like (ZF4) cells. The activation of *Dr*IRF4 promoter was regulated by overexpression of STAT6 and c-Rel in a cooperation manner, which could be inhibited by mutation of the putative STAT6 and c-Rel binding sites in *Dr*IRF4 promoter region. The overexpression of *Dr*IRF4 inhibited the activation of its own promoter under induction of STAT6 and c-Rel, which was the result of that *Dr*IRF4 bound to STAT6 and c-Rel directly. In addition, cellular location analysis showed that *Dr*IRF4 was located in nuclear region. These data indicate that the signaling pathway of IRF4 upregulated by STAT6 and c-Rel is well conserved within vertebrates.

P-69. Comparative anesthetic effects of eugenol and benzocaine in juveniles of red tilapia (*Oreochromis sp.*)

Santiago Rucinque G[1], Gina Polo [2], Javier Borbón[1], Jaime F. González [1]*

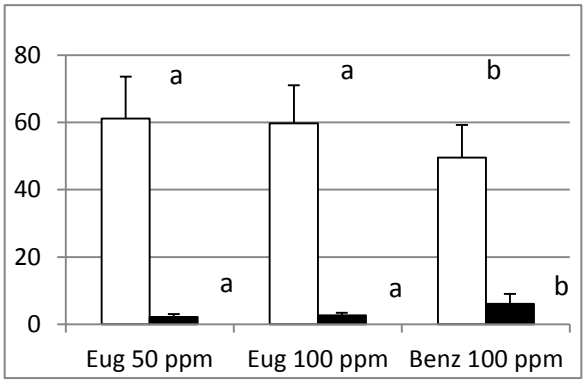
[1] AQUÁTICA: Research Group in Aquatic & Environmental Toxicology, Laboratory of Aquatic Toxicology, School of Veterinary Medicine and Animal Science, Universidad Nacional de Colombia, Bogotá. jfgonzalezma@unal.edu.co.
[2] DVM, M.Sc. Universidade de São Paulo, Brazil.

Anesthetics are needed for fish handling, shipping and surgical procedures. Eugenol (2-methoxy-4-(2-propenyl) phenol) is a phenylpropanoid obtained from clove oil and benzocaine is an ethyl ester derived from PABA. Thirty juveniles of red tilapia (56.3 g body weight, 14.3 cm total length) were randomly assigned to 3 treatments: eugenol 50 ppm, eugenol 100 ppm and benzocaine 100 ppm. Fish were put in each anesthetic solution individually. Times for induction, total anesthesia and recovery were recorded. Heparinised blood from caudal vein was used to measure glucose and plasma cortisol as indicators of stress. One fish in eugenol 50 ppm died during the 24-h monitoring after anesthesia. Eugenol 50 and 100 ppm had the best response of induction and recovery time (Table 1). These treatments also showed reduced stress based on lower cortisol levels as compared to benzocaine (Figure 1). Based on present results, eugenol could be used as an adequate choice for anesthesia in tilapia in addition to benzocaine.

Table 1. Average (mean ± S.D.) time (min.) spent during each phase of anesthesia (n=10 fish/ treatment)

Phase	Eugenol 50 ppm	Eugenol 100 ppm	Benzocaine 100 ppm
Induction time	1.3 ± 0.4 [a]	1.2 ± 0.5 [a]	2.9 ± 0.8 [b]
Total time anesthesia	9.2 ± 2.7 [a]	10.0 ± 0.0 [a]	9.7 ± 0.9 [a]
Time for recovery	3.9 ± 1.3 [a]	5.3 ± 1.0 [b]	4.9 ± 2.6 [b]

Figure 1. Blood glucose (mg/dL) and cortisol (µg/dL) in fish after anesthesia (mean ± S.D.). Glucose = white bars, cortisol = black bars. (n=10 fish/treatment).



p-70. Evaluation of five commercial kits for isolation of quantifiable, polymerase chain reaction-quality genomic DNA from commercial catfish ponds

Cynthia Ware[1], Matt J. Griffin[1], Michael J. Mauel[1, 2]*

[1]Thad Cochran National Warmwater Aquaculture Center, College of Veterinary Medicine, Mississippi State University, 127 Experiment Station Road, Stoneville, MS 38776 griffin@cvm.msstate.edu (M. Griffin), cware@cvm.msstate.edu (C. Ware)
[2]Current address: Mississippi Veterinary Research and Diagnostic Laboratory, College of Veterinary Medicine, Mississippi State University, P.O. Box 97813, Pearl, MS 39288, mmauel@mvrdl.msstate.edu

This work evaluated five different commercial DNA extraction kits for their ability to reliably and repeatedly recover quantifiable, polymerase chain reaction quality genomic DNA (gDNA) from straight broth culture, as well as water and sediment collected from catfish aquaculture ponds. To determine the yield and reproducibility of the isolation kits, gDNA was isolated from sample matrices spiked with known quantities of *Edwardsiella ictaluri* cells and analyzed using a quantitative PCR assay developed for the detection and quantification of *E. ictaluri* gDNA. The kits used in this comparison included the MoBio PowerSoil® DNA Isolation Kit, the MoBio UltraClean® Soil DNA Isolation Kit, the Epicentre Biotechnologies SoilMaster™ DNA Extraction Kit, the Qiagen DNEasy® Blood and Tissue Kit and the Gentra Puregene® Tissue Kit. All kits were successful in isolating PCR quality DNA, although this success varied with substrate and number of bacteria. The MoBio PowerSoil® and UltraClean® DNA Isolation Kits demonstrated the highest level of reliability and sensitivity regardless of substrate. Results suggest the MoBio PowerSoil® and UltraClean® kits offer the highest degree of DNA recovery with the greatest reproducibility for the sample types evaluated in this study.

P-71. Non-lesions, misdiagnoses, missed diagnoses, and other interpretive challenges in fish histopathology studies

Jeffrey C. Wolf[1]*, Wes A. Baumgartner[2], Vicki S. Blazer[3], Alvin C. Camus[4], Jeffery A. Engelhardt[5], John W. Fournie[6], Salvatore Frasca Jr.[7], David B. Groman[8], Michael L. Kent[9], Lester H. Khoo[10], Jerry M. Law[11], Eric D. Lombardini[12], Christine Ruehl-Fehlert[13], Helmut E. Segner[14], Stephen A. Smith[15], Jan M. Spitsbergen[16], Klaus Weber[17], Marilyn J. Wolfe[18]

[1]Experimental Pathology Laboratories, Inc., Sterling, Virginia, USA
[2]Department of Pathobiology/Population Medicine, College of Vet. Med., Mississippi State, MI, USA
[3]U.S. Geological Survey, Kearneysville, West Virginia, USA
[4]Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA
[5]Experimental Pathology Laboratories, Inc., Camarillo, California, USA
[6]U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, Gulf Breeze, Florida, USA
[7]Connecticut Veterinary Medical Diagnostic Laboratory, Department of Pathobiology and Veterinary Science, University of Connecticut, Storrs, Connecticut, USA
[8]Aquatic Diagnostic Services, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada
[9]Departments Microbiology & Biomedical Sciences, Oregon State University, Corvallis, Oregon, USA
[10]Mississippi State University, College of Veterinary Medicine, Stoneville, Mississippi, USA
[11]Aquatic Ecotox, North Carolina State University College of Veterinary Medicine, Raleigh, NC, USA
[12]Divisions of Comparative Pathology and Veterinary Medical Research Department of Veterinary Medicine, Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand
[13]Bayer HealthCare AG, Wuppertal, Germany
[14]Centre for Fish and Wildlife Health, University of Bern, Bern, Switzerland;
[15]Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia, USA
[16]Fish Disease Research Group, Department of Microbiol, Oregon State Univ., Corvallis, Oregon, USA
[17]AnaPath GmbH, Oberbuchsiten, Switzerland
[18]Sterling, Virginia, USA

Differentiating salient histopathologic changes from normal anatomic features or tissue artifacts can be especially challenging for the novice fish pathologist. Consequently, findings of questionable accuracy may be reported inadvertently. The objectives of this project were to identify specific morphologic findings in commonly examined fish tissues that are frequently either misdiagnosed or underdiagnosed, and to illustrate such findings through the use of photomicrographic examples. A number of highly-trained, veteran fish pathologists were tasked with assembling lists of histopathologic diagnoses that often appeared questionable based on evaluations of published morphologic descriptions and figure illustrations. For the current project, photomicrographic examples of normal and abnormal specimens were acquired from the personal slide collections of the authors, or obtained by permission from prior studies. Histopathologic findings that appeared to be commonly over-diagnosed or misdiagnosed in the literature included nine types of gill diagnoses, six kidney diagnoses, four liver diagnoses, and five additional diagnoses in various other tissues. Additionally, the authors identified nine types of findings that tend to be under-reported. Histopathology continues to be a valuable tool for investigating the morphologic features and extent of both naturally-occurring and experimentally-induced disease. The authors describe practical measures that can be instituted to safeguard against the publication of dubious histopathologic results. The fundamental goal of this effort is to elevate the science and practice of fish histopathology, which has become an increasingly important discipline in fields that include basic biomedical research, aquaculture, environmental resource management, and ecotoxicology.
Doctorate student supported by FAPESP scholarship (Proc. nº 2011/10738-1 - J. Naldoni).
Doctorate student supported by FAPESP scholarship (Proc. nº 2011/08549-6 – T. Milanin)

P-72. Fish Deformities in African Catfish *Heterobranchus bidorsalis* in relation to Chromosome set manipulation techniques

Olubunmi T. Agbebi[1]*, Samuel O. Otubusin[2], Samuel O. Olufeagba[3]

[1]Department of Aquaculture and Fisheries Management, Federal University of Agriculture, PMB 2240, Abeokuta, Ogun State, Nigeria. agbebi20@yahoo.com

[2]Department of Aquaculture & Fisheries Management, Federal University of Agriculture, PMB 2240, Abeokuta, Ogun State, Nigeria. sam-oluotubusin@yahoo.com.

[3]National Institute for Freshwater Fisheries Research, PMB 6006, New Bussa, Niger State, Nigeria. olabodekainji@yahoo.com

Triploidy was induced by cold-shocking at 5^0C for 40 minutes, and the hatchlings from diploid and triploid treatments were distributed into three aquaria to monitor the daily survival and weekly growth rate for six weeks. The percentage survival was determined by ratio of the number of hatchling survived to the total number of fry stocked. The triploids experienced low hatchability and survival at the incubation period due to cold shocking effect on their eggs. After the initial low survival rate of triploid 50% and 74% for diploid, survival rate was improved and stabilized after the sixth day in the incubator. Percentage survival of both diploid and triploid were equal during the growing stage in the aquaria tanks. Histological examination of gonads at ten month in outdoor tanks showed that diploid males had well-developed testes unlike the rudimentary testes in the triploid. Ovaries of diploid showed presence of numerous ova unlike the triploid ovary which showed total degenerated vitellogenic follicles. This suggested high fertility in the diploids while the triploids tended to be sterile. 7% abnormalities were observed in triploid strains showing missing left pelvic fin and post juvenile stage showing the irregular dark spots. The two challenges faced in triploid production (high mortality of embryos and deformities in triploids strains) should pose no threat, could be genetic, environmental or associated with extremely low temperature (5^oC) coupled with long duration of shock. The low hatchability of triploidy can be solved with the production of interploid progeny which involves cross between the diploid individual and induced tetraploid parents. The sterile situation in triploids is a very good tool in Aquaculture development. The use of triploid fish in stocking ponds would help to preserve the genetic integrity of wild population of sterile fish. It would eliminate uncontrolled reproduction and subsequently increase growth rate.

Registered Attendees

Maria Aamelfot
Norwegian Veterinary Institute
Oslo, Norway
472-321-6000 maria.aamelfot@vetinst.no

Hossam Abdelhamed
College of Veterinary Medicine
Mississippi State University, MI, USA
662-325-1125 hossam_amr8@yahoo.com

Mark Adkison
California Department of Fish and Wildlife
Davis, CA, USA
916-358-2830 Mark_Adkison@sbcglobal.net

Edson Adriano
Federal University of Sao Paulo
Sao Paulo, Brazil
55-19-992330364 edapadriano@gmail.com

Olubunmi Agbebi
Federal University of Agriuculture
Abeokuta, Nigeria
agbebi20@yahoo.com

Bermejo Alama
Institute of Parasitology
South Bogemie, Czech Republic
42 0387775408 gema.alama@gmail.com

Neely Alberson
College of Veterinary Medicine
Mississippi State University, MI, USA
662-325-1154 nrw2@msstate.edu

Julie Alexander
Oregon State University
Corvallis, OR, USA
541-737-1849 alexanju@science.oregonstate.edu

Takashi Aoki
Waseda University
Tokyo Japan
81-80-3410-5271 aokitaka@aoni.waseda.jp

Stephen Atkinson
Oregon State University
Corvallis, OR, USA
541-737-1849 atkinsos@science.oregonstate.edu

Betsy Bamberger
CA Dept of Fish and Wildlife,
Rancho Cordova, CA, USA
916-358-2824 betsy.bamberger@wildlife.ca.gov

Jerri Bartholomew
Oregon State University
Corvallis, OR, USA
541-737-1856 bartholj@science.oregonstate.edu

Bill Batts
USGS - Western Fisheries Research Center
Seattle, WA, USA
206-526-2289 bbatts@usgs.gov

Wes Baumgartner
College of Veterinary Medicine
Mississippi State, MS, USA
662-325-2167 baumgartner@cvm.msstate.edu

Rachel Beecham
Mississippi Valley State University
Greenville, MS, USA
662-820-8301 rvbeecham@yahoo.com

Marco Belo
Sao Paulo State University
Sao Paulo, Brasil
55 16999617299 maabelo@hotmail.com

Jim Bertolini
Northwest Indian Fisheries Commission
Olympia, WA, USA
360-528-4355 jbertoli@nwifc.org

Reginald Blaylock
University of Southern Mississippi
Ocean Springs, MS, USA
228-818-8003 reg.blaylock@usm.edu

Vicki Blazer
U.S. Geological Survey
Kearfenysville, WV, USA
304-724-4434 vblazer@usgs.gov

Hogne Bleie
MSD Animal Health Norway
Bergen, Norway
47 909 58 026 hogne.bleie@merck.com

Anne Bolick
US Fish and Wildlife Service
Anderson, CA, USA
530-736-6668 anne_bolick@fws.gov

Joanna Borucinska
University of Hartford
West Hartford, CT, USA
860-768-4586 borucinsk@hartford.edu

Ryan Braham
West Virginia University
Kearneysville, WV, USA
304-724-4451 rbraham@mix.wvu.edu

Andrew Bridle
University of Tasmania
Launceston, Tasmania, Australia
61402113617 Andrew.Bridle@utas.edu.au

Nicholas Brown
Ross University School of Veterinary Medicine
Corvallis, OR, USA
360-972-5699 nicholasbrown@students.rossu.edu

David Burbank
Idaho Fish and Game/PSMFC
Boise, ID, USA
208-939-2413 david.burbank@idfg.idaho.gov

Kenneth Cain
University of Idaho
Moscow, ID, USA
208-885-7608 kcain@uidaho.edu

Wade Cavender
Utah Division of Wildlife
Logan, UT, USA
435-720-2784 wadecavender@utah.gov

Carolyn Chang S
State University of New York
Syracuse, NY, USA
315-416-6252 ctchang@syr.edu

Luciano Chiaramonte
Idaho Department of Fish and Game
Eagle, ID, USA
208-939-2413 luciano.chiaramonte@idfg.idaho.gov

Calin-Decebal Cojocaru
National Sanitary Veterinary and Food Safety Authority
TIMISOARA TIMIS Romania
40726860247 c_cojocaru_d@yahoo.com

Carla Conway
USGS - Western Fisheries Research Center
Seattle, WA, USA
206-526-2042 cmconway@usgs.gov

Emily Cornwell
Cornell University College of Veterinary Medicine
Ithaca, NY, USA
971-241-8389 erc58@cornell.edu

Ruth Cox
Atlantic Veterinary College
Charlottetown, Prince Edward Island, Canada
902-566-0815 rucox@upei.ca

Tina Crosby
FDA Center for Veterinary Medicine
Laurel, MD, USA
310-210-8889 Tina.Crosby@fda.hhs.gov

David Cummins
CSIRO Australian Animal Health Laboratory (AAHL)
Geelong, VIC, Australia
6135-227-5777 david.cummins@csiro.au

Chris Darnall
Missouri Dept. of Conservation
Warsaw, MO, USA
660-438-4465 x 6409 chris.darnall@mdc.mo.gov

Ariel Diamant
Israel Oceanographic & Limnological Research Institute
Eilat, Israel
972-505373759 diamant@ocean.org.il

Lidiya Dubytska
Louisiana State University
Baton rouge, LA, USA
225-578-9668 ldubyt1@lsu.edu

Robert Durborow
Kentucky State University
Frankfort, KY, USA
502-597-6581 robert.durborow@kysu.edu

Diane Elliott
USGS - Western Fisheries Research Center
Seattle, WA, USA
206-526-6591 dgelliott@usgs.gov

Evi Emmenegger
USGS - Western Fisheries Research Center
Seattle, WA, USA
206-526-2276 eemmenegger@usgs.gov

Kevin Erickson
CQ University Australia
Gladstone, Queensland, Australia
61 7 4970 7309 K.Erickson2@cqu.edu.au

Edit Eszterbauer
Hungarian Academy of Sciences
Budapest, Hungary
0036 1 4674067 eszterbauer.edit@agrar.mta.hu

Jason Evenhuis
USDA-ARS-NCCCWA
Kearneysville, WV, USA
304-724-8340 jason.evenhuis@ars.usda.gov

Tyson Fehringer
Idaho Department of Fish and Game
Eagle, ID, USA
208-939-2413 tyson.fehringer@idfg.idaho.gov

Jayde Ferguson
Alaska Department of Fish & Game
Anchorage, AK, USA
907-267-2394 jayde.ferguson@alaska.gov

Ivan Fiala
Institute of Parasitology, Biology Centre, AVCR
Kraj, Czech Republic
42 0608232278 fiala@paru.cas.cz

Jeannine Fischer
Ministry for Primary Industries NZ
Wellington, New Zealand
0064-48940876 jeannine.fischer@mpi.govt.nz

Ines Fontes
Natural History Museum London
London , UK
44(0)2079425671 i.fontes@nhm.ac.uk

Scott Foott
US Fish and Wildlife Service
Anderson, CA, USA
530-365-4271 Scott_Foott@fws.gov

Mike Foreman
Institute of Ocean Sciences
Sidney, BC, Canada
250-363-6306 mike.foreman@dfo-mpo.gc.ca

Mark Freeman
University of Malaya
Kuala Lumpur, KL, Malaysia
6032374325 mark@um.edu.my

Kathleen Frisch
Cermaq Canada
Campbell River, BC, Canada
604-773-4978 kathleenf@mac.com

Alkhateib Gaafar
National Research Centre
El Buhouth St., Dokki, Cairo, Egypt
Higashihiroshima, Hiroshima Prefecture, Japan
81 9013353677 alkhateibyg@yahoo.com

Ian Gardner
Atlantic Veterinary College
Charlottetown, PEI, Canada
902-620-5059 iagardner@upei.ca

Kyle Garver
Fisheries and Oceans Canada
Nanaimo, BC, Canada
250-756-7340 kyle.garver@dfo-mpo.gc.ca

Patricia Gaunt
Mississippi State University
Stoneville, MI, USA
662-686-3237 gaunt@cvm.msstate.edu

Carissa Gervasi
VIMS
Gloucester Point, VA, USA
804-684-7218 cgervasi@vims.edu

Rod Getchell
Cornell University
Ithaca, NY, USA
607-253-3393 rgg4@cornell.edu

Bikramjit Ghosh
University of Tasmania Launceston
Tasmania, Australia
61412198939 bikramjit.ghosh@utas.edu.au

Charlie Gieseker
FDA Center for Veterinary Medicine
Laurel, MD, USA
301-210-4217 Charles.Gieseker@fda.hhs.gov

Cem Giray
Kennebec River Biosciences, Inc.
Richmond, ME, USA
207-737-2637 (ext 207) cgiray@kennebecbio.com

Mona Gjessing
Norwegian Veterinary Institute
Oslo, Norway
4723216000 mona.gjessing@vetinst.no

Christopher Good
The Conservation Fund's Freshwater Institute
Shepherdstown, WV, USA
304-876-2815 x279 c.good@freshwaterinstitute.org

Tom Goodrich
AquaTactics LLC
Kirkland, WA, USA
425-922-4208 tomg@aquatactics.com

Stacey Gore
FDA/CVM
Rockville, MD, USA
240-402-0591 stacey.gore@fda.hhs.gov

Jacob Gregg
USGS - Marrowstone Field Station
Nordland, WA, USA
360-385-1007 jgregg@usgs.gov

David Groman
Atlantic Veterinary College
Charlottetown, Prince Edward Island, Canada
902-566-0864 groman@upei.ca

Zemao Gu
Huazhong Agricultural University
Hubei, China
86 13277065487 guzemao@mail.hzau.edu.cn

David Angelo Guanzon
University of Santo Tomas
Manila NCR, Philippines
632-406-1611 Loc. 4056
gelo.guanzon@gmail.com

Qingxiang Guo
Huazhong Agriculture University
Wuhan Hubei, China
541-602-8883 530qx@hotmail.com

Lori Gustafson
USDA APHIS VS
Fort Collins, CO, USA
970-494-7297 Lori.L.Gustafson@aphis.usda.gov

Olga Haenen
NRL for Fish, Crustacean and Shellfish Diseases
Flevoland, the Netherlands
31-320-238352 olga.haenen@wur.nl

Cassidy Hahn
West Virginia University
Kearneysville, WV, USA
304-724-4482 Cassidy.Hahn@gmail.com

Sascha Hallett
Oregon State University
Corvallis, OR, USA
541-737-4721 halletts@science.oregonstate.edu

John Hansen
USGS - Western Fisheries Research Center
Seattle, WA, USA
206-526-6588 jhansen@usgs.gov

Larry Hanson
Mississippi State University
Mississippi State, MS, USA
662-325-1130 hanson@cvm.msstate.edu

Lucas Hart
USGS – Marrowstone Marine Field Station
Nordland, WA, USA 3
60-797-3966 lhart@usgs.gov

Ashlie Hartigan
Institute of Parasitology, AVCR Ceske Budejovi
Jihoceske Kraj, Czech Republic
42 0775365509 ashlie.hartigan@paru.cas.cz

Hanna Hartikainen
ETH Zurich & Eawag Duebendorf
Zurich, Switzerland
41 791354633 Hanna.Hartikainen@eawag.ch

Nicholas Hasbrouck
FDA Center for Veterinary Medicine
Laurel, MD, USA
301-210-8887 Nicholas.Hasbrouck@fda.hhs.gov

John Hawke
Louisiana State University
Baton Rouge, LA, USA
225-578-9705 jhawke1@lsu.edu

William (Bill) Hemstreet
Auburn University
Greensboro, AL, USA
334-624-4016 hemstwi@auburn.edu

Jayme Hennenfent
House Paws Mobile Veterinary Services
Arlington, VA, USA
847-909-8109 jayme.hennenfent@gmail.com

Tharangani Herath
University of Stirling
Stirling, Stirlingshire, UK
44 1786 358209 t.k.herath@stir.ac.uk

Daniel Hernandez
University of Washington
Seattle, WA, USA
707-502-6285 dh38@uw.edu

Paul Hershberger
USGS - Marrowstone Marine Field Station
Nordland, WA, USA
360-385-1007 phershberger@usgs.gov

Jun-ichi Hikima
University of Miyazaki
Miyazaki, Japan
81-985-58-7230 jhikima@cc.miyazaki-u.ac.jp

Rich Holt
Dept. of Microbiology, Oregon State University
Corvallis, OR, USA
541-760-0904 holtr@onid.orst.edu

Astrid Holzer
Institute of Parasitology, AVCR Ceske Budejovice
Jihoceske, Kraj, Czech Republic
42 0775545862 astrid.holzer@paru.cas.cz

Marcia House
Northwest Indian Fisheries Commission
Olympia, WA, USA
360-528-4344 mhouse@nwifc.org

Hui-Min Hsu
Wisconsin Vet Diagnostic Lab
Madison, WI, USA
608-262-5432 hui-min.hsu@wvdl.wisc.edu

Tzu-Ming Huang
Animal Health Research Institute
New Tapei City , Taiwan
886-2-26212111 ext.232 tmhuang@mail.nvri.gov.tw

Sue-Min Huang
Animal Health Research Institute
Tamshui, Taipei, Taiwan
886-2-26212111 Ext. 207 smhuang@mail.nvri.gov.tw

Charlene Hurst
Oregon State University
Corvallis, OR, USA
541-737-9664 hurstch@onid.orst.edu

Amberly Huttinger
USFWS - Bozeman Fish Health Center
Bozeman, MT, USA
406-582-8656 Amberly_Huttinger@fws.gov

Luke Iwanowicz
U.S. Geological Survey - Leetown Science Center,
Kearneysville, West Virginia, USA
304-724-4439 liwanowicz@usgs.gov

David Jackson
Marine Institute (Foras na Mara)
Galway, Ireland
3 53876993259 dave.jackson@marine.ie

Kym Jacobson
NOAA Fisheries
Newport, OR, USA
541-867-0375 kym.jacobson@noaa.gov

Michelle Jakaitis
Oregon State University
Corvallis, OR, USA
303-882-8159 jakaitim@onid.oregonstate.edu

Diana Jaramillo-Martinez
University of Sydney
Camden, NSW, Australia
61293511610 diana.jaramillo@sydney.edu.au

Luo Jia
Huazhong Agricultural University
Wuhan Hubei, China
86 18062422330 luojack1988@gmail.com

Simon Jones
Fisheries and Oceans Canada
Nanaimo, BC, Canada
250-729-8351 simon.jones@dfo-mpo.gc.ca

Jorunn Jorgensen
Norwegian College of Fishery Science
Troms, Norway
479-509-5754 jorunn.jorgensen@uit.no

Andrew Kane
University of Florida
Gainesville, FL, USA
352-273-9090 kane@ufl.edu

Norie Kaneshige
Tokyo University of Marine Science and Technology
Tokyo, Japan
81-3-5463-689 kcmkq562@yahoo.co.jp

Myron Kebus
Wisconsin Department of Agriculture
Madison, WI, USA
608-224-4876 myron.kebus@wisconsin.gov

Michael Kent
Oregon State Univesity
Corvallis, OR, USA
541-737-8652 michael.kent@oregonstate.edu

Megan Kepler
Pennsylvania State University
University Park, PA, USA
814-482-8032 mvk10@psu.edu

Jong-Oh Kim
Chonnam National University
Yeosu Chollan Namdo , South Korea
82-62-659-6947 jongoh.kim77@gmail.com

Rae Knight
Novartis Animal Health
Hamilton, ON, Canada
905-516-3424 rae.knight@novartis.com

Keigo Kobayashi
Tokyo University of Marine Science and Technology
Minato Tokyo, Japan
81-3-5463-689 k.kobayashi1206@gmail.com

Richard Kocan
University of Washington
Seattle, WA, USA
360-620-2373 kocan@uw.edu

Hidehiro Kondo
Tokyo University of Marine Science and Technology
Minato Tokyo, Japan
81354630174 h-kondo@kaiyodai.ac.jp

Tomoya Kono
University of Miyazaki
Miyazaki, Japan
81985-587866 tkono@cc.miyazaki-u.ac.jp

Arni Kristmundsson
University of Iceland
Reykjavik, Iceland
354 5855100 arnik@hi.is

Sunil Kumar
University of Minnesota
Saint Paul, MN, USA
612-625-8263 kumars@umn.edu

Gael Kurath
USGS - Western Fisheries Research Center
Seattle, WA, USA
206-526-6583 gkurath@usgs.gov

Jerome La Peyre
Louisiana State University Agricultural Center
Baton Rouge, LA, USA
225-578-5419 jlapeyre@agcenter.lsu.edu

Benjamin LaFrentz
USDA, ARS
Auburn , AL, USA
334-887-3741 benjamin.lafrentz@ars.usda.gov

Eric Landis
US Food and Drug Administration for Veterinary Medicine
Rockville, MD, USA
240-402-0574 eric.landis@fda.hhs.gov

Scott LaPatra
Clear Springs Foods, Inc.
Buhl, ID, USA
208-543-3465 scott.lapatra@clearsprings.com

Iske Larkin
University of Florida
Gainesville, FL, USA
352-294-4095 ivlarkin@ufl.edu

Mark Lawrence
Mississippi State University
Mississippi State, MS, USA
662-325-1205 lawrence@cvm.msstate.edu

Teresa Lewis
USFWS
Dexter, NM, USA
575-734-5910, ext 24 teresa_lewis@fws.gov

Ai-Hua Li
Chinese Academy of Sciences
Wuhan Hubei, China,
8 613871108782 liaihua@ihb.ac.cn

John Han-You Lin
National Cheng Kung University
Tainan, Taiwan
886-920894777 hanyou@mail.ncku.edu.tw

Amy Long
Oregon Department of Fish and Wildlife
Corvallis, OR, USA
541-737-1863 longam@onid.orst.edu

Steve Lord
University of Guelph
Guelph, Ontario, Canada
519-824-4120 ext 53577 rstevens@uoguelph.ca2

Jan Lovy
New Jersey Division of Fish & Wildlife
Oxford, NJ, USA
908-637-4173 ext. 120 jan.lovy@dep.state.nj.us

Geoff Lowe
Fisheries and Oceans Canada
Nanaimo, BC, Canada
250-756-7131 Geoff.Lowe@dfo-mpo.gc.ca

Kay Lwin Tun
University of Yangon, The University of Tokyo
Tokyo, Japan
81-70-1322-2567 kaylwintun@gmail.com

Lone Madsen
DTU National Veterinary Institute
Frederiksberg C, Denmark
0045 35 88 68 26 loma@vet.dtu.dk

Forgivemore Magunda
Washington State University-VMP/WADDL
Pullman, WA, USA
509-335-9696 magundaf@vetmed.wsu.edu

Heather Maness
University of Florida
Gainesville, FL, USA
352-294-4198 htdaniel@ufl.edu

Mary Beth Maningas
University of Santo Tomas
Manila, Philippines
6327314031 marybethmaningas@yahoo.com

David Marancik
USDA-ARS-NCCCWA
Kearneysville, WV, USA
304-724-8340 david.marancik@ars.usda.gov

Hannah Martin
Chesapeake Research Consortium
Annapolis, MD, USA
410-267-9830 martin.hannah@epa.gov

Mauricio Martins
Federal University of Santa Catarina
Florianopolis, SC, Brasil
55 4832093605 mauricio.martins@ufsc.br

Gary Marty
BC Animal Health Centre
Abbotsford, BC , Canada
604-556-3123 Gary.Marty@gov.bc.ca

Dante Mateo
Atlantic Veterinary College
Charlottetown, Prince Edward Island, Canada
902 5660668 dmateo@upei.ca

Michael Mauel
Mississippi State University
Pearl, MS, USA
601-420-4790 mmauel@mvrdl.msstate.edu

Sarah McConnachie
Atlantic Veterinary College
Charlottetown, Prince Edward Island, Canada
902-394-6747 smcconnachie@upei.ca

Charles McGurk
Skretting
Rogaland, Norway
4795961326 charles.mcgurk@skretting.com

Douglas McKenney
USGS - Western Fisheries Research Center
Seattle, WA, USA
206-526-2275 dmckenney@usgs.gov

Connie McKibben
USGS – Western Fisheries Research Center
Seattle, WA, USA
206-526-2045 cmckibben@usgs.gov

Theodore Meyers
Alaska Department of Fish and Game
Juneau, AK, USA
907-465-3577 ted.meyers@alaska.gov

Tiago Milanin
Universidade Estadual de Campinas (Unicamp)
Pirassununga, SP, Brasil
55 18997150240 tiago_milanin@hotmail.com

Vicki Milano
Colorado Parks and Wildlife
Brush, CO, USA
970-380-7013 vicki.milano@state.co.us

Jessica Miller
Oregon State University
Newport, OR, USA
541-867-0381 jessica.miller@oregonstate.edu

Timothy MIller-Morgan
Oregon Sea Grant/Oregon State University
Newport, OR, USA
541-270-4218 tim.miller-morgan@oregonstate.edu

Alexander Munson
Idaho Department of Fish and Game
Eagle, ID, USA
208-939-2413 doug.munson@idfg.idaho.gov

Katy Murray
Zebrafish International Resource Center
Eugene, OR, USA
541-346-6028, x14 katy@zebrafish.org

Lucy Mutharia
University of Guelph
Guelph, Ontario, Canada
519-824-4120 ext 53577 lmuthari@uoguelph.ca

Ryusuke Nagamine
University of Miyazaki
Miyazaki, Japan
81985587866 r.naga0816@gmail.com

Michael Ness
Grieg Seafood BC Ltd.
Campbell River, BC, Canada
250-203-2013 michael.ness@griegseafood.com

Thu Nguyen
University of Tasmania,
Newnham, Tasmania, Australia
61 469749710 Diem.Nguyen@utas.edu.au

Du Nguyen
Mississippi State University
Mississippi State, MS, USA
662-325-1130 troberson@cvm.msstate.edu

Beth Okamura
Natural History Museum
London , Middlesex, UK
44 207 9426631 b.okamura@nhm.ac.uk

Niels Jorgen Olesen
DTU National Veterinary Institute
Frederiksberg, Copenhagen, Denmark
45 29 24 43 10 njol@vet.dtu.dk

Craig Olson
NW Indian Fisheries
Olympia, WA, USA
360-528-4343 colson@nwifc.org

Bukola Oyebanji
Department of Animal Sciences Ile-Ife
Osun, Nigeria
2 347032765674 oyebanji.bukola44@gmail.com

Anjali Pande
Ministry for Primary Industries
Lower Hutt, Wellington, New Zealand
00 64 298945621 anjali.pande@mpi.govt.nz

Anna Papadopoulou
Abo Akademi University
Turku, Varsinais-Suomi, Finland
35841-754-0518 apapadop@abo.fi

Sneha Patra
Institute of Parasitology, Biology Centre AVCR
Ceske Budejovice, South Bohemia,Czech Republic
42 0387775423 snehampatra@gmail.com

Edmund Peeler
Cefas
Weymouth, Dorset, UK
4 41305206746 ed.peeler@cefas.co.uk

Denise Petty
North Florida Aquatic Veterinary Services
Fort White, FL, USA
386-344-8363 pettyd@windstream.net

Nicholas Phelps
University of Minnesota
St. Paul, MN, USA
612-624-7450 phelp083@umn.edu

Mark Polinski
University of Tasmania
Launceston, TAS, Australia
645-734-5053 mark.polinski@utas.edu.au

Sudheesh Ponnerassery
University of Idaho
Moscow, ID, USA
208-310-2526 sudheesh@uidaho.edu

Linda Pote
Mississippi State University,
Mississippi State, MS, USA
662-312-4724 lpote@cvm.msstate.edu

Sarah Poynton
Johns Hopkins University School of Medicine
Baltimore, MD, USA
410-502-5065 spoynton@jhmi.edu

Timothy Pridmore
Wyoming Game and Fish
Laramie, WY, USA
307-772-1967 Tim.pridmore@gmail.com

Maureen Purcell
USGS - Western Fisheries Research Center
Seattle, WA, USA
206-526-2052 mpurcell@usgs.gov

Doug Ramsey
Rangen Aquaculture Research Center
Hagerman, ID, USA
208-837-6191 dramsey@rangen.com

Melinda Raymond
University of Guelph
Guelph, Ontario, Canada
519-824-4120 ext 53577 rstevens@uoguelph.ca1

Stephen Reichley
Mississippi State University
Mississippi State, MS, USA
662-469-6096 sreichley@cvm.msstate.edu

JoLynn Reno
Washington State University
Pullman, WA, USA
509-335-1841 jreno@vetmed.wsu.edu

Linda Rhodes
NOAA Fisheries / NW Fisheries Science Center
Seattle, WA, USA
206-860-3279 linda.rhodes@noaa.gov

Espen Rimstad
Norwegian university of Life Sciences
Oslo, Norway
22-96-47-66
espen.rimstad@nmbu.no

Jill Rolland
USGS – Westrn Fisheries Research Center
Seattle, WA, USA
206-526-6292 jrolland@usgs.gov

Sean Roon
Oregon State University
Corvallis, OR, USA
541-737-2977 roons@onid.orst.edu

Thomas Rosser
Mississippi State University
Mississippi State, MI, USA
662-325-1154 tgr49@msstate.edu

Masahiro Sakai
University of Miyazaki
Miyazaki, Japan
81-90-7151-0401 m.sakai@cc.miyazaki-u.ac.jp

Irene Salinas
University of New Mexico
Albuquerque, NM, USA
505-277-0039 isalinas@unm.edu

George Sanders
University of Washington
Seattle, WA, USA
206-543-4652 gsander@uw.edu

Justin Sanders
Oregon State University
Corvallis, OR, USA
541-737-1859 Justin.Sanders@oregonstate.edu

A. David Scarfe
American Veterinary Medical Association
Schaumburg, IL, USA
847-285-6634 dscarfe@avma.org

Paula Schaffer
Colorado State University
Fort Collins, CO, USA
970-297-5123 aluapa@gmail.com

Nataliia Sergeenko
Kamchatka Research Institute of Fisheries and Oceanography
Petropevlovsk-Kamchatsky, Russia
79146235907 nvsergeenko@gmail.com

Iwona Skulska
University of Guelph
Guelph, Ontario, Canada
519-824-4120 ext 53577 rstevens@uoguelph.ca3

Pedro Smith
Universidad de Chile
Santiago, Chile
56-2-29785577 psmith@uchile.cl

Kevin Snekvik
Washington State University
Pullman, WA, USA
509-335-9696 ksnek@vetmed.wsu.edu

Esteban Soto
Ross University-Veterinary School
Basseterre, St. Kitts
732-898-0078 esoto@rossvet.edu.kn

Sean Spagnoli
Oregon State University Environmental Health Sciences
Corvallis, OR, USA
845-820-0679 sean.spagnoli@oregonstate.edu

Roselynn Stevenson
University of Guelph
Guelph, Ontario, Canada
519-824-4120 ext 53577 rstevens@uoguelph.ca

Bruce ‘Lumpy’ Stewart
Northwest Indian Fisheries Commission
Olympia, WA, USA
360-528-4338 bstewart@nwifc.org

Cindy Stine
FDA Center for Veterinary Medicine
Laurel, MD, USA
301-210-4658 Cynthia.Stine@fda.hhs.gov

Chen Su
Taichung City, Taiwan
886-4-2463-9869 caroline@genereachbiotech.com

Oriol Sunyer
University of Pennsylvania
Philadelphia, PA, USA
215-573 8592 sunyer@vet.upenn.edu

Csaba Szekely
Hungarian Academy of Sciences
Budapest, Hungary
36-1-4674065 szekely.csaba@agrar.mta.hu

Lauren Taneyhill
Chesapeake Research Consortium
Annapolis, MD, USA
410-267-9839 ltaneyhill@chesapeakebay.net

Brandon Taro
Wyoming Game and Fish Department
Laramie, WY, USA
307-399-9806 Brandon.Taro@Wyo.Gov

Hasan Tekedar
Mississippi State University
Mississippi State, MS, USA
662-325-1130 hct37@msstate.edu

Jayne Theurer
Aimee Noelle Reed
Corvallis, OR, USA
541-737-6921 jayne.theurer@oregonstate.edu

Joan Thomas
Washington Department of Fish and Wildlife
Olympia, WA, USA
360-902-2667 Joan.Thomas@dfw.wa.gov

Ronald Thune
Louisiana State University
Baton Rouge, LA, USA
225-578-9680 thune@vetmed.lsu.edu

Ole Torrissen
Institute of Marine Research
Bergen, Norway
47 90839556 Olet@imr.no

Kimberly True
USFWS
Anderson, CA, USA
530-365-4271 kimberly_true@fws.gov

Shigehiko Urawa
Hokkaido National Fisheries Research Institute
Sapporo, Hokkaido, Japan
81-11-822-2349urawa@affrc.go.jp

Vikram Vakharia
University of Maryland
Baltimore, MD, USA
301-717-1883 vakharia@umbc.edu

Jed Varney
Pacific Aquaculture
Sedro Woolley, WA, USA
360-420-3029 jvarney@pacseafood.com

Leticia Vidal
Maria Imaculada Poblete Vidal e Eduardo Antonio Poblete Sepulveda
Volta Redonda, RJ, Brasil
55 24992198356 let_vidal@yahoo.com.br

Wolfgang Vogelbein
Virginia Institute of Marine Science
Gloucester Point, VA, USA
804-684-7261 wolf@vims.edu

Sarah Vojnovich
Lebanon, OR, USA
541-405-5523 sarah.vojnovich25@gmail.com

Eric Wagner
Utah Division of Wildlife Resources
Logan, UT, USA
435-752-1066 x202 ericwagner@utah.gov

Rui Wang
LSU vet school
Baton Rouge, LA, USA
225-578-9204 rwang@tigers.lsu.edu

Janet Warg
USDA-APHIS-NVSL
Ames, IA, USA
515-337-7551 Janet.V.Warg@aphis.usda.gov

A. Michelle Wargo Rub
NOAA Fisheries NWFSC
Seattle, WA, USA
503-861-1818 ext. 32 michelle.rub@noaa.gov

Timothy Welch
USDA-ARS-NCCCWA
Kearneysville, WV, USA
304-724-8340 tim.welch@ars.usda.gov

Jennifer Welsh
Department of Marine Ecology
Den Burg, Noord Holland, Netherlands
31 (0) 222 369 300 (Ext. 558) jennifer.welsh@nioz.nl

Brent Whitaker
National Aquarium
Baltimore, MD, USA
410-576-3852 bwhitaker@aqua.org

Gregory Wiens
USDA-ARS-NCCCWA
Kearneysville, WV, USA
304-724-8340 greg.wiens@ars.usda.gov

Tom Wiklund
Laboratory of Aquatic Pathobiology
Turku, Finland
358 400 75 8957 twiklund@abo.fi

Chris Wilson
Utah Division of Wildlife Resources
Logan, UT, USA
435-757-7493 chriswilson@utah.gov

James Winton
USGS – Western Fisheries Research Center
Seattle, WA, USA
206-526-6587 jwinton@usgs.gov

Jeffrey Wolf
Experimental Pathology Laboratories, Inc.
Sterling, VA, USA
703-471-7060 jwolf@epl-inc.com

Thierry Work
US Geological Survey
Honolulu, HI, USA
808-792-9520 thierry_work@usgs.gov

Dehai Xu
USDA, ARS
Auburn, AL, USA
334-887-3741 dehai.xu@ars.usda.gov

Jiayun Yao
Zhejiang institute of freshwater fishery
Huzhou Zhejiang, China
86572-2046195 yaojiayun@126.com

Tomoyoshi Yoshinaga
The University of Tokyo
Bunkyo, Tokyo, Japan
81-3-5841-5284 atyoshi@mail.ecc.u-tokyo.ac.jp

Yong-An Zhang
Academy of Sciences
Wuhan Hubei, China
86-27-68780393 yzhang@ihb.ac.cn

Author Index

FLOOR PLAN
FOR CONFERENCE & EVENT ROOMS

FLOOR MAPS