ISAAH

9Th

Sept, 2022 Santiago, Chile

SCIENTIFIC PROGRAM

9th International Symposium on Aquatic Animal Health (ISAAH 9th)

"*Enhancing aquatic animal health towards One Health*"

Centro de Extensión, Universidad Católica de Chile
5-8 de Septiembre, 2022

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ISAAH NETWORKING & SOCIAL EVENTS

For the first time, the ISAAH community will meet outside of North America! For such a special occasion we wanted to build an equally special program of social events. Delegates attending ISAAH9 will have the opportunity to participate in several exciting social functions where they can meet, mingle and enjoy the company of their professional colleagues in a relaxed setting. Let ISAAH and Santiago show you all they have to offer:

"Student/Early Career - Faculty Mixer"

Monday, September 5
Main Ballroom (Salón Fresno)
17:00 – 18:30 (5:00 - 6:30 pm)

ISAAH9 will host a "Speed Networking" Mixer for students and early career professionals to build their professional network. Faculty and other professionals will be seated at assigned tables. Students and early career scientists will rotate around the room in 5 minute "sessions". The goal is to meet and connect with as many people as you can in this 1.5-hour session.

The Speed Networking strategy facilitates multiple, rapid introductions in an informal, low-pressure environment as the short session/rotation time restricts conversations to the who, what and where variety of questions/answers. Who are you? What are you interested in? Where are you now?

This approach allows students and early career folks to meet and introduce themselves to a wide variety of professionals from varied agencies and institutions, which is often difficult to do at coffee breaks and organized dinners. It's also allot of fun.

Anyone interested in attending this event (students or faculty/professionals) please notify the organizing committee at isaah9@uc.cl so we can plan accordingly.

Looking forward to seeing you at ISAAH9!

ISAAH 9Th Sept, 2022 Santiago, Chile

PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE

ISAAH Welcome Reception, *"A Taste of Pisco"*

Monday, September 5
Chipe-Libre Restaurant (@republicachipe)
José Victorino Lastarria 282, Barrio Lastarria, Santiago
19:00 – 21:00 (7:00 – 9:00 pm)

All attendees are welcome to join us at 19:00h (7:00 pm) at the Chipe Libre Restaurant. Chipe Libre is at the core of the historic Barrio Lastarria neighborhood, a cultural center in the heart of Santiago. The restaurant is within walking distance of our conference venue and participating hotels. During this social hour you can try the "Pisco Sour", one of Chile's national drinks. The event will also feature local dishes and locally sourced seafood including a mix of tasty empanadas and fine red wines that Chile is known for. Take this opportunity to try as many things as you like and discover the wonderful culinary delights Santiago and the Barrio Lastarria neighborhood has to offer.

ISAAH 9Th Sept, 2022 Santiago, Chile

PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE

OECD Social, *"Fire Walk with Me"*

Wednesday, September 7
Quitral (@quitral_resto)
Jose Victorino Lastarria 70 Local 4 Boulevard Lastarría, Santiago
19:00 – 21:00 (7:00 – 9:00 PM)

ISAAH9 attendees are invited to join us at 19:00h (7:00 pm) on Wednesday, September 7th, at the Quitral Fuego & Cava Restaurant, also located in the Barrio Lastarria district. "Quitral" means "Fire" in the language of the Mapuche people. Quitral Fuego & Cava will be the second destination in our culinary journey through Santiago and Chilean cuisine. Quitral is known for their "Sopaipillas" with "Pebre" (like Mexican "Pico de Gallo"), Ceviche, Steak Kebabs, and Shrimp/Cheese empanadas; as well as Pisco Sour, sparkling and red wine. Please join us for an evening of food and fellowship!

Closure *"The ISAAH Banquet"*

Thursday, September 8
19:30 pm – 22:30 (7:30 – 10:30 PM)

Av. Libertador Bernardo O´Higgins 816, Santiago

The ISAAH Banquet will be held at the Hotel Plaza San Francisco, a unique location with international allure and exceptionally fantastic food. An alluring union of classic elegance and contemporary luxury, Hotel Plaza San Francisco stands at the gateway to downtown Santiago as the only 5-star hotel in the city center; a short walk (0.5-mile, 10 min walk) from our conference venue and most hotels in the downtown core.

With a privileged setting just moments from world-class museums, restaurants and cultural attractions and mere steps from a metro station, the Hotel Plaza San Francisco is a beacon of urban sophistication, impeccable service, and award-winning gastronomy. Step inside and be swept into an ambience of English-style intimacy and warmth, where history and modern style are perfectly intertwined to create a truly personalized experience for each guest.

The ISAAH Banquet will consist of a three-course meal, a keynote address (TBA) and the Awards Ceremony. Attire is business casual and we recommend comfortable shoes suitable for dancing. We hope you will attend!

Keynote Speakers

Dr. Alicia Gallardo

Dr. Alicia Gallardo Lagno graduated as a veterinary at the Universidad of Chile and she also holds a Doctorate in Veterinary Sciences at the same institution. She has been working for twenty years in the public service and at the moment to be assigned to the position of Undersecretary of Fisheries and Aquaculture she was the National Director of the National Fisheries and Aquaculture Service (Sernapesca). At Sernapesca, she leaded the process of modernizing the organization, controlling the ISA virus at the salmon farming centers and implementing a national and international strategy against illegal fishing.Some of her areas of expertise include biosecurity in the aquaculture production, aquatic animal health and the prevention, monitoring and elimination of diseases. Gallardo has carried out duties in both academic and international fields. She is the Vice-President of the Aquatic Animal Commission at the World Organization for Animal Health (OIE) and in 2020 was appointed to be the president of the APEC Ocean and Fisheries Working Group for the term 2021-2022.

Facing Aquaculture in 2030:
How to Deal with Main Health Challenges in Aquaculture

Alicia Gallardo Lagno[1]

[1]First Vice-president Aquatic Animal Health Standards Commission (AAHC);
World Organization for Animal Health (WOAH)

According to the report on the status of Fisheries and Aquaculture (SOFIA, 2022) of the Food and Agriculture Organization of the United Nations (FAO), an intensification and expansion of sustainable aquaculture production is expected in the next decade, to address the food security needs of the world population. By 2030, aquatic food production is expected to increase by a further 15%. However, this growth must preserve the health of aquatic ecosystems, prevent pollution, and protect biodiversity and social equality. World Organization for Animal Health (WOAH) has established in 2021, an strategy to improve aquatic animal health with four objectives: standard setting, capacity building, leadership and resilience. Emerging diseases and changes in the behavior of prevalent diseases will be the next challenges, for which veterinary services (public and private) need to be prepared in epidemiological surveillance, biosecurity and timely response to contingencies. Also, preventing AMR, through prudent and responsible use of antimicrobials and following the One Health approach is a priority challenge. The academia involvement and public-private partnerships will be key in advances in aquatic animal health. Faced with these challenges, FAO promotes the blue transformation for the development and adoption of sustainable aquaculture practices; integrate aquaculture into development strategies and food policies at national, regional and global levels; expand and intensify aquaculture production to meet the growing demand for aquatic food and foster inclusive livelihoods, and enhance capacities at all levels to develop and adopt innovative technologies and management practices for a more efficient and resilient aquaculture industry.

Dr. Irene Salinas

Dr. Irene Salinas is an Associate Professor at the University of New Mexico, Department of Biology. Her laboratory investigates the mucosal immune system of teleost and sarcopterygian fish and its interactions with microbiota and pathogens. Recently, her laboratory has developed an interest on neuroimmune communication at fish mucosal barriers. Dr. Salinas is committed to actively make science more equal and inclusive to everyone.

The Hidden Sophistication of Teleost Mucosal Immune Systems

Irene Salinas[1]

Center for Evolutionary and Theoretical Immunology; University of New Mexico, Albuquerque, NM87108

Most fish pathogens initiate infection at mucosal barrier tissues and, therefore, mucosal immunity is vital for host survival. The presence of innate and adaptive immune defense mechanisms at teleost mucosal barriers has been known for almost a century, but its anatomical and cellular complexity continues to be unraveled. My presentation will summarize the body of work that has been conducted by many laboratories as well as our own group pertaining teleost mucosal immunity. Mucosa-associated lymphoid tissues were previously thought to be associated with the gastrointestinal tract, skin and gills but has now been expanded to also the olfactory organ, oral cavity and swim bladder. The current dogma postulates that teleosts lack organized lymphoid structures and therefore there is no division between inductive and effector mucosal sites in teleosts. Using examples from the teleost nasal cavity, I will challenge this dogma and propose the presence of mucosal lymphoid structures where maturation of the adaptive immune response may occur in response to infection or vaccination. Finally, I will highlight knowledge gaps in the field and how these fundamental gaps need to be filled for optimal design of mucosal vaccines in aquaculture.

Dr. Ian Gardner

Dr. Ian Gardner is an infectious disease epidemiologist with a research focus on the control and prevention of aquatic and terrestrial animals that includes aspects of ecosystem health. From 2011 to 2019, he was the Canada Excellence Research Chair (CERC) in Aquatic Epidemiology at the University of Prince Edward Island (UPEI). He is now a Professor Emeritus at both UPEI and the University of California, Davis where he worked for 23 years. His research interests include validation of diagnostic tests for aquatic infectious diseases in the absence of perfect reference tests, health interactions between farmed and wild fish, infection disease modelling and evaluation of the cost-effectiveness of surveillance and disease control strategies. He has published more than 320 peer-reviewed papers and book chapters, and recently co-edited a Special Issue for the World Organisation of Animal Health (OIE) on Test Validation Science. Dr. Gardner obtained his veterinary degree at the University of Sydney in 1975 and completed post-graduate training (Master of Preventive Veterinary Medicine and PhD) at U.C. Davis in 1988.

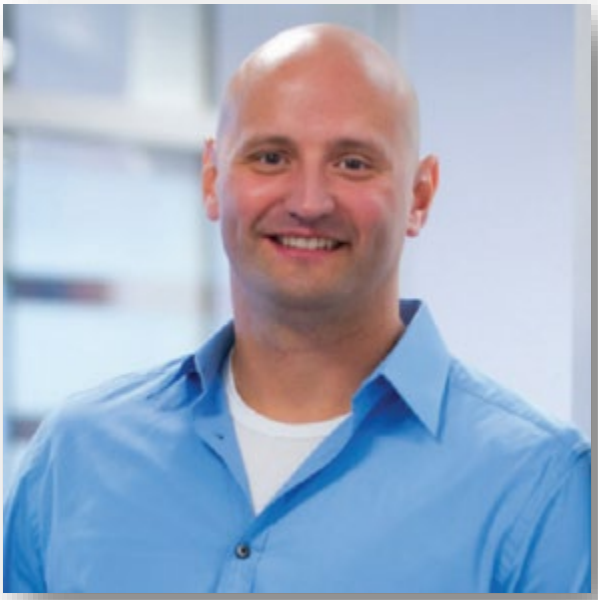
qPCR Tests for Animal Pathogens Never Make Mistakes Until They Do: Rigorous Validation Studies, Adherence to Quality Standards, Peer Review, and Proficiency Testing Reduce Risk of Errors

Ian A. Gardner[1]

[1]Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, Canada

Molecular diagnostics, especially quantitative PCR (qPCR), are the cornerstone of current diagnostic algorithms for important pathogens in aquatic animal health, and for use in epidemiologic studies (e.g. prevalence surveys, risk factor studies) and disease surveillance systems. From a decision-making perspective, qPCR tests should be accurate, reproducible, cost-effective, and minimally invasive – perfect accuracy all of the time is impossible. The limitations of qPCR are well documented and include effects of exogenous and endogenous inhibitors and the fact that they only reflect detection of genetic material (DNA/RNA) rather than live organisms. Furthermore, labs implementing qPCR do so on a range of platforms, using different detection chemistries, extraction methods, primers and probes, and cut-off values for declaring a qPCR result as positive or negative. This induces additional variability in qPCR results and may lead to incorrect classification of population infection status. The paucity of proficiency testing (ring trials) in aquatic animal health, including for qPCR, is in stark contrast to accreditation requirements of laboratories testing for human infectious diseases. Failures of qPCR testing occur in human, plant, crop, and terrestrial animal health so the aforementioned factors are not unique to aquatic animal species.

The World Organisation for Animal Health (WOAH, formerly known as OIE) has a recommended assay validation pathway with four stages that should be followed for detection of listed diseases of aquatic animals (fish, crustaceans, molluscs, and amphibians) before an assay can be considered "fit for purpose" in the species in which the test will be used. For aquatic animals, the purposes are presumptive diagnosis, confirmatory diagnosis, and surveillance. The validation metrics include analytical and diagnostic performance characteristics, and reproducibility among laboratories. In addition, use of point-of-care tests and portable qPCR equipment for on-farm diagnosis needs to be supported by assessments of ruggedness under extreme environmental conditions (e.g., temperature and humidity). Operator training is essential to ensure that performance in the field is comparable to that in controlled laboratory conditions, which are quality assured. The consequences of test errors depend on the prevalence of disease in sampled populations and the costs and benefits of testing. False-positive results in disease-free populations that lead to transboundary trade restrictions can be especially costly. However, there are few published studies that assess the economic value of decisions to test versus not to test for regulated and non-regulated diseases in farmed aquatic animals. Validation of qPCR takes time and is costly, but without validation data and consideration of the limitations of the method, the risk of misuse and misinterpretation of qPCR test results is increased. Rigorous validation studies, adherence to quality standards, peer review of results, and proficiency testing can reduce the risk of test errors. In this talk, I provide a veterinary epidemiologist's perspective of the last decade of progress and achievements in diagnostic validation studies of qPCR in aquatic animal health, using white spot syndrome virus (WOAH-listed), tilapia lake virus and salmon rickettsial syndrome (both not listed by WOAH) as examples. Finally, I briefly discuss the possible role of new technologies, e.g., environmental DNA (eDNA), for enhancing our understanding of the population dynamics of important aquatic infectious diseases. I provide a cautionary note about the likely complexity of requirements for comprehensive validation and reproducibility assessments of eDNA sampling methods and platforms, if used for regulated diseases.



Dr. Mark Fast

Dr. Mark Fast has as worked on fish health, comparative immunology and host-pathogen interactions, with a major focus on sea lice since 1999. In 2007, Dr. Fast joined the School of Marine and Atmospheric Sciences at Stony Brook University, NY, as an Assistant Professor and in 2010 took up the Novartis Research Chair in Fish Health at the Atlantic Veterinary College (AVC)-UPEI until 2015. Dr. Fast is currently a Full Professor at AVC-UPEI, researching new methods for controlling sea lice infection in Atlantic salmon, comparative immunology, vaccinology and feed associated immunomodulation of teleosts and chondrosteans.

Tackling Global Sea Lice Issues with Genomics

Mark D. Fast[1]

[1]Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, C1A 4P3, Canada

Sea lice, namely *Lepeophtheirus salmonis* in the Northern Hemisphere and *Caligus rogercresseyi* in the Southern Hemisphere, have caused major impacts to wild and cultured salmonids for decades. In response, research consortia have come together across the globe through initiatives such as the TREAT, Sea Lice Research Centre (SLRC), GenoLice, CRISPResist, Lice Resist and NoLice, to tackle these issues. Over the last few years this has resulted in immense amounts of knowledge being gained on salmonids and sea lice, through applications of 'Omics' technologies such as genomics, transcriptomics, proteomics, nutrigenomics, and immunomics. The Atlantic salmon (*Salmo salar*) genome has been mapped, and several other salmonid species genomes have been assembled, and the same is true for *L. salmonis* and *C. rogercresseyi*. Genetic maps have been created and allowed us to identify chromosome regions within *L. salmonis* responsible for different forms of drug resistance, allowing large scale genotyping of lice to occur in several farming regions with single nucleotide polymorphism panels or through biomarker testing for specific resistance gene mutations. Whole genome sequencing and genome wide association studies have also allowed us to identify genomic regions associated with resistance to sea lice in Atlantic salmon, so that we may enhance industry breeding programs to carry lice-resistant traits. Knockdown studies using RNAi have allowed us to characterize important genes in the louse life cycle to target for new drug therapies and vaccine candidates, and now CRISPR-Cas9 technology is being examined for the ability to test different gene manipulations and whether these can deliver lice resistance in a more efficient manner to Atlantic salmon, through the use of comparative genomics and examination of more resistant phenotypes in coho (*Oncorhynchus kisutch*) and pink (*O.gorbuscha*) salmon. This seminar will discuss the application of these technologies, how it has improved our biological understanding of the host-parasite relationship, informed industry and policy decisions, and where it will go into the future.

9th International Symposium on Aquatic Animal Health

ORAL PROGRAM

Monday, September 5th
Main Ballroom (*Salón Fresno*)

8:00 AM **Registration**

Welcome
9:00 AM **Fernando Mardones,** Local Organizing Chair
Gary Marty, President**,** American Fisheries Society- Fish Health Section
Dr. Ignacio Sánchez Díaz, President, Pontificia Universidad Católica de Chile

Keynote Address
9:15 AM **Dr. Alicia Gallardo:** Facing Aquaculture in 2030: How to Deal with Main Health Challenges in Aquaculture

10:00 AM Refreshments

General Session I: Matt Griffin/Sherri Kasper
10:15 AM **Johnsen**: The OIE Aquatic Animal Health Strategy (2021-2025)
10:30 AM **Algüerno**: Strengthening Veterinary and Aquatic Animal Health Services: The Contribution of the World Organization for Animal Health
10:45 AM **Piñeros-Duque**: Perception of Tilapia Farm Workers about Knowledge of Fish Diseases with a One Health Approach in Five Departments of Colombia during 2019
11:00 AM **Kohli**: Red Sore Disease of American Eels in Chesapeake Bay: Etiology and Epidemiology
11:15 AM **Kasper**: Harmful Algal Blooms Effects, Diagnosis and Mitigation in Aquaculture
11:30 AM **Kasper**: Increasing Threat of Harmful Algal Blooms Caused by Global Climate Change and Increased Migration

Virology: Esteban Soto/Eva Quijano Cardé
1:00 PM **Soto**: Susceptibility of Lake Sturgeon (*A. fulvescens*) to Acipenserid Herpesvirus 2, White Sturgeon Iridovirus, and Ranaviruses
1:15 PM **Quijano Cardé**: Design and Validation of a qPCR Assay for Diagnosis of Acipenserid Herpesvirus 2 in White Sturgeon (*Acipenser transmontanus*) Tissues
1:30 PM **Soto**: Susceptibility of Acipenserid Herpesvirus 2, White Sturgeon Iridovirus, and Ranaviruses to Buffered Povidone-Iodine Complex, Chlorine and Virkon Aquatic®
1:45 PM **Getchell**: Viral Hemorrhagic Septicemia Endemic in St. Lawrence River Round Goby and Lake Ontario Gizzard Shad
2:00 PM **Patel**: Infectious Salmon Anemia Virus Directly Modulates the Red Cell Surface
2:15 PM **Zawisza**: CEV-Infection Induced Cortisol Release and Immunosuppression Are Associated With High Mortality of Susceptible Koi Carp
2:30 PM **Kebus:** Risk Assessment of Fish Movements from Great Lakes Region Fish Farms and Hatcheries to Natural Waters or Other Premises During a Viral Hemorrhagic Septicemia Outbreak

Microbiomes: Matt Griffin/Divya Rose
3:15 PM **Jimenez-Lopez**: Microbial Communities of Salmonids, a Meta-Analysis
3:30 PM **Coca Rives**: Characterization of the Intestinal Microbiota of *Salmo salar* Smolt During an Infectious Outbreak of *Aeromonas salmonicida* subsp. *salmonicida*
3:45 PM **Kent**: Intestinal Lesions and Microorganisms Associated with Senescence and Prespawn Mortality in Chinook Salmon *Onchorynchus tshawtscha*
4:00 PM **Coca Rives**: Intestinal Microbiota Characterization in *Salmo salar* With Clinical Signs of *Piscirickettsia salmonis* in Chilean Salmon Farming

Monday, September 5th
Breakout Room A (*Sala Matte*)

Flavobacterium: Tom Loch/Taylor Heckman
10:15 AM **Loch**: Enhancing Bacterial Coldwater Disease Diagnosis and Prevention by Elucidating the Predominant *Flavobacterium psychrophilum* Serovariants in the USA
10:30 AM **Heckman**: Flavors of Flavobacteriales: Characterizing Atypical Flavobacterial Pathogens in Aquaculture
10:45 AM **Valdés**: Phenotypic, Serological and Genetic Characterization of Tenacibaculum Strains Isolated from Chilean Salmon Farms
11:00 AM **Ilardi**: Intraspecific Diversity of *Flavobacterium psychrophilum* Isolated from Salmonids Farms in Chile
11:15 AM **Poblete**: Expert elicitation to identify risk factors for Tenacibaculosis outbreaks in farmed Atlantic salmon in Chile
11:30 AM **Ilardi**: Flavobacteria Isolated From BCWD Outbreaks in Chilean Salmon Farms

Tilapia Health: Paola Barato/ Inácio Assane
1:00 PM **Adamek**: Nile Tilapia Strain Resistant to Tilapia Lake Virus Disease – Immunological and Implementation Considerations
1:15 PM **Vela**: Acute Toxicity Evaluation of Practical Diets with *Erythrina edulis* as a source of adhesion glycoinhibitors for *Streptococcus agalactiae* in tilapia (Oreochromis sp.)
1:30 PM **Delphino**: Economic Appraisal of Using Genetically Selected Nile Tilapia Fingerlings to Control *Streptococcus agalactiae* Under Cage and Pond Farming System
1:45 PM **Cruz**: Characterization of Polyclonal Antibodies Generated Against Interferon Gamma in Nile Tilapia (*Oreochromis niloticus*) by Western Blot, ELISA and Flow Cytometry
2:00 PM **Assane**: Phenotypic and Genotypic Characterization of *Aeromonas jandaei* Involved in Mass Mortalities of Cultured Nile tilapia, *Oreochromis niloticus* (L.) in Brazil
2:15 PM **Peña**: Histological and Molecular Biomarkers Applied to the Study of Vaccine Immune Modulation and Hepatic Function to Diets in Nile Tilapia
2:30 PM **Assane**: *Enterogyrus* spp. (Monogenea Ancyrocephalinae) and *Aeromonas jandaei* Co-infection Associated with High Mortality Following Transport Stress in Cultured Nile Tilapia

Monday, September 5th
Breakout Room B (*Salón e 204*)

Virtual Microscopy: Dave Groman
10:15 AM **Groman**: Introduction to the Virtual Microscopy Session
10:30 AM **Groman**: Overview of Digital Pathology's Current State: With Comments on Use in Aquatic Diagnostics
11:00 AM **Sandoval**: Practical Operation of a Digital Fish Pathology Service - How it Works
11:30 AM **Ildefonso**: Advantages and Disadvantages of using Digital Pathology vs Traditional Histopathology
12:00 PM Lunch
1:00 PM **Practical Session**: Virtual Laboratory Session I
3:00 PM Refreshments
3:15 PM **Practical Session**: Virtual Laboratory Session II

Tuesday, September 6th
Main Ballroom (*Salón Fresno*)

Keynote Address
8:30 AM **Dr. Irene Salinas**: The Hidden Sophistication of Teleost Mucosal Immune Systems

World Organization for Animal Health Session on Antibiotic Use and Resistance: Alicia Gallardo/Dante Mateo`
9:30 AM **Mateo**: Contribution of the World Organization for Animal Health (WOAH) to Prevent AMR on Aquatic Animals
9:45 AM **Gallardo**: New WOAH Collaborating Center for Antimicrobial Stewardship in Aquaculture
10:00 AM **Lara**: Health Management in Aquaculture Program (PGSA): Strengthening Responsible and Prudent Use of Antimicrobials (AMU) in Salmon Production.
10:15 AM **Burgos**: An Experience in the Implementation of Title 6 of Aquatic Animal Health Code: The Chilean Case
10:30 AM Refreshments
10:45 AM **Contreras-Lynch**: Research Program for Monitoring Bacterial Resistance in Chilean Salmon Farming
11:00 AM **Navarro**: Implementing Effective Monitoring and Surveillance of Antimicrobial Use from Farmed Salmon in Chile
11:15 AM **Ojasanya**: Antimicrobial Susceptibility Patterns of Bacteria Commonly Isolated from Farmed Salmonids in Atlantic Canada (2000–2021)
11:30 AM **San Martin**: Closing Remarks and Discussion

Emergent Diseases: Al Camus/Taylor Heckman
1:00 PM **Camus**: Viral Discoveries in Elasmobranch Fishes
1:15 PM **Kannimuthu**: Temporal Dynamics of Piscine Orthoreovirus-1 (PRV-1) Infection During Pre-smolt Stages of Atlantic Salmon (*Salmo salar*)
1:30 PM **Solano-Iguaran**: First Detection of Infectious Spleen and Kidney Necrosis Virus (ISKNV) and the Parasite *Centrocestus* sp. in Chile: Co-Infection in the Ornamental Fish Platy (*Xiphophorus maculatus*)
1:45 PM **Gaete-Carrasco**: Epidemiological Analysis and Determination of Risk Factors for Tenacibaculosis in the Chilean Salmon Farming
2:00 PM **Rodger**: Complex Gill Disease (CGD) of Atlantic salmon (*Salmo salar*): Our Current State of Knowledge
2:15 PM **Dale**: Infectious Salmon Anemia Causes New Challenegs in Norway
2:30 PM **Heckman**: For *Lac* of a better name: Redefining Piscine Lactococcosis
2:45 PM **Ness**: Emerging Variants of *Moritella viscosa*
3:00 PM Refreshments
3:15 PM **Mora-Salas**: Interlaboratory Ring Trial to Evaluate a Real-Time PCR Assay for the Detection of *Renibacterium salmoninarum* in Chile
3:30 PM **Godoy**: Macroscopic and Histopathological Morphological Spectrum of Muscle Melanosis in Salmon Farming in Chile
3:45 PM **Adamek**: When Sleep Meets Death - How Gill Disease Can Induce Secondary Pathology in the Brain

Tuesday, September 6th
Breakout Room A (*Sala Matte*)

General Session II: Matt Griffin/Fanny Giudicelli

9:30 AM **Okwuosa**: Diets Influencing Hematological Profile as Fitness and Genetic Bioindicator of Fish health
9:45 AM **Salazar**: Expression Analysis of Estrogen Receptors Genes, Immune Response Genes and Immune Selected miRNAs, in a *Salmo salar* Cell Line Induced with Xenoestrogens: 17α-Ethynyl Estradiol and 4-Nonyphenol.
10:00 AM **Schumann**: Physiological Stress Response Induced by Different Hydrodynamic Conditions with Varying Group Size in *Telestes multicellus*
10:15 AM **Jungers**: Pharmacokinetic Analysis of Ceftazidime in Signal Crayfish (*Pacificastacus leniusculus*) Following Intravascular and Intramuscular Administration
10:30 AM Refreshments
10:45 AM **Quidel**: Severity Classification of Salmonid Rickettsial Syndrome Outbreaks on Atlantic Salmon Reared in Chilean Aysén Region. A Predictor Model
11:00 AM **Curotto-Zola**: Biosecurity Characterization of Rainbow trout (*Oncorhynchus mykiss*) Production Farms in Puno, Peru
11:15 AM **Michnowska**: Horizontal Transmission of Disseminated Neoplasia in the Widespread Clam *Macoma balthica* from Southern Baltic Sea
11:30 AM **Michnowska**: Novel Study of Metabolism of Bivalve Transmissible Neoplasia (BTN) Mitochondrial Respiration and Free Amino-acids Profile of Contagious Cancer Cells in *Macoma balthica*

Microbiology/Bacteriology: Tim Bruce/Allison Wise

1:00 PM **Barato**: Advance in phage therapy to control *Weissellosis* by *Weissella ceti* in rainbow trout (Onchorynchus mikiis) in Colombia
1:15 PM **Yunis-Aguinaga**: Characterization and Preliminary Vaccine Trial Against *Yersinia ruckeri* in Cage-Reared Rainbow Trout in Peru
1:30 PM **Patel**: Pasteurellosis; a Serious, Emerging Disease in Atlantic Salmon Farmed in the North-Atlantic
1:45 PM **Yunis-Aguinaga**: Biochemical and Molecular Identification of *Aeromonas* spp. Isolated from Diseased Amazon Fish Cultured in Peru
2:00 PM **Mora-Salas**: New PCR Method for Lineage Typing of Epidemic *Renibacterium salmoninarum* in Chilean Salmon Farms
2:15 PM **Yunis-Aguinaga**: Isolation and primary characterization of *Chryseobacterium* spp. in outbreaks in farmed rainbow trout in Peru
2:30 PM **Omeje**: Drug resistant profiles of *Aeromonas hydrophila* isolated from cultured African catfish *Clarias gariepinus* in the Kainji Lake area, Nigeria.
2:45 PM **Yunis-Aguinaga**: Susceptibility of *Colossoma macropomum* to Experimental Infection with Aeromonas Species Isolated from Ornamental Fish
3:00 PM Refreshments
3:15 PM **Divya**: Phenotypic, Genotypic and Serological Comparison of *Edwardsiella ictaluri* Isolates Derived from Catfish and Ornamental Fish Species
3:30 PM **Wise**: Polymicrobial Infection Dynamics with *Edwardsiella ictaluri* and *Flavobacterium covae* in Channel Catfish Ictalurus punctatus
3:45 PM **Dubytska**: *Edwardsiella ictaluri* T3SS Effector EseN is Involved in Regulation of Apoptosis in Infected Head-Kidney-Derived Macrophages
4:00 PM **Hanson**: Microcystin-LR Exposure Predisposes Channel Catfish to Bacterial Diseases

Tuesday, September 6th
Breakout Room B (*Salón e 204*)

World Aquatic Veterinary Medical Association/American Association of Fish Veterinarians: Nora Hickey

9:30 AM **Soto**: Phenotypic and Genetic Diversity Amongst the Etiological Agents of Columnaris Diseases: *Flavobacterium columnare*, *F. covae*, *F. davisii* and *F. oreochromis*
10:30 AM Refreshments
10:45 AM **Reichley**: Successes and Failures of Combating Columnaris Disease at a Large-Scale Rainbow Trout Farm
12:00 PM Lunch
1:00 PM **Bruce**: An Overview of Columnaris Disease in Cultured U.S. finfish: Experimental Infections, Disease Diagnostics, and Current Treatments
2:00 PM **Kasper**: Columnaris Disease: Prevalence, Prevention and Treatment of Non-Food Species
3:15 PM **Onofryton:** Vaccination for Columnaris Disease: A Brief History and Future Prospects
4:15 PM **Stilwell:** Pathology of Columnaris Disease in Catfish and Ornamental Fish

Wednesday, September 7th
Main Ballroom (*Salón Fresno*)

Keynote Address
8:30 AM **Dr. Ian Gardner:** qPCR Tests for Animal Pathogens Never Make Mistakes, Until They Do: Rigorous Validation Studies, Adherence to Quality Standards, Peer Review, and Proficiency Testing Reduce Risk of Errors

Big Data: Ian Gardner/Grace Kerreman/Jon Grant
9:30 AM **Grant:** Big Data and the Implementation of Precision Fish Farming
10:00 AM **Vanderstichel:** Longitudinal Dissolved Oxygen Patterns in Atlantic salmon Aquaculture Sites in British Columbia, Canada
10:15 AM **Midtlyng:** A Proposed Improvement of Real-time Monitoring of Cause-specific Mortality and Losses in Industrial Salmon Farming
10:30 AM Refreshments
10:45 AM **Nygren**: Fishing for Answers: Using Big Data Analytics to Predict and Manage Environmental Risks at BC Salmon Farms
11:00 AM **López-Riveros**: Precision Biometrics Data of Atlantic Salmon (*Salmo salar* L.) in Commercial Grow-out Sea-Cages: Manual Sampling and Infrared Diode frames Compared to Processing Plant
11:15 AM **Burciaga-Robles**: Connect, Digitize and Exchange of Information: Creating Better Producer to Consumer Outcomes in Aquaculture. What Can We Learn from the Feedlot Industry?
12:00 PM Lunch
1:00 PM **Valdes Donoso**: What Does Big Data Mean for Aquaculture? Current Development, Limitations, and Future Challenges
1:15 PM **Bravo**: Big Data Analytics to Support Evidence-based Strategic Planning in Salmon Farming in Chile
1:30 PM **Panel Discussion**

Modeling Applications in Aquatic Animal Health: Ian Gardner/Bradley Richardson
1:45 PM **Romero**: Overview of a Simulation Framework for Evaluation of Mitigation Strategies to Reduce Waterborne Spread of Viral Diseases in Marine Aquaculture
2:00 PM **Grant**: Farming in Natural Systems (FINS): A Provincial-wide Modelling Tool for Environmental and Infectious Disease Risks in Farmed Atlantic salmon and Oysters in Nova Scotia, Canada (Part I)
2:30 PM **Delphino**: Cost-effectiveness of longitudinal surveillance for *Piscirickettsia salmonis* using qPCR in Atlantic salmon farms (*Salmo salar*) in Chile
2:45 PM **Romero**: Use of Simulation Modelling for Cost-effectiveness Analysis of Infectious Disease Management Options in Marine Salmon Aquaculture
3:00 PM Refreshments
3:15 PM **Rivera**: Epidemiological Genetic Model through Bioassays with Genetically Improved Families in Atlantic Salmon (*Salmo salar*) in the Presence of SRS (*Piscirickettsia salmonis*)
3:30 PM **Gaete-Carrasco**: Identification of Risk Factors Associated with Piscirickettsiosis Outbreaks in *Salmo salar* in Chile
3:45 PM **Gardner**: Use of a Waterborne-Spread Model of Infectious Salmon Anaemia virus (ISAV) to Inform Management Decisions Following Detection of a Pathogenic Strain in a Newfoundland Salmon Hatchery
4:00 PM **Gardner:** Closing Remarks/Discussion

Wednesday, September 7th
Breakout Room A (*Sala Matte*)

Zebrafish/Lab Animal Models: Mike Kent
10:45 AM **Kent**: Common Laboratory Diets Differentially Impact Fitness, Health, and the Gut Microbiome in Zebrafish (Danio rerio)
11:00 AM **Schuster**: Use of Occupancy Modeling to Understand Pathogen Diagnostic Efficacy in a Zebrafish Facility
11:15 AM **Rakus**: Tilapia Lake Virus Infection in Zebrafish: A Model to Study Antiviral Immune Response and Host-Pathogen Interaction in Fish
11:30 AM **Peterman**: Enhanced Immunity and Gut Nccrp-1+ and Mpeg-1+ Cell Populations in rag1-/- Zebrafish

Parasitology: Graham Rosser/Celene Slifka
1:00 PM **Nguyen**: A Morphological and Molecular Comparison of *Clinostomum Metacercariae* and Adults of the United States
1:15 PM **Slifka**: What's in the Water: Identifying *Planorbella trivolvis* and *Biomphalaria havanensis* DNA Using Real-Time qPCR
1:30 PM **Powell**: Overview and Future Research Directions of *Bolbophorus damnificus* Trematode Pathogenesis in Channel (*Ictalurus punctatus*) and Hybrid (Channel X Blue Catfish *Ictalurus furcatus*) Catfish
1:45 PM **Garcia**: Estimation of Genetic Parameters and Genetic Co-variation Between *Piscirickettsia salmonis* Resistance and Sea Lice (*Caligus rogercresseyi*) Susceptibility in Atlantic Salmon (*Salmo salar*) Using Genomic Information

Wednesday, September 7th
Breakout Room B (*Salón e 204*)

Pathology of Fish and Shellfish: Paola Barato
9:30 AM **Barato:** Introduction and Opening Remarks
9:45 AM **Groman:** Histopathological Responses in Primary Organ Systems of Salmonids to Bacterial and Viral Disease Conditions
10:30 AM Refreshments
10:45 AM **Stilwell:** Infectious Disease Pathology of U.S. Catfish Aquaculture
12:00 PM Lunch
1:30 PM **Camus:** Pathology of Main Diseases of Elasmobranchs
2:15 PM **Barato:** Select Diagnostic Cases of Tilapia
3:00 PM Refreshments
3:15 PM **Ildefonso**: Histoscore in Tilapia to Evaluate Substances and Vaccines
4:00 PM **Ferguson:** Selected Diagnostic Cases of Shellfish
4:45 PM **Sandoval:** Aluminum Intoxication in Atlantic Salmon Fingerlings

Wednesday, September 7th
Breakout Room C (*Sala Colorada*)

Food Security Workshop: Fernando Mardones

9:30 AM **Bouchon/Mardones/Szécáks:** Introduction and Opening Remarks
10:00 AM **Boden:** Role and Importance of Intangible Heritage on Food Security, Sustainable Development and Planetary Health
10:30 AM Refreshments
10:45 AM **Mardones:** Enhancing Aquatic Animal Health towards One Health
11:15 PM **Reichley:** Utilizing Biosecurity Practices to Increase Resilience
12:00 PM Lunch
1:00 PM **Geers:** Global Seafood Sustainability
1:30 PM **Szécáks**: Environmental Safety and a Key to Food Safety
2:00 PM **Crossley:** Aquaculture: The Missing Contributor in the Food Security Agenda
2:30 PM **Gelcich:** Global Change and the Future of Fisheries Management
3:00 PM Refreshments
3:15 PM **Paredes**: Conserving and Sustaining Life Below Water; Critical Parts of The Global Health Paradigm
3:45 PM **Barcos:** Aquatic Animal Health Strategy in 2021

Thursday, September 8th
Main Ballroom (*Salón Fresno*)

Keynote Address
8:30 AM **Dr. Mark Fast:** Tackling Global Sea Lice Issues with Genomics

Genomic Applications in Fish Health: Phillip Dettleff/Sebastian Escobar
9:30 AM **Dettleff**: Introduction and Welcome
9:45 AM **Dettleff**: Transcriptomic Applied to Fish Health: Pathogen and Stress Response in Fish.
10:00 AM **Yáñez**: On the Use of Ultra-dense Genome-wide Information to Boost Host Response Against Diseases in Aquaculture
10:15 AM **Escobar**: CRISPR-Cas9: A Genetic Tool to Study Gene Functions in Fish.
10:30 AM Refreshments
10:45 AM **Valenzuela-Munoz**: Genomics Applied to Understand the *Caligus rogercresseyi*-Atlantic salmon Interaction.
11:00 AM **Valdés**: Functional Genomics Applied to Aquaculture and Teleost Muscle Growth
11:15 AM **Martinez**: Developing Genomic Resources of Non-model Species. The Case of the Diversification Program of *Seriola lalandi* for a Better Management of Fish Health.
11:30 AM **Dettleff**: Evaluating High Temperature Effects on Red Cusk-eel (*Genypterus chilensis*) Trough Gill De Novo Transcriptome Assembly.
11:45 AM **Roh**: Endogenous DNA Is Highly Dynamic Constituent of Skin Mucus in Atlantic Salmon
12:00 PM Lunch
1:00 PM **Piña-Elgueda**: Description of the Genetic Basis of Sea Lice (*Caligus rogercresseyi*) Counts Using a Repeated Measures Genome-Wide Association Study (GWAS) in Atlantic Salmon
1:15 PM **Tapia**: Differential Gene Expression Patterns of Early Response Against Sea Lice Infestation in the Parasitized Skin of Coho and Atlantic Salmon
1:30 PM **Vidal**: Unveiling the Role of Differential Alternative Splicing Between Resistant and Susceptible Atlantic Salmon to Sea Lice, *Caligus rogercresseyi*
1:45 PM **Cáceres**: Meta-analysis of GWAS for Sea Lice Load in Atlantic salmon
2:00 PM **Núñez-Acuña**: Duplicated Genome Regions in *Caligus rogercresseyi* Associated with Pharmacological Resistance and Evaluation at Sea Lice Populations
2:15 PM **Marin-Nahuelpi**: Meta-analysis of GWAS for *Piscirickettsia salmonis* resistance in Atlantic salmon
2:30 PM **Tekedar**: Tad Operon Contributes to Virulence of Epidemic Isolate *Aeromonas hydrophila* ML09-119
2:45 PM **Chicoski**: Genomic Features of Fish Pathogens *Edwardsiella* spp. Isolated in Brazil Insight About Genomic, Antibiotic Resistance and Virulence Factors
3:00 PM Refreshments
3:15 PM **Dubytska**: *Edwardsiella ictaluri* T3SS Effector EseN Modulates Expression of Host Genes Involved in the Immune Response
3:30 PM **Valdés**: Role of Mineralocorticoid and Glucocorticoid Receptors in Teleost Somatic Growth and Stress
3:45 PM **Gallardo-Hidalgo**: Genome-Wide Association Study for Growth Traits Under Upper and Lower Thermal Rearing Conditions Using Genome-wide Imputation, Multi-trait Analysis and Gene-Based Association Approach in Rainbow Trout (*Oncorhynchus mykiss*)

Thursday, September 8th
Breakout Room A (*Sala Matte*)

Myxozoa: Jerri Bartholomew/Ethan Woodyard
9:30 AM **Bartholomew**: *Ceratonova shasta*: Biological and Artistic Insights on What Drives Host-Myxozoan Interactions in Large River Systems
9:45 AM **Americus**: The Myxozoan Parasite *Ceratonova shasta* Uses a Minimal Genetic Repertoire to Infect Its Fish and Invertebrate Hosts
10:00 AM **Ghai**: Morphological and Molecular Identification of Myxozoan Parasites and Its Effect on Cultured Indian Major Carps in Panjab, India
10:15 AM **Kaur**: Diversity of Myxozoan Parasites Associated with Diseases in Aquaculture and Wild Fish Stocks in India
10:30 AM Refreshments
10:45 AM **Gorgoglione**: Discovery of *Tetracapsuloides bryosalmonae* Infecting Salmon in the Great Lakes
11:00 AM **Stilwell**: Massive Branchial Henneguyosis: A Distinctive Myxozoan-Induced Gill Disease of Catfish Caused by Massive Interlamellar Infection of *Henneguya exilis*
11:15 AM **Woodyard**: *Myxidium mollismum* n, Sp., a Novel Myxozoan from the Common Elder *Samoteria moillissima*
11:30 AM **Ferguson**: Proliferative Kidney Disease and Surveilance in Wild Alaska Salmon

Immunology/Vaccinology: Lora Petrie-Hanson/Beth Peterman
1:00 PM **Adamek**: Vaccination Protects the Skin Barrier and Gill Function from Disruption Caused by Cyprinid Herpesvirus 3
1:15 PM **Soto-Davila**: Effect of Feeding Strategy of Jameison® Probiotic on Growth Performance and Immune Response of Chinook Salmon (*Oncorhynchus tshawytscha*) Challenged with *Vibrio anguillarum*
1:30 PM **Liu**: Evaluating the Efficacy of New Oral Vaccine Feeds Against Salmonid Novirhabdovirus in Rainbow Trout (*Oncorhynchus mykiss)*
1:45 PM **Jones**: Evaluating a Novel Oral Vaccine Delivery Platform in Rainbow Trout *Onchorhynchus mykiss*
2:00 PM **Thorarinsson**: Effect of Vaccines Against Pancreas Disease in Atlantic Salmon Challenged with Salmonid Alphavirus, Subtype 2
2:15 PM **Thorarinsson**: Effect of Vaccines Against Pancreas Disease on Viral Shedding and Disease Transmission from Atlantic Salmon Challenged with Salmonid Alphavirus, Subtype 2
2:30 PM **Giudicelli**: Beneficial Effects of Marine Bacillus Multi-Strains Consortium Encapsulated in Algae on Growth Performance, Mucosal Microbiota Modulation, Density Stress Resistance and Immunity Gene Expressions of Atlantic Salmon *Salmo salar*
2:45 PM **Cortes**: The Phagosome–Lysosome Fusion Is the Target of a Purified Quillaja saponin Extract (PAQ-Xtract) in Reducing Infection of Fish salmon Macrophages by the Bacterial Pathogen *Piscirickettsia salmonis*
3:00 PM Refreshments
3:15 PM **Aedo**: Early Regulation of Immune-Related Genes Mediated by Cortisol in Rainbow Trout (*Oncorhynchus mykiss*) Gills
3:30 PM **Petrie-Hanson**: Epigenetic Changes Associated with Increased Phagocyte Functions Demonstrate Trained Immunity in Catfish Leukocytes
3:45 PM **Saez**: Evaluation of Iron Metabolism-Related Genes Post-Vaccination of Atlantic Salmon.
4:00 PM **Casadei**: Antimicrobial Peptide Modulation in Rainbow Trout During Acute Stress

Thursday, September 8th
Breakout Room B (*Salón e 204*)

Myxozoa: Jerri Bartholomew/Matt Griffin
1:30 PM Roundtable Discussion

Thursday, September 8th
Breakout Room C (*Sala Colorada*)

Food Security Workshop: Fernando Mardones
9:30 AM **Bouchon/Mardones/Szécáks:** Introduction and Opening Remarks
10:00 AM **Román:** Soil Security for Food Security
10:30 AM Refreshments
10:45 AM **Marquet:** Macroecology, Global Change, and Complex System Science
11:15 PM **Barja:** Food Security and Nutrition
12:00 PM Lunch
1:00 PM **Villanueva:** Food Security: One Health Beyond Zoonoses
1:30 PM **Moreno**: Antimicrobial Resistance and One Health
2:00 PM **Varela:** Food Safety Towards Food Security
2:30 PM **Ferres: I**nfectious Diseases, Zoonosis and Food Security
3:00 PM Refreshments
3:15 PM **Fellenberg**: Promoting Food Security in Rural Farmers
3.45 PM **Mardones:** Roundtable Discussion

Monday, September 5th
Main Ballroom

General Session I: Matt Griffin/Sherri Kasper

10:15 AM **Johnsen**: The OIE Aquatic Animal Health Strategy (2021-2025)

10:30 AM **Algüerno**: Strengthening Veterinary and Aquatic Animal Health Services: The Contribution of the World Organization for Animal Health

10:45 AM **Piñeros-Duque**: Perception of Tilapia Farm Workers about Knowledge of Fish Diseases with a One Health Approach in Five Departments of Colombia during 2019

11:00 AM **Kohli**: Red Sore Disease of American Eels in Chesapeake Bay: Etiology and Epidemiology

11:15 AM **Kasper**: Harmful Algal Blooms Effects, Diagnosis and Mitigation in Aquaculture

11:30 AM **Kasper**: Increasing Threat of Harmful Algal Blooms Caused by Global Climate Change and Increased Migration

The OIE Aquatic Animal Health Strategy (2021-2025)

Stian Johnsen[1]

[1]Standards Department, World Organisation for Animal Health (WOAH) Headquarters

Aquatic animal production is growing rapidly and contributes significantly to human nutrition, poverty alleviation and sustainable development. It is essential to achieving many of the Unites Nations Sustainable Development Goals. Disease outbreaks are the greatest threat to aquatic animal production globally. Efforts to manage aquatic animal health and welfare worldwide have not kept pace with the rapid growth of aquatic animal production and the increased risk of disease. This threat is shared and requires collaborative actions by the OIE and its Members, in collaboration with relevant stakeholders, to protect and improve aquatic animal health globally. The OIE Aquatic Animal Health Strategy was launched at the 88th General Session in May 2021 and sets priorities for collaborative actions to protect aquatic animal health and welfare, and to fully realise the potential of aquatic animal production. The Strategy was developed by the organisation's Secretariat in cooperation with the Aquatic Animal Health Standards Commission. Member Countries and partners were asked to contribute to its content by providing their views through a survey on what OIE initiatives they consider the most valuably to them; the biggest opportunities to improve aquatic animal health and welfare the next 5-10 years; and what they consider to be the biggest threats to a sustainable growth in aquatic animal health productions.

The Strategy has three main outcomes:
Outcome 1. Competent Authorities have improved aquatic animal health management in place, supporting increased aquatic animal production and reduced disease risk.
Outcome 2. Regions are supported to collaborate on aquatic animal health issues of common concern, improving the overall health, productivity and resilience of the region.
Outcome 3. The OIE provides global leadership and in partnership with the OIE Community, builds a stronger and more resilient global aquatic animal health system.

This Strategy addresses **FOUR OBJECTIVES: STANDARDS, CAPACITY BUILDING, RESILIENCE and LEADERSHIP** and contains a total of 23 specific actives to improve aquatic animal health and welfare are national, regional and global level.

You are strongly encouraged to take active part in the implementation of **The Strategy**.

Strengthening Veterinary and Aquatic Animal Health Services: The Contribution of the World Organisation for Animal Health

Mario I. Algüerno[1], Valentyna Sharandak[1], Stian Johnsen[2], Jennifer Lasley[1], Maud Carron[3], Barbara Alessandrini,

[1]Capacity-Building Department, World Organisation for Animal Health (OIE); [2]Standards Department, World Organisation for Animal Health (OIE); [3]Canadian Food Inspection Agency (CFIA).

The OIE is a unique intergovernmental organisation that works alongside its partners towards a healthier and safer planet. It has built international consensus on the principles of good governance and the quality of Veterinary Services (VS) and Aquatic Animal Health Services (AAHS), as embodied by its international Standards. Based on these principles, the OIE has developed the Performance of Veterinary Services (PVS) Pathway, the OIE's flagship capacity building platform for the sustainable improvement of national VS and AAHS. The PVS Pathway empowers national animal health Services by providing them with a comprehensive understanding of their strengths and weaknesses using a globally consistent methodology – a useful external perspective that can reveal gaps, inefficiencies and opportunities for innovation. This enables countries to take ownership and prioritise improvements to their animal health system. The PVS Tool − Aquatic forms the fundamental methodological basis of the OIE's multi-stage PVS Pathway cycle of AAHS support. An OIE PVS Evaluation using the PVS Tool − Aquatic is a mechanism to ensure full coverage of the aquatic animal health domain, including all of the activities that are directly or indirectly related to aquatic animals, their products and by- products, such as feed production, farming, killing, processing, waste disposal, transport, import and export, which help to protect, maintain and improve the health and welfare of humans, by means of the protection of aquatic animal health and welfare, and food safety. A PVS Evaluation of the AAHS mission raises awareness among stakeholders and supports continuous improvement by assessing 47 Critical Competencies that comprise several cross-cutting or transversal elements that are the basic requirements for functioning AAHS. These include adequate human and financial resources, quality veterinary/aquatic animal health education, effective laboratory services, interaction with stakeholders and supporting legislation. The latest edition of the PVS Tool – Aquatic 2021 addresses current aquatic animal health issues relating to antimicrobial resistance and antimicrobial use, the One Health approach, bio-threat reduction, and welfare of farmed fish. It also expands the evaluation of biosecurity for aquaculture establishments, a critical factor in disease prevention, recently adopted in a new chapter of the OIE Aquatic Animal Health Code, as well as disease investigation and tracing, food safety in export and domestic markets, and public−private partnerships, all elements of major importance for the development of aquaculture, understanding that aquatic animal products are and will be even more crucial for human nutrition, livelihoods, food security and poverty alleviation for a better and more sustainable future.

Perception of Tilapia Farm Workers About Knowledge of Fish Diseases with a One Health Approach in Five Departments of Colombia During 2019

Ricardo Javier Piñeros Duque[1,2,3]; Jairo Palomares[3], Paola Barato[1,2,3]

[1]Doctorado en Agrociencias, Facultad de Ciencias Agropecuarias, Universidad de La Salle, Bogotá, Colombia; [2]Corporación Patología Veterinaria, CORPAVET, Bogotá, Colombia; [3]MolecularVet SAS, Neiva, Huila, Colombia.

Colombia is one of the top ten tilapia producers in the world. By 2021 the country had 36,464 aquaculture farms, generating around 57,756 direct jobs and 173,269 indirect jobs, with a production of 179,351 tons. To know the perception of tilapia farm workers about knowledge of fish diseases with a One Health approach, a survey was conducted on 212 workers from 53 farms in the departments of Huila (33), Meta (8), Córdoba (4), Valle del Cauca (7), and Atlántico (1). The information obtained was transcribed by 3 professionals in Google Forms to verify the quality of the information and analysis was performed in Excel for descriptive statistics. Most workers were men (85%) mainly between 26-45 years old (51.4%). Most of them had high school (37.3%), or elementary school (21.7%) degrees and had less than 3 years of experience in the sector (36.9%). Activities known and carried out by the workers to maintain the health of the fish included disinfection and management of equipment and ponds (73.9%), quarantine of the fish (47.1%), monitoring and management of the physicochemical quality of the water (38.6%), treatment with antibiotics (37.7%), antiparasitic treatment (37.7%), use of vitamins (30.2%), monitoring, and regular integral diagnosis (29.4%), use of prebiotics, probiotics and immunomodulators (11.2%) and vaccination (5.9%). They use antibiotics in all production stages, but the highest proportion was in fingerlings (53.2%), and pre-growth and growth (45%). It was asked if clinical evaluation of the fish was carried out daily with signalment, physicochemical and microbiological analysis of the water, necropsy, histopathology, PCR, bacteriology, and/or antibiograms, from 60% to 70% of workers did not answer the questions, and 8% to 24% never did any of these activities. Workers considered that the most important source of diseases is contaminated water (74.8%), followed by entry of unauthorized personnel to the farm (52.7%), poor management practices (49.0 %), entry of other animal species (44.9%), contaminated food (44.9%), native fish in the system (38.6%). They consider that the most important diseases were external mycosis (50.0%), external parasites (46.6%), TiLV (36.6%), internal parasites (32.7%) hypoxia (31.5%), streptococcosis (31.3%), among other. Other aspects related to the impact measure of disease, training or education in fish health and biosecurity, and knowledge about zoonotic diseases were also evaluated and will be presented.

Red Sore Disease of American Eels in Chesapeake Bay: Etiology and Epidemiology

Amanpreet K. Kohli[1], Wolfgang K. Vogelbein[1], Andrew R. Wargo[1]

[1]Aquatic Health Sciences, Virginia institute of Marine Science, William & Mary, Gloucester Point, Virginia 23062

American eel (*Anguilla rostrata*) is an ecologically and economically important finfish distributed along the Atlantic coast of the United States and throughout the Gulf of Mexico. American eel aquaculture in Chesapeake Bay region is based largely on temporarily holding wild-caught eels, for variable time periods, in primitive recirculating systems lacking effective water polishing capabilities. Such aquaculture operations experience significant disease-associated mortality (10-20%) from Red Sore Disease (RSD), an infectious disease characterized by severe skin lesions. RSD may also be contributing to the declining wild eel stocks. Extensive studies have been carried out for similar diseases in European and Japanese eels but research on pathogenic lesions of American eels is limited. The specific aims of our research were to: i) Identify the microbial agents associated with RSD in aquaculture and the wild eels using standard bacteriological and molecular identification methods (16S rRNA gene sequencing), ii) Quantify select epidemiological parameters (prevalence, infection intensity) and environmental correlates of RSD in the York river, Virginia by bi-weekly sampling, and iii) Quantify the impact of selected environmental stressors (temperature) on clinical severity of RSD using laboratory challenges by injecting clinically healthy fish with a field bacterial isolate and exposing fish to different temperatures. RSD was recorded from both aquaculture and wild eels, with a higher prevalence in the wild during the hotter months. We isolated and identified species of opportunistic bacteria *Vibrio* and *Aeromonas* from the external lesions and internal organs of diseased eels. *Vibrio* was more often associated with severe and systemic infections in eels than *Aeromonas*. In our laboratory challenges, clinical signs of RSD and mortality were observed in fish injected with *V. vulnificus* and mortality was increased at higher temperatures. Re-isolation and identification of bacteria from experimentally infected fish confirmed *V. vulnificus* as a causative agent of RSD in American eels. High temperatures were found to be both stressful to eels and favorable to *Vibrio* growth. These results indicate that bacterial infections due to *Vibrio* in eels may occur more frequently in warmer environments, which may have implications for climate change. Our next efforts are focused on further biochemical and genomic characterization of the *Vibrio* isolates associated with disease, histopathological examination of the affected tissues, and exploring other environmental drivers of the disease. These results will be instrumental in effective disease mitigation and management of eel losses, both in aquaculture and the wild.

Harmful Algal Blooms Effects, Diagnosis and Mitigation in Aquaculture

Sherri Kasper[1]

[1]Gulf Coast Aquatic Veterinary Services, Tallahassee, Fl 32311

Aquaculture is one of the largest growing areas of food production in the world. This growth can be affected by harmful algal blooms found in both fresh water and marine environments. Aquaculture can also influence the occurrence of HAB events if placement, population size, water quality are not mitigated properly. HAB events can cause high mortality in all aquatic animals due to decreased oxygen saturation, damage to gills and toxicity. Reporting of fish acclimation to toxins is based on low mortality when exposed to many of the toxins; however, newer research has shown that these toxins at low levels may inhibit growth, reproduction and larval development. The effects of the toxins can be minimized through early detection of the toxins and the type of bloom. This presentation will discuss testing and different forms of mitigation to decrease the hazards of HABS.

Increasing Threat of Harmful Algal Blooms Caused by Global Climate Change and Increased Migration

Sherri Kasper[1]

[1]Gulf Coast Aquatic Veterinary Services, Tallahassee, Fl 32311

Blooms of phytoplankton, diatoms and cyanobacteria have been increasing in frequency and size over the past several decades. Many of these blooms are harmful to the ecosystem and to populations of aquatic and terrestrial animals. There have been laboratory experiments looking at a large variety of species and their response to increased water temperatures. This research has shown that many species of these blooms will increase in growth, frequency and percentage of toxic species. Species of harmful algal blooms have been found at latitudes that have not been recorded in centuries due to increased shipping containers and migration in warmer waters. This presentation will look at the research performed that will help to predict future harmful algal blooms and discuss further research necessary to improve the mitigation and adaptation necessary to decrease high mortality events and chronic disease due to harmful algal blooms.

Monday, September 5th
Main Ballroom

Virology: Esteban Soto/Eva Quijano Cardé

1:00 PM **Soto**: Susceptibility of Lake Sturgeon (*A. fulvescens*) to Acipenserid Herpesvirus 2, White Sturgeon Iridovirus, and Ranaviruses

1:15 PM **Quijano Cardé**: Design and Validation of a qPCR Assay for Diagnosis of Acipenserid Herpesvirus 2 in White Sturgeon (*Acipenser transmontanus*) Tissues

1:30 PM **Soto**: Susceptibility of Acipenserid Herpesvirus 2, White Sturgeon Iridovirus, and Ranaviruses to Buffered Povidone-Iodine Complex, Chlorine and Virkon Aquatic®

1:45 PM **Getchell**: Viral Hemorrhagic Septicemia Endemic in St. Lawrence River Round Goby and Lake Ontario Gizzard Shad

2:00 PM **Patel**: Infectious Salmon Anemia Virus Directly Modulates the Red Cell Surface

2:15 PM **Zawisza**: CEV-Infection Induced Cortisol Release and Immunosuppression Are Associated with High Mortality of Susceptible Koi Carp

2:30 PM **Kebus:** Risk Assessment of Fish Movements from Great Lakes Region Fish Farms and Hatcheris to Natural Waters or Other Premises During a Viral Hemorrhagic Septicemia Outbreak

Susceptibility of Lake Sturgeon (*A. fulvescens*) to Acipenserid Herpesvirus 2, White Sturgeon Iridovirus, and Ranaviruses

Esteban Soto[1], Zeinab Yazdi[1]; Eva M. Quijano Cardé[1]; Kelsey Anenson[1]; Susan Yun[1]; Eileen Henderson[1,2]; Amber Johnston[3], Kim Scribner[4], Thomas Loch[3]

[1]University of California-Davis; [2]California Animal Health and Food Safety Laboratory; [3]Aquatic Animal Disease Ecology Program, Michigan State University – Aquatic Animal Health Laboratory, [4]Molecular Ecology Laboratory, Michigan State University

Lake sturgeon (LA), *Acipenser fulvescens*), is an ancient and iconic fish of the Great Lakes. Regrettably, they are listed as a threatened species in multiple states and provinces in the USA and Canada due to dramatic declines in both numerical abundance and distribution range. White sturgeon (WS), (*Acipenser transmontanus*), is another native North American sturgeon species, cultured both for conservation programs and for production of high-quality caviar and sturgeon meat. Infectious diseases pose a major threat to aquaculture production, causing millions of dollars in annual losses. Acipenserid herpesvirus 2 (AciHV-2), White sturgeon iridovirus (WSIV), and Ranaviruses like Frog virus 3 (FV3) and Rana catesbeiana virus (RCV) have been associated with outbreaks of diseases in cultured sturgeon in North America. Although some laboratory-controlled challenges have been conducted using the WS as a model of infection, the susceptibility of LS to these viruses is unknown. In this study, LS and WS fingerlings were immersion-exposed to AciHV2, WSIV, FV3 and RCV to gain a better understanding of virus susceptibility and the pathogenesis of these infections. Following a 1h static exposure to the viruses, fish were monitored for 30d for clinical signs of diseases and mortality. Mortalities and clinically affected animal were subjected to histological and microbiological examination, including quantification of viral DNA using quantitative PCR and virus isolation using different fish cell lines. Thirty days post-challenge, a subset of survivors from each treatment group was collected and similarly evaluated. The rest of the animals were subjected to a netting stressor event, and returned to their respective systems for further monitoring. Mortalities and clinically affected animal were analyzed as previously described. Two weeks post netting stressor event, all surviving fish were collected and evaluated as previously described. Different susceptibility to the viruses was observed. White sturgeon fingerlings presented significantly lower survival when exposed to AciHV-2 and WSIV compared to LS fingerlings; in contrast, LS presented lower survival to FV3 and RCV compared to exposed WS ($p<0.05$). White sturgeon fingerlings exposed to AciHV-2 and WSIV presented significantly lower survival when compared to the control treatment group ($p<0.05$). Although greater mortality was detected in WS treatments exposed to FV3 and RCR, it wasn't significantly different to those observed in negative controls. On the other hand, LS fingerlings exposed to FV3 and RCV presented significantly lower survival compared to the control treatment group ($p<0.05$). Similar survival was observed in AciHV-2 and WSIV exposed LS and non-exposed controls. Viral DNA detection also differed between exposed LS and WS. Whereas AciHV-2 and WSIV DNA was detected in dead, moribund, and surviving WS; no AciHV-2 nor WSIV DNA was detected in any of the exposed lake sturgeon. On the other hand, FV3 DNA was detected in dead, moribund, and surviving WS and LS; whereas RCV was only detected in dead and moribund exposed WS and LS. In WSIV and AciHV2 infected sturgeon histologic findings were limited to WS. While both FV3 infected WS and LS exhibited necrosis of a number of tissues (predominantly hematopoietic tissues), these lesions were noted in a larger number of examined LS than WS. Altogether, the results suggest a marked difference in susceptibility to these viruses by WS and LS fingerlings. At the conditions tested, WS appear highly susceptible to WSIV and AciHV-2, moderately susceptible to FV3, and mildly susceptible to RCV; whereas LS appear highly susceptible to FV3 and RCV, and not susceptible to WSIV and AciHV-2.

Design and Validation of a qPCR Assay for Diagnosis of Acipenserid Herpesvirus 2 in White Sturgeon (*Acipenser transmontanus)* Tissues

Eva M. Quijano Cardé[1]; Kelsey M. Anenson[1]; Eric Littman[1]; Susan Yun[1]; Geoffrey Waldbieser[4]; Mark D. Fast[2]; Matt Griffin[3]; C. Titus Brown[1]; Esteban Soto[1]

[1]University of California-Davis; [2]University of Prince Edward Island; [3]Mississippi State University; [4]United States Department of Agriculture – Agricultural Research Service

White sturgeon (*Acipenser transmontanus*) is the primary species used for high-quality caviar and sturgeon meat production in the United States. These aquaculture practices provide substantial revenues to producers, generation of local employment opportunities, and wild population conservation by offering a sustainable alternative to wild caught sturgeon. Infectious diseases pose a major threat to aquaculture production, causing millions of dollars in annual losses. An important pathogen of white sturgeon is Acipenserid herpesvirus 2 (AciHV-2), which causes up to 10% mortality in adult and 80% mortality in juvenile white sturgeon. A current limiting factor in the research efforts is that a complete genome is not currently available for the white sturgeon or for AciHV-2. In addition, only a few methods are available for the diagnosis of AciHV-2 in white sturgeon, including viral culture and non-specific conventional Polymerase Chain Reaction (PCR). Unfortunately, the currently available degenerate primers cross-react with white sturgeon tissues. Thus, the purpose of this study was to assemble the complete genome of AciHV-2 and design and validate a quantitative PCR (qPCR) assay for the diagnosis of AciHV-2 in white sturgeon tissues. Four isolates from previous natural outbreaks in California throughout the past 30 years (UCD3-30, OCR, SR-WSHV, and R20-11) were sequenced via Illumina and Nanopore technologies. After filtering out contaminants, the obtained annotated assemblies of approximately 134 Kb cluster in a similarity matrix with the published partial AciHV-2 sequence and separate from other herpesviruses. Candidate open reading frames (ORFs) for qPCR were selected based on being conserved among all four isolates of AciHV-2 and sharing very little homology with AciHV-1. Blast of the top candidate ORF suggest this may be the terminase small subunit open reading frame. Primers produced a single band after conventional PCR for all four isolates near the expected 105 bp length. Analytical specificity *in silico* of the primers using NCBI Primer Blast revealed no significant off-target match. A standard curve was generated from 4 biological replicates and 3 technical replicates. Efficiency was 96% with an R^2 of 0.9885. Spiking with 250 ng of white sturgeon fin DNA revealed a decrease in efficiency to 88% but no significant difference in the Ct values obtained per dilution. Internal Positive Control (IPC) analysis revealed no significant inhibition by white sturgeon fin DNA. The limit of detection for this assay was 10^4 copies/reaction with a protocol of 35 cycles. Analytical specificity assessment revealed no cross-reaction with other known viruses that affect white sturgeon and other closely related sturgeon species. Diagnostic sensitivity was assessed from fin clip samples obtained from mortalities of an immersion challenge with 10^2 $TCID_{50}$/mL of AciHV-2 UCD3-30 passage number 8. Diagnostic specificity was assessed from the negative control group of the same immersion challenge. Viral culture was used as the gold standard diagnostic test for comparison. Both the True Negative and True Positive Rates were 100%, giving this assay a 100% relative accuracy when compared to viral culture during an active outbreak. Survivors were tested by qPCR, but only 6% (3 out of 49 fin clips collected 82 days post infection) tested positive via this assay. This study demonstrated that the developed qPCR assay is highly sensitive and specific for the identification and quantification of AciHV-2 from white sturgeon fin samples during an active outbreak.

Susceptibility of Acipenserid Herpesvirus 2, White Sturgeon Iridovirus, and Ranaviruses to Buffered Povidone-Iodine Complex, Chlorine and Virkon Aquatic®

Esteban Soto[1]; Susan Yun[1]; Thomas Loch[2]

[1]Department of Medicine & Epidemiology, School of Veterinary Medicine, University of California, Davis, CA, United States; [2]Department of Fisheries and Wildlife, College of Agriculture and Natural Resources, Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University, East Lansing, MI, United States

Acipenserid herpesvirus 2 (AciHV-2), White sturgeon iridovirus (WSIV), and the ranaviruses Frog virus 3 (FV3) and Rana catesbeiana virus (RCV) are important viral pathogens of the sturgeon aquaculture industry. Mortality in affected farms can reach 80-90% in severe outbreaks and effective vaccines and chemotherapeutic treatments are currently unavailable. The aim of this study was to identify the virucidal efficacy of three commonly used disinfectants, namely household bleach (free-chlorine), a Buffered Povidone-Iodine (PVP Iodine) complex, and Virkon Aquatic® (Virkon), against these important pathogens. Archived virus isolates were cultured on white sturgeon (*Acipenser transmontanus*) skin (WSSK1; AciHV-2, Rana catesbeiana), white sturgeon spleen (WSS-2; WSIV), or epithelioma papulosum cyprini (EPC; FV3) cell lines. Disinfectants were diluted on the day of the assay in filtered well water collected from an aquaculture facility in California, USA and added to viral cultures for 0, 10, 30 and 60min. At each respective timepoint, sodium thiosulphate or fetal bovine serum were added to inactivate the available iodine and free chlorine or Virkon, respectively. All aliquots were then titrated on respective cell lines to determine the $TCID_{50}$/ml, revealing differential susceptibility to disinfectants among the viruses. Virucidal reductions in titer >4 $\log_{10}$ $TCID_{50}$/ml for AciHV-2 were obtained with 25 ppm free chlorine after 10min, but immediately (i.e., 0 min) with 100 ppm. Similarly, virucidal reductions in titer >2 $\log_{10}$ $TCID_{50}$/ml for WSIV were obtained with 50ppm free chlorine after 30min and immediately following 100 ppm free chlorine. On the contrary, greater resistance to chlorine was appreciated for the two ranaviruses. Virucidal reductions in titer >4 $\log_{10}$ $TCID_{50}$/ml for RCV were obtained with 500 ppm free chlorine, but FV3 persisted even after incubation with 500 ppm free chlorine for 1h. Buffered Povidone-Iodine inactivated AciHV-2 after 10 min incubation in 50ppm and immediately at 100ppm. Similarly, WSIV was immediately inactivated after incubation in 50ppm PVP Iodine. On the other hand, although RCV was inactivated after a 10 min incubation in 50 ppm PVP Iodine; some FV3 isolates still persisted in75ppm PVP Iodine after 60 min. Among the disinfectants tested, Virkon was the most consistent and effective, inactivating AciHV-2, WSIV and the two ranaviruses immediately at the recommended concentration of 0.5%, and even lower doses significantly decreased viral load in <10min. This information should be taken into consideration when developing biosecurity protocols in captive sturgeon programs to prevent the spread of these important viruses.

Viral Hemorrhagic Septicemia Endemic in St. Lawrence River Round Goby and Lake Ontario Gizzard Shad

Anna Conklin[1], Rodman G Getchell[2], Loredana Locatelli[2], Web Pearsall[3], Andrew D Noyes[4], and John M. Farrell[1]

[1]Thousand Islands Biological Station, SUNY College of Environmental Science and Forestry, Syracuse, NY 13210 USA; [2]Aquatic Animal Health Program, Department of Microbiology and Immunology, College of Veterinary Medicine, 930 Campus Road, Cornell University, Ithaca, NY, 14853 USA; [3]Region 8 Division of Fish and Wildlife, New York State Department of Environmental Conservation, 6274 E. Avon-Lima Rd, Avon, NY 14414 USA; [4]Rome Field Station, New York State Department of Environmental Conservation, 8314 Fish Hatchery Road, Rome, NY 13440 USA

Viral Hemorrhagic Septicemia Virus (VHSV) is a World Organization for Animal Health (OIE) reportable fish pathogen found across the northern hemisphere. A novel lineage, VHSV-IVb, was detected in the Great Lakes basin following sizeable fish kills in the mid-2000's. Through IVb's history, researchers have documented more than 30 species vulnerable to infection, high rates of genetic mutation, and expanding geographic range in New York (e.g., Finger Lakes). Sensitivity to infection is extremely variable between fishes, and factors that influence VHSV persistence are not well understood. The emergence of VHSV coincides with dramatic declines of spotted muskellunge (*Esox masquinongy masquinongy*), an apex predatory fish in the St. Lawrence River (SLR). One threat to their recovery is invasion of nursery bays by large abundances of round gobies (*Neogobius melanostomus*). These invasive fish harbor VHSV, and are regarded as essential for the amplification, spread, and evolution of the virus. VHSV surveillance of round gobies from 2010 to 2022 demonstrates their endemic status in the SLR. Further investigation into reservoir hosts that maintain the pathogen is underway to assess the continued risk associated with VHSV-IVb in the Great Lakes watershed. New York fishery biologists also continue to monitor outbreaks of VHSV involving thousands of dying gizzard shad (*Dorosoma cepedianum*) occurring in Lake Ontario embayments such as Irondequoit Bay (2013, 2018, 2022). From the outset of the first VHS outbreaks in the Great Lakes in the mid-2000's, we have investigated many aspects of this pathogen's epidemiology, including host susceptibility, modes of viral transmission, anatomical pathology, and viral evolution. We will discuss the VHSV persistence and continued ecological impacts in the St. Lawrence River basin by measuring susceptibility and abundance of different hosts, and directly compare how several potential reservoir species compare in their viral characteristics (i.e., prevalence, viral load, viral genetic sequence).

Infectious Salmon Anemia Virus Directly Modulates the Red Cell Surface

Johanna H. Fosse[1], Adriana M. S. Andresen[1], Knut Falk[1], Raoul V. Kuiper[1], Inger A. Heffernan[1], Anita Solhaug[1], Sonal Patel[1], Simon Weli[1]

[1]Norwegian Veterinary Institute, 1433 Ås, Norway

Infectious salmon anemia virus (ISAV) is an important pathogen of farmed Atlantic salmon that has caused national epidemics with severe economic consequences in most salmon-producing countries. Pathogenic ISAV replicates in vascular endothelial cells before it buds from the luminal cell surface into the blood stream and attaches to red blood cells. Consumptive anemia is a characteristic sign of disease in ISAV-infected fish, but the mechanisms that trigger the premature removal of red blood cells from the circulation have not yet been characterized. Interestingly, ISAV attaches to cell surface 4-*O*-acetylated sialic acids, and the surface level of sialic acids contributes to determine the circulating life span of red blood cells. We characterized the viremia in ISAV-infected fish and showed that ISAV extensively coats the surface of circulating red blood cells. The permissiveness of red blood cells to productive infection is minimal, and significant viral RNA production is limited to endothelial cells of solid organs. We further reveal that the red blood cell surface is modulated in infected fish. Red blood cells isolated from ISAV-infected fish have an increased affinity for wheat germ agglutinin, a lectin that attaches to sialic acids and N-acetylglucosamine. This modulation of the red cell surface co-incides with increased erythrophagocytosis in spleen and head kidney and progressive anemia. Enhanced binding of wheat germ agglutinin and another sialic acid- recognizing lectin, the sambucus nigra lectin, was also observed when red blood cells isolated from healthy Atlantic salmon were exposed to ISAV in the laboratory. The modulation of lectin- binding was dose-dependent and rapid, occurring within 60 minutes of ISAV-exposure. The change in lectin-binding could be prevented by blocking cellular ISAV attachment by two distinct monoclonal antibodies targeting the ISAV haemagglutinin esterase. In conclusion, we show that ISAV extensively binds the red blood cell surface in infected fish, that this binding directly modulates the availability of sialic acids on the cellular surface, and that it takes place in the same time period when red blood cells are removed from the circulation. Together, our findings suggest a mechanism that could trigger the removal of red blood cells in infectious salmon anaemia.

CEV-Infection Induced Cortisol Release and Immunosuppression are Associated with High Mortality of Susceptible Koi Carp

Maria Zawisza[1], Alexander Rebl[2], Veronica Piackova[3], David Gela[3], Martin Kocour[3], Magdalena Chadzinska[1], Krzysztof Rakus[1], and Mikołaj Adamek[4]

[1]Department of Evolutionary Immunology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland; [2]Genetics Unit, Research Institute for Farm Animal Biology (FBN), Institute of Genome Biology, Dummerstorf, Germany, [3]South Bohemian Research Centre of Aquaculture and Biodiversity of Hydrocenoses, Faculty of Fisheries and Protection of Waters, University of South Bohemia in Ceske Budejovice, Vodnany, Czech Republic [4]Fish Disease Research Unit, Institute for Parasitology, University of Veterinary Medicine, Buenteweg 17, 30559 Hannover, Germany

Gill diseases seriously affect fish health and have a very negative impact on aquaculture, mainly because of the multifunctional properties of gills in fish physiology. Carp edema virus (CEV) is a large DNA poxvirus, which predominantly infects the gills of common carp (*Cyprinus carpio* L.), and causes a highly contagious and fatal disease known as koi sleepy disease (KSD). Our previous studies demonstrated that during experimental infections with CEV genogroup IIa different strains of common carp show high (Amur wild carp - AS) or low (Koi carp) resistance to this virus. The increased susceptibility of koi results with sever impairment of gill function. In the present work we studied blood parameters, viral load and expression of selected immune-related genes in the gills in both carp strains. Experiments were performed in two temperatures: 12 and 18 °C. Moreover, in case of Koi carp we implemented a salt rescue model based on the supplementation of 0.5% NaCl into the tank water, which prevents mortality of the fish. The nano-scale qPCR analysis of the panel of 40 genes showed that in the gills CEV induced clear antiviral response in all infected groups of fish compared to non-infected controls. Moreover, we found that in all studied groups of fish, viral load was higher at 18 °C than at 12 °C and that at both temperatures, the highest viral load was demonstrated in Koi carp as compared to Koi salt rescue group and AS carp. Interestingly, at both temperatures CEV-infected Koi carp had high glucose and cortisol level and low level of sodium in the blood plasma than Koi salt rescue group and AS carp. This clearly indicate that CEV infection in susceptible strain is correlated with high stress parameters what can induce immunosuppression. And indeed, we observed that in Koi carp the severity of the disease and higher stress response was associated with immunosuppression manifested by downregulation of T-cell responses connected with down-regulation of *cd4* and *tcra2* expression. Our data suggest that there is tangible link between CEV-induced gill disorders, stress and immunosuppression in susceptible Koi carp.

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Risk Assessment of Fish Movements From USA Great Lakes Region Fish Farms and Hatcheries to Natural Waters or Other Premises During a Viral Hemorrhagic Septicemia Outbreak

Myron J. Kebus[1], Catherine Y. Alexander[1], Rosemary A. Marusak[1], Kaitlyn M. St. Charles[1], Marie R. Culhane[1], Carol J. Cardona[1]

[1]Secure Food Systems Team, Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, 55108

When a foreign animal disease (FAD) strikes, animal health regulatory officials (i.e., regulators) and producers face the often-conflicting goals of preventing disease spread, safely maintaining business continuity, and public water restocking capabilities. Previous FAD responses - including the outbreak response to viral hemorrhagic septicemia (VHS) in Great Lakes (GL) fish in 2006 in the United States - have had serious unintended economic consequences to uninfected premises, due to a lack of available, consistent, science-based guidance to inform animal movement decisions for VHS in finfish. To address this need, a newly funded project has been launched. Still in its initial phases, the project activities thus far have been to convene a workgroup of federal, state, and tribal nation fish health regulators, fish farmers, hatchery producers, and other subject matter experts to develop science-based assessments of the risk of moving susceptible fish species from apparently uninfected premises to natural waters or other premises during a VHS outbreak in the GL region. The risk assessments (RAs) will be translated into movement guidances, which provide regulators with a consistent risk-based framework to guide fish movement decisions in a VHS outbreak, and direct-action guides for fish producers, which facilitate the implementation of disease mitigation measures such as biosecurity on their premises. These materials will be widely distributed through a dedicated website, professional, trade, and academic conferences, and professional and lay publications. The workgroup will be the foundation for consensus among differing agencies, and the science-based RAs, movement guidances, and direct-action guides will facilitate the movement of fish in commerce during a large-scale fish disease outbreak, namely VHS in the GL, while concurrently controlling disease spread.

Monday, September 5th
Main Ballroom

Microbiomes: Matt Griffin/Divya Rose

3:15 PM **Jimenez-Lopez**: Microbial Communities of Salmonids, a Meta-Analysis

3:30 PM **Coca Rives**: Characterization of the Intestinal Microbiota of *Salmo salar* Smolt During an Infectious Outbreak of *Aeromonas salmonicida* subsp. *salmonicida*

3:45 PM **Kent**: Intestinal Lesions and Microorganisms Associated with Senescence and Prespawn Mortality in Chinook Salmon *Onchorynchus tshawtscha*

4:00 PM **Coca Rives**: Intestinal Microbiota Characterization in *Salmo salar* with Clinical Signs of *Piscirickettsia salmonis* in Chilean Salmon Farming

Microbial Communities of Salmonids, a Meta-Analysis

Omar A. Jimenez-Lopez[1], Nicholas Jacob[2], Tara Gaire[1], Alex E. Primus[3], Noelle Noyes[1]

[1]Veterinary Population Medicine Department, College of Veterinary Medicine, University of Minnesota; [2]College of Biological Sciences, University of Minnesota; [3]HUBBS SeaWorld Research Institute.

The microbiome plays a critical role in health and disease across farmed species, but microbiomes associated with farmed salmonids are not well understood. Further, salmon farms employ diverse management practices, and it is unknown whether this heterogeneity impacts the fish microbiome. The aim of this study was to describe the mucosal microbiomes of Salmonidae species based on relevant existing datasets. A literature search was conducted through MEDLINE and Scopus databases (2010 to January 2021). Studies were restricted to English and screened in two stages: abstract screening and full- text screening. Studies were included in the analysis if they met these criteria: were peer reviewed, presented data on salmonid species, performed high-throughput 16S rRNA gene sequencing, and made the sequence data publicly available. Additional studies were screened from NCBI's Short Read Archive (SRA). A meta-analysis will be performed using the publicly available datasets from studies that met the inclusion criteria. A total of 523 studies were screened, 112 were eligible for full-text screening, and 52 were included in the meta-analysis (MEDLINE and Scopus: 40; SRA: 12). Of those, 83% used Illumina as their sequencing platform, 36% targeted the V3-V4 region, and 25% the V4 region. No studies were included from 2010-2014, while 12 studies were included from 2020. The most represented species were the Atlantic salmon (*Salmo salar*) (53%) and the Rainbow trout (*Oncorhynchus mykiss*) (34%). Sample type was dominated by gut content (65%), while others such as skin and gill were scarce (5 and 4% respectively). Analysis of the hypervariable regions and factors explaining different microbiome patterns is ongoing. Microbiome studies of farmed Salmonidae species have increased recently, with a focus on gut samples. Further research should characterize the microbiome of other Salmonidae species and other sample types like gill or skin, as these mucosal surfaces are critical against pathogens. In addition, there should be an effort to include the relevant information to each study design and sequence data to repositories such as the SRA.

Characterization of the Intestinal Microbiota of Salmo salar Smolt During an Infectious Outbreak of Aeromonas salmonicida subsp. salmonicida

Yoandy Coca Rives[1,2,3], Marcos Godoy[3], Diego Caro Troncoso[3], César A. Sáez-Navarrete[2], Juan P. Pontigo[4], Vinicius Maracaja[5], Raúl Arias[5], Karina Kusch[3], Leonardo A. Córdova Rodríguez[2]

[1]Doctorado en Ciencias de la Ingeniería. Área Ingeniería Química y Bioprocesos. Escuela de Ingeniería, Pontificia Universidad Católica de Chile, Avenida Vicuña Mackenna 4860, Macul, Santiago de Chile, 7820436, Chile; [2]Laboratorio de Energía Renovables y Residuos (LERR-UC), Escuela de Ingeniería Pontificia Universidad Católica de Chile, Avenida Vicuña Mackenna 4860, Macul, Santiago de Chile, 7820436, Chile; [3]Centro de Investigaciones Biológicas Aplicadas (CIBA), Lago Panguipulli 1390, Puerto Montt, Región de Los Lagos; [4]Laboratorio de Biotecnología Aplicada, Facultad de Medicina Veterinaria, Universidad San Sebastián, Sede Patagonia, Lago Panguipulli 1390, Puerto Montt, Chile; [5]Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago de Chile; [6]Escuela de Ingeniería Civil Industrial, Facultad de Ingeniería, Universidad Santo Tomás, Chile

Aeromonas salmonicida subsp. *salmonicida* is the infectious agent responsible for the disease known as Furunculosis. It is estimated that it can reach 89% to 90% during the initial stages of salmon farming. Although this disease has low mortality rates in other stages, because *A. salmonicida* is found in a wide range of hosts in both wild and farmed fish of all ages, and its infections occur in freshwater environments, brackish and marine, constitute a latent risk. Atypical Furunculosis is a bacterial infection caused by atypical *Aeromonas salmonicida*, affecting Atlantic Salmon (*Salmo salar*) in Chile during the culture phase in freshwater and seawater. The first record of the disease in Chile was described in 1995 (Bravo, 2000). Subsequently, the infection spread mainly in estuarine and freshwater systems. As of 2002, the antigen is incorporated in the vaccination of Atlantic Salmon (*S. salar*), which allowed efficient infection control, restricting its geographical distribution mainly to freshwater centers. Through this research, our team analyzes the behavior of the intestinal microbiome of Atlantic salmon smolt (*Salmo salar*) during an infectious outbreak of Furunculosis. The sampled fish were under a clinical picture of severe Furunculosis. Twenty salmon with clinical signs were selected, showing erratic and superficial swimming, exophthalmos, fin erosion, and skin hemorrhages. Necropsy reveals hemorrhagic symptoms (perirenal, pyloric caeca, and swim bladder) associated with pale internal organs and swollen spleen and liver. Samples of the intestinal mucosa and digesta were taken from the affected fish. As a control group for this analysis, fish were taken from the same farm that did not show clinical signs of infection. The study of the gastrointestinal material was sequenced using Illumina technology, analyzing the V3-V4 region of the 16S rRNA gene. The sequencing data of the diseased fish is being analyzed bioinformatically. The expected results of this research are to identify how the intestinal microbiota is affected in *Salmo salar* smolt infected with the pathogen *A. salmonicida.*

Intestinal Lesions and Microorganisms Associated with Senescence and Prespawn Mortality in Chinook Salmon *Oncorhynchus tshawtscha*

Michael L. Kent[1,2], Stephane Nervino[1], Julie D. Alexander[1], Tamsen Polley[1], Carl B. Schreck[3], James T. Peterson[3]; Claire E. Couch[3]

[1]Department of Microbiology, Oregon State University, Corvallis, OR 97331; [2]Department of Biomedical Sciences, Oregon State University, Corvallis, OR 97331; [3]Department of Fisheries, Wildlife and Conservation, Oregon State University, Corvallis, OR 97331

Pre-spawning mortality (PSM) is a major problem for population recovery of spring Chinook Salmon (*Oncorhynchus tshawytscha*) in the Willamette River basin, Oregon. In certain reaches and years, PSM can exceed 90%. We have been conducting pathologic investigations on these fish since 2009 to elucidate the underlying causes. Adult Pacific salmon are semelparous and are severely immune compromised after they return to freshwater, spawn, and then die shortly thereafter. High burdens of some well-recognized salmon parasites have been documented prior to and after spawning, and some have been associated with PSM. We have been using histology as primary diagnostic method to investigate PSM in the Chinook Salmon for the last decade, and we have examined a variety of life stages of the adult fish; midsummer healthy and PSM fish, as well as post-spawned fish in September. One consistent change that we have seen is degeneration of the intestinal epithelium with concurrent severe inflammation in the lamina propria of the intestine and pyloric caeca. These lesions progress through the summer; 1) intestines are essentially normal, usually with mild inflammation, in most fish shortly after they return to the river in late spring, then, 2) varying degrees of lesion severity are observed in in midsummer in fish that appear to be clinically normal fish and 3) almost all PSM fish collected in summer and many of the post-spawned fish from both the wild and from a hatchery in September show profound manifestations of the lesions. When accounting for Julian date, a lower proportion of the epithelium remaining was strongly associated with being a PSM fish ($F=59.26$, $p<0.0001$; estimate -4.853 SE=0.499). Two common intestinal pathogens; *Ceratonova shasta* (Myxozoa) and a new microsporidium *Enterocytozoon schreckii* were not statistically correlated with PSM, but both were positively associated with increase in Julian date. We are investigating microbiome profiles of these adult salmon as well as juveniles that were immunosuppressed via slow-release corticosteroid implants (cortisol or dexamethasone). 16S rDNA amplicon sequencing of gut swabs from both adults and experimental juveniles had microbiome compositions correlated with intestinal integrity in the former and morbidity and gill disease in the latter. We analyzed similarities between whole gut communities (PERMANOVA) and abundances of individual genera (generalized linear mixed models). In both juveniles and adults, affected fish had different microbiome profiles than their healthier counterparts. Hence, immunosuppression resulted in significant restructuring of the gut microbial community. Among immunosuppressed juveniles, morbidity correlated with overall gut microbiome community composition, as well as abundance of several specific microbial biomarkers. Adult fish with severe intestinal degeneration had different microbiome communities than those with mild intestinal degradation, and all adult fish had microbiomes that were more similar to immunosuppressed juveniles than healthy juveniles. Ultimately, we plan to investigate the possibility of using a microbiome signature or perhaps a host factor as a non-lethal predictive test for PSM.

Intestinal Microbiota Characterization in *Salmo salar* with Clinical Signs of *Piscirickettsia salmonis* in Chilean Salmon Farming

Yoandy Coca Rives[1,2,3], César A. Sáez-Navarrete[2], Marcos Godoy[3], Juan P. Pontigo[4], Diego Caro Troncoso[3], Vinicius Maracaja[5], Raúl Arias[5], Karina Kusch[3], Leonardo Rodríguez-Córdova[6]

[1]Doctorado en Ciencias de la Ingeniería. Área Ingeniería Química y Bioprocesos. Escuela de Ingeniería, Pontificia Universidad Católica de Chile, Avenida Vicuña Mackenna 4860, Macul, Santiago de Chile, 7820436, Chile; [2]Laboratorio de Energía Renovables y Residuos (LERR-UC), Escuela de Ingeniería Pontificia Universidad Católica de Chile, Avenida Vicuña Mackenna 4860, Macul, Santiago de Chile, 7820436, Chile; [3]Centro de Investigaciones Biológicas Aplicadas (CIBA). Lago Panguipulli 1390, Puerto Montt, Región de Los Lagos; [4]Laboratorio de Biotecnología Aplicada, Facultad de Medicina Veterinaria, Universidad San Sebastián, Sede Patagonia, Lago Panguipulli 1390, Puerto Montt, Chile; [5]Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago de Chile; [6]Escuela de Ingeniería Civil Industrial, Facultad de Ingeniería, Universidad Santo Tomás, Chile

In Chile, SERNAPESCA (the National Fisheries Service) has identified *Piscirickettsia salmonis* (SRS; Salmonid Rickettsial Septicemia infection) as the most severe health problem facing the Chilean salmon industry owing to its highly aggressive nature, recurrent outbreaks, and widespread transmission, among other cultivated salmonid species. The total mortality of salmon due to specific diseases in Chile is associated with SRS and is in the order of 50% to 97%, which translates into direct and indirect annual losses of close to 700 million dollars. The changes caused by the pathogen *Piscirickettsia salmonis* in the gastrointestinal microbiota in *Salmo salar* during infectious outbreaks due to this pathogen in Chile are unknown and not widely researched. Therefore, knowing which microbial species in the gut are affected or favored by the pathogen *Piscirickettsia salmonis* is the main objective of this research. Microbiome analysis could be a valuable tool in the Chilean salmon industry to establish future diagnoses and take prophylactic measures. Our team presents in the study one of the few characterizations of the intestinal microbiome of *Salmo salar* cultured in Chile during an infectious outbreak of the pathogen *Piscirickettsia salmonis* based on high-throughput sequencing of the 16S rRNA gene's V3-V4 region. Twenty-three infected *Salmo salar* were sampled in four salmon farms (six salmon per farm) in southern Chilean Patagonia. Intestine microbiota and associated digesta microbiota were analyzed in each salmon. The control of this investigation was 12 *Salmo salar* without clinical signs of infection. The research found that the control groups in the study (*Salmo salar* without clinical signs of *P. salmonis* infection) have concentrations of this pathogen in their gastrointestinal system. The study also found a high concentration of Proteobacteria in the gut of all *Salmo salar* sampled. Although the main phylogenetic groups found in Actinobacteria, Firmicutes, and Proteobacteria coincide with those reported by other researchers for this same species, we can say that the salmon sampled could be suffering from a state of dysbiosis.

Monday, September 5th
Breakout Room A

Flavobacterium: Tom Loch/Taylor Heckman

10:15 AM **Loch**: Enhancing Bacterial Coldwater Disease Diagnosis and Prevention by Elucidating the Predominant *Flavobacterium psychrophilum* Serovariants in the USA

10:30 AM **Heckman**: Flavors of Flavobacteriales: Characterizing Atypical Flavobacterial Pathogens in Aquaculture

10:45 AM **Valdés**: Phenotypic, Serological and Genetic Characterization of *Tenacibaculum* Strains Isolated from Chilean Salmon Farms

11:00 AM **Ilardi**: Intraspecific Diversity of *Flavobacterium psychrophilum* Isolated from Salmonids Farms in Chile

11:15 AM **Poblete**: Expert Elicitation to Identify Risk Factors for Tenacibaculosis Outbreaks in Farmed Atlantic Salmon in Chile

11:30 AM **Ilardi**: Flavobacteria Isolated from BCWD Outbreaks in Chilean Salmon Farms

Enhancing Bacterial Coldwater Disease Diagnosis and Prevention by Elucidating the Predominant *Flavobacterium psychrophilum* Serovariants in the USA

Sean M. G. Lennox[1,2], Megan A. Shavalier[1,2], Travis O. Brenden[2], Christopher K. Knupp[1,2], Douglas R. Call[3], Esteban Soto[4], Thomas P. Loch[1,2,5]

[1]Michigan State University - Aquatic Animal Health Laboratory, Aquatic Animal Disease Ecology Program; [2]Department of Fisheries and Wildlife, Michigan State University; [3]Department of Veterinary Microbiology, Washington State University; [4]Department of Medicine and Epidemiology, University of California, Davis; [5]Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University

Flavobacterium psychrophilum (*Fp*), the causative agent of bacterial coldwater disease (BCWD), generates substantial losses in trout and salmon (Family Salmonidae) aquaculture facilities and hatcheries in the USA and abroad, thereby negatively affecting food security and fishery conservation efforts. The recently uncovered and substantial diversity of BCWD-causing *Fp* strains in the USA may be an important factor behind the elusiveness of effective BCWD prevention and control strategies to date. Unfortunately, little is known about the serotypic diversity of *Fp* in the USA. To address this knowledge gap and subsequently enhance vaccine development and BCWD diagnosis, 324 *Fp* isolates, recovered from infected fishes across the USA over a span of four decades, were analyzed using a multiplex polymerase chain reaction-based serotyping scheme. Based upon this data set, serotype 0 was the most common of the five currently described molecular serotypes, followed by serotypes 1, 2, 4, and 3, respectively. Analyses further revealed apparent associations of some *Fp* serotypes with certain host species or genera (some of which have been reported in other regions of the world) and also identified several intriguing geographical and temporal trends. These new data, in conjunction with *Fp* multilocus sequence typing data, were subsequently used to guide the development of a new loop-mediated isothermal amplification (LAMP) assay that has proven capable of rapidly detecting all US *Fp* variants tested thus far, and, after further optimization, will enhance BCWD diagnosis in laboratory and field (i.e., "pondside") settings. This large-scale analysis of *Fp* serological diversity within the USA has highlighted important nationwide epidemiological trends that are critical not only for ongoing BCWD vaccine development, but also for improving on-site and rapid *Fp* diagnostic techniques. Collectively, the culmination of these improvements will mitigate BCWD associated losses in US hatcheries and aquaculture facilities.

Flavors of Flavobacteriales: Characterizing Atypical Flavobacterial Pathogens in Aquaculture

Taylor I. Heckman[1], Zeinab Yazdi[1], Eric K. Pomaranski[1], Fernanda de Alexandre Sebastião[1,2], Brent M. Vuglar[3], Kenneth D. Cain[3], Thomas P. Loch[4], Esteban Soto[1]

[1]Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California-Davis, CA, USA; [2]Universidade Federal do Amazonas, Amazonas, Brazil; [3]Department of Fish and Wildlife Sciences, College of Natural Resources, University of Idaho, ID, USA; [4]Department of Fisheries and Wildlife, College of Agriculture and Natural Resources, Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University, MI, USA

Flavobacterial diseases, caused by bacteria in the order Flavobacteriales, are responsible for devastating losses in farmed and wild fish populations worldwide. The genera *Flavobacterium* and *Chryseobacterium* encompass the most well-known agents of fish disease in the order, but the full extent of piscine-pathogenic species within these diverse groups is unresolved, and likely unappreciated. To identify emerging agents of flavobacterial disease in US aquaculture, 186 isolates of suspected *Flavobacterium* and *Chryseobacterium* were collected from clinically affected animals representing 20 host types, from across five western states. Isolates were phylogenetically characterized by 16S rRNA and *gyrB* sequencing, and antimicrobial susceptibility profiles were compared between representatives from each major clade. Of the isolates, 54 were identified as *Chryseobacterium* species and 126 as *Flavobacterium*. The majority of *Chryseobacterium* strains fell into six clades (A-G) consisting of ≥5 isolates with ≥70% bootstrap support, and *Flavobacterium* into nine (A-I). Phylogenetic clades showed distinct patterns in antimicrobial susceptibility. Two *Chryseobacterium* clades (A & B), and four *Flavobacterium* clades (F-I) had comparably high minimal inhibitory conditions (MICs) for 11/18 antimicrobials tested. Multiple clades in both genera exhibited MICs surpassing the established *F. psychrophilum* breakpoints for oxytetracycline and florfenicol, indicating potential resistance to two of the three antimicrobials approved for use in finfish aquaculture. Further work to investigate the virulence and antigenic diversity of these genetic groups will improve our understanding of flavobacterial disease, with applications for treatment and vaccination strategies.

Phenotypic, Serological and Genetic Characterization of *Tenacibaculum* Strains Isolated from Chilean Salmon Farms

Sara Valdés[1], Pedro Ilardi[1], Javier Rivera–Bohle[1], Johanna Gajardo–Córdova[1], Rute Irgang[2], Ruben Avendaño–Herrera[2]

[1]Farmacología en Aquacultura Veterinaria FAV S.A. Santiago, Chile, [2]Laboratorio de Patología de Organismos Acuáticos y Biotecnología Acuícola and Interdisciplinary Center for Aquaculture Research (INCAR), Universidad Andrés Bello, Viña del Mar, Chile

Tenacibaculum species generally present as long rod and/or filamentous Gram-negative cells and are widespread in marine environments, some of them are recognized as fish pathogens. In Chilean salmon industry, Tenacibaculosis, an ulcerative and emerging marine disease, is the second most important causes of mortality for Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus* mykiss) and Coho salmon (*Oncorhynchus kisutch*). The disease typically occurs following the transfer of smolts to sea-cages, causing external lesions such as skin ulcers, mouths erosion, frayed fins, and tail-rot. The present study aimed at investigating the phenotypic, serological, and genetic characteristics of 40 Chilean *Tenacibaculum* isolates, including *T. dicentrarchi, T. finnmarkense, T. maritimum* and *T. piscium*. These isolates were recovered from outbreaks occurred from 23 sea farms between 2018–2021. All isolates were identified at level species by specific PCR and multilocus sequence typing. Interestingly, two or more *Tenacibaculum* species were recovered from the same outbreak. The phenotypic characterization of the isolates showed that they are a homogeneous group, except for tolerance to freshwater, where *T. finnmarkense* isolates were more tolerant. Serological studies can be grouped *T dicentrarchi*, *T. finnmarkense* and *T. maritimum* isolates, respectively. Different serological groups were observed within *T. dicentrarchi* isolates. Virulence assays in Atlantic salmon smolts shown differences between *T. dicentrarchi*, *T. finnmarkense* and *T. maritimum*, being the *T. maritimum* isolates the highest pathogenic followed by *T. dicentrarchi*. No mortality could be generated with the isolates of *T. finnmarkense* and very low with *T. piscium*. Future studies need to include determining pathogen-host interactions and identifying possible antigens for novel development vaccines against Tenacibaculosis.

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Intraspecific Diversity of *Flavobacterium psychrophilum* Isolated From Salmonids Farms in Chile

Pedro Ilardi[1], Sara Valdes–Huerta[1], Javier Rivera–Bohle[1], Johanna Gajardo–Córdova[1], Ruben Avendaño–Herrera[2]

[1]Farmacología en Aquacultura Veterinaria FAV S.A. Santiago, Chile, [2]Laboratorio de Patología de Organismos Acuáticos y Biotecnología Acuícola and Interdisciplinary Center for Aquaculture Research (INCAR), Universidad Andrés Bello, Viña del Mar, Chile.

Salmonids infections caused by *Flavobacterium psychrophilum,* the aetiological agent of Bacterial Cold-Water Disease (BCWD) and Rainbow Trout Fry Syndrome (RTFS), are the responsible for the major losses for Chilean freshwater aquaculture facilities. Previous antigenic and genetic studies reported the existence of intra-specific diversity using *F. psychrophilum* isolated before 2018. To developed vaccine candidates, knowledge of more recent bacterial isolates is essential. The present study aimed at investigating the serological and genetical diversity of 113 Chilean *F. psychrophilum* isolates collected from eight freshwater farms between 2018-2020 from external and internal lesions from farmed Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*) and Coho salmon (*Oncorhynchus kisutch*). The genetic characterization using mPCR-based procedure indicates the presence of four *F. psychrophilum* serotypes: 29% Type 0; 8% Type 1; 44% Type 2 and 17% Type 4. However, serological characterization using antisera defined a total of 12 serological group previously described and other groups that not fit in any classification. The distribution of the two16S rRNA alleles was also examined, whereas the 67.25% contained only the virulent genogroup CSF 259-93. Based on the findings reported here, we distinguish the existence of predominant serotypes according to fish farming and species, Type 0 and 4 in Atlantic salmon, Type 2 in rainbow trout, and Type 0 in Coho salmon. However, several serological groups can coexist at the same rearing site, which justifies and supports the use of auto-vaccines for the prevention and control of the disease.

Expert Elicitation to Identify Risk Factors for Tenacibaculosis Outbreaks in Farmed Atlantic Salmon in Chile

Alejandro Poblete[1], Álvaro Pérez[2], Patricio Peña[2], Joel Quidel[2], Claudia Venegas[2], Manuel Salas[1], Sebastián Pérez[1], Mauro Araneda[2], Juan Carlos López[1], Roberto Martin[1], Daniela Vargas[2], Francisco Vallejos[2], Fernando O. Mardones[3]

[1]Directorio Mevea Chile AG, Asociación Gremial Chilena de Médicos Veterinarios vinculados a la acuicultura, Puerto Montt, Chile; [2]Comité Técnico Mevea Chile AG, Asociación Gremial Chilena de Médicos Veterinarios vinculados a la acuicultura, Puerto Montt, Chile; [3]Escuela de Medicina Veterinaria, Facultad de Agronomía e Ingeniería Forestal, Facultad de Ciencias Biológicas y Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile.

Tenacibaculum spp. infections are known as "tenacibaculosis," an ulcerative disease observed in farmed salmon dominantly caused by *T. dicentrarchi*, *T. maritimum* and *T. piscium*. Tenacibaculosis is one of the most prevalent bacterial infections affecting farmed salmon in Chile, ranking third in 2019 for diseases impacting farmed rainbow trout (12.9% of mortalities) and Atlantic salmon (9.8% of mortalities). To date, no vaccine against tenacibaculosis is available, and veterinarians prescribe antimicrobials to treat and control outbreaks. Unfortunately, epidemiological data is limited to quantify the patterns of the disease and there is still uncertainty regarding the case definition for tenacibaculosis. In this study, an expert elicitation process was performed to characterize the disease and identify risk factors associated with severe outbreaks of tenacibaculosis during a production cycle of Atlantic salmon. A qualitative based-expert approach was used to generate risk estimators for a total of 31 hypothesized risk factors. In the expert elicitation process, each fish health specialist independently estimated factors in two rounds (n = 75). Most experts reported that the disease occurs throughout the production cycle and correlated tenacibaculosis mainly with piscirickettsiosis and in a lesser extent with bacterial kidney disease (BKD). Experts identified novel factors that may be associated to an increased risk for severe tenacibaculosis outbreaks, such as increased antiparasitic bath treatments, sealions attacks and extended time of smolts transportation between freshwater to saltwater rearing phases. Results slightly varied between expert' characteristics and farmed salmon production regions. Using these results, a preliminary model is proposed to provide a better understanding leading to strategic decision making and the need to generate and analyze epidemiological data.

Flavobacteria Isolated from BCWD Outbreaks in Chilean Salmon Farms.

Pedro Ilardi[1], Sara Valdes–Huerta[1], Javier Rivera–Bohle[1], Johanna Gajardo–Córdova[1]

[1]Farmacología en Aquacultura Veterinaria FAV S.A. Pedro de Valdivia 295, Santiago, Chile

Flavobacterial diseases in fish are caused by multiple bacterial species belonging to the family Flavobactericidae, that negatively affects wild and cultures fishes worldwide. Chile is considered the second largest producer of farmed salmonids in the world and this intensive fish farming has resulted in growing problems as bacterial diseases and infections caused by the fish pathogen *Flavobacterium psychrophilum*, during the freshwater phase. We report the presence of a large and diverse group of flavobacteria, many of which were associated with skin lesions in various salmonid farms in Chile, associated with outbreaks of RTFS or BCWD. We obtain 25 isolates of Yellow Pigmented Bacteria (YPB) from three Atlantic salmon (*Salmo salar*) and four Rainbow trout (*Oncorhynchus mykiss*) farms, that presented outbreaks of *Flavobacterium psychrophilum* during the years 2018 and 2019. The 25 YPB isolates were identified using 16S rRNA gene sequence analysis and phylogenetic analyses based upon neighbor-joining and Bayesian methodologies. The results of the analysis indicated that nine isolates correspond to *Chryseobacterium chaponense*, 1 isolate was identified as *C. piscicola,* both species were described in Chile in 2009 and 2011 from outbreaks of *F.psychrophilum* affecting Atlantic salmon and Rainbow trout. Two isolates were identified as *C. vrystatnese*. while the 5 isolates identified as *Flavobacterium* spp, were not like any previously described species affecting fish or salmonids. We can conclude that the results will highlight the increasing number and heterogeneity of flavobacteria that are related to BCWD outbreaks and that are capable of infecting fish.

Monday, September 5th
Breakout Room A

Tilapia Health: Paola Barato/ Inácio Assane

1:00 PM **Adamek**: Nile Tilapia Strain Resistant to Tilapia Lake Virus Disease – Immunological and Implementation Considerations

1:15 PM **Vela**: Acute Toxicity Evaluation of Practical Diets with *Erythrina edulis* as a source of adhesion glycoinhibitors for *Streptococcus agalactiae* in tilapia (*Oreochromis* sp.)

1:30 PM **Delphino**: Economic Appraisal of Using Genetically Selected Nile Tilapia Fingerlings to Control *Streptococcus agalactiae* Under Cage and Pond Farming System

1:45 PM **Cruz**: Characterization of Polyclonal Antibodies Generated Against Interferon Gamma in Nile Tilapia (*Oreochromis niloticus*) by Western Blot, ELISA and Flow Cytometry

2:00 PM **Assane**: Phenotypic and Genotypic Characterization of *Aeromonas jandaei* Involved in Mass Mortalities of Cultured Nile tilapia, *Oreochromis niloticus* (L.) in Brazil

2:15 PM **Peña**: Histological and Molecular Biomarkers Applied to the Study of Vaccine Immune Modulation and Hepatic Function to Diets in Nile Tilapia

2:30 PM **Assane**: *Enterogyrus* spp. (Monogenea Ancyrocephalinae) and *Aeromonas jandaei* Co-infection Associated with High Mortality Following Transport Stress in Cultured Nile Tilapia

Nile Tilapia Strain Resistant to Tilapia Lake Virus Disease – Immunological and Implementation Considerations

Mikolaj Adamek[1], Alexander Rebl[2], Marek Matras[3], Christian Lodder[4], Sahar El Rahman[5], Magdalena Stachnik[3], Krzysztof Rakus[6], Julia Bauer[1], Alberto Falco[7], Verena Jung-Schroers[1], Chutchai Piewbang[8], Somporn Techangamsuwan[8], Win Surachetpong[9], Michal Reichert[3], Jens Tetens [4,10] Dieter Steinhagen[1]

[1]Fish Disease Research Unit, Institute for Parasitology, University of Veterinary Medicine Hannover, Hannover, Germany; [2]Fish Genetics Unit, Institute of Genome Biology, Research Institute for Farm Animal Biology (FBN), Dummerstorf, Germany; [3]Laboratory of Fish Diseases, National Veterinary Research Institute, Pulawy, Poland; [4]Department of Animal Sciences, Georg-August-University of Göttingen, Göttingen, Germany; [5]Department of Virology, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt; [6]Department of Evolutionary Immunology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Krakow, Poland; [7]Institute of Research, Development, and Innovation in Healthcare Biotechnology in Elche (IDiBE), Miguel Hernández University (UMH), 03202 Elche, Spain; [8]Animal Virome and Diagnostic Development Research Group, Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand; [9]Department of Veterinary Microbiology and Immunology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand; [10]Center for Integrated Breeding Research, Georg-August-University of Göttingen, Göttingen, Germany

The emergence of viral diseases causing very high mortality of fish can lead to a disruption of aquaculture production. Recently, this occurred in Nile tilapia aquaculture after emergence of a disease caused by tilapia lake virus (TiLV) which dramatically affect tilapia farms worldwide. In virus endemic areas, three strategies can be implemented to limit losses caused by the infection: 1) biosecurity, 2) vaccination programmes and 3) selective breeding increasing the resistance. Exploring the third strategy, we studied the natural resistance to TiLV in three genetic strains of tilapia which were kept in Germany. We used two strains originating from Nilotic regions (Lake Mansala (MAN) and Lake Turkana (ELM)) and one from an unknown location (DRE). Nile tilapia from these strains were infected with TiLV by intraperitoneal injection or cohabitation. The immune responses were measured by a Fluidigm array and linked with viral load and pathological changes. Infection by injection resulted in 100% mortality of fish from all three strains, however, using cohabitation as infection model, we found the ELM strain that did not develop any clinical signs of the infection and had nearly 100% survival rate. The other two strains showed severe clinical signs and much lower survival rates of 29.3% in the DRE strain and 6.7% in the MAN strain. The disease resistance of tilapia from the ELM strain was correlated with lower viral loads both in mucosa and internal tissues. The lower virus spread was linked with a higher magnitude of an *mx1*-based antiviral response in the initial phase of infection in ELM strain. Furthermore lower pro-inflammatory responses in the resistant strain might additionally contribute to its protection from developing pathological changes related to the disease. In conclusion, our results indicate the possibility of using TiLV-resistant strains as an *ad hoc*, cost-effective solution to the TiLV challenge. However, it has to pointed that the fish from the disease-resistant strain still retained significant virus loads in liver and brain for 28 days post infection and they could become persistent virus carriers spreading the virus to naïve population. Therefore, we suggest that the resistant strain should be used within an integrative approach also combining biosecurity, diagnostics and vaccination measures when required.

Acute Toxicity Evaluation of Practical Diets with *Erythrina edulis* as a Source of Adhesion Glycoinhibitors for *Streptococcus agalactiae* in Tilapia (*Oreochromis* sp.)

Hernán A. Vela M.[1,2], Adriana Muñoz[1], Carlos Iregui[1,3], Paola Barato[1,3,4]

[1]Facultad de Medicina Veterinaria y de Zootecnia, Universidad Nacional de Colombia, Bogotá, Colombia; [2]Italcol SA, Bogotá, Colombia; [3]Corporación Patología Veterinaria, CORPAVET, Bogotá, Colombia; [4]MolecularVet SAS, Neiva, Huila, Colombia.

Tilapia (*Oreochromis sp.*) is the second most cultured finfish in the world and streptococcosis is the most important bacterial disease in the industry with high losses mainly at the end of the culture. *Streptococcus agalactiae* (GBS by Group B *Streptococcus*) is the most common bacteria that cause streptococcosis in tilapia and several risk factors such as hypoxia, high density, and high temperature of the water have been associated with the disease. Vaccination and antibiotics are used to prevent and control the illness, respectively. Vaccination is effective to reduce mortality, but it does not have cross protection between serotypes. Antibiotics such as oxytetracycline have been demonstrated that cause subclinical conditions without elimination of the pathogen in the fish. For these reasons, it is necessary to develop new strategies to complement the approximation to reduce the impact of the disease. Glycoinhibitors of adherence have been proposed to avoid adhesion and infection of GBS to the intestine in tilapia. Lectins from *Erythrina cristagallyi* (ECL) with an affinity for galactose have been demonstrated, *in vivo* and *in vitro*, that reduce the adhesion of GBS in the tilapia intestine. In this study, we evaluated the presence of ECL biochemical characteristics in *Erythrina edulis* lectins (EEL), and its acute toxicity in a practical diet with the inclusion of EEL in tilapia alevins. Intragastrical inoculation and *ad libitum* feeding three times per day with practical diets, in flour and extruded food, with two concentrations of EEL (150ug and 300ug) and one control group were performed for 96 hours in tilapia of 8 to 15g. No mortalities were observed, fish keep their initial weight, and the consumption was good and permanent during the experiment. No significant differences were observed in the quantity mean consumption between groups. Regurgitation did not observe in any fish. The histological findings will be presented. The acute toxicity essay is one of the steps to developing a functional diet with EEL inclusion to reduce the adhesion of GBS in tilapia and therefore avoid the presentation of streptococcosis.

Economic Appraisal of Using Genetically Selected Nile Tilapia Fingerlings to Control *Streptococcus agalactiae* Under Cage and Pond Farming System

Marina K.V.C Delphino[1], Rajesh Joshi[2], Alejandro T. Alvarez[3]

[1]GenoMar Genetics Group, 105N, Al. das Aroeiras, Lote 4, Ap.1402, Palmas, Tocantins, Brasil; [2]GenoMar Genetics Group, Oluf Thesens vei 6, 1433 Ås, Norway; [3]GenoMar Genetics Group, Bolette Brygge 1, 0252 Oslo, Norway

Streptococcosis is one of the most important infectious diseases in commercial Nile tilapia (*Oreochromis niloticus*) farming resulting in important economic losses in the industry. Farmers have few control measures at hand to control these outbreaks. Injectable vaccines have been one of the few innovations to arrive to market in recent years however the adoption of this technology globally is limited particularly under pond farming conditions in Asia where animals are stocked in ponds at 0,2-1 grams and are not moved until harvest. Genetic selection for disease resistance have been proposed as alternative strategy to control infectious disease in livestock and aquaculture since the last decade. Economic analysis for such strategies is lacking and this study assesses the economic worth of using tilapia fingerlings resistant to Streptococcosis in cage and pond production system. Further, the paper assesses the profitability of paying the higher price for tilapia fingerlings resistant to Streptococcosis at different levels of infection. Partial-budgeting was used to develop a stochastic simulation model that considers the benefits and costs that are likely to be associated with the adoption of tilapia fingerlings resistant to Streptococcosis at the farm level, in one production cycle. The probability of break-even (benefits≥costs) for a combination of the additional cost of genetics and *Streptococcus* related mortality, given two production system scenarios, and sensitivity analysis to assess how changes in an input variable impact the net results were also carried out. In both ponds and cage production systems of Nile tilapia, the use of genetically selected *Streptococcus* resistant tilapia fingerlings was found to be profitable where *Streptococcus* infection is prevalent. The higher the mortality due to *Streptococcus* infection, the higher the economic profitability of using such *Streptococcus* resistant tilapia fingerlings. In the cages and ponds where *Streptococcus* related mortality was ≥10%, the Nile tilapia aquaculture was found to be profitable even if the amount paid for genetically selected *Streptococcus* resistant tilapia fingerlings was 100% higher than the amount paid for standard fingerlings.

Characterization of Polyclonal Antibodies Generated Against Interferon Gamma in Nile Tilapia (*Oreochromis niloticus*) by Western Blot, ELISA and Flow Cytometry

Lynn Cruz[1], Janet Velázquez[1], Geysi Pérez[1], Antonio Morales[1], Fidel Herrera[1], Osmany González[1], Mario Pablo Estrada[1], Yamila Carpio[1]

[1]Veterinary Immunology Project, Animal Biotechnology Department, Center for Genetic Engineering and Biotechnology (CIGB), P.O. Box 6162, Havana 10600, Cuba.

The validation of a polyclonal and monoclonal antibody is a process that is carried out using analytical techniques, to demonstrate its sensitivity, specificity and reproducibility in the context in which they are going to be used. In previous work, the IFNγ of the Nile tilapia (*Oreochromis niloticus*) was isolated and cloned for the first time. Also, polyclonal antiserum was obtained in rabbits against tilapia IFNγ using a peptide- based strategy. The specificity and utility of the purified anti-p65 polyclonal antibody was evaluated by Western blot and ELISA. Flow cytometry analysis was carried out to study the spleen, head kidney and peripheral blood IFN+ lymphocytes populations. Furthermore, an *in vivo* assay was performed using a model antigen for sea lice control to study the impact on IFN gamma production after booster injecting. Samples of spleen, head kidney and peripheral blood were taken at 3 and 7 days post-booster and the percentages of IFNγ+ cells were determined by flow cytometry after *ex-vivo in vitro* stimulation with the pP0 antigen at 72 hours post-stimulation. This pAbs allowed us to determine for the first time the percentage of IFNγ+ cells in basal conditions and after immune stimulation in Nile tilapia.

Phenotypic and Genotypic Characterization of *Aeromonas jandaei* Involved in Mass Mortalities of Cultured Nile Tilapia, *Oreochromis niloticus* (L.) in Brazil

Inácio M. Assane[1,2], Elielma L. de Sousa[3], Gustavo M. R. Valladão[4], Geovana Dotta Tamashiro[1], Eduardo Criscoulo-Urbinati[5], Diogo Teruo Hashimoto[6], Fabiana Pilarski[1,3]

[1]Laboratory of Microbiology and Parasitology of Aquatic Organisms, São Paulo State University (Unesp), Aquaculture Center of Unesp, Jaboticabal, SP 14884-900, Brazil; [2]Faculdade de Ciências Agrárias, Universidade Zambeze (UniZambeze), Ulónguè, Tete 0713-02, Mozambique; [3]Graduate Program in Agricultural and Livestock Microbiology, São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Jaboticabal, SP 14870-00, Brazil; [4]Postgraduate Program in Aquaculture, Nilton Lins University, Manaus, AM 69058-030, Brazil; [5]SOCIL Nutrição Animal, ADM, Brazil; [6]Laboratory of Aquaculture Genetics and Conservation, São Paulo State University (Unesp), Aquaculture Center of Unesp, SP 14884-900, Brazil.

Aeromonas jandaei is an emerging fish pathogen associated with massive mortalities in cultured freshwater fish. This study investigated the morphological, biochemical, molecular, virulence, pathogenicity, and antimicrobial susceptibility characteristics of four strains of *A. jandaei* involved in the occurrence of mass mortalities of cultured Nile tilapia in an earthen pond farm and laboratory fibreglass tanks in Brazil. Isolates were identified by morphological, biochemical, and molecular analyses. Bacteria morphology was assessed under light and scanning electron microscopes, biochemical profile by conventional biochemical tests, and molecular identification by nucleotide sequencing and phylogenetic analysis of the highly conserved 16S rRNA gene, as well as the housekeeping genes, *gyrB*, and *rpoB*. The virulence and pathogenicity were confirmed by screening for 12 virulence genes and induced experimental infection. Broth microdilution method was used to assess susceptibility to enrofloxacin, florfenicol, oxytetracycline, and thiamphenicol. All isolates were confirmed as *A. jandaei*. Haemolysin, temperature-sensitive protease, haemolysin-aerolysin, and nuclease were the most predominant virulence genes. Lateral flagella B and cytolytic enterotoxin/cytotonic enterotoxin were only detected in strains isolated from fish from fibreglass tanks. In the experimental challenge, doses above 4.3×10^7 CFU mL^{-1} resulted in mass mortality (100%) in a short period (less than 12 h) without remarkable external clinical signs. Typical clinical signs of disease, including lethargy, inappetence, surface swimming, exophthalmia, cloudy eyes, haemorrhagic patches, and redness of the skin, below the opercula, and at the base of all fins, fin rot, and pale body surface were observed in fish challenged with doses below 4.0×10^7 CFU mL^{-1} (10–100% mortality). The most predominant histopathological changes in the internal organs of diseased fish were melano-macrophage centres, vascular congestion with thrombus, haemorrhage, and necrosis. Strains isolated from fish from the earthen pond farm were resistant to oxytetracycline, while the strains from fibreglass tanks were sensitive to all antimicrobials. This study provides pertinent data on *A. jandaei* host-pathogen interactions, susceptibility to antimicrobials, morphological, biochemical, and molecular characteristics, and geographical distribution, which may serve as a guideline for *A. jandaei* isolation and identification in aquaculture.

Histological and Molecular Biomarkers Applied to the Study of Vaccine Immune Modulation and Hepatic Function to Diets in Nile Tilapia

Andrea Peña[1], Ricardo Ildefonso[1,2], Marco Rozas-Serri[1,2], Miguel Fernandez[2], Mateus Cardoso[2], Victoria Jaramillo[1], Catalina Rodriguez[1], Estefanía Peñaloza[1], Lucerina Maldonado[1], Ariel Muñoz[1], Hugo Roa[2]

[1]Laboratorio Pathovet Chile, Puerto Montt, Región de Los Lagos, Chile; [2]Laboratorio Pathovet Brasil, Riberao Preto, Sao Paulo, Brasil

Tilapia aquaculture has shown significant development in the last 5 years in Brazil, becoming the 5th largest producer of this species in the world. Intensive fish farming implies several management challenges in the health and nutrition fields. To allow timely and properly decision-making biological indicators are required. In the health framework, there are several infectious diseases that increase the use of antibiotics and generate quality losses in fillets due to muscle injuries. A prophylactic measure is the administration of effective vaccines along with having diets that help strengthen the immune system and improve production yields. The aim of the present study was to show the application of predictive and quantitative phenotypic and transcriptomic indicators for the evaluation of vaccines and diets in Nile Tilapia (*Oreochromis niloticus*). In the first case a trial under controlled conditions was carried out injecting fish intraperitoneally with an adjuvant and a commercial vaccine against Streptococcus agalactiae, respectively, to evaluate the innate and adaptive immune response. Histological score analysis for head kidney and spleen were performed, as well as gene expression analysis in head kidney for il1b, il8, infg, cd3e, cd4 and cd8. Histoscore results indicate that both the vaccine and the adjuvant do not generate negative impacts on these organs. Meanwhile, gene expression results show a strong activation of the innate immune response during the first 24 hours after injection followed by an activation of the cell-mediated immune response 5 days after injection. For the evaluation of diets, a food restriction challenge was carried out under controlled conditions and its impact on growth indicators, histoscore and expression of oxidative stress related genes in the liver was evaluated. The results show the impact of restrictions of food over 40% of the recommended daily dose in the liver from day 7 showing tissue injuries that intensified over time as the percentage of restriction increased. At the gene expression level an increase in the expression of oxidative stress related genes (gpx and sod1) can be seen from day 7 in fish subjected to food restriction over 40%. In the same way, an increase in the expression of genes related to liver function such as lbsep and a decrease in igf1 can be seen. We can conclude from these studies that the application of histological and transcriptomic tools is useful to provide a comprehensive diagnosis of the immunological and physiological status of the fish facilitating timely and strategic decision-making.

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Enterogyrus spp. (Monogenea: Ancyrocephalinae) and *Aeromonas jandaei* Co-infection Associated with High Mortality Following Transport Stress in Cultured Nile Tilapia

Inácio M. Assane[1,2], Karen D. Prada-Mejia[1], Sílvia U. Gallani[3], Natasha F. Weiser[1], Gustavo M. R. Valladão[3], Fabiana Pilarski[1,4]

[1]Laboratory of Microbiology and Parasitology of Aquatic Organisms, São Paulo State University (Unesp), Aquaculture Center of Unesp, Jaboticabal, SP 14884-900, Brazil; [2]Faculdade de Ciências Agrárias, Universidade Zambeze (UniZambeze), Ulónguè, Tete 0713-02, Mozambique; [3]Postgraduate Program in Aquaculture, Nilton Lins University, Manaus, AM 69058-030, Brazil; [4]Graduate Program in Agricultural and Livestock Microbiology, São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Jaboticabal, SP 14870-00, Brazil.

Monogenean infection of the internal organs is extremely rare when compared to external infections. This study describes mass mortality of Nile tilapia (*Oreochromis niloticus* L.) originating from co-infection with *Enterogyrus* spp. and *Aeromonas jandaei* following transport stress. The first fish deaths occurred on day 1 post-transport, while cumulative mortality reached approximately 90% by day 10 post-stocking. An atypical amount of pale (whitish) faeces floating on the surface of the water as well as typical clinical signs of motile *Aeromonas* septicemia, were reported. Adult monogeneans and countless eggs of monogeneans were found in the stomachs and the intestines of both moribund and dead fish, respectively. Two strains of *A. jandaei* were isolated from the kidneys. Scanning electron microscope microphotographs of the stomach revealed the presence of numerous monogeneans penetrating deep into the gastric tissue, and diffuse lesions filled with bacilliform bacteria. Histopathological examination showed multifocal eosinophilic infiltrate, gastric gland, and epithelial necrosis with sloughed necrotic debris in the lumen. This is the first report of co-infection by *Enterogyrus* spp. and *A. jandaei* in Nile tilapia and the first report of *Enterogyrus coronatus*, *Enterogyrus foratus*, and *Enterogyrus malbergi* parasitizing tilapia in Brazil. These findings indicate that synergic co-infection by Monogenean stomach parasites (*E. coronatus*, *E. foratus*, and *E. malbergi*) and *A. jandaei* may induce high mortalities in tilapia following transport stress.

Monday, September 5th
Breakout Room B

Virtual Microscopy: Dave Groman

10:15 AM **Groman**: Introduction to the Virtual Microscopy Session

10:30 AM **Groman**: Overview of Digital Pathology's Current State: With Comments on Use in Aquatic Diagnostics

11:00 AM **Sandoval**: Practical Operation of a Digital Fish Pathology Service - How it Works

11:30 AM **Ildefonso**: Advantages and Disadvantages of using Digital Pathology vs Traditional Histopathology

12:00 PM Lunch

1:00 PM **Practical Session**: Virtual Laboratory Session I

3:00 PM Refreshments

3:15 PM **Practical Session**: Virtual Laboratory Session II

Overview of Digital Pathology's Current State: With Comments on Use in Aquatic Diagnostics

David B. Groman[1]

[1]Aquatic Diagnostic Services, Atlantic Veterinary College, University of Prince Edward Island, 550 University Ave., Charlottetown, Prince Edward Island, Canada. C1A 4P3

This presentation will provide an introduction to Digital Pathology (Virtual Microscopy) and discuss the current state of Digital Pathology systems use by pathologists and biologist. Additionally, it will detail how Digital Pathology is currently being used for morphologic diagnosis and will touch on the limitations of Digital Pathology and barriers to adoption in the laboratory setting. Finally, current opportunities for the use of in Digital Pathology in aquatic diagnostics and research will be discussed.

Practical Operation of a Digital Fish Pathology Service – How It Works

Carlos G Sandoval[1]

[1]Veterinary Histopathology Center - VeHiCe – Research and Development

Digital pathology is a new tool for histological diagnosis of fish, which is based on the digital scanning of slides and their storage on web platforms or local servers for subsequent observation and analysis through these platforms. This digital system for diagnosis in fish pathologies is user-friendly, since it allows a better overview and better exploration of the tissues, which improves the diagnosis of pathologies. Digital pathology has not geographical barriers, which allows access to this material to different pathologists in different countries, who can discuss a particular case simultaneously. Some difficulties of digital pathology are the cost of implementing the system, the operational maintenance of web platforms and the resistance of pathologists to new technologies.

Advantages and Disadvantages of Using Digital Pathology vs Traditional Histopathology

Ricardo S. Ildefonso[1]

[1]Pathovet, Palena 280, Puerto Montt, Chile, and Rua Eliseu Guilherme 1086, Ribeirão Preto, São Paulo, Brasil.

In recent years diagnostic tools in veterinary pathology have evolved side by side with advances in science and technology as well as with information technology. These advances allow obtaining more objective, homogeneous, and faster results, especially, with more defined criteria promoting standardization of histopathological diagnosis and the possibility of increasing the interaction between people and institutions dedicated to the practice and development of pathology, being also the support for the application of artificial intelligence. These events can be grouped under the name of digital pathology.

Tuesday, September 6th
Main Ballroom

World Organization for Animal Health Session on Antibiotic Use and Resistance: Alicia Gallardo/Dante Mateo

9:30 AM **Mateo**: Contribution of the World Organization for Animal Health (WOAH) to Prevent AMR on Aquatic Animals

9:45 AM **Gallardo**: New WOAH Collaborating Center for Antimicrobial Stewardship in Aquaculture

10:00 AM **Lara**: Health Management in Aquaculture Program (PGSA): Strengthening Responsible and Prudent Use of Antimicrobials (AMU) in Salmon Production.

10:15 AM **Burgos**: An Experience in the Implementation of Title 6 of Aquatic Animal Health Code: The Chilean Case

10:30 AM Refreshments

10:45 AM **Contreras**-**Lynch**: Research Program for Monitoring Bacterial Resistance in Chilean Salmon Farming

11:00 AM **Navarro**: Implementing Effective Monitoring and Surveillance of Antimicrobial Use from Farmed Salmon in Chile

11:15 AM **Ojasanya**: Antimicrobial Susceptibility Patterns of Bacteria Commonly Isolated from Farmed Salmonids in Atlantic Canada (2000–2021)

11:30 AM **San Martin**: Closing Remarks and Discussion

Contribution of the World Organisation for Animal Health to Prevent AMR on Aquatic Animals

Dante R. Mateo[1], Luis O. Barcos[2], María E. Chimenti[2], María Mesplet[2], Olafur Vasson[1], Javier Yugueros-Marcos[1]

[1]Antimicrobial Resistance and Veterinary Medicinal Products Department, World Organisation for Animal Health (WOAH), Paris, France; [2]Regional Representation for the Americas of the World Organisation for Animal Health (WOAH)

The World Organisation for Animal Health (WOAH, founded as OIE) is an intergovernmental organisation that sets standards on animal health, to its 182 Member countries, based on scientific evidence. One of our global initiatives is to support curbing antimicrobial resistance (AMR) by promoting the prudent and responsible use of antimicrobials in the veterinary field under a One Health approach. The Strategy on Antimicrobial Resistance and the Prudent Use of Antimicrobials, the Aquatic Animal Health Strategy and the workplan on AMR in Aquaculture lead the path of our current and future activities oriented to AMR and aquatic animals. Regional efforts are led by our Regional and Sub-Regional Representations. Reference Laboratories and Collaborating Centres (CC) provide us supportive expertise in multiple fields, including our new CC, Center for Antimicrobial Stewardship in Aquaculture CASA. The current and future activities at WOAH, aimed to develop and enhance tools to support our members to address AMR in aquaculture will be presented, from a global and a regional perspective. Among others, the development of a list of antimicrobials for aquatic species to address specific needs, a refined AMU database to identify trends within aquatic animals, training seminars for Focal Points to build capacity, new communication resources for behaviour change, and updated technical publications for disseminating current knowledge, are considered. Gaps on future research that would support the development of useful standards for our Members on AMR in aquaculture will be discussed.

New OIE Collaborating Center for Antimicrobial Stewardship in Aquaculture (CASA)

Alicia Gallardo[1], Betty San Martín[1,2], Javiera Cornejo[1,3], Lisette Lapierre[1,3], José Manuel Yañez[1,4], Jurij Wacyk[1,4], Rodrigo Pulgar[1,4] José Miguel Burgos[1], Hernán Rojas[1], Marcela Lara[5]

[1]Center for Antimicrobial Stewardship in Aquaculture (CASA) for the Americas; [2]Laboratory of Veterinary Pharmacology (FARMAVET), Veterinary and Livestock Science Faculty, University of Chile, Santiago, Chile; [3]Laboratory of Food Safety (LIA) Veterinary and Livestock Science Faculty, University of Chile, Santiago, Chile; [4]Center for Research in Aquaculture (CRIA), University of Chile, Santiago, Chile; [5]National Fisheries and Aquaculture Service (SERNAPESCA), Chile.

Chile, in the hands of the University of Chile and supported by Sernapesca (Aquatic Animal Health Authority) has a new OIE Collaborating Center with the aim to address all areas of AMR in aquaculture that contribute to improved antimicrobial stewardship such as research, expertise, standardization of techniques and dissemination of knowledge. CASA gives support to competent authorities and the aquaculture sector throughout public private partnerships in the countries, being a reference in prudent and responsible use of antimicrobials in aquaculture. It furthermore, gives support to build capacities in countries, allowing them to develop antimicrobial stewardship programs in Aquaculture. One of the main goals of the center is to promote a better understating of the OIE standards in the Aquatic Code (Section VI) to prevent development of antimicrobial resistance (AMR) including: Principles for responsible and prudent use of antimicrobial agents in aquatic animals; Monitoring of the quantities and usage patterns of antimicrobial agents used in aquatic animals; Development and harmonization of national antimicrobial resistance surveillance and monitoring programs for aquatics animal and; Risk analysis for antimicrobial resistance arising from the use of antimicrobial agents in aquatic animals.

Health Management in Aquaculture Program (PGSA): Strengthening Responsible and Prudent use of Antimicrobials (AMU) in Salmon Production.

Marcela Lara[1], Alicia Gallardo [2], Roberto Montt [3]

[1]Aquatic animal health epidemiology consultant, OMSA Colaborative Center for Antimicrobial Stewardship in Aquaculture (CASA), Chile; [2]OMSA Colaborative Center for Antimicrobial Stewardship in Aquaculture (CASA), Chile; [3]National Fisheries and Acuaculture Service, Chile

Program for Health Management in Aquaculture (PGSA), is a public-private initiative, executed by the National Fisheries and Aquaculture Service, with funding from the Ministry of Economy, Development and Tourism and the Chilean Salmon Industry Association G.A. The initiative carried out 47 research projects, which involved the participation of 40 institutions and 194 researchers both nationally and internationally. These research projects focused on epidemiology, pharmacology, genomics, microbiology, parasitology, and host response. The main focus was address piscirickettsiosis and sea lice, main diseases that affect Chilean Salmon production. In relation with Piscirickettsiosis, a bacterial disease in salmon production, the main outcomes were materialized in 19 final reports and 6 manuals, available to the general public at the link www.pgsa.sernapesca.cl, with an impact on the number of national scientific publications related to piscirickettsiosis, rising from a median of 5 per year to 16 annual publications and generating more than 20 events to disseminate results. In this context, a significant number of *P. salmonis* strains were sequenced. The results were used for the development of new treatments for piscirickettsiosis, such as phage therapy and iron chelators; guidelines were generated to improve practices in the use of antimicrobials and the epidemiology and the factors that affect the life cycle of *P. salmonis* both pathologies, among others, were studied in depth. This information has been the basis for the improvement of practices at the industry level and for the modification and elaboration of normative tools aimed at optimizing the use of antimicrobials. PGSA represented a milestone in public-private collaboration, which, through applied research projects, managed to generate management tools at both the industry and public administration levels, contributes to reduction of antimicrobial use in Salmon production in Chile.

An Experience in the Implementation of Title 6 of the Aquatic Animal Health Code: The Chilean Case

Jose Miguel Burgos[1,2], Eugenio Zamorano[3]

[1]Manager Acuiestudios; [2]Advisor Laboratory of Veterinary Pharmacology (FARMAVET), Veterinary and Livestock Science Faculty, University of Chile, Santiago, Chile; [3]Advisor Acuiestudios

The implementation of these standards is related to the capacity of public institutions to be able to record data on the use of antimicrobials (AMU) and thus be able to monitor it. Since 2017, a very useful tool has been incorporated, the Online Veterinary Medical Prescription (PMV) for antimicrobials in seawater production, and the treatments carried out in the freshwater phases of national salmon farming have recently been incorporated. This data on AMU is relevant to improve the performance of therapies and a better knowledge of the results of each of the treatments and, perhaps, more importantly, to know the failures of the treatments. The research of the host, the agent and the epidemiological relationships and also pharmacokinetics and pharmacodynamics studies of the main antimicrobials most widely used in the national salmon industry are very important for the success of the treatments, incorporating more information that must be collected by the private veterinarians and transforming them into best practices and better results of antimicrobial therapies, to prevent the antimicrobial resistance (AMR). All these aspects must be supported or guided by public policies that include regulatory facilities for research in these fields and the research necessary to test technology and management.

Research Program for Monitoring Bacterial Resistance in Chilean Salmon Farming

Sergio Contreras-Lynch[1], M. Eugenia Loy[1], Jaiber Solano[1], Felipe Pontigo[1].

[1]Departamento de Salud Hidrobiológica, Instituto de Fomento Pesquero, Puerto Montt, Chile.

The production of aquatic animals contributes significantly to human nutrition, poverty reduction, and sustainable development, being essential to achieving the "Sustainable Development Goals" of the United Nations Organization. Disease outbreaks are the greatest threat to aquatic animal production worldwide, and, in the medium term, antimicrobials will continue to be the main therapeutic tools for their control. The reduction of the susceptibility to antimicrobials, usually called "bacterial resistance", has, among other generating factors, the selection pressure that can produce incorrect antimicrobial therapies. Since 2015, IFOP has developed a research program for the Chilean State, which addresses from different perspectives, the phenomenon of changes and trends in the susceptibility of the main pathogenic microorganisms in Chilean salmon farming, to the main antimicrobial agents used in this industry, representing decision-making support to address the problem of disease control in aquaculture. This work presents the background and main results of the research program for the surveillance of bacterial resistance of *Piscirickettsia salmonis* and other important pathogens in the salmon industry, detailing the bacterial isolates obtained, the establishment of epidemiological cut-off values (COwt), the determination of the Minimum Inhibitory Concentration (MIC) values, the detection of resistance genes and determinants, and finally, their classification for epidemiological purposes. Finally, an advance is given in the study of the antimicrobial susceptibility of bacteria from the intestinal microbiota of farmed fish, using microbiological and molecular biology methodologies, to obtain a broad perspective of the bacterial susceptibility to antimicrobials in the ecosystems where aquaculture is developed.

Implementing Effective Monitoring and Surveillance of Antimicrobial Use from Farmed Salmon in Chile

[1]Carlos Navarro, [1]Roberto Montt, [2]Fernando O. Mardones

[1]National Fisheries Services (Sernapesca), Valparaíso, Chile; [2]Escuela de Medicina Veterinaria, Facultad de Agronomía e Ingeniería Forestal, Facultad de Ciencias Biológicas y Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile.

Antimicrobial is an indispensable part of veterinary medicine used for the treatment and control of bacterial diseases in farmed salmon production. Frequent use of antimicrobials (AMU) in veterinary practices may lead to the residue in animal originated products and creates some potential problems for human health, including antimicrobial resistance (AMR). Better surveillance is an essential feature of AMR control, thus there is an urgent call to increase the capacity to develop surveillance and monitoring of AMU and AMR in food and agriculture provided by the World Health Organization in collaboration with the Food and Agriculture Organization (FAO) and World Organization for Animal Health (WOHA). Chile has taken positive strides in the development of a comprehensive database, known as SIFA, to track antimicrobial usage. In this study, we describe the key aspects in the implementation of an effective monitoring and surveillance of AMU from farmed salmon in Chile that may be as a good model for other countries. Results from the SIFA includes the identification of trends of antimicrobials use, identification of high and low risk farms, etc. It will also show the benefit of implementing electronic prescription processes as an effective strategy for improving antibiotic use in aquaculture.

Antimicrobial Susceptibility Patterns of Bacteria Commonly Isolated from Farmed Salmonids in Atlantic Canada (2000-2021)

Rasaq A. Ojasanya[1], Ian A. Gardner[1], David Groman[2], Sonja Saksida[1], Matthew E. Saab[2], Krishna K. Thakur[1]

[1]Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Canada; [2]Aquatic Diagnostic Services, Atlantic Veterinary College, University of Prince Edward Island, Canada.

Bacterial infection and antimicrobial resistance are important constraints in the production and sustainability of farmed salmonids. This retrospective study aimed to describe the frequency of bacterial isolates and antimicrobial resistance patterns in salmonid aquaculture in Atlantic Canada. Bacterial isolates and antimicrobial susceptibility testing (AST) results assessed by disk diffusion testing were summarized for 18,776 Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) samples from 2,291 unique cases submitted to the Atlantic Veterinary College, Aquatic Diagnostic Services Bacteriology Laboratory from 2000 to 2021. Kidney was the most commonly submitted tissue (60.0%, n=11,320), and these specimens were mostly submitted as swabs (63.7%, n=11,957). The most prevalent pathogens detected in these cases were *Yersinia ruckeri* type 1 (5.5%, n=127), *Renibacterium salmoninarum* (2.1%, n=48), *Aeromonas salmonicida* (atypical) (1.7%, n=38), and *Pseudomonas fluorescens* (1.2%, n=28). Most bacterial isolates tested (n=918) showed various degrees of resistance to florfenicol, oxytetracycline, ormetoprim-sulfadimethoxine, trimethoprim-sulfamethoxazole, ampicillin, but not to enrofloxacin. None of the bacteria tested showed an increase in antimicrobial resistance trends. This report provides baseline data for antimicrobial surveillance programs that investigate emerging antimicrobial resistance trends in salmonid aquaculture in Atlantic Canada.

Tuesday, September 6th
Main Ballroom

Emergent Diseases: Al Camus/Taylor Heckman

1:00 PM **Camus**: Viral Discoveries in Elasmobranch Fishes

1:15 PM **Kannimuthu**: Temporal Dynamics of Piscine Orthoreovirus-1 (PRV-1) Infection During Pre-smolt Stages of Atlantic Salmon (*Salmo salar*)

1:30 PM **Solano-Iguaran**: First Detection of Infectious Spleen and Kidney Necrosis Virus (ISKNV) and the Parasite *Centrocestus* sp. in Chile: Co-Infection in the Ornamental Fish Platy (*Xiphophorus maculatus*)

1:45 PM **Gaete**-**Carrasco**: Epidemiological Analysis and Determination of Risk Factors for Tenacibaculosis in the Chilean Salmon Farming

2:00 PM **Rodger**: Complex Gill Disease (CGD) of Atlantic salmon (*Salmo salar*): Our Current State of Knowledge

2:15 PM **Dale**: Infectious Salmon Anemia Causes New Challenges in Norway

2:30 PM **Heckman**: For *Lac* of a better name: Redefining Piscine Lactococcosis

2:45 PM **Ness**: Emerging Variants of *Moritella viscosa*

3:00 PM Refreshments

3:15 PM **Mora**-**Salas**: Interlaboratory Ring Trial to Evaluate a Real-Time PCR Assay for the Detection of *Renibacterium salmoninarum* in Chile

3:30 PM **Godoy**: Macroscopic and Histopathological Morphological Spectrum of Muscle Melanosis in Salmon Farming in Chile

3:45 PM **Adamek**: When Sleep Meets Death - How Gill Disease Can Induce Secondary Pathology in the Brain

Viral Discoveries in Elasmobranch Fishes

Alvin C. Camus[1], Ashley L. Powell[1], Abigail R. Armwood[1], Justin M. Stilwell[1], John H. Leary[1], Andrew J. Davison[2], Tom B. Waltzek[3], and Terry Fei Fan Ng[1]

[1]Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA; [2]MRC-University of Glasgow Center for Virus Research, Glasgow, Scotland, UK, G61 1QH; [3]Live Animal Imports & Exports, VS, APHIS, USDA, Gainesville, FL 32606, USA

Few infectious diseases, particularly viral, are documented in elasmobranch fishes compared to teleosts. However, as concern grows over wild elasmobranch populations and elasmobranchs are increasingly displayed in public aquaria, reports of viral diseases will likely increase. Since 2016, three viral agents have been characterized by the Aquatic Diagnostic Service at the University of Georgia's College of Veterinary Medicine. Before then, reports were limited to histopathologic and transmission electron microscopic (TEM) descriptions of dermatitis in smooth and spiny dogfish, *Mustelus canis* and *Squalus acanthias*, respectively, associated with herpesvirus-like viral particles, as well as branchitis and dermatitis attributed to adenovirus-like particles in the smooth dogfish. Intracytoplasmic inclusions in erythrocytes, resembling those of viral erythrocytic necrosis, have been reported in the small-spotted catshark *Scyliorhinus canicula* and little skate *Raja erinacea*. Although no supporting evidence exists, viruses are speculated to cause papillomas in additional elasmobranch species. While no viruses have been isolated in culture, the first molecular evidence of viral infection came from papillomatous skin lesions in a giant guitarfish *Rhynchobatus djiddensis* with epithelial cells containing nuclear inclusions filled by 75 nm icosahedral particles. Molecular sequencing using Illumina MiSeq revealed a 22-kb circular ds-DNA genome and in situ hybridization (ISH) localized the virus to affected epithelial cell nuclei. The virus, guitarfish adomavirus (GAdoV), shared distant homology with Japanese eel endothelial cell infecting virus and marbled eel virus. The helicase and capsid genes of these viruses share a complex evolutionary history with polyomaviruses, papillomaviruses, and adenoviruses and define a newly proposed family of DNA viruses. Additional adomaviruses have since been identified from skin and gill lesions in smallmouth bass *Micropterus dolomieu* and American eels *Anguilla rostrata*, respectively. Prior to submission, a second elasmobranch adomavirus genome was assembled from skin lesions in a sand tiger shark *Carcharias taurus*. The virus appeared to induce proliferation of epithelial elements within dermal denticles producing malformed structures resembling odontogenic neoplasms in other vertebrates. The genome of this virus is smaller than that of GAdoV, did not produce inclusion bodies, and virus particles were not observed with TEM. An RNAscope ISH assay is currently in development. The third viral condition involved necrotizing branchitis in a tiger shark *Galeocerdo cuvier*. Histologic examination revealed intranuclear and intracytoplasmic inclusion bodies within gill lamellar and esophageal epithelial cells. Arrays of intracytoplasmic enveloped virus particles (148 nm) with icosahedral nucleocapsids (84 nm) and electron dense cores were observed by TEM. Illumina Hi-Seq molecular sequencing performed on formalin fixed tissue produced the 293 kb genome of a novel herpesvirus. The genome contained the 12 core genes conserved in members of the family *Alloherpesviridae* (i.e., herpesviruses infecting fish and amphibians), including the DNA polymerase, major capsid protein, and terminase genes. Phylogenetically, an unrooted maximum likelihood tree generated using a concatenation of the 12 core genes indicated the virus forms a distinct branch most closely related to the anguillid and cyprinid alloherpesviruses. Lamellar epithelial cells positively labelled with RNAscope probes to the polymerase and major capsid protein gene sequences. Findings demonstrate that emerging viruses in elasmobranchs are productive areas for the study of viral evolution in vertebrate species.

Temporal Dynamics of Piscine Orthoreovirus-1 (PRV-1) Infection During Pre-smolt Stages of Atlantic Salmon (*Salmo salar*)

Dhamotharan Kannimuthu[1], Ma Michelle Demogina Penaranda[1], HyeongJin Roh[1], Øystein Wessel[2], Ghebretnsae Dawit Berhe[1], Håkon Berg-Rolness[1], Stig Mæhle[1], Craig Morten[1], Bjørn Olav Kvamme[1], Søren Grove[1]

[1]Institute of Marine Research, P.O. Box 1870, Nordnes, N-5817 Bergen, Norway; [2]Faculty of Veterinary Medicine, Norwegian University of Life Sciences, 1433, Ås, Norway

Piscine orthoreovirus (PRV) causes the disease heart and skeletal muscle inflammation (HSMI) in farmed Atlantic salmon and it is predominantly observed after seawater transfer. Surveillance and longitudinal studies have reported the occurrences of PRV-1 in freshwater broodstock farms and hatcheries. However, the viral kinetics and disease development during pre-smolt stages is not known. In this study, we conducted a long-term PRV-1 challenge experiment to examine the temporal profile of viral load, shedding and/or clearance in Atlantic salmon over the course of its growth (and development) from fry to parr stage. Fish (mean weight: 1.1 ±0.19g) infected with PRV-1 (high virulent variant) via intraperitoneal (IP) injection reached peak viral RNA load at 2 weeks post-challenge (WPC) in heart and muscle tissues. The viral RNA load was maintained at relatively high levels in whole blood, spleen, and head kidney tissues even after 36 WPC. Heart lesions typical of HSMI, including pericarditis and myocarditis, were observed at 6 and 8 WPC, but heart inflammation already began to resolve by 10 WPC. Despite achieving high viremia, PRV infection failed to cause any mortality during the 36-week virus challenge period*. However, significant growth differences between infected and control fish were observed during the experimental period. Cohabitation of persistently infected fish (12 and 26 WPC) with naïve Atlantic salmon fry did not result in successful infection, suggesting that fish during this phase of infection are not shedding significant amount of virus to initiate horizontal transmission. Moreover, stress exposures did not affect the viral load or shedding of PRV at 12 and 26 WPC. These findings confirm the persistence of PRV-1 for a long period in blood and lymphoid organs of Atlantic salmon pre-smolts similar to post-smolts.

*Tentative data - monitoring will continue until August 2022

First Detection of Infectious Spleen and Kidney Necrosis Virus (ISKNV) and the Parasite *Centrocestus sp*. in Chile: Co-Infection in the Ornamental Fish Platy (*Xiphophorus maculatus*).

Jaiber J. Solano-Iguaran[1], Margarita P. Gonzalez[1], Claudia Spinetto[1], Renato Oyarzun[1], Katerine Subiabre[1], Mario Rivas[1], Noelia Vega[1], Felipe I. Pontigo[1], Dennis Cisterna[1], Sergio Contreras-Lynch[1,2]

[1]Departamento de Salud Hidrobiológica, División de Investigación en Acuicultura, Instituto de Fomento Pesquero, Puerto Montt, Chile; [2]Doctorado en Acuicultura, Programa Cooperativo Universidad de Chile, Universidad Católica del Norte, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile.

Infectious Spleen and Kidney Necrosis Virus (ISKNV) is an important fish pathogen, mainly distributed in East and South-East Asian countries, although it has been reported in several countries around the world (i.e., Germany, Australia, United States, Brazil, etc.), principally associated with the translocation of ornamental fish. ISKNV belongs to the genus Megalocytivirus of the family Iridoviridae and more than 50 susceptible fish species has been reported, including ornamental fish as Angelfish (*Pterophyllum spp.*), Guppy (*Poecilia reticulata)*, Zebrafish (*Danio rerio)*, Platy (*Xiphophorus maculatus)*, etc., and others economically important species such as *Seriola dumeril* and *Seriola lalandi*. Here, we detected ISKNV for the first time in Chile in ornamental fish obtained from quarantine place. We sampled 24 ornamental fish species from quarantine places in Chile. The samples were pooled with maximum 3 fishes for pool and, PCR or qPCR/RT-qPCR (hereafter just PCR) assays were run for the viral diseases listed in OIE and the List 1 of High-Risk Diseases in Fishes in Chile (List 1; Res. 1741/2013, SUBPESCA). Positive samples by PCR were sequenced to confirm identity of the virus. Only ISKNV were positive in two quarantine places, representing a positivity of 0.15 (0.03 – 0.44; Agresti-Coull confidence limits), and these detections were related only to Platy. Regarding this specie, 19/74 individuals were positive to ISKNV (0.26, 0.17 – 0.37). Both quarantines are located in the Metropolitana district, and the fish were shipped by the same supplier in United States. In addition, in 2 fish (ISKNV positives) from the same quarantine place, we found the parasite *Centrocestus sp.* (Opisthorchiida: Heterophyidae) lodged in the gills of Platys observed in the microscope. These findings represent the first report of this zoonotic parasite in Chile. Our results highlight the necessity to create a surveillance program of high-risk pathogens for ornamental fish in Chile (e.g., List 1), to safeguard the sanitary patrimony and prevent the propagation of emerging pathogens which are not established in this country, and could have potentially serious consequences on wild and farmed organisms.

Epidemiological Analysis and Determination of Risk Factors for Tenacibaculosis in the Chilean Salmon Farming

Alvaro Gaete-Carrasco[1,2], Carla Rosenfeld[3] and Marcela Lara[1]

[1]National Fisheries and Aquaculture Service, Department of Animal Health, Victoria No. 2832, Valparaiso, Chile; [2]Escuela de Medicina Veterinaria, Facultad de Recursos Naturales y Medicina Veterinaria, Universidad Santo Tomás, Viña del Mar, Chile; [3]Institute of Veterinary Preventive Medicine, Austral University of Chile, Isla Tejas Campus S/N, Valdivia, Chile.

The National Fisheries and Aquaculture Service (Sernapesca) of Chile has classified tenacibaculosis as an emerging disease in salmon farming. Knowing the behavior and risk/protection factors of its presentation is essential for its control. We carried out a descriptive and case-control analysis of the mortalities declared by the marine farms to Sernapesca from week 30 of 2018 to week 30 of 2021. We obtained 802 cycles (60%) that reported mortality due to tenacibaculosis, the disease occurs in the three species and regions of sea farming in the country. The disease causes higher than average mortality in the winter months in Los Lagos, in the autumn-winter period in Aysén, and in Magallanes during the months of January, May, September, October and November. The cycles of rainbow trout farmed in Magallanes had the highest mean (0.5%) of percentage accumulated mortality due to tenacibaculosis. The case-control analysis, which evaluated 163 closed cycles, determined that the Aysén region, the species *Salmo salar* and *Oncorhynchus mykiss*, the stocking season in autumn, number of fish stocked >1,000,000 and ≤ 1,200,000, high SRS mortalities and cycles classified CAD (high dissemination centers) of caligidosis have a significant association and risk of presenting high mortalities due to tenacibaculosis. The results of this study contribute to understanding the epidemiology and risk factors of tenacibaculosis in chilean salmon farming.

Complex Gill Disease (CGD) of Atlantic salmon (*Salmo salar*): Our Current State of Knowledge

Hamish Rodger[1] and Ana Herrero[2]

[1]VAI Consulting, Kinvara, Co. Galway, Ireland; [2]VAI Consulting, Oban, Argyll, Scotland

Gill disease of marine stage farmed Atlantic salmon (*Salmo salar*) has emerged to become one of the most significant health and welfare challenges for farms in Europe. Complex gill disease (CGD) is the name of the disorder where clinical, gross and specific histopathological findings have been described. There are an increasing number of infectious agents that have been investigated in CGD, however, a core of *Candidatus, Branchiomonas cysticola*, *Desmozoon lepeophtherii* (syn. *Paranucleospora theridion*) and salmon gill pox virus (SGPV) have been consistently reported associated with the condition. The causal agent of amoebic gill disease (AGD), *Neoparamoeba perurans*, is also involved in many cases. Non-infectious elements and farm management procedures may also be involved in the development of the condition, and these include harmful phytoplankton, cnidarians, net washing debris as well as mechanical and thermal delousing procedures. The current state of our knowledge regarding the pathology, pathogenesis, aetiologies, risk factors, methods to reduce the impacts from the disorder and gaps in our knowledge will be presented.

Infectious Salmon Anemia Cause New Challenges in Norway

Sonal Patel[1], Mona Dverdal Jansen[1], Torfinn Moldal[1], Hilde Sindre[1], Simon Weli[1], Haitham Tartor[1], Bjørn Spilsberg[1], Ole Bendik Dale[1]

[1]Norwegian Veterinary Institute, 1433 Ås, Norway

ISA in Atlantic salmon is a serious orthomyxoviral disease, listed in Norway as well as internationally by OIE and EU. The wild type of ISAV called HPR0 is associated with asymptomatic gill or skin infections, while the virulent HPRdel variant infect cardiovascular endothelium resulting in severe disease with anemia and circulatory disturbances. A good control of ISA is a necessity for successful farming of Atlantic salmon as shown by devastating epidemics in several countries. In Norway, the introduction of multiple biosecurity measures curbed such an epidemic around 1990 and have served to keep ISA under good control. However, during the past two years there has been an increase in the number of outbreaks especially in Northern Norway compared to the previous years. Analyses of sequences for segment 5 and segment 6 link several of the outbreaks to hatcheries that have delivered smolt to the sea sites, and last year there also was an outbreak in a hatchery itself. A method to sequence the whole virus genome have recently been developed. Sequence data produced with this method will enable a better resolution in phylogenetic analyses. The changes in disease outbreak development and our increased focus and activities relating to ISA will be presented and discussed.

For *Lac* of a Better Name: Redefining Piscine Lactococcosis

Taylor I. Heckman[1], Zeinab Yazdi[1], Matt J. Griffin[2], Geoffrey C. Waldbieser[3], Timothy J. Welch[4], Taylor Abraham[1], Eric M. Littman[1], Alexander M. Chow[1], Khalid Shahin[1], Kaveramma Mukkatira[5], Christine Richey[5], Kevin Kwak[5], Bill Keleher[6], Ruben Avendaño-Herrera[7], Cesar Ortega[8], Hugh Mitchell[9], Haitham H. Mohammed[10], Véronique LePage[11], Guillermo Risatti[12], Michael W. Hyatt[13], Salvatore Frasca, Jr. [12], Mark Adkison[5], Esteban Soto[1]

[1]Department of Medicine & Epidemiology, School of Veterinary Medicine, University of California, Davis, CA, USA; [2]College of Veterinary Medicine, Mississippi State University, MS, USA; [3]USDA-ARS, Warmwater Aquaculture Research Unit, MS, USA; [4]USDA-ARS, National Center for Cool and Coldwater Aquaculture, WV, USA; [5]California Department of Fish and Wildlife, CA, USA; [6]Kennebec River Biosciences, ME, USA; [7]Facultad de Ciencias Biológicas and Interdisciplinary Center for Aquaculture Research (INCAR), Universidad Andrés Bello, Chile; [8]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, México; [9]AquaTactics Fish Health, WA, USA; [10]Department of Aquatic Animal Medicine and Management, Faculty of Veterinary Medicine, Assiut University, Egypt; [11]Department of Pathobiology, University of Guelph, Ontario, Canada; [12]Connecticut Veterinary Medical Diagnostic Laboratory, Department of Pathobiology and Veterinary Science, University of Connecticut, CT, USA; [13]Wildlife Conservation Society, New York Aquarium, NY, USA

Lactococcosis is one of the most serious emerging diseases in fish worldwide. Outbreaks are associated with high mortality rates and significant economic losses, and effective tools for treatment and prevention are limited. A wide range of host species are susceptible, including wild and cultured fish from cold, temperate, and warm fresh or marine water systems. Infections have also been reported in humans and other terrestrial species, making it a disease of concern in aquaculture, conservation, and human and animal health. Historically, cases of piscine lactococcosis have been attributed to the gram-positive bacterium *Lactococcus garvieae*. Our recent work, however, has revealed that the disease is caused by multiple species - *L. garvieae, L. petauri,* or a yet undescribed third species – that are indistinguishable by conventional diagnostic methods. Sequencing of the *gyrB* gene, multilocus sequence analysis, or whole genome comparisons are necessary to identify isolates to the species level. Epizootics in rainbow trout (*Oncorhynchus mykiss*) in California and Mexico previously credited to *L. garvieae* were retroactively found to be caused by *L. petauri,* which appears to be the primary agent of lactococcosis in the Americas. Representative isolates of *L. petauri* were strong biofilm formers *in vitro,* showed differential resistance to antimicrobials, and caused significantly higher mortality in experimental challenges of salmonids compared to the other species. This suggests differences in species physiology, virulence, and host specificity that will impact management of lactococcosis and inform development of cross-protective vaccines.

Emerging Variants of *Moritella viscosa*

Monica G. Tingbø[1], Lars-Gaute Jørgensen[1], Michael G. Ness[1], Siv H. Tunheim[1], Ane L. Sandtrø[1] and Anette Furevik[1]

[1]Pharmaq part of Zoetis, Harbitzalleen 2A, 0275 Oslo

Winter ulcer disease caused by *M. viscosa* is a major problem for the salmon farming industry as it causes animal welfare challenges and significant economic losses due to mortality and downgrade at slaughter. All commercial basis vaccines with a winter ulcer disease component currently available across markets contain a classic/typical strain of this pathogen. During the past 5-6 years, it has become evident that vaccines with antigens based on classic *M. viscosa* isolates may not protect the fish sufficiently from disease caused by emerging atypical strains of this bacterium. A summary of the field situation in Norway as well as Canada shows a complex disease picture. In this study we have compared the efficacy of experimental vaccines against classic as well as variant *M. viscosa* challenge. Furthermore, we have characterized a number of field isolates using gyrB- and OMP sequencing as well as with antibody-based methods. Our results show that there are likely several serogroups of *M. visocsa*. Experimental vaccines formulated using gyrB-variant *M. viscosa* isolates show promising results against challenge by the emerging *M. viscosa* strains.

Interlaboratory Ring Trial to Evaluate a Real-Time PCR Assay for the Detection of *Renibacterium salmoninarum* in Chile

Patricia Mora-Salas[1], Cristóbal Verdugo[2], Ricardo Enríquez[3], Isabel M. Aguirre[3], Edith A. Mancilla[3], Marcela A. Lara[4], Carlos A. Navarro[4], Carla F. Anguita[4], Fernando O. Mardones[1]

[1]Escuela de Medicina Veterinaria, Facultad de Agronomía e Ingeniería Forestal, Facultad de Ciencias Biológicas y Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile; [2]Instituto de Medicina Preventiva Veterinaria, Universidad Austral de Chile, Valdivia, Chile; [3]Laboratorio de Biotecnología y Patología Acuática, Universidad Austral de Chile, Valdivia, Chile; [4]Servicio Nacional de Pesca y Acuicultura, Valparaíso, Chile.

Renibacterium salmoninarum (*Rs*) is the etiological agent of Bacterial Kidney Disease, an endemic disease of farmed salmon of the Chilean industry. Current disease surveillance is based on passive and active sampling, where different diagnostic assays are available. The most used diagnostic assay is the Real Time Polymerase Chain Reaction (qPCR), mainly used for the active surveillance of broodstock. However, despite numerous private laboratories that offer this diagnostic technique, there is no standardization of the diagnostic protocol. This work aims to evaluate the Chilean laboratories' performance through an interlaboratory ring test to estimate 'result' concordance between laboratories and the diagnostic performance from a sample panel with known *Rs* status. The sensitivity (Se), specificity (Sp), and Cohen's kappa (*K*) coefficient, to measure the interrater agreement values or concordance, were calculated for each fifteen laboratories by comparison with the National Reference Laboratory (NRL) for *Rs*. The NRL prepared batches of samples containing *Rs*' DNA (positive controls) and without *Rs*' DNA (negative control). Each laboratory received a set of three groups of samples that had to be analyzed in duplicate in three different runs. The samples consisted of different DNA dilutions of two isolates of *Rs* held by the reference laboratory. The negative control sample, one within each group, corresponds to the *Arthrobacter* bacterium's DNA dilutions. Additionally, a survey was submitted together with the panel, gathering information about the brand of qPCR equipment and qPCR reagents, genomic segment analysis and laboratory DNA extraction method. Twelve of the fifteen (80%) participant laboratories had a 100% *K*, Se, and Sp. Whereas the remaining three showed problems in *K* and Se. Laboratory 13 evidenced 95.2% *K* and 94.4% Se, while laboratories 14 and 15, exhibited 85.7% *K* with 83.3% Se. Accordingly, the surveillance capacity for detecting *Rs* in the country may be considered as adequate but still errors in Se may lie in the cut-off Ct given for the technique or the sample load's failure. DNA extraction process from *Rs* was not evaluated, representing another potential bias source to consider in subsequent interlaboratory trials.

Macroscopic and Histopathological Morphological Spectrum of Muscle Melanosis in Salmon Farming in Chile

Marcos Godoy[1,2], Fred Kibenge[3], Rudy Suarez[1], Yoandy Coca[4], Diego Caro[1], Karina Kush[1,] Juan Pablo Pontigo[1,5]

[1]Laboratorio de Biotecnología Aplicada, Facultad de Medicina Veterinaria, Universidad San Sebastián, Sede de la Patagonia, Lago Panguipulli 1390, Puerto Montt, Chile. [2]Centro de Investigaciones Biológicas Aplicadas (CIBA), Lago Panguipulli 1390, Puerto Montt, Chile. [3]Departament of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, 550 University Ave, Charlottetown, PEI C1A 4P3 Canada. [4]Laboratorio de Energía Renovables y Residuos (LERR-UC). Escuela de Ingeniería Pontificia Universidad Católica de Chile. Avenida Vicuña Mackenna 4860, Macul, Santiago de Chile, 7820436, Chile. Laboratorio Institucional, Universidad San Sebastián, Sede de la Patagonia, Lago Panguipulli 1390, Puerto Montt, Chile. [5]Laboratorio Institucional, Universidad San Sebastián, Sede de la Patagonia, Lago Panguipulli 1390, Puerto Montt, Chile.

Pigmentary disorders result from various pathophysiological mechanisms that include quantitative or qualitative variations in the melanin pigment, abnormalities in the distribution of melanin or hemoglobin derivatives, and the abnormal presence of pigment of endogenous or exogenous origin. Melanosis is called any hyperpigmentation from gray to the black of ectopic location, which can occur in the muscles, internal organs, or peritoneum because of the accumulation of melanin. In fish, melanosis in the tissues is generally a consequence of the proliferation of highly pigmented phagocytic cells called melanomacrophages, usually found in the spleen and kidney, among other organs. The presentation of muscular melanosis observed in farmed salmonids can take different morphological patterns. The lesions are gray to black in color, variable in size, location, and degree of infiltration into the musculature. In this work, melanosis's macroscopic and histopathological morphological patterns correspond to 50 cases of the Atlantic Salmon (*Salmo salar*) and Coho Salmon (*Oncorhynchus kisutch*) species, described during the years 2020 to 2022, are described.

When Sleep Meets Death – How Gill Disease Can Induce Secondary Pathology in the Brain

Mikolaj Adamek[1], Franziska E. Müller[2], Urszula Orzechowska[3], Maria Zawisza[3], Krzysztof Rakus[3], Magdalena Chadzińska[3], Verena Jung-Schroers[1], Felix Teitge[1], Veronika Piackova[4], David Gela[4], Martin Kocour[4], Dieter Steinhagen[1]

[1]Fish Disease Research Unit, Institute for Parasitology, University Of Veterinary Medicine Hannover, Hannover, Germany; [2]Department of Cellular Neurophysiology, Center of Physiology, Hannover Medical School, Hannover, Germany; [3]Department of Evolutionary Immunology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Krakow, Poland; [4]South Bohemian Research Centre of Aquaculture and Biodiversity of Hydrocenoses, Faculty of Fisheries and Protection of Waters, University of South Bohemia in Ceske Budejovice, Vodnany, Czech Republic

Gill diseases impair respiration, excretion of harmful metabolites and osmoregulation. Therefore a dysfunction of this organ may have consequences for other tissues including the brain. Carp edema virus (CEV), primarily infecting the gills of common carp (*Cyprinus carpio* L.), and causes koi sleepy disease (KSD). The final stage of the disease is related to clear neurological clinical signs which include loss of body balance and sleepiness (constant lying at one side of their body without any motoric response). Therefore, to better elucidate the cause for the neurological clinical signs, we studied selected aspects of the brain pathology in koi infected with CEV genogroup IIa. Water and ammonia content, histopathological changes, the integrated density of the brain and the brain immune response were studied. CEV-infected fish displayed clinical signs of KSD and had typical abnormalities of blood parameters attributed to this gill disease: the sodium concentration was significantly lower (136.6 mmol/L in controls, 88.9 mmol/L in CEV infected koi) while the ammonia concentration was significantly elevated from 211.8 µmol/L to 616.4 µmol/L. Although the brains of infected koi harboured an over 100-fold lower virus load than the gills, it still showed an increased antiviral response manifested by an upregulation of the expression of genes encoding antiviral proteins: *gig1*, *vig1*, *mx2*. Moreover, upregulation of the expression of genes encoding microglia/macrophage markers (*aif*, *csf1r*) and pro-inflammatory cytokines (*il1b*) were also observed. In the brains of infected fish down-regulation of the expression of *cfos* - marker for neuron activity as well as upregulation of astrocytic marker *gfap* was recorded. These changes correlated with altered integrated brain density recorded on astrocyte and neuronal level by the immunocytochemistry. Furthermore, in the brains of CEV-infected animals, the increase of glutamate dehydrogenase and glutamine synthetase expression was found, what, most probably, was a consequence of the exposure to high level of ammonia (indicated by Nessler's staining). In the brain, CEV infection caused also a significant accumulation of water by 20 % from 4.09 mL/g dry weight to 5.09 mL/g dry weight indicating a brain swelling. In conclusion, the studies on KSD show that a gill disease can have a severe implication for the functioning off-target tissues such as the brain. Clinical signs observed in koi affected by KSD, in particular, the disturbed righting behaviour and the lying on one body side, most probably are related to the brain pathology induced by hyponatremia and hyperammonemia resulting from the gill function impairment.

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Tuesday, September 6th
Breakout Room A

General Session II: Matt Griffin/Fanny Giudicelli

9:30 AM **Okwuosa**: Diets Influencing Hematological Profile as Fitness and Genetic Bioindicator of Fish health

9:45 AM **Salazar**: Expression Analysis of Estrogen Receptors Genes, Immune Response Genes and Immune Selected miRNAs, in a *Salmo salar* Cell Line Induced with Xenoestrogens: 17α-Ethynyl Estradiol and 4-Nonyphenol.

10:00 AM **Schumann**: Physiological Stress Response Induced by Different Hydrodynamic Conditions with Varying Group Size in *Telestes multicellus*

10:15 AM **Jungers**: Pharmacokinetic Analysis of Ceftazidime in Signal Crayfish (*Pacificastacus leniusculus*) Following Intravascular and Intramuscular Administration

10:30 AM Refreshments

10:45 AM **Quidel**: Severity Classification of Salmonid Rickettsial Syndrome Outbreaks on Atlantic Salmon Reared in Chilean Aysén Region: A Predictor Model

11:00 AM **Curotto-Zola**: Biosecurity Characterization of Rainbow trout (*Oncorhynchus mykiss*) Production Farms in Puno, Peru

11:15 AM **Michnowska**: Horizontal Transmission of Disseminated Neoplasia in the Widespread Clam *Macoma balthica* from Southern Baltic Sea

11:30 AM **Michnowska**: Novel Study of Metabolism of Bivalve Transmissible Neoplasia (BTN) Mitochondrial Respiration and Free Amino-acids Profile of Contagious Cancer Cells in *Macoma balthica*

Diets Influencing Hematological Profile as Fitness and Genetic Bioindicator of Fish Health

Okwuosa Obinna Ben[1,2,3], Eyo Joseph E[2]. and Hon Jung Liew[3]

[1]Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana, Nigeria;[2]Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Nigeria; [3]Institute of Tropical Aquaculture and Fisheries, Universiti Malaysia, Terengganu.

Hematology analysis is one of the validated techniques used in fish health evaluation and welfare in veterinary practice for; aquaculture and scientific research. Studies have shown that hematological parameters have proven to be highly relevant to various environmental factors including water quality, nutrition, stress or pathogens, etc. Blood analysis provides essential information about the physiological aspects of fish health indicators such as the activation status of the neuroendocrine and immune system, acute and long-term stress impacts due to adverse husbandry conditions, potential diseases infection, and genetic predispositions. Hematological indices include leukocytes (white blood cells (lymphocytes specifically) for immunity, thrombocytes (platelets) for blood clotting, erythrocytes (red blood cells) for oxygen circulation, neutrophils, monocytes, and packed cell volume. Other calculable indices include mean corpuscular volume, mean corpuscular hemoglobin concentration, and mean corpuscular hemoglobin. The aim of this review was to summarize the hematological profile of fish as a physiological and genetic bioindicator in fish influenced by diets and health status. We, therefore, recommend that more attention should be paid to the relationship between fish hematological indices, diets, and the influence of genetic makeup on welfare and health. We believe that scientific investigation on the effect of fish diets on hematological profiles in fish health and molecular assessment should be encouraged.

Expression Analysis of Estrogen Receptors Genes, Immune Response Genes and Immune Selected miRNAs, in a *Salmo salar* Cell Line Induced with Xenoestrogens: 17α-Ethynyl Estradiol and 4-Nonyphenol.

Carolina C. Salazar[1] and Luis A. Mercado[1]

[1]Laboratorio de Genética e Inmunología Molecular, Instituto de Biología, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso.

Xenoestrogens are a type of Endocrine Disruptor Compounds (EDCs) which can be present in aquatic ecosystems. This type of EDCs can possess estrogenic activity and disrupt the normal estrogen signaling. Their toxic effects is exerted by diverse mechanisms: mainly, they affect the normal estrogen signaling by directly binding to nuclear Estrogen Receptors (ERs) or by binding to the cell-surface receptor G-protein linked estrogen receptor (GPER), and, therefore, modify their genomic or nongenomic activity, respectively. Two of the most relevant xenoestrogens are 4-nonylphenol (NP) and ethinylestradiol (EE2). NP is a non-ionic surfactant widely used in domestic and industrial applications such as detergents, emulsifiers, dispersants, cosmetics, pesticides, and plasticizer. NP is found in the aquatic environment worldwide since it is discharged in high concentrations in wastewater effluents. EE2 is a synthetic hormone used in female contraceptive pills; human and animal excretions are the primary sources for the presence of these xenoestrogens in water bodies. ERs receptors are expressed in teleost fish. Therefore, estrogens recognized by these receptors can modulate a variety of functions including the innate and adaptative immune system processes in teleost. MicroRNAs (miRNAs) are small non-coding RNAs of approx. 19-24 nt in length. miRNAs regulate mRNA expression at the posttranscriptional level, primarily, by binding to the 3' untranslated region (UTR) and decreasing messenger RNA (mRNA) stability; this provokes mRNA degradation and translational repression. Transcriptional regulation by nuclear receptors is the primary level of control for miRNA expression. The regulation of miRNA expression by nuclear receptors, especially ERs, occurs at different levels: 1) by affecting the canonical biogenesis, which could be related with downregulation of miRNAs expression; 2) directly, by binding to the promoter of the miRNAs genes; 3) through the induction of transcription factors that can bind to the promoter of miRNAs genes. Nowadays, there is little information about how expression of miRNAs can be altered after EDC or xenoestrogen exposure in teleost fish. Considering that most piscicultures of Atlantic salmon in Chile are in places where the exposition to xenoestrogen in effluxes can be possible, it is crucial to understand the effect, if any, of the xenoestrogens over the immune response of *Salmo salar*, and its possible molecular mechanisms. In this context, the present study aims to study how the exposition to xenoestrogens affects the expression of estrogen receptors, immune genes and selected immune related miRNAs in an *in vitro* assay using *S. salar* induced with heat inactivated *Flavobacterium psychrophilym* (one of the principal pathogens in freshwater). ASK-1 and SHK- 1 *Salmo salar* cell lines were exposed to NP or EE2 and heat inactivated *F. psychrophilum*, after 24 and 48 hours ARN were extracted, and cDNA synthesis (for mRNA and small RNA) were performed. qRT-PCR analysis was performed to measure the expression of selected genes. Our analysis indicated a differential expression of the estrogen receptors, estrogen- regulated genes (Vtg and zona pellucida), immune genes and selected miRNA, suggesting that xenoestrogens can affects the correct immune response in *Salmo salar* cell lines.

Physiological Stress Response Induced by Different Hydrodynamic Conditions with Varying Group Size in *Telestes muticellus*

Sophia Schumann[1], Gloria Mozzi[2], Costantino Manes [2], Claudio Comoglio[2], Elena Negrato[4], Elisabetta Piva[1], Andrea Marion[3], Daniela Bertotto[4], Gianfranco Santovito[1]

[1]Department of Biology, University of Padova, Italy; [2]Department of Environment, Land and Infrastructure Engineering, Politecnico di Torino, Italy; [3]Department of Industrial Engineering, University of Padova, Italy; [4]Department of Comparative Biomedicine and Food Science, University of Padova, Italy

Artificial barriers in rivers cause a substantial decline in endemic and migratory fish as habitat fragmentation, changed reproduction environments, and blocked migratory routes. Fishways can allow safe passage across these obstructions while also preserving river biodiversity. Hydrodynamic variables influence fish swimming ability and behaviour, which must be understood to construct appropriate fishways. When fish cannot consist or balance, high velocities at passages result in physical stress. School formation results in a hydrodynamic benefit and improves individual performance. The hydrodynamic use of swimming alongside other people is observed in schools, improving performance. A multidisciplinary research strategy was used to investigate the effects of group size at various hydraulic conditions. As a result, collective behaviour in physiological stress reactions was investigated. In a portable flume, wild vairone (*Telestes muticellus*) was examined in groups of 1, 2, and 6 fish. The stress response was studied by analysing the hypothalamic-pituitary-interrenal axis and the cellular antioxidant defence system. Lipid peroxidation was studied through malondialdehyde as an oxidative stress marker in the muscle. Non-enzymatic oxidative changes were also studied by evaluating the amount of advanced oxidative proteins. Preliminary results suggest that oxidative damage is lower in grouped fish than in single fish. Furthermore, increased oxidative stress risk in the tissue will likely enhance cytoplasmatic and mitochondrial gene expression of various antioxidant enzymes, assessed by qRT PCR. Advanced antioxidant responses result in lower cell damage and a higher homeostatic capacity.

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Pharmacokinetic Analysis of Ceftazidime in Signal Crayfish (*Pacifastacus leniusculus*) Following Intravascular and Intramuscular Administration

Hali T. Jungers[1], Heather K. Knych[2], Taylor N. Abraham[1], Taylor I. Heckman[1], Eva M. Quijano Cardé[1], Zeinab Yazdi[1], Diem Thu Nguyen[1], Isabella Medina Silva[1], Lana Krol[3], Freeland H. Dunker[3], Esteban Soto[1]

[1]Department of Medicine & Epidemiology, School of Veterinary Medicine, University of California Davis, Davis, CA 95616; [2]K.L. Maddy Equine Analytical Pharmacology Laboratory, School of Veterinary Medicine, University of California Davis, Davis, CA 95616; [3]Steinhart Aquarium at California Academy of Sciences, San Francisco, CA 94118

Crustaceans are becoming increasingly popular as pets and are housed by many aquatic and zoological institutions. When disease occurs involving shell lesions, often implicating bacterial infections, clinicians typically implement treatment plans that include empirical antibiotic therapy. Unfortunately, few controlled studies have been done to assess common broad spectrum antibiotics such as ceftazidime, which are used in aquatic animal medicine. Therefore, this study aimed to determine and compare the pharmacokinetics of a single 22 mg/kg dose of ceftazidime following intravascular (IV) and intramuscular (IM) administration in signal crayfish (*Pacifastacus leniusculus*). A total of 198 wild caught, signal crayfish were obtained from a local vendor and used as part of this research study. Six crayfish were haphazardly selected and designated as untreated, negative controls. Twelve served as route of administration controls (six crayfish for each route) and were injected with an equivalent quantity of sterile water. The remaining 180 crayfish were assigned to 30 ceftazidime treatment groups with 6 animals/group and 15 timepoints (5, 15, 30, and 45 minutes and 1, 2, 6, 12, 24, 36, 48, 72, 120, 168, and 240 hours post ceftazidime administration). Crayfish were given a single dose of ceftazidime (22 mg/kg) either IV by access of the joint between the carpus and merus or IM in the ventral tail musculature. Hemolymph samples were collected from the ventral tail vasculature of each group at the appropriate timepoint. Plasma drug concentrations were determined by liquid chromatography with tandem mass spectrometry using a noncompartmental pharmacokinetic model of a sparse data set. The maximum ceftazidime plasma concentration for IM injection (124.6 ± 14.7 μg/mL) was reached approximately 5 minutes after ceftazidime administration, with approximately 80% bioavailability. The hemolymph terminal half-life was 10.2 and 8.03 hours after IV and IM administration, respectively. Ceftazidime was last detected at 120 and 72 hours after IV and IM administration, respectively. Results suggest that ceftazidime reaches a maximum plasma concentration quickly and has good bioavailability when administered IM. For crustaceans undergoing antibiotic treatment, an IV route could be considered due to its longer half-life but if this is not feasible, IM administration remains a good option.

Severity Classification of Salmonid Rickettsial Syndrome Outbreaks on Atlantic Salmon Reared in Chilean Aysén Region: A Predictor Model

Joel A Quidel[1], Marcela I Galanti[1]

[1]Australis Seafoods, Puerto Varas, Chile

Piscirickettsiosis (SRS) is the most prevalent bacterial disease in Chilean salmon aquaculture and is responsible for high economic losses. SRS outbreaks fluctuate greatly on severity and extent. There are currently no studies that describe the severity, behavior, and duration of this disease outbreaks after the beginning of antibiotic treatments. The aim of this study was to characterize, through daily mortality curves, different types of SRS outbreaks on Chilean salmon farms, so they will help as a prediction guide, of the possible behavior of new outbreaks and help for taking the appropriate control decisions. Between years 2016 and 2019 mortality information of 56 Atlantic Salmon SRS outbreaks were retrospectively collected. The information was grouped in four patterns according to severity and duration of high daily mortality (over acute, acute, medium, and low). Since year 2020, each new outbreak was followed referring it to the obtained patterns and once the outbreak ended, it was incorporated to the base obtained according to its own classification. 70% of the analyzed outbreaks resulted on low and medium level, 24% were acute and only 6% showed an over acute pattern. Average curves reached daily mortalities ranged from 0.008% on low outbreaks to 0.4% on the over acute ones. The duration of the uprising daily mortality varied from 41 to 60 days for low and over acute curves respectively. For all cases, the mortality recovery period was close to three weeks. This study shows that it is required a long period of time to recover the normal levels of daily mortality after ending oral antibiotic treatment. This should be known and considered before deciding a new therapy, thus avoiding unnecessary antibiotic consumption.

Biosecurity Characterization of Rainbow Trout (*Oncorhynchus mykiss*) Production Farms in Puno, Peru

Jesús Curotto-Zola[1,2], Dora Rodríguez-Huaraca[1], Katherine Zapata-Lovera[1], Fernando O. Mardones[3]

[1]Colectivo digital Comunidad Peruana de Acuicultura (CPACUI), Lima, Perú, [2]Acuivetlife E.I.R.L., Ca. García y García 514, Lima, Perú, [3]Escuela de Medicina Veterinaria, Facultad de Agronomía e Ingeniería Forestal, Facultad de Ciencias Biológicas y Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile.

In this study, we characterized rainbow trout (*Oncorhynchus mykiss*) production farms in the Puno region of Peru considering the progressive management pathway for improving aquaculture biosecurity program proposed by the Food and Agriculture Organization of the United Nations (FAO). Field visits and surveys were carried out for 40 producers during 2020 with emphasis on the implemented biosecurity measures for each risk factors for the introduction and spread of pathogens throughout the rainbow trout production cycle. Of all surveyed producers, 90% were classified as "low biosecurity" characterized by a high share of waters from ponds and lakes and closely distant (less than 5 kilometers) between production units. In addition, there is no transport control and/or animal movement records between farms from regional authorities. The region is limited in terms of the participation of veterinarians and consequently the use and administration of antimicrobials is critically suboptimal. There is also lack in the control and verification of key biosecurity measures in most production units. The study highlights the urgent need to intervene the Puno region to increase biosecurity and control disease awareness.

Horizontal Transmission of Disseminated Neoplasia in the Widespread Clam *Macoma balthica* from Southern Baltic Sea

Alicja Michnowska[1], Samuel F. M. Hart[2,3], Katarzyna Smolarz[1], Anna Hallmann[4], Michael J. Metzger[2,3]

[1]Department of Marine Ecosystems Functioning, Institute of Oceanography, Faculty of Oceanography and Geography, University of Gdańsk, Piłsudskiego 46, 81-378 Gdynia; [2]Pacific Northwest Research Institute, 720 Broadway, Seattle, WA 98122; [3]Molecular and Cellular Biology Program, University of Washington, 1959 NE Pacific Street, HSB T-466, Seattle, WA 98195; [4]Department of Pharmaceutical Biochemistry, Medical University of Gdańsk, Dębinki 1, 80-211 Gdańsk

Disseminated neoplasia (DN) is one of the most challenging and unrecognised diseases occurring in aquatic fauna. It has been diagnosed in four bivalve species from the Gulf of Gdańsk (Southern Baltic Sea) with the highest frequency in *Macoma balthica*, reaching up to 94%. The aetiology of DN in the Baltic Sea has not yet been identified, with earlier studies trying to link its occurrence with environmental pollution. Taking into account recent research providing evidence that DN is horizontally transmitted as clonal cells between individuals in some bivalve species, we aimed to test whether DN is a transmissible cancer in the population of *M. balthica* from the Gulf of Gdańsk highly affected with cancer. We examined mitochondrial cytochrome c oxidase I (mtCOI) and elongation factor 1α (EF1α) sequences of genomes obtained from haemolymph and tissues of neoplastic and healthy individuals. Sequence analysis resulted in detection of an independent transmissible cancer lineage occurring in 4 neoplastic clams that is not present in healthy animals. In this presentation we describe the first case of transmissible DN in the clam *M. balthica* providing further insights for studies on this disease.

Novel Study of Metabolism of Bivalve Transmissible Neoplasia (BTN) - Mitochondrial Respiration and Free Amino-acids Profile of Contagious Cancer Cells in *Macoma balthica*

Anna Hallmann[1], Alicja Michnowska[2], Agnieszka Chomiczewska[1,3], Marcin Lipiński[1], Katarzyna Smolarz[2]

[1]Department of Pharmaceutical Biochemistry, Medical University of Gdańsk, Dębinki 1, 80-211 Gdańsk; [2]Department of Marine Ecosystems Functioning, Institute of Oceanography, Faculty of Oceanography and Geography, University of Gdańsk, Piłsudskiego 46, 81-378 Gdynia; [3]Department of Biochemistry, Medical University of Gdańsk, Gdańsk, Poland

Occurring in marine bivalves, bivalve transmissible neoplasia (BTN), a disease of infectious nature, arises from genome instabilities leading to multilevel malfunctions and unregulated cell division of presumably haemocyte precursors. As its biochemical characterisation remains unknown, we here present results describing selected physiological and biochemical aspects of the disease measured in model clams *Macoma balthica*. We assessed free amino acids (FAAs) profile for both healthy and neoplastic *M. balthica* clams' haemolymph using high performance liquid chromatography-mass spectrometry (LC/MS). In both clam groups nine FAAs were detected with Asp, Glu, Pro, Ser constituting over 90% of total FAA content. Significantly higher Gln level was detected in cancerous clams suggesting an essential role of this FAA in cancer energy production. To assess mitochondrial metabolism of the cancer we used oxygen electrodes system, Seahorse XFp. In neoplastic cells, an impairment of mitochondrial metabolism was observed as a decrease in mitochondrial oxygen consumption and lower cytochrome c oxidase activity in comparison to healthy clams. Observed Warburg effect of cancer cells suggest that BTN in *M. balthica* is characterized with mostly anaerobic respiration. This observation, along with potential Gln role in energetic metabolism, provides a new insight into BTN biochemistry and physiology.

Tuesday, September 6th
Breakout Room A

Microbiology/Bacteriology: Tim Bruce/Allison Wise

1:00 PM	**Barato**: Advance in phage therapy to control *Weissellosis* by *Weissella ceti* in rainbow trout (Onchorynchus mikiis) in Colombia
1:15 PM	**Yunis-Aguinaga**: Characterization and Preliminary Vaccine Trial Against *Yersinia ruckeri* in Cage-Reared Rainbow Trout in Peru
1:30 PM	**Patel**: Pasteurellosis; a Serious, Emerging Disease in Atlantic Salmon Farmed in the North-Atlantic
1:45 PM	**Yunis-Aguinaga**: Biochemical and Molecular Identification of *Aeromonas* spp. Isolated from Diseased Amazon Fish Cultured in Peru
2:00 PM	**Mora-Salas**: New PCR Method for Lineage Typing of Epidemic *Renibacterium salmoninarum* in Chilean Salmon Farms
2:15 PM	**Yunis-Aguinaga**: Isolation and primary characterization of *Chryseobacterium* spp. in outbreaks in farmed rainbow trout in Peru
2:30 PM	**Omeje**: Drug resistant profiles of *Aeromonas hydrophila* isolated from cultured African catfish *Clarias gariepinus* in the Kainji Lake area, Nigeria.
2:45 PM	**Yunis-Aguinaga**: Susceptibility of *Colossoma macropomum* to Experimental Infection with Aeromonas Species Isolated from Ornamental Fish
3:00 PM	Refreshments
3:15 PM	**Divya**: Phenotypic, Genotypic and Serological Comparison of *Edwardsiella ictaluri* Isolates Derived from Catfish and Ornamental Fish Species
3:30 PM	**Wise**: Polymicrobial Infection Dynamics with *Edwardsiella ictaluri* and *Flavobacterium covae* in Channel Catfish Ictalurus punctatus
3:45 PM	**Dubytska**: *Edwardsiella ictaluri* T3SS Effector EseN is Involved in Regulation of Apoptosis in Infected Head-Kidney-Derived Macrophages
4:00 PM	**Hanson**: Microcystin-LR Exposure Predisposes Channel Catfish to Bacterial Diseases

Advances in Phage Therapy to Control Weissellosis by *Weissella ceti* in Rainbow Trout (*Onchorynchus mykiss*) in Colombia

Viviana Clavijo[1,2], Zulma Hernández[3], Alejandro Franco[4], Angela Holguin-Moreno[5], Juan Carmilo Farfán[5], Laura Tatiana Camacho[5], Martha-Josefina Vives[5], Paola Barato[1,2,6]

[1]MolecularVet SAS, Neiva, Huila, Colombia; [2] Ciencia y Tecnología de Fagos SciPhage, Bogotá, Colombia; [3]Corporación Universitaria del Huila, CORHUILA, Neiva, Huila, Colombia; [4]Fundación Universitaria de Colombia, UNIAGRARIA, Bogotá, Colombia; [5]Universidad de los Andes, Bogotá, Colombia. [6]Corporación Patología Veterinaria, CORPAVET, Bogotá, Colombia.

One important emerging pathogen for rainbow trout (*Onchorynchus mykiss*) production is *Weissella ceti*. This pathogen is rapidly gaining importance due to the high mortality reported in grow-out fish causing mortalities up to 40%. Bacteriophages, acting as host-specific parasites of bacterial cells, have gathered interest as an alternative strategy to antibiotic treatment or as a prophylactic measure against pathogens important to the aquaculture industry. The aim of the study was to evaluate the safety and feasibility of using a phage cocktail for controlling *W. ceti* infections in rainbow trout. To do so, two bacteriophages W2 and W7 were isolated and characterized from *W. ceti* isolates from diseased rainbow trout, and water ponds. Furthermore, the potential of delivering a two-phage cocktail orally to rainbow trout for controlling *W. ceti* in aquariums was investigated. Characterization of phages W2 and W7 showed that they formed small and round-transparent plaques on a double-layer plate and they have activity against six *W. ceti* strains isolated from rainbow trout farming in Colombia. *In vivo* experiment comprised, first, the validation of the model with experimental infections using rainbow trout (4 to 7g, and 5 to 8 cm) infected by intragastrical (IG) and by immersion (IMM) with $1x10^6$ UFC/ml of *W. ceti.* After 7 days, mortality of 85% for IG inoculation and 40% for IMM exposition were recorded. Typical clinical signs, gross, and histological lesions were observed in 100% of inoculated fish, and *W. ceti* was successfully recovered from both death and survivor fish. Then, using this infection model, the experimental design with phages was achieved using five experimental groups for 15 days: 1. Fish infected with *W. ceti* without treatment, 2. Fish treated prophylactically with phages and then infected with *W. ceti*, 3. Fish infected with *W. ceti* and treated with antibiotic, 4. Fish without infection treated with phages, and 5. Fish without any intervention. Phages were supplied on feed pellets by spraying (1.6×10^8 PFU g^{-1}) at days 1 to 7. *Weissella ceti* counts, mortality, signalment, gross, and histology lesions were recorded for all the groups and statistical analysis was performed. Altogether, the results represent important information for the development of phage therapy to control weissellosis in the trout industry.

Characterization and Preliminary Vaccine Trial Against *Yersinia ruckeri* in Cage-reared Rainbow Trout in Peru

Carla Fernandez-Espinel[1]; Marco Medina[1]; Giovanna Sotil[2]; Violeta Flores-Dominick[1]; Jefferson Yunis-Aguinaga[1]

[1]Laboratorio de Patobiología Acuática, Instituto del Mar del Perú (IMARPE), Callao, Perú. [2]Laboratorio de Genética Molecular, Instituto del Mar del Perú (IMARPE), Callao, Perú.

Yersinia ruckeri is the causative agent of enteric redmouth disease. In Peru, it is probably the most common disease in this species and still does not count with commercial vaccines in the country. For this, in the current study we characterized twenty-nine isolates from seven cage-reared farms in Puno region, the main aquaculture area in Peru. We found that not only biotype 1 of *Y. ruckeri* was present in the country; most of the isolates in this study were biotype 2. Besides the isolates showed different API profiles 5307100 (21 isolates), 1307100 (4 isolates), 1305100 (2 isolates), 1307120 (1 isolate), and 5305100 (1 isolate) with main differences in the lysine decarboxylase, gelatine hydrolysis, and D-saccharose fermentation tests. Then, two intraperitoneal vaccines were tested (bacterin with and without adjuvant) in 3 cages of ten thousand rainbow trouts each (including a control group). After 6 months, it was found that in vaccinated groups, biomass were higher than the control group. Besides, the vaccine without adjuvant presented the highest biomass due to likely that some fish in vaccinated group + adjuvant presented adherences in the coelomic cavity. Further analysis is needed to confirm the vaccines efficacy in field conditions.

Pasteurellosis; a Serious, Emerging Disease in Atlantic Salmon Farmed in the North-Atlantic

Sonal J. Patel[1], Duncan J. Colquhoun[1,2], Matilde Holmeset[1,2], Aslaug Ness[3], Eirik A. Nævdal[1,2], Anne-Berit Olsen[1], Hanne K. Nilsen[1], Snorre Gulla[1]

[1]Norwegian Veterinary Institute, Bergen and Ås, Norway; [2]University of Bergen, Bergen, Norway; [3]MSD Animal Health Innovation, Bergen, Norway.

Although the term 'pasteurellosis' has been and continues to be erroneously used to describe infections caused by the bacterium *Photobacterium damselae* subsp. *piscicida* (previously known as *Pasteurella piscicida*) around the world, infections in fish caused by true members of the *Pasteurellaceae* appear to be uncommon. To date only a single officially described bacterial species i.e. *Pasteurella skyensis,* has been associated with outbreaks of systemic disease in sea-farmed salmon in Scotland. *P. skyensis*-like isolates have, however, also been associated with a common serious systemic disease in farmed lumpfish in Norway and Scotland and (until recently) with infrequent outbreaks of disease in sea-farmed salmon in Norway. As these bacteria have until recently represented only a minor threat to farmed salmon in Norway, they have not been studied in depth or officially classified. The situation has recently changed however, with an epizootic in Atlantic salmon, first recognised in 2018 (7 outbreaks) continuing to impact the industry today (41 outbreaks in 2021). Due to concerns over the possible future impact of pasteurellosis to the Norwegian salmon farming industry, we have initiated a research project focusing on development of diagnostic methodologies, non-lethal detection of sub-clinical infections and development of infection models for future vaccine testing. Some of the results from the research project will be presented.

Biochemical and Molecular Identification of *Aeromonas* spp Isolated from Diseased Amazon Fish Cultured in Peru

Marco Medina Morillo[1]., Jefferson Yunis-Aguinaga[1]., Giovanna Sotil[2], Gino Rengifo[3], Mery Vazquez[3]., German Murrieta Morey[3], Carla Fernández Espinel[1], & Violeta Flores[1]

[1]Laboratorio de Patobiología Acuática – Instituto del Mar del Perú; [2]Laboratorio de Genética Molecular– Instituto del Mar del Perú; [3]Laboratorio de Sanidad Acuícola y Parasitología – Instituto de Investigaciones de la Amazonía Peruana

Aeromonas spp is world widely distributed bacteria with currently concern due to cause important losses in tropical aquaculture. In particular, species like *Aeromonas hydrophila*, *A. jandaei*, among other, have being reported as pathogenic to Amazonian fish. In this context, we identified biochemical and molecularly, different isolates of the genus *Aeromonas*, obtained from captive Amazonian fish with clinical signs. Fish cultured in aquariums and ponds from fish farms in Iquitos, Peru, were collected. We included samples from *Colossoma macropomum* (gamitana), *Arapaima gigas* (paiche), *Brycon* sp (sabalo), *Otocynclus* sp. (otocynclus), *Myleus schomburgkii* (black band), *Trachelyopterus galeatus* (bride), *Apistogramma* sp. (apistograma) and *Calophysus macropterus* (mota). Internal organs were aseptically collected, homogenized, and streaked on base agar selective for Pseudomonads and Aeromonads (GSP), supplemented with 100′000 IU/mL Penicillin G, tryptone soybean (TSA) and TSA supplemented with blood (TSAB). Plates were incubated at 28 °C for 24 - 48 h. Fourteen yellow colonies surrounded by yellow zones in GSP agar, Gram negative, catalase and oxidase positive and showed to be alpha and beta hemolytic on TSAB were selected. API 20E profiles identified all 14 isolates within the *A. hydrophila/caviae/sobria* complex with different percent of identification (between 85 and 99 %). For the molecular identification, we amplified, sequenced and analyzed the 16S rRNA and gybB genes. The fourteen isolates were identified at the species level, as *A. jandaei* (1), *A. veronii* (1), *A. hydrophila* (2), *A. taiwanensis* (2), *A. caviae* (8). According to the phylogenetic analysis based on NJ method (K2P model), the five species were clearly discriminated, showing bootstrap values >99%. In addition, two subgroups were observed for *A. caviae*. Therefore, this *Aeromonas* isolates are possibly involved in some infectious processes of Amazonian fish, from aquaculture farms in Iquitos - Peru. Also, it is possible that these potential bacterial pathogens could be transferred from ornamental to commercial cultured fish and vice versa causing mortality. This assumption has to be tested in further pathogenicity studies to satisfy Koch's postulates that will be important to determine the potential of these bacterial isolates to cause diseases in commercial Amazonian fish.

New PCR Method for Lineage Typing of Epidemic *Renibacterium salmoninarum* in Chilean Salmon Farms

Patricia Mora-Salas[1], Sebastián Zapararte[2], Pamela Villouta[2], Fernando O. Mardones[1], Ruben Avendaño-Herrera[3], Francisco Melo[2,4]

[1]Escuela de Medicina Veterinaria, Facultad de Agronomía e Ingeniería Forestal, Facultad de Ciencias Biológicas y Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile; [2]Laboratorio de Bioinformática Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile; [3]Laboratorio de Patología de Organismos Acuáticos y Biotecnología Acuícola and Interdisciplinary Center for Aquaculture Research (INCAR), Universidad Andrés Bello, Viña del Mar, Chile; [4]Instituto de Ingeniería Biológica y Médica, Facultades de Ingeniería, Medicina y Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile.

Chile is the second salmon producer behind Norway, and *Renibacterium salmoninarum* (*Rs*) is the etiological agent of Bacterial Kidney Disease (BKD), one of the oldest diseases of farmed salmon worldwide. Although the whole genome of *Rs* (~3.1 million nucleotides) is highly conserved, genomic epidemiology analyses have identified four lineages of *Rs* in Chile. To understand BKD epidemiology, it is highly relevant to establish a simple and cost-effective method for typing the lineage of *Rs* isolates to trace their geographic origin and establish potential relationships with their virulence. A total of 94 *Rs* genomes from the GenBank database were aligned and compared using the MUMmer software package, identifying 2,199 independent single nucleotide polymorphisms (SNPs) spread along the genome. A detailed analysis of the distribution of the SNPs showed five local zones of a length in the range of 10-15 kbp that should be used to unambiguously identify a specific lineage (*i.e.,* the zone contains SNPs with unique nucleotides that are specific for at least 3 out of the 4 identified lineages). One of them was selected, and three regions containing lineage-specific nucleotides were identified and denominated polymorphic sites (PS): PS1, PS2, and PS3. Based on the reference *Rs* strain (ATCC 33209), we designed specific PCR primers that gave amplification products of 567 bp for PS1, 368 bp for PS2, and 334 bp for PS3. For the genetic typing, we evaluated 28 *Rs* isolates recovered from BKD outbreaks in different fish species. The assignment of the specific lineage was confirmed by Sanger sequencing of each PCR amplicon. Based on the findings reported here, we propose the PCR approach as a valuable tool for the rapid and reliable studying of the relationships between *Rs* isolates and the different lineage without requiring the sequencing of the entire genome.

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Isolation and Primary Characterization of *Chryseobacterium* spp. in Outbreaks in Farmed Rainbow Trout in Peru

Carla Fernandez-Espinel[1]; Marco Medina[1]; Giovanna Sotil[2]; Violeta Flores-Dominick[1]; Jefferson Yunis-Aguinaga[1*]

[1]Laboratorio de Patobiología Acuática, Instituto del Mar del Perú (IMARPE), Callao, Perú. [2]Laboratorio de Genética Molecular, Instituto del Mar del Perú (IMARPE), Callao, Perú.

In the process of identifying the main infectious agents that affect rainbow trout (*Oncorhynchus mykiss*) cultivated in Puno and Junin, Peru, samples of external fish lesions were taken. The lesions were characterized by being focal, circular in shape, which had some ulcerative appearance with muscular involvement. Samples were taken from these by swabbing and planted on tryptone yeast extract salts media (TYES, pH = 7.2) in incubated at 15 ° C for 72 h. Eleven isolates that presented yellow coloration were recovered from rainbow trout lesions. Identification using 16 S rRNA gene sequencing was performing confirming the genus *Chryseobacterium*. Isolation were *Chryseobacterium yeoncheonense / C. limigenitum* (ID= 97.05 %), *C. aquaticum* (ID=97.74 %), *C. scophthalmum / C. balustinum* (ID=98.99 %), *C. carnis / treverense / solincola* (ID= 97 %), *C. xinjiangense / palustre* (ID= 96.7 / 96.5 %), *C. psychrotolerans / tenax* (ID= 98.5 %), *C. indoltheticum* (ID = 99.82 %), *C. vrystaatense* (ID= 99.82 %), *C. indoltheticum* (99.63 %), *C. vrystaatense* (ID= 99.63 %), and *C. psychrotolerans* (ID = 98.99 %). Bacteria of the genus Chryseobacterium have been reported as potential pathogens of marine and freshwater fish. Further studies are necessary to demonstrate that rainbow trout skin lesions isolates could represent a pathological hazard for the species in culture in Peru and if they are primarily involved in the lesions found.

Drug Resistant Profiles of *Aeromonas hydrophila* Isolated from Cultured African Catfish *Clarias gariepinus* in the Kainji Lake area, Nigeria

Victor O. Omeje[1] and Stanley S. Eze[2]

[1]Department of Veterinary Medicine, University of Nigeria, Nsukka, Enugu state, Nigeria; .[2]Aquaculture and Biotechnology Division, National Institute for Freshwater Fisheries Research, New Bussa, Niger state, Nigeria.

The area bordering the Lake Kainji, a manmade lake created when River Niger was dammed for hydroelectricity generation is reputed to host the highest concentration of homestead fish farms when compared to other parts of Nigeria. The high concentration of fish farms apart from the availability of water may be due to the sitting of the National Institute for Freshwater Fisheries Research, New Bussa there. The area is the major supplier of cultured food fish and fisheries product to the rest of Nigeria and even to some Sub-Saharan African countries. Motile aeromonas septicaemia (MAS) caused by the Gram negative bacterium *Aeromonas hydrophila* is one of the most prevalent fish diseases while catfish especially *Clarias gariepinus* is the most cultivated species in the Kainji Lake area. The aim of the study was to isolate, characterize and determine the in-vito antimicrobial susceptibilities of *A. hydrohila* isolated from cultured *C. gariepinus* in the study area. A total of 1,200 fish samples collected from different fish farms selected using simple random sampling techniques were used for the study. The gut, muscle and skin of the fish samples were cultured following standard microbiological techniques. *Aeromonas hydropila* suspected isolates were characterized by phenotypic and molecular methods. Antibiotics susceptibility test of the *A. hydrophila* isolates were carried out using the Kirby-Bauer disc diffusion method. Eight antibiotics commonly available in the study area were used to test for the susceptibility of the isolates, they include; Ampicillin, Tetracycline, Streptomycin, Co-trimoxazole Colistin, Gentamicin, Nalidixic acid and Nitrofurantoin. Our result shows that out of the 1,200 adult *C. gariepinus* examined, a total of 186 were positive for *A. hydrophila*, giving a prevalence rate of 15.5%. All the isolates (100%) were resistant to ampicillin and colistin while 60% were resistant to co-trimoxazole. The isolates were moderately resistant to gentamycin (40%), nalidixic acid (37.7%) and nitrifurantoin (33.3%) while 26.7% were resistant to streptomycin. Forty seven (25.2%) isolates were resistant to 4 or more antibiotics indicating multidrug resistant. High percentage of antimicrobial resistance and emergence of multiple drug resistance among the *A. hydrophila* strains observed in the study is of serious concern to fish farmers since the efficacy of chemotherapy which is the major practical method of control of MAS of catfish is threatened by the emergence of drug resistant *A. hydrophila* variants. It is also of grave concern to consumers of such fish and fisheries product because of the health implications.

Susceptibility of *Colossoma macropomum* to Experimental Infection with *Aeromonas* Species Isolated from Ornamental Fish

Marco Medina Morillo[1], Jefferson Yunis-Aguinaga[1], Mery Vazquez[2], Gino Rengifo[2], German Murrieta Morey[2], Carla Fernández Espinel[1], & Violeta Flores Dominick[1]

[1]Laboratorio de Patobiología Acuática – Instituto del Mar del Perú; [2]Laboratorio de Sanidad Acuícola y Parasitología – Instituto de Investigaciones de la Amazonía Peruana

Aeromonas spp causes one of the most common bacterial infections in Amazon fish. In Peru, there is a lack of information about the species and its pathogenicity in reared fish such as *Colossoma macropomum* (gamitana), the main native species in aquaculture. Considering the importance of pathogen dissemination through the global trade of ornamental fish, where Peru is involved, and its relationship with aquaculture fish, here we show the pathogenicity of *Aeromonas* spp. isolated from ornamental fish and infecting Amazon reared fish *C. macropomum*. *Aeromonas hydrophila*, *A. veronii*, *A, jandaei*, *A. caviae*, and *A. taiwanensis* isolated from *Otocynclus* sp. (otocynclus), *Myleus schomburgkii* (black band), *Trachelyopterus galeatus* (bride), *Apistogramma* sp. (apistograma) and *Calophysus macropterus* (mota) were used for experimental infections. All *Aeromonas* species were previously identified using 16 sRNA and GyrB sequencing genes. Prior to infection, fish were anesthetized in 90 mg/L of MS-222. Eighty healthy gamitanas (*C. macropomum*) (~9 cm) were intraperitoneally infected with 0.1 µl of 10^4 CFU, and maintained in 90 L tanks at 28 ± 0.4 °C for 10 days. Fish injected with 0.1 ml of PBS was considered as control group. Isolation and identification of bacteria from moribund fish were performed. At the end of the experiment, 90 %, 60%, 31.25%, 37.5% 45% of average mortality was recorded in each treatment. All *Aeromonas* species isolated from ornamental fish cause mortality in *C. macropomum,* which demonstrates the high possibility that ornamental fish can transmit diseases to aquaculture species.

Phenotypic, Genotypic and Serological Comparison of *Edwardsiella ictaluri* Isolates Derived from Catfish and Ornamental Fish Species

Divya Rose[1], Matt J. Griffin[1,2], Geoffrey C. Waldbieser[2,3], Hasan Tekedar[4], Benjamin R. LaFrentz[5], Ethan T. Woodyard[1], Cynthia C. Ware[1], Roy P. E. Yanong[5], John P. Hawke[6], Lester H. Khoo[1,2,] David J. Wise[2,8] and Suja Aarattuthodi[2,8]

[1]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Stoneville, MS 38776; [2]Thad Cochran National Warmwater Aquaculture Center, Delta Research and Extension Center, Mississippi State University, Stoneville, MS 38776; [3]U.S. Department of Agriculture, Agriculture Research Service, Stoneville, MS 38776; [4]Department of Comparative Biomedical Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762; [5]Aquatic Animal Health Research Unit, United States Department of Agriculture – Agricultural Research Service (USDA-ARS), Auburn, AL 36832; [5]Tropical Aquaculture Laboratory, School of Forest, Fisheries, and Geomatics Sciences, Institute of Food and Agricultural Sciences, University of Florida, Ruskin, FL, 33570; [6]School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803; [7]Department of Wildlife, Fisheries, and Aquaculture, College of Forest Resources, Mississippi State University, MS 38776

The gram negative enteric bacteria *Edwardsiella ictaluri* causes significant economic losses in U.S. farm-raised catfish. Historically regarded as host-specific to catfish, *E. ictaluri* outbreaks have also been reported in other aquacultured species, including ornamental fish from the Southeastern U.S. Consequently, a comprehensive phenotypic and genotypic characterization of *E. ictaluri* isolates from U.S. catfish (n=50) and ornamental (n=42) aquaculture is undertaken. Morphological, biochemical, and protein profiles among isolates are largely similar, although growth in broth varies between groups. Catfish isolates remained turbid in static broth, while ornamental isolates auto-aggregateed to the bottom of the tube. A select group of catfish-derived isolates displayed reduced sensitivity to Aquaflor®, Terramycin®, and Romet® (as reported by the Aquatic Research and Diagnostic Laboratory, Stoneville, MS). Native plasmid profiles are largely homogeneous and consistent with previous published reports within groups. However, between host groups, there are stark differences in plasmid profiles of these isolates. Within the catfish strains demonstrating reduced sensitivity to antibiotics, two distinct multi-drug resistant (MDR) plasmids are identified, one (135,268 bp) previously reported from *E. ictaluri* and another (117,449 bp) reported from the closely related *E. piscicida*. These results indicate these MDR plasmids can be trafficked between *Edwardsiella* congeners and further highlight the importance of judicious antibiotic use. Whole genome analysis reveals discrete genomic differences of *E. ictaluri* among host groups, including the presence of a unique Type 4 secretory pathway and putative phage elements in ornamental fish derived isolates. Phylogenetic signal assessment of individual gene components from previously established MLST schemes identifies optimal MLST gene targets for delineating *E. ictaluri* strains. Serological analysis of select strains reveals marked differences in serological profiles for catfish and ornamental isolates. Sera from catfish exposed to catfish isolates reacted positively with catfish strains, with limited signal present when probed against ornamental strains suggesting limited antibody recognition in catfish to ornamental strains. Combined, these data evince catfish and ornamental fish-derived isolates represent two discrete phyletic lineages, yet within their respective industries, *E. ictaluri* isolates are largely clonal with a high degree of genetic stability.

Polymicrobial Infection Dynamics with *Edwardsiella ictaluri* and *Flavobacterium covae* in Channel Catfish (*Ictalurus punctatus*)

Allison L. Wise[1], Benjamin R. LaFrentz[2], Anita M. Kelly[1], Mark R. Liles[3], Benjamin H. Beck[2], and Timothy J. Bruce[1]

[1]Auburn University, School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn, AL 36849; [2]USDA-ARS Aquatic Animal Health Unit, Auburn, AL 36830; [3]Auburn University, Department of Biological Sciences, Auburn, AL 36849

Catfish farming is of major economic importance for Southern U.S. agriculture and is the largest sector of U.S. aquaculture. *Edwardsiella ictaluri* and *Flavobacterium covae* are prevalent bacterial pathogens that can cause high mortality in production ponds. The occurrence of bacterial co-infections in catfish production may often go unreported or misdiagnosed, resulting in a lack of proper mitigation for the co-infective bacteria. Polymicrobial infections may increase disease severity of the constituent pathogens and increase mortality. A preliminary assessment of *in vivo* bacterial co-infection with *E. ictaluri* (S97-773) and *F. covae* (ALG-00-530) was conducted using 10 g juvenile channel catfish (*Ictalurus punctatus*). Following tank acclimation, triplicate tanks of catfish were divided into five groups: 1) mock control; 2) *E. ictaluri*; 3) *F. covae*; 4) a half dose of *E. ictaluri* followed by a half dose *F. covae* at 48 h post-initial infection; and 5) a half dose of *F. covae* followed by a half dose *E. ictaluri* at 48 h post-initial infection. In addition to the mortality assessment, three separate tanks per treatment group were also used to collect kidney and spleen tissues to evaluate immune responses and quantify bacterial load using qPCR. At 21 days post-challenge (dpc), the full *E. ictaluri* infection (dosed with 5.4 $x10^5$ CFU mL^{-1} inoculum in 10 L of rearing water) resulted in a CPM of 90.0 ± 4.1 % while the *F. covae* group (dosed with of a 3.6 x 10^6 CFU mL^{-1} inoculum in 10 L of rearing water) was found to be 13.3 ± 5.9 %. Concerning the reduced-dose co-infection treatments, similar mortality was noted in catfish initially challenged with either *E. ictaluri* (93.3 ± 5.4%) or *F. covae* (93.3 ± 2.7 %). Despite similarities in final CPM within the co-infection groups, the onset of peak mortality appeared to be delayed in fish first exposed to *F. covae*. Serum lysozyme concentrations were analyzed across treatments and were found to be different at 48 h ($P<0.001$), 96 h ($P<0.001$) and 7 dpc ($P<0.001$). Catfish administered a full dose of S97-773 and a co-infective dose of S97-773 followed by ALG-00-530 had the greatest serum lysozyme content at 48h and 96 h post-challenge. The synthesis of these mortality and health metrics will aid both fish health diagnosticians and channel catfish producers in developing therapeutants and prevention methods to better control bacterial co-infections.

Edwardsiella ictaluri T3SS Effector EseN is Involved in Regulation of Apoptosis in Infected Head-Kidney-Derived Macrophages

Lidiya Dubytska[1*], Ronald Thune[2,3]

[1]Department of Biology and Chemistry, Southern University and A & M College, Baton Rouge, LA 70813; [2]Department of Pathobiological Sciences, Louisiana State University School of Veterinary Medicine, Baton Rouge, LA 70803; [3] School of Animal Science, Louisiana State University Agricultural Experiment Station, Baton Rouge, LA 70803

Edwardsiella ictaluri (*E. ictaluri*) is a Gram-negative bacterial pathogen that causes enteric septicemia of catfish (ESC), is a major disease of farm-raised channel catfish, *Ictalurus punctatus*. *E. ictaluri* is able to invade and replicate in phagocytic and non-phagocytic cells. The *E. ictaluri* Type-Three-Secretion-System (T3SS) plays an essential role in this process. Previously we reported that *E. ictaluri* T3SS is also important in the suppression of programmed cell death and cytotoxicity in infected head-kidney-derived macrophages (HKDM). Here we report that the T3SS effector EseN is an important factor in that process. Essential components in the initiation and activation of programmed cell death through apoptosis are the cysteine-aspartic acid proteases (caspases). Caspase-9 triggers intrinsic, or mitochondrial, signaling pathways of apoptosis, while caspase-8 triggers extrinsic or cell surface receptor pathways. Initiation of either of these pathways leads to activation of the executioner caspases-3, -6, and -7, which activate substrates that mediate the changes that characterize apoptotic cells, including cell shrinkage and blebbing, as well as genomic DNA and nuclear fragmentation. Previously we demonstrated that *E. ictaluri* invasion activates extracellular signal-regulated kinases 1 and 2 (ERK1/2) early in the infection, which are subsequently inactivated by T3SS effector EseN. Previously we demonstrated that the *E. ictaluri* T3SS acts to repress caspase-8 activity at 1-h and 3-h of post-infection (PI), caspase-9 at 3-h and 5-h of PI, and prevents the subsequent release of LDH in the cytotoxicity assay. Our data demonstrate that EseN is involved in inhibition of initiator caspase-8 at 1-h and 3-h PI, does not affect caspase-9 activity. In addition, *E. ictaluri* EseN has no effect on release of LDH at 3-h and 5-h PI. Interestingly, executioner caspase-3/7 activity is significantly increased at 1 h PI for WT compared to T3SS mutant (65ST) but then is significantly reduced compared to 65ST at 3-h and 5-h PI. Caspase-3/7 activity is significantly increased only at 3 h PI in the EseN mutant compared to the WT, indicating that EseN inhibits executioner caspase-3/7 activity only at 3-h PI. Collectively our results indicate that *E. ictaluri* EseN is an important player in modulation of apoptosis.

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Microcystin-LR Exposure Predisposes Channel Catfish to Bacterial Diseases

Alison Marchant[1], Courtney Baugher[1], Lorelei Ford[1], Ann Peterman[1], Lora Petrie-Hanson[1], Larry Hanson[1]

[1]Department of Comparative Biomedical Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762

Cyanobacteria blooms have intensified with increasing catfish production density and can produce toxins that affect fish. Microcystins are among the most common of these toxins. They cause substantial liver damage. Given the importance of the liver in the immune system, we hypothesize sublethal microcystin exposure predisposes channel catfish to infectious diseases. To study this, we exposed fish to a sublethal dose of microcystin-Leucine-Arginine (MC-LR) and evaluated pathology, the effects on innate immune cells and the changes in susceptibility to challenge with two bacterial pathogens. Fish were given a single intracoelomic dose of 500ng/g bw MC-LR and histopathology and serum chemistry were compared to saline injected controls over a 6-day period. In ex-vivo studies, channel catfish leukocytes were exposed to 0, 10, 100 or 1000 ng/ml of MC-LR for 6 hours and evaluated for phagocytic ability. In the third part, survival of fish that were injected with 500 ng/g bw MC-LR were compared to fish injected with saline and after exposure to a LD20 dose of a virulent strain of *Aeromonas hydrophila* (vAh) and in another trial they were exposed to an LD20 dose of *Edwardsiella piscicida*. The MC-LR exposed fish appeared normal but stopped eating. Serum AST and ALT levels were significantly elevated from 6 hours through 96 hours post-exposure and histology confirmed diffuse hepatic injury in the treated fish and substantial recovery by 6 days post exposure. In immunohistochemistry staining using an ADDA specific antibody, strong diffuse MC-LR specific staining was present in the liver during early sampling periods and the staining became more focused to isolated cells during later periods. In leukocyte studies, MC-LR exposure decreased the number of cells that endocytosed dextran 40, and dextran 80 and that phagocytosed the bacterial pathogen *Edwardsiella ictaluri*. In the vAH- MC-LR challenge study, fish that received microcystin experienced 67.3% mortality after vAh exposure compared to 22.4 % mortality in fish that were given saline injections and vAh exposure. In the In the *E. piscicida*- MC-LR challenge study, fish that received microcystin experienced 98% mortality after *E. piscicida* exposure compared to 0 % mortality in fish that were given saline injections and *E. piscicida* exposure. There were no losses in the MC-LR only or the saline only injected fish. These data demonstrate that sublethal MC-LR exposure cause transient hepatic changes, suppress phagocyte function, and make channel catfish more susceptible to bacterial pathogens. This suggests that managing cyanobacterial blooms may reduce infectious disease outbreaks in aquaculture.

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Tuesday, September 6th
Breakout Room B

World Aquatic Veterinary Medical Association/American Association of Fish Veterinarians: Nora Hickey

9:30 AM	**Soto**: Phenotypic and Genetic Diversity Amongst the Etiological Agents of Columnaris Diseases: *Flavobacterium columnare*, *F. covae*, *F. davisii* and *F. oreochromis*
10:30 AM	Refreshments
10:45 AM	**Reichley**: Successes and Failures of Combating Columnaris Disease at a Large-Scale Rainbow Trout Farm
12:00 PM	Lunch
1:00 PM	**Bruce**: An Overview of Columnaris Disease in Cultured U.S. Finfish: Experimental Infections, Disease Diagnostics, and Current Treatments
2:00 PM	**Kasper**: Columnaris Disease: Prevalence, Prevention and Treatment of Non-Food Species
3:15 PM	**Onofryton:** Vaccination for Columnaris Disease: A Brief History and Future Prospects
4:15 PM	**Stilwell:** Pathology of Columnaris Disease in Catfish and Ornamental Fish

Phenotypic and Genetic Diversity Amongst the Etiological Agents of Columnaris Diseases: *Flavobacterium columnare*, *F. covae*, *F. davisii* and *F. oreochromis*

Esteban Soto[1], Zeinab Yazdi[1], Diem Thu Nguyen[1], Taylor I. Heckman[1]; Benjamin R. LaFrentz[2], Matt J. Griffin[3]; Thomas P. Loch[4]

[1]School of Veterinary Medicine, University of California, Davis, California, United States; [2]Aquatic Animal Health Research Unit, United States Department of Agriculture – Agricultural Research Service (USDA-ARS), Auburn, Alabama, United States; [3]College of Veterinary Medicine, Mississippi State University, Stoneville, Mississippi, United States; [4]College of Veterinary Medicine, Michigan State University, East Lansing, Michigan, United States

Columnaris disease is an important disease of freshwater fish resulting in substantial losses in global aquaculture production as well as in wild fish populations. Historically, *F. columnare* was regarded as the only etiological agent of columnaris disease; however, recent research demonstrated four different bacterial species within the genus *Flavobacterium* cause columnaris disease in fish. The level of genetic diversity observed among these different species offers an explanation why an effective vaccine for columnaris diseases has been so elusive. Additionally, inter-species diversity partially explains different virulence and fish species susceptibility. In this talk, we will cover some of the "classic" and novel diagnostic methods used to identify the different agents causing columnaris disease in fish. Additionally, we will discuss different methods used to type *Flavobacterium* spp. An improved understanding on the phenotypic and genotypic diversity of flavobacteria will provide enhanced specific and sensitive diagnostic methods and facilitate the development of effective therapeutic and prophylactic methods against this important disease.

Successes and Failures of Combating Columnaris Disease at a Large-Scale Rainbow Trout Farm

Stephen R. Reichley[1]

[1]Mississippi State University College of Veterinary Medicine, 240 Wise Center Drive, Mississippi State, MS 39762 USA

Rainbow trout farming has a long history in the United States. Before their sale in 2020, Clear Springs Foods was the leading producer of premium rainbow trout, processing over 20 million pounds a year with over 300 employee-owners. It was a vertically-integrated company with brood operations, farm operations, feed manufacturing, processing plants, refrigerated truck distribution fleet, sales and marketing, and research and development. While seafood companies face many challenges, fish health and infectious diseases are one of the major impediments to global aquaculture. Columnaris disease, caused by *Flavobacterium columnare*, was one of the major bacterial diseases impacting the farm. This talk with provide an overview of the columnaris challenges. Prevention and control strategies for this disease will also be discussed.

An Overview of Columnaris Disease in Cultured U.S. Finfish: Experimental Infections, Disease Diagnostics, and Current Treatments

Timothy J. Bruce[1]

Auburn University, School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn, AL 36849

Columnaris disease (CD) is a major bacterial pathogen within many segments of U.S. aquaculture and global fish culture operations. Of particular note, this pathogen is associated with high mortality within the U.S. catfish industry, as well as within rainbow trout and tilapia production. The hallmark signs include saddleback, fin fray, gill lesions, and lethargy. Aside from its prevalence as a primary pathogen, CD is often also associated with polymicrobial infections. The gram-negative bacteria, *F. columnare,* has traditionally been responsible for disease outbreaks, and four major genetic groups were associated with different host species. Recently, separate *Flavobacterium* spp. have been identified and detailed as disease agents. Current disease management tools for this dynamic pathogen include dietary additives, probiotic applications, stress mitigation, and changes to water quality. As with other *Flavobacterium* spp., vaccination development has been a challenging arena. This CE presentation will discuss the CD-associated clinical signs, pathogen isolation, and currently available therapeutics in the industry. Experimental *in vivo* pathogen challenge results will be presented, along with vaccine design and strategy review.

Columnaris disease: Prevalence, Prevention and Treatment of Non-food Species

Sherri L. Kasper[1]

[1]Gulf Coast Aquatic Veterinary Services, Tallahassee, Fl 32317

There are many names for the diseases in aquatic animals caused by *F. columnare*. They range from descriptive to the organs affected including "saddle back", "bacterial gill disease", "cotton wool", "mouth fungus". These are not defined disease, but diseases caused by bacteria. The most common bacteria causing these diseases includes Aeromonas, Streptococcus and *F. columnare*. Columnaris has been found as the cause in high mortality of aquatic species since the early 1900's. In 2003 the USDA listed columnaris as the cause of 70% of economic loss in catfish(Hamza et al. 2019). In Egypt, a study showed that 42.8% of tilapia had *F. columnare*. Columnaris is a ubiquitous worldwide disease (Hamza et al. 2019). It effects fish in the wild, aquaculture, aquariums and the hobbyist's ponds. Studies have shown that *F. columnare* is pathogenic at temperatures greater the 15C and will increase in virulence as temperatures increase from subacute disease to acute mortality (Noga 2019) This is important to consider for both prevention and treatment of aquatic species who are susceptible to columnaris. *F. columnare* can live in a planktonic free living form in the environment for months and is still viable on dead fish; therefore, treatment through oral antibiotics is limited without treatment of the population and the environment (Smith 2019). Research into treatments of this bacteria are ongoing, but treatment is very difficult due antibiotic restrictions and environmental concerns. Prevention is the most effective. Prevention can include environmental controls as well as vaccines in some fish. Treatment may range from oral antibiotics to bath combinations. Repeated infections in any environment are common due to antibiotic resistance, reinfection and poor husbandry. This discussion will review the potential difficulties of treating non-feed fish including ornamentals, bass, and koi. We will review the most effective diagnostics and how they can be used efficiently in private practice. We will also discuss the best ways to prevent this disease and how to adapt to changes in climate that will make the bacteria more prevalent and more virulent.

Vaccination for Columnaris Disease: A Brief History and Future Prospects

Katharine M. Onofryton[1] and Hugh M. Mitchell[1]

[1]AquaTactics Bimeda, Kirkland, WA 98034

The aquatic bacterium *Flavobacterium columnare*, the agent associated with "columnaris" disease, has not only wreaked havoc upon fish health and can result in high mortality for warm- and cold-water species, but also has caused high economic losses in cultured and wild fish populations around the world loss for decades. A commercially available, safe, and effective vaccine would be a great tool to use to mitigate the impacts of this disease in multiple species. A few autogenous attempts have been produced for certain life stages and certain species, but there is not one that is used widely, especially in salmonids. Oftentimes, columnaris disease presents following the risk factors of stress, loss of mucosa, and abrasions to the epidermis from water quality, increasing temperatures, handling, and high densities, or a combination thereof. Since *F. columnare* is primarily an external pathogen in most species (which can lead to septicemia), it is important to have antibodies present in the mucosal and epidermal layers to fight infection. Once the mucosa and epidermis are compromised or lost, it can be difficult to regain the upper hand with regards to providing a robust immunological response to columnaris disease. It has been shown that although injectable vaccines are typically more effective in fish with most diseases, with columnaris disease, immersion or oral vaccines may be a better choice as there are reports of more favorable stimulation of mucosal immunity. Strain differences as related to cross protection has not been fully investigated. This presentation will provide an overview of the history, the current state, and a brief foray into the future of vaccine technology for columnaris disease. It will review fish vaccine principles, illustrate why it has been difficult to develop a columnaris vaccine, and provide fish clinical practitioners with information regarding current columnaris disease vaccine technology.

Pathology of Columnaris Disease in Catfish and Ornamental Fish

Justin M. Stilwell[1], Natalie K. Stilwell[1]

[1]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762

Flavobacterium columnare, the namesake and major causative agent of columnaris disease, elicits significant clinical morbidity and mortality across a variety of warmwater fish species. Epidemiological factors predisposing to columnaris outbreaks are multifactorial resulting from a combination of environmental and handling stressors, traumatic injury, bacterial species and strain virulence, and fish immune status. Gross lesions typically exhibit characteristic yellow to brown discoloration of erosive to ulcerative, necrotic cutaneous, oral, ocular, and branchial lesions. Systemic disease can also occur. Microscopic lesions often exhibit dense lawns of filamentous bacteria colonizing areas of necrosis, collagenolysis, and edema with little to no inflammatory response. Columnaris may occur as a solitary primary pathogen or as a coinfection with other agents. This talk will summarize the pathologic lesions and processes associated with the disease in U.S. channel catfish aquaculture and highlight cases in freshwater ornamental fish.

Wednesday, September 7th
Main Ballroom

Big Data: Ian Gardner/Grace Kerreman/Jon Grant

9:30 AM **Grant:** Big Data and the Implementation of Precision Fish Farming

10:00 AM **Vanderstichel:** Longitudinal Dissolved Oxygen Patterns in Atlantic salmon Aquaculture Sites in British Columbia, Canada

10:15 AM **Midtlyng:** A Proposed Improvement of Real-time Monitoring of Cause-specific Mortality and Losses in Industrial Salmon Farming

10:30 AM Refreshments

10:45 AM **Nygren**: Fishing for Answers: Using Big Data Analytics to Predict and Manage Environmental Risks at BC Salmon Farms

11:00 AM **López-Riveros**: Precision Biometrics Data of Atlantic Salmon (*Salmo salar* L.) in Commercial Grow-out Sea-Cages: Manual Sampling and Infrared Diode frames Compared to Processing Plant

11:15 AM **Burciaga**: Connect, Digitize and Exchange of Information: Creating Better Producer to Consumer Outcomes in Aquaculture. What Can We Learn from the Feedlot Industry?

12:00 PM Lunch

1:00 PM **Valdes Donoso**: What Does Big Data Mean for Aquaculture? Current Development, Limitations, and Future Challenges

1:15 PM **Bravo**: Big Data Analytics to Support Evidence-based Strategic Planning in Salmon Farming in Chile

1:30 PM **Panel Discussion**

Big Data and the Implementation of Precision Fish Farming

Jon Grant[1], Meredith Burke[1], Ramon Filgueira[2]

[1]Department of Oceanography, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4R2;
[2]Marine Affairs Program, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4R2

Big Data consisting of large data sets requiring specialized handling and analysis have become central to fish farming in recent years. Similar to precision agriculture, precision aquaculture involves sensor networks to track cultured animals and their environment. The advent of wireless sensors to determine ocean variables such as oxygen and temperature have led to prolific amounts of data at each fish farm as well as collectively at multiple farm sites. In addition, novel fish tags to detect movement and additional variables such as heart rate provide data on fish health and welfare. These sources of information can provide input to farm management decisions which are dependent on fish condition, including feeding, stocking, harvesting, and pathogen treatment. We conducted studies utilizing both environmental sensors and fish tags at salmon farms in Nova Scotia, in collaboration with Cooke Aquaculture. In addition to using sensors at the cages, we undertook an oceanographic study of the inner continental shelf using an unmanned underwater vehicle (Slocum glider) to characterize 3D oxygen and temperature in the water column which influence water quality in coastal bays. Oxygen content is characterized by several scales of variation which at the farm level include tides, cage position, and fish respiration, while at the larger scale wind-based upwelling affects oxygen content of water advected into bays. Simulation of these effects with circulation models indicate that as the oceans warm and oxygen content declines, risk of hypoxia to fish may increase. Prediction of poor water quality through either mechanistic or big data driven modelling and apps will provide decision support tools for further farm management capability.

Longitudinal Dissolved Oxygen Patterns in Atlantic Salmon Aquaculture Sites in British Columbia, Canada

Jaewoon Jeong[1,], Babafela Awosile[1, 2], Krishna K. Thakur[1], Henrik Stryhn[1], Diane Morrison[3], Mykolas Kamaitis[3], Raphaël Vanderstichel[1, 4]

[1]Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, Canada [2]Texas Tech University, Amraillo, Texa, USA [3] MOWI Canada West, Campbell River, British Columbia, Canada [4]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Long Island University, Brookville, NY, USA

Dissolved oxygen (DO), an important water-quality parameter required to support aquatic life, is a critical factor for determining the general biological health of the aquatic ecosystem. The concentration of DO is a critical factor in determining salmon growth and welfare. This study used longitudinal DO concentration, recorded hourly from 21 aquaculture sites in four areas in British Columbia, Canada, between 2015 and 2017. The measurements were evaluated based on the recommended DO concentrations for protection of salmonids from hypoxia. Using a two-stage time-series analysis, we described variations in DO concentrations measured over the study period and their associations with environmental factors. Based on the water quality criteria for DO concentration, 42.3, 56.5, and 1.2 % of the hourly DO data from the overall 21 aquaculture sites were 'optimal', 'sub-optimal', and 'stressed', respectively. The frequency of hypoxic episodes differed substantially among seasons, aquaculture sites and even among cages within a site. Also, it was found that the effects of environmental variables on DO concentration have markedly different patterns depending on the season. The time-series regression model results showed dominant associations of temperature and moderate associations of wind speed and remotely-sensed (sea surface) estimated absorption due to phytoplankton with DO concentration. Describing DO measurements at these aquaculture sites provided an understanding of the biological state and how much they deviated from the recommended DO concentrations, as well as provide baseline information for future water resource planning, including continued and improved water quality monitoring.

A Proposed Improvement in Real-Time Monitoring of Cause-Specific Mortality and Losses in Industrial Salmon Farming

Paul J. Midtlyng[1], Arnfinn Aunsmo[1], David Persson[1], Marit Stormoen[1]

[1]Clinical Fish Health Group, Institute of Production Animal Medicine, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Oslo.

Current academic research on mortality and losses in salmon farming are increasingly focusing on pathology, reduced fish welfare and molecular evidence for the presence of pathogens. For the operational improvement of health, well-being and survival of the fish, however, rapid and reliable knowledge on what caused the illness or mortality are essential. We have therefore designed a new and improved system for classification and real-time monitoring of mortality and losses in industrial salmon culture, that seeks to avoid the recording of convenience (causally ambiguous) categories and to maintain high specificity on population level. The system focuses on the underlying cause of death as the principal variable to monitor daily. The classification code has a hierarchical structure with three levels: six main causal categories, several causal subcategories at level 2, and specified causes of death at level three. The hierarchy allows both entry and reporting of data at all three levels, including recording of unspecified causal categories at level one and two. Establishing a uniform code, allows to standardize monitoring outcomes between sites within a company, and by anonymizing the data also between companies, regions, and finally also between countries. A uniform code will enable the fish farmer to monitor the total effect of the underlying causes, both in biological and monetary terms. In this way it will be possible to target limited resources in the direction of losses with the highest impact and prioritize interventions with the highest pay-back. The "International Classification of Disease" (ICD) code in human medicine has served as a model for designing the system we propose. The ICD was established more than 120 years ago and has demonstrated its operational feasibility and sophistication for setting priorities and allocating resources for improving human health world-wide. Secondly, the classification of mortality in humans shows that health and disease information originating from diverse sources, (named "Verbal Autopsy") can be used to monitor mortality with acceptable specificity on population level. We think that the concept of mortality monitoring based on diversified sources of information will be equally applicable to industrialized aquaculture.

Fishing for Answers: Using Big Data Analytics to Predict and Manage Environmental Risks at BC Salmon Farms

Kirstyn N. Nygren[1] and Liam E. Peck[1]

[1]Grieg Seafood British Columbia, Environmental Monitoring Department, Campbell River BC V9W 5P7

Grieg Seafood BC Ltd, in collaboration with Scoot Science, have developed a start-of-the-art ocean monitoring platform designed to provide Grieg a unique opportunity to collect and observe real-time data on ocean environmental conditions to better predict ocean trends and reduce exposure to marine risks. The Environmental Monitoring Department at Grieg Seafood has designed and implemented one of the most robust harmful algal monitoring programs in the world, with more than 1.7 million data points on multi-dimensional ocean conditions and harmful algal presence collected annually. All this data seamlessly flows into Grieg's monitoring platform, SeaState, which gives salmon farmers improved oceanographic situational awareness and reduces Grieg's exposure to marine risks through rigorous data analysis and accurate subsurface ocean forecasting.

Precision Biometrics Data of Atlantic Salmon (*Salmo salar* L.) in Commercial Grow-Out Sea-Cages: Manual Sampling and Infrared Diode Frames Compared to Processing Plant

Cesar A. Lopez-Riveros[1,2], German E. Merino[3], Hector Flores-Gatica[3]

[1]Programa De Magíster En Acuicultura, Facultad De Ciencias Del Mar, Universidad Católica Del Norte, Larrondo, 1281, Coquimbo, Chile; [2]Vaki Chile Ltda., Parcela 53, Loteo El Mirador S/N, Puerto Varas, Chile; [3]Departamento De Acuicultura, Facultad De Ciencias Del Mar, Universidad Católica Del Norte, Larrondo, 1281, Coquimbo, Chile

One of the critical challenges that the global salmon farming industry will confront when upscaling production is accurate biomass control. Commercial salmon farming requires a significant level of certainty regarding fish count, average weight measurement, live weight distribution, and other production indicators. A reliable control system for assessing the biomass of farmed Atlantic salmon is essential for sustainable and cost-effective precision aquaculture. A study was done in four production sea-cages in a Chilean Atlantic salmon marine grow-out farm to estimate the average weight and frequency distribution utilizing the VAKI Biomass Daily® diode frames as an alternate technology to manual weight measurement. From post-smolt reception to fish harvest, diode frames were put in each sea-cage in a secure position for 15 months. There were no significant changes in length or average weight between manual sampling and frame estimate. The mean degree of accuracy for the average weight estimation was 98.83% for the frames utilized in the four sea cages. The diode frames also achieved a high degree of precision in predicting the frequency distribution of fish. There were no statistically significant variations between the distribution variances of the diode frame measurements and the distribution variances of the fish received at the fish processing facility (fpf). The maximum difference between the average weight calculated by the frames and the average weight of the fish received in the processing facility was 2.4%, with 99.66% being the highest accuracy with only 19 g of difference. We determined that diode frames might replace manual weight assessments with greater reliability for growth monitoring and production management. To assure the optimal performance of the diode frames in terms of accuracy and precision for future commercial scale validations in the salmon farming business, the development of a standard best practice manual is necessary.

Connect, Digitize and Exchange of Information: Creating Better Producer to Consumer Outcomes in Aquaculture
What Can We Learn from the Feedlot Industry?

Luis O. Burciaga-Robles[1], Mathew L. May[1], Matthew Quinn[1], Calvin W. Booker[1], Tye Perret[1], and Kee Jim[1]

[1]Feedlot Health Management Services by TELUS Agriculture, Okotoks, Alberta, Canada.

With the widespread use of technology to collect data in animal and agricultural production systems, the industry has become data rich, but information poor. This has increased the need for individuals with capabilities to synthesize, summarize, interpret the data and put information to work. However, this human resource is scarce, as it requires a multidisciplinary approach and advanced training. At Feedlot Health Management Services, we provide consulting services to feedlots and calf grower operations, with a yearly throughput of 1.7M of animals with individual records around the world. Data collected at the individual, lot, facility, and specific production system information collected using different technologies (from weigh scales to GPS and satellite images) has allowed us to improve the sustainability of our clients' operations, in addition to improving animal well-being of the cattle under our care and decreasing the carbon footprint of the industry. In this session we will cover some practical examples on how we have used technology and big data for generating hypothesis and using research to validate the biological, environmental and economic outcomes of our clients. As part of TELUS Agriculture, (a major Canadian and international communications company) FMHS has been able to leverage the connectivity, technology innovation and digitization of the food supply chain. This includes the significant challenge of meeting increasing consumption needs while improving the quality, safety and sustainability of the food supply.

What does Big Data Mean for Aquaculture? Current Development, Limitations, and Future Challenges

Pablo Valdes Donoso [1,2]

[1]Faculty of Veterinary Medicine, Department of Clinical Sciences. University of Montreal. Saint-Hyacinthe, QC, Canada. [2]Platform of AI for Agri-food and Animal Health (PIAAS). Faculty of Veterinary Medicine. University of Montreal. Saint-Hyacinthe, QC, Canada.

Big data refers to a large volume of information collected at a high velocity and composed of various features. The use of big data paired with an improvement in computing power and cloud storage growth is facilitating the development of complex algorithms that may assist with various human activities, including food production. For example, pairing agriculture and environmental data can be used to train and test algorithms that predict crop yields, which may help optimize resource allocation and promote economic and environmental sustainability of production. The predictive capacity of these algorithms strongly depends on data quality and quantity. Aquaculture operations generate and store data from several sources. Depending on the scale and type of aquaculture production systems, data are commonly collected in handwritten logbooks, Excel files, and production software. In addition, nearly real-time sensor data can be used to evaluate tasks, such as specific times of feeding. Despite the rich environment for data generation in many aquaculture systems, producers do not integrate the information in common repositories, so they cannot visualize nor evaluate their data comprehensively. In this talk, I discuss the recent research and development of aquaculture big data and its possible applications for the development of AI algorithms, as well as its limitations and future challenges.

Big Data Analytics to Support Evidence-Based Strategic Planning in Salmon Farming in Chile

Francisco Bravo[1], Patricio Bernal[1], Rodrigo H. Bustamante[2], Scott Condie[3], Ramon Filgueira[6], Jonathan Grant[6], Bec Gorton[3], Mike Herzfeld[3], Daniel Jimenez[4], Fernando O. Mardones[5], Farhan Rizwi[3], Jatinder P.S. Sidhu[2], Andy D.L. Steven[2], Ingrid van Putten[3]

[1]Fundación CSIRO Chile Research. Apoquindo 4700, Las Condes, Santiago, Chile; [2]CSIRO Oceans and Atmosphere, University of Queensland BioSciences Precinct (QBP), 306 Carmody Rd, St Lucia, Brisbane, QLD 4072, Australia; [3]CSIRO Oceans and Atmosphere, GPO Box 1538, Hobart, Tasmania 7001, Australia; [4]Instituto Tecnológico del Salmón, Av. Juan Soler Manfredini 41, OF 1802, Puerto Montt, Chile; [5]Escuela Medicina Veterinaria, Pontificia Universidad Católica de Chile, Santiago, Chile; [6] Department of Oceanography, Dalhousie University, Halifax, NS, Canada, B3H 4J1.

Big data analytics can contribute to more accurate planning and operation of open-cage marine fish farms, leading to better prevention of diseases and mortality, reduced use of antibiotics or other chemical treatments, and reduced local and far field environmental impacts. In this presentation we explore different models and tools built in conjunction by epidemiologist, economists, and oceanographers to gain understanding and support strategic planning (not day-to-day operation) at marine open-cage fish farms. Models operates at different spatial and temporal scales from individual farm and farming cycles to spatially aggregated scales of planning (e.g. bay, group of leases, etc.). The integration of mechanistic and probabilistic modelling has allowed us to explore interactions and dependencies between fish farmed practices (e.g. cultured densities, coordination among farms, oxygen supplementation system, etc.) and the broad range of environmental conditions under which fish farms operate (temperature, salinity, hydrodynamic connectivity, oxygen availability, etc.), with fish welfare and bioeconomic performance. Special emphasis will be given to the bacterial pathogen *Piscirickettsia salmonis*, leading cause of mortality and the main target of antimicrobials used in the Chilean industry, and to the effects of oceanographic conditions fish behaviour (and ultimately welfare) using highly resolved positioning datasets. Examples of Chile and Canada will be considered.

Wednesday, September 7th
Main Ballroom

Modeling Applications in Aquatic Animal Health:
Ian Gardner/Bradley Richardson

1:45 PM **Romero**: Overview of a Simulation Framework for Evaluation of Mitigation Strategies to Reduce Waterborne Spread of Viral Diseases in Marine Aquaculture

2:00 PM **Grant**: Farming in Natural Systems (FINS): A Provincial-wide Modelling Tool for Environmental and Infectious Disease Risks in Farmed Atlantic salmon and Oysters in Nova Scotia, Canada

2:30 PM **Delphino**: Cost-effectiveness of longitudinal surveillance for *Piscirickettsia salmonis* using qPCR in Atlantic salmon farms (*Salmo salar*) in Chile

2:45 PM **Romero**: Use of Simulation Modelling for Cost-effectiveness Analysis of Infectious Disease Management Options in Marine Salmon Aquaculture

3:00 PM Refreshments

3:15 PM **Rivera**: Epidemiological Genetic Model through Bioassays with Genetically Improved Families in Atlantic Salmon (*Salmo salar*) in the Presence of SRS (*Piscirickettsia salmonis*)

3:30 PM **Gaete-Carrasco**: Identification of Risk Factors Associated with Piscirickettsiosis Outbreaks in *Salmo salar* in Chile

3:45 PM **Gardner**: Use of a Waterborne-Spread Model of Infectious Salmon Anaemia virus (ISAV) to Inform Management Decisions Following Detection of a Pathogenic Strain in a Newfoundland Salmon Hatchery

4:00 PM **Gardner**: Closing Remarks/Discussion

Overview of a Simulation Framework for Evaluation of Mitigation Strategies to Reduce Waterborne Spread of Viral Diseases in Marine Aquaculture

João F. Romero[1], Ian A. Gardner[1], Sonja Saksida[1], Derek Price[2], Kyle A. Garver[3], Krishna K. Thakur[1]

[1]Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island C1A 4P3, Canada; [2]Aquaculture Environmental Operations Management Division, Fisheries and Oceans Canada, Canada; [3]Pacific Biological Station, Fisheries and Oceans Canada (DFO), Nanaimo, British Columbia V9T 6N7, Canada.

The COVID-19 epidemic has showcased the utility of infectious disease modelling to predict spatial and temporal spread of the SARS-CoV-2 virus and to evaluate the effects of interventions. In this regard, simulation models (e.g., compartmental models such as the SEIR (Susceptible, Exposed, Infected, Recovered)) have been used to forecast hospitalizations and fatalities, thereby informing public health decisions, including vaccination and masking. However, in the aquaculture context there are limited published examples of applications of simulation models in infectious disease epidemiology. In this presentation, we provide an overview of the components of a waterborne disease spread model, developed by our team in collaboration with the Danish Technical University, for within- and among-site spread of economically important pathogens. Briefly, our model uses an SEIR component to simulate pathogen spread within net-pens and an agent-based model (ABM) for spread among net-pens within and between sites, with seaway-distance driving the among-site transmission. Seaway distance among marine sites is used as a proxy for hydrodynamic connectivity, however the model can be informed by particle tracking data from hydrodynamic models, incorporating pathogen survival behavior and the effect of currents, winds, tides, and freshwater flow. Our model is stochastic and spatiotemporally explicit, and output parameters can be tabulated or shown in graphs for ease of communication of results. Components within the model allow for the evaluation of alternate surveillance, detection, vaccination, and culling (removal) strategies at the pen and farm level. Model code is written in R (www.r-project.org) and is freely available (https://github.com/upei-aqua/DTU-DADS-Aqua). In the second part of the presentation, we provide two examples of model applications for infectious hematopoietic necrosis virus (IHNV) and infectious salmon anaemia virus (ISAV) in farmed Atlantic salmon (*Salmo salar* L.) populations on the west and east coasts of Canada, respectively. We consider scenarios with modelling of IHNV spread in farmed salmon with interventions such as vaccination; also, in the context of marine spatial planning, we assess how readily ISAV might spread within and among bay management areas (BMA) separated by varying distances. Sensitivity analyses are presented as they are critical to assess the effects of changes in model input parameters. Model outputs provide vital information to assist the aquaculture industry and regulatory agencies in supporting decision-making for siting of marine production sites and designing protocols to enhance disease mitigation efforts. The model can be adapted to other farmed aquatic species and waterborne viral and bacterial pathogens.

Farming in Natural Systems (FINS): A Provincial-Wide Modelling Tool for Environmental and Infectious Disease Risks in Farmed Atlantic Salmon and Oysters in Nova Scotia, Canada

Jon Grant[1*], Ramon Filgueira[2], Ian Gardner[3], Gregor Reid[4], Leah Lewis-McCrea[4] and Joao Ferreira[5]

[1]Dept. of Oceanography, Dalhousie University, Halifax NS B3H 4R2; [2]Marine Affairs, Dalhousie University, Halifax NS B3H 4R2; [3]Dept. of Health Management, Atlantic Veterinary College, University of Prince EdwardIsland, Charlottetown PEI C1A 4P3; [4]Centre for Marine Applied Research, Dartmouth NS B2Y 4T5; [5]Longline Environment, 64 New Cavendish Street, London, W1G 8TB

The development of ecosystem models in coastal marine waters containing fish farms has matured to the extent that predictive modelling can be applied to dissolved and particulate waste dispersal, benthic deposition and diagenesis, and the spread of pathogens. These model results are critical to both siting and ongoing monitoring of fish farms. Nonetheless, systematic application of these models to fish farm management is less common, i.e. modelling capability and results are neither widely available or user-friendly. Drawing on decades of model development, we have developed a Windows-based application known as FINS (Farming in Natural Systems) to integrate multiple carrying capacity criteria into aquaculture decision support (Figure 1). FINS is effectively a GIS framework allowing a marine spatial planning approach to management in terms of cage density and location. The following models are included for carrying capacity: (a) Particulate deposition and mapped footprints, (b) sediment diagenesis including sulfides, (c) nutrient plumes, (d) disease risk, and (e) chlorophyll depletion (bivalve culture). Due to the overall forcing of marine environments with circulation via wind and tides, numerical circulation models are the foundation of ecosystem models. We utilize FVCOM as the physical platform to enable diffusion-advection of particles and solutes, and include its particle tracking module to create numerical experiments with respect to non-conservative pathogens such as sea lice. Although all of the component models in FINS are fully developed and groundtruthed, several steps remain including the establishment of carrying capacity thresholds, and integration into the software platform. Among the models, disease risk requires further consideration of epidemiological criteria associated with location of multiple farm sites. A targeted project of the Atlantic Fisheries Fund and the province of Nova Scotia will lead to completion of the FINS framework and extension to multiple sites. Compilation of GIS, bathymetry, river flow, wind fields, and other data is proceeding as input to application to multiple sites in Nova Scotia based on aquaculture development priorities. A calibrated FVCOM model is required for each new location. Although each case study is site-specific, FINS can be developed for any site worldwide.

Cost-effectiveness of Longitudinal Surveillance for *Piscirickettsia salmonis* Using qPCR in Atlantic Salmon Farms (*Salmo salar*) in Chile

Marina K. V. C. Delphino[1], Fernando O. Mardones[2], Joaquin N. Heise[2], Alicia Gallardo[3], Daniel Jimenez[4], Andrea Peña[5], Marco Rozas-Serri[5], Ian A. Gardner[1]

[1]Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island C1A 4P3, Canada; [2] School of Veterinary Medicine, Pontifical Catholic University of Chile, Santiago, Chile; [3] Servicio Nacional de Pesca y Acuicultura, Valparaíso, Chile; [4] Instituto Tecnológico del Salmón, Puerto Montt, Chile; [5] Pathovet Laboratory Ltd, Puerto Montt, Chile.

Costs of diagnostic testing including sample collection, sampling frequency and sample size are an important consideration in the evaluation of the economic feasibility of alternative surveillance strategies for detection of infectious diseases in aquatic animals. In Chile, *Piscirickettsia salmonis* is the primary reason for antibiotic treatments in farmed Atlantic salmon. In 2012, a surveillance and control program for piscirickettsiosis was established with an overall goal of reducing antibiotic use. The objectives of the present study were to: (a) estimate the pen-level and farm-level sensitivities and specificity of qPCR testing for *P. salmonis* in Atlantic salmon for sample sizes between 5 and 30 fish, and (b) assess the cost-effectiveness of sampling frequency, sampling strategy effect (random versus risk-based sampling) and sample size to achieve 95% confidence of detecting *P. salmonis* at the netpen and farm levels using a validated qPCR test. We developed a stochastic Monte Carlo simulation model in @RISK (Palisade, Raleigh, North Carolina) that incorporated variability in test accuracy, within-pen prevalence, and sampling costs in a three-level hierarchical model (fish, netpen and farm) to reflect the population structure of Atlantic salmon farmed in marine sites in southern Chile. Our findings indicated that the current piscirickettsiosis surveillance program based on risk-based sampling of 5 moribund or dead fish from 2 to 3 netpens is cost-effective and gives a high probability (>95%) of detection of *P. salmonis* in Atlantic salmon farms in Chile at both the netpen and farm levels. Results from this study should incentivize salmon farmers to establish cost-effective strategies for early detection of *P. salmonis* infection and the methods used in this study could be applied to other highly infectious diseases of farmed fish.

Use of Simulation Modelling for Cost-Effectiveness Analysis of Infectious Disease Management Options in Marine Salmon Aquaculture

João F. Romero[1,2], Ian A. Gardner[1,2], Pablo Valdes-Donoso[3], David Groman[4], Krishna K. Thakur[1,2]

[1]Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island C1A 4P3, Canada; [2]Centre for Veterinary Epidemiological Research, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island C1A 4P3, Canada; [3]Département de sciences cliniques, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec J2S 2M2, Canada; [4]Aquatic Diagnostic Services, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island C1A 4P3, Canada

Infectious diseases have caused substantial production and economic losses in global salmon aquaculture due to fish mortality, reduced growth rates, restrictions on trade, and costs of outbreak prevention and mitigation. To prevent pathogen spread among farmed salmon populations, regulatory agencies outline disease management and contingency plans, and licensing requirements that impose restrictions on production capacity and define the spatial arrangement of marine salmon farms. However, comparison of alternatives in disease prevention and management strategies is difficult to achieve due to ethical or logistic constraints. This study aimed to assess the cost-effectiveness of disease outbreak mitigation strategies considering different salmon aquaculture capacity levels in Atlantic Canada. Therefore, we used an epidemiological model to simulate the waterborne spread of an economically relevant viral pathogen – infectious salmon anaemia virus (ISAV) – among marine salmon farms and disease prevention and mitigation protocols implemented in the region. Different scenarios were explored, reflecting varying percentages of active salmon farms in the region (40%, 65%, and 65% of licensed farms) and two distinct depopulation approaches: (i) pre-emptive depopulation of all net-pens in ISAV-diagnosed farms and (ii) depopulation of ISAV-infected net-pens only. Model outcomes included the number of surveillance visits to marine farms, number of ISAV-infected net-pens and farms, and number of culled fish. Each scenario quantified the magnitude of hypothetical ISAV outbreaks, and ISAV surveillance and depopulation interventions. Direct costs of ISAV prevention, surveillance and detection, and depopulation of infected stocks were estimated from model outcomes. Simulation results indicated that the pre-emptive depopulation of all net-pens in ISAV-positive farms was more effective in limiting viral spread, particularly with higher percentages (90% vs. 65% vs. 40%) of active farms in Bay Management Areas (BMAs). Nevertheless, this depopulation approach resulted in higher direct costs of outbreak mitigation. Moreover, the close proximity of farms contributed to the waterborne transmission of ISAV, indicating that production capacity considerations should be consolidated with spatial planning of farm sites (both within and between BMAs). This study quantified potential economic implications of ISAV outbreak mitigation in salmon aquaculture. The model and approach presented herein can be used to support decision-making in the planning of aquaculture operations, licensing of additional farms, and disease outbreak management.

Epidemiological Genetic Model Through Bioassays with Genetically Improved Families in Atlantic Salmon (*Salmo salar*) in the Presence of SRS (*Piscirickettsia salmonis*)

Luis Rivera[1], Alejandro Alert[1], Rodrigo Torrijo[1], Daniel Rubio[2], Natalia Zimin-Veselkoff[2] and Fernando O. Mardones[2]

[1]Hendrix Genetics Aquaculture Chile, Pucon, Chile [2]School of Veterinary Medicine, Pontificia Universidad Católica de Chile

Piscirickettsia salmonis is the etiological agent of Salmonid Rickettsial Septicaemia (SRS), the most important infectious disease affecting farmed salmon in Chile. There is an urgent need to develop non-pharmaceutical technologies such as genetic improvements to prevent and control this infection. To evaluate the benefit of genetics we collected information from 281 families from 10.800 individual Atlantic salmon (Salmo salar) from five bioassays between 2015 and 2020. From the studies we estimated epidemiological parameters including the latency, subclinical, incubation, mortality and recovery rates which where integrated into a compartmental epidemiological model for SRS. From the model, it was determined that genetics can effectively modulate the shape of the epidemic curve for SRS including changes in the onset of clinical signs and severity. After exposure from intra-peritoneal injection (pi) with *P. salmonis*, incubation period was determined as 12-15 days pi where infectiousness started at day 6 pi. The duration of the infectiousness period varied between 10 to 14 days pi. The estimated reproduction number was estimated in 1.67 (95% CI 1.58-1.78). The study is unique in integrating genetics and epidemiology to evaluate the value of non-pharmaceutical interventions in farmed salmon.

Identification of Risk Factors Associated with Piscirickettsiosis Outbreaks in *Salmo salar* in Chile

Alvaro Gaete-Carrasco[1], Carla Rosenfeld[2], and Marcela Lara[1]

[1]National Fisheries and Aquaculture Service, Department of Animal Health, Victoria No. 2832, Valparaiso, Chile; [2]Institute of Veterinary Preventive Medicine, Austral University of Chile, Isla Tejas Campus S/N, Valdivia, Chile.

Piscirickettsiosis is the infectious disease that causes the highest mortality in marine salmon farms in Chile. Since 2013, Chile has maintained a health program for the surveillance and control of the disease. Identifying risk factors and quantifying their influence on the disease will help to better characterize and improve control measures. The aim of this work was to identify the risk factors associated with piscirickettsiosis outbreaks in *Salmo salar* in Chile. A total of 492 production cycles from 2013 to 2019 were included in this study. On average the median time elapsed before issuing of the alert category (farm ≥ 1 cages and ≥ 0.35% per week with SRS mortality) was 38 weeks (95% CI: 36–41), the spring seeding season was found to significantly prolong the time to first issuing of alert category with a median time of 46 weeks (95% CI: 32-49), and the starting of treatment with ≥0.03% mortality associated to SRS was significantly reduce the time to issuing of alert category with a median time of 26 weeks (95% CI: 24-28). Our model identified that farms that begin to apply antimicrobial treatment with a mortality rate of SRS <0.01% per week and fish that are seeded in winter delay the issue of the Alert category to the 45th week of the production cycle. This study contributes to understanding outbreaks of this disease and provides an epidemiological evaluation of the pathology within the framework of an official health program.

Use of a Waterborne-Spread Model of Infectious Salmon Anaemia Virus (ISAV) to Inform Management Decisions Following Detection of a Pathogenic Strain in a Newfoundland Salmon Hatchery

Ian A. Gardner[1], Joao F. Romero[1]

[1]Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island C1A 4P3, Canada.

This study addressed the risk question of whether stocking of Atlantic salmon (*Salmo salar* L.) smolts from a single hatchery in Newfoundland with a pathogenic ISAV genotype (HPR 19.11, North American) into one bay management area (BMA) would pose a risk of ISAV infection in 4 new marine sites in the BMA to farms in 2 adjoining BMAs. To answer the risk question, 2 sequential steps were completed: 1) a semi-quantitative ISAV infection assessment for ISAV introduction into the newly-populated marine cage sites using ISAV PCR data from hatchery testing, and 2) waterborne-spread simulation modelling at the recipient sites, assuming that ISAV infection was present in smolts at the time of stocking**.** The hatchery infection assessment showed that the probability that smolts were infected with pathogenic ISAV was very low. Although ISAV was originally detected at a prevalence of 10% in one recirculation system (which was depopulated), subsequent PCR testing of 875 smolts yielded negative results indicating that the populations in the 5 remaining systems (source fish to be moved to sea) were likely non-infected but if infection was present, the prevalence would be no more than 6 infected fish per thousand with 99% certainty. Enhanced biosecurity practices at the source hatchery were put in place after initial detection of ISAV in smolt building 2 to further mitigate the risk of transmission to other systems, if the pathogenic genotype was still present. The risk that the transferred population of smolts would experience clinical signs and elevated mortality with the pathogenic strain of ISAV soon after stocking into the marine sites was considered to be low by veterinarians because of the low virulence of the genotype. The waterborne ISAV-spread model (https://github.com/upei-aqua/DTU-DADS-Aqua) showed that if ISAV infection was introduced into a single net-pen of salmon at each of the 4 sites in the BMA, infection would spread among the 4 sites (separated by 5 to 8 km) but not to 2 adjoining BMAs (15 km and 50 km distant). This conclusion was based on the seaway distances between sites within 1 km of each other having the highest risk, sites that are separated by 10 to 15 km having very low risk and sites greater than 30 km having a zero or negligible risk. After transfer, it was proposed that strengthened ISAV protocols be implemented at the 4 new-populated marine sites to enhance timely detection of ISAV spread and investigation of causes of increased weekly mortality, if that was to occur.

Wednesday, September 7th
Breakout Room A

Zebrafish/Lab Animal Models: Mike Kent

10:45 AM **Kent**: Common Laboratory Diets Differentially Impact Fitness, Health, and the Gut Microbiome in Zebrafish (Danio rerio)

11:00 AM **Schuster**: Use of Occupancy Modeling to Understand Pathogen Diagnostic Efficacy in a Zebrafish Facility

11:15 AM **Rakus**: Tilapia Lake Virus Infection in Zebrafish: A Model to Study Antiviral Immune Response and Host-Pathogen Interaction in Fish

11:30 AM **Peterman**: Enhanced Immunity and Gut Nccrp-1+ and Mpeg-1+ Cell Populations in rag1-/- Zebrafish

Common Laboratory Diets Differentially Impact Fitness, Health, and the Gut Microbiome in Zebrafish (*Danio rerio*)

Michael L. Kent[1], Michael J. Sieler Jr[1], Kristin Kasschau[1], Thomas J. Sharpton[1,2]

[1]Department of Microbiology, Oregon State University, Corvallis, Oregon, USA; [2]Department of Statistics, Oregon State University, Corvallis, Oregon, USA

There is now an established history of the use of zebrafish in biomedical and fish biology research, along with an increasing popularity in microbiota-targeted studies and research on infectious diseases using the model. There is a lack of consensus regarding zebrafish husbandry practices involving type of diet. This is a concern because diet influences gut microbiota composition, and may influence physiological variation and susceptibility to infectious diseases. It is not known whether standard zebrafish diets manifest in distinctive gut microbiota composition as well as fitness, nor is it known how diet influences pathogen burdens in zebrafish. Whereas zebrafish are maintained in a laboratory setting, certain pathogens are remarkably prevalent in these research fish. Pertaining to this study, we are particularly interested in *Mycobacterium chelonae* because infections are very common in zebrafish and it is transmitted by the oral route of exposure. We conducted experiments with three zebrafish laboratory diets; two commercial diets Gemma and Ziegler + *Spirulina)* and a defined diet (Watts). Fish fed the Zielger diet showed significantly great weight and condition factor, driven by large females. We included an experiment in which fish were experimentally exposed to *M. chelonae* by intraperitoneal injection, and the fish from the Zielger group showed a higher prevalence of infection compared to the other two diet groups when examined at 15 wk post exposure. Microbiome analysis showed that Watts fed fish had the lowest alpha diversity scores compared to Gemma and Ziegler, and body condition score was negatively associated with alpha diversity score in the Ziegler group. Watts fed fish had the highest beta diversity scores and Gemma fed fish had the lowest. The microbiome composition of ZIRC fed fish did not differ after 3 months feeding, but at 6 months they stratified by body condition score. Additionally, 421 bacterial taxa were differentially abundant across diets. Our results demonstrate that variation in husbandry practices relating to diet impacts the successional development of the zebrafish gut microbiome. Collectively, our results indicate researchers should carefully consider the role of diet in their zebrafish in their research, particularly with disease and microbiome studies.

Use of Occupancy Modeling to Understand Pathogen Diagnostic Efficacy in a Zebrafish Facility

Corbin J. Schuster[1], Michael L. Kent[2,3], James T. Peterson[4], and Justin L. Sanders[3]

[1]The Zebrafish International Resource Center, University of Oregon, Eugene, OR 97403, USA; [2]Department of Microbiology, Oregon State University, Corvallis, OR 97331, USA; [3]Department of Biomedical Sciences, Oregon State University, Corvallis, OR 97331, USA; [4]U.S. Geological Survey, Oregon Cooperative and Wildlife Unit, Department of Fish and Wildlife, Oregon State University, Corvallis, OR 97331, USA

Detecting the presence of important pathogens within a host and its environment is critical to understanding the dynamics that influence a pathogens ability to persist, while accurate detection is also essential for implementation of effective control strategies. *Pseudoloma neurophilia* is the most common pathogen reported in zebrafish (*Danio rerio*) research facilities and although this microsporidium presents largely asymptomatic, it may cause emaciation and skeletal deformities. Moreover, it causes non-protocol induced variation in research. The only assays currently available for *P. neurophilia* entail lethal sampling, often requiring euthanasia of the entire population for accurate estimates of prevalence in small populations. Thus, we first developed a nonlethal assay utilizing environmental screening of tank water using water filtration coupled with digital PCR (dPCR). Using an in vivo lab study, we then implemented a multi-state occupancy model to predict the probability of detection in tank water under different flow regimes and pathogen prevalence. Occupancy models are frequently utilized in wildlife ecology to quantify presence/absence of a species, but this is the first implementation to understand disease status in a zebrafish vivarium. Importantly, the occupancy model revealed that samples collected in static conditions were more informative than samples collected from flow-through conditions, with a probability of detection at 80% and 47%, respectively. There was also a positive correlation with the prevalence of infection in water and prevalence in the fish. Overall, the occupancy model revealed several key dynamics about using molecular diagnostic tests which could be extended to aquaculture facilitates beyond zebrafish.

Tilapia Lake Virus Infection in Zebrafish: a Model to Study Antiviral Immune Response and Host-Pathogen Interaction in Fish

Miriam Mojzesz[1], Magdalena Widziolek[1], Mikolaj Adamek[2], Piotr Podlasz[3], Tomasz K. Prajsnar[1], Niedharsan Pooranachandran[1], Anna Pecio[4], Anna Michalik[5], Win Surachetpong[6], Jean-Pierre Levraud[7], Pierre Boudinot[8], Magdalena Chadzinska[1], and Krzysztof Rakus[1]

[1]Department of Evolutionary Immunology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Krakow, Poland; [2]Fish Disease Research Unit, Institute for Parasitology, University of Veterinary Medicine, Hannover, Germany; [3]Department of Pathophysiology, Forensic Veterinary Medicine and Administration, Faculty of Veterinary Medicine, University of Warmia and Mazury, Olsztyn, Poland; [4]Department of Comparative Anatomy, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Krakow, Poland; [5]Department of Invertebrate Development and Morphology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Krakow, Poland; [6]Department of Veterinary Microbiology and Immunology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand; [7]Macrophages et Développement de l'Immunité, Institut Pasteur, CNRS, Paris, France; [8]University of Paris-Saclay, INRAE, Jouy-en-Josas, France

Tilapia lake virus (TiLV; genus: *Tilapinevirus*, family *Amnoonviridae*) is as an etiological agent of highly contagious and emerging disease of wild and farmed tilapia species including Nile tilapia (*Oreochromis niloticus*), the third main cultured freshwater fish species in the world. TiLV causes a high mortality of infected fish and has a very negative impact on tilapia aquaculture worldwide. The virus was detected in many countries of Asia, Africa, South and North America, including the main tilapia producing countries. We recently established a zebrafish (*Danio rerio*) model to study immune response and host-pathogen interactions during TiLV infection. Upon TiLV infection, increased viral load was demonstrated in the brain, eye, spleen, kidney and liver of zebrafish. Interestingly, we demonstrated that TiLV persists at a high level in the zebrafish brain for at least 3 months, when the virus is already not detectable in the periphery organs. We also observed histopathological abnormalities in the brain of TiLV-infected zebrafish larvae. TiLV induced up-regulation of the expression of genes encoding proteins involved in type I interferon (IFN) response such as pathogen recognition receptors involved in sensing of viral dsRNA (*rig-I, tlr3, tlr22*), transcription factors (*irf3, irf7*), type I interferon (*infφ1*), and antiviral protein (*mxa*) as well as pro-inflammatory cytokines (*il-1β, tnf-a, il-8, ifnγ1-2*) in the spleen, kidney and brain of adult fish and in the zebrafish larvae. We also demonstrated the protective role of the recombinant zebrafish IFNφ1 on the survival of zebrafish larvae during TiLV infection. Moreover, in the brain of infected adult zebrafish, up-regulation of the expression of macrophages/microglia markers (*csf1r* and *cd68*) was observed, while confocal microscopy analysis showed the changes in microglia morphology from a resting ramified state in mock-infected to a highly ameboid active state in TiLV-infected larvae from *Tg(mpeg1.1:mCherryF)ump2* transgenic line. Furthermore, in adult zebrafish, TiLV induced sickness behavior manifested as reduced food intake which led to the weight loss, as well as decreased locomotor activity associated with the presence of infected fish near to the bottom zone of aquaria. All together our data indicate the importance of type I IFN response against TiLV infection and reveal that TiLV infection in zebrafish is a good model to study the neuroinflammation, microglia activation and sickness behavior during virus infection.

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Enhanced Immunity and Gut Nccrp-1+ and Mpeg-1+ Cell Populations in *rag1*$^{-/-}$ Zebrafish

Ann E. (Beth) Peterman[1] and Lora Petrie-Hanson[1]

College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762

Mucosal surfaces provide fish with a first line of defense against threats in their environment. At gut surfaces, host nutrition and immunity, and the microbiome combine to impact pathogen prevalence, fish growth and fish health. Immune stimulants can provide predictable protection. The gut has the largest aggregation of immune cells in the vertebrate body. Zebrafish gut associated immune cells include macrophages, neutrophils, eosinophils, dendritic cells, natural killer cells, and several populations of innate lymphocyte-like cells. *Rag1-/-* zebrafish were exposed to phosphate buffered saline (PBS) or beta-glucan by intracoelomic injection and after four weeks, gut samples were collected to analyze cell type distribution and protein expression of Nccrp-1 (NCC cells), Mpeg-1 (macrophages/monocytes) and Nitr9 (NK cells) by flow cytometry and western blot analyses. There were significantly more NCCs and macrophages in *rag1-/-* zebrafish gut tissue 4 weeks post beta-glucan injection when compared to PBS injected control tissues. There were significant differences in the relative protein expression of Mpeg-1 and Nitr9 after stimulation with beta-glucan. A separate group of *rag1-/-* zebrafish was gavage challenged with *Edwardsiella ictaluri* at 4 weeks post PBS or beta-glucan injection and survival and cell type distribution were analyzed. Following gavage bacterial challenge, *rag1-/-* zebrafish previously exposed to beta-glucan had significantly higher survival than *rag1-/-* zebrafish previously exposed to PBS and significantly more NCCs and macrophages in the gut. These findings demonstrate that resident gut NCCs and macrophages in *rag1-/-* zebrafish increase in number and undergo immune-modulation or training and contribute to increased survival after exposure to an enteric pathogen four weeks post immune-modulation. This study suggests that the TLR ligand β-glucan induced long-term immune function changes in innate immune cell populations in the *rag1* mutant zebrafish gut.

Wednesday, September 7th
Breakout Room A

Parasitology: Graham Rosser/Celene Slifka

1:00 PM **Nguyen**: A Morphological and Molecular Comparison of *Clinostomum Metacercariae* and Adults of the United States

1:15 PM **Slifka**: What's in the Water: Identifying *Planorbella trivolvis* and *Biomphalaria havanensis* DNA Using Real-Time qPCR

1:30 PM **Powell**: Overview and Future Research Directions of *Bolbophorus damnificus* Trematode Pathogenesis in Channel (*Ictalurus punctatus*) and Hybrid (Channel X Blue Catfish *Ictalurus furcatus*) Catfish

1:45 PM **Garcia**: Estimation of Genetic Parameters and Genetic Co-variation Between *Piscirickettsia salmonis* Resistance and Sea Lice (*Caligus rogercresseyi*) Susceptibility in Atlantic Salmon (*Salmo salar*) Using Genomic Information

A Morphological and Molecular Comparison of *Clinostomum* Metacercariae and Adults of the United States

Jonah A. Nguyen[1], Bradley M. Richardson[2], Ethan T. Woodyard[3], Justin M. Stilwell[3], Celene M. Slifka[1], Divya Rose[1], Chris McAllister[4], Thomas G. Rosser[1]

[1]Department of Comparative Biomedical Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762; [2]U.S. Department of Agriculture, Agricultural Research Service, Warmwater Aquaculture Research Unit, Thad Cochran National Warmwater Aquaculture Research Center, 141 Experiment Station Road, Stoneville, Mississippi, 38776 USA; [3]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762; [4]Science and Mathematics Division, Eastern Oklahoma State College, Idabel, OK, 74745, USA.

The genus *Clinostomum* is a common trematode infecting many species of freshwater fish and amphibians. The adult stage is found in the oral cavity or esophagus of predatory birds, the cercariae develop in freshwater snails, and the metacercariae, commonly known as "yellow grubs", encyst in freshwater fish or amphibians. Naming new species of *Clinostomum* is complicated by the lack of morphological data from metacercariae and the lack of molecular data for some pre-described species, the wide distribution of many *Clinostomum* species and their respective bird hosts, as well as the idea that metacercariae, although typically easier to collect than adults, are less reliable for morphologically distinguishing species compared to adults. Herein, a morphological and molecular comparison of *Clinostomum* metacercariae and adults is presented in order to better understand the utility of metacercariae morphology as a way of distinguishing species of *Clinostomum*. A total of 110 metacercariae belonging to 4 species that infect both fish and amphibian intermediate hosts were used: *Clinostomum marginatum, C. album* and 2 metacercariae of unknown identity (*C.* sp. 1 and *C.* sp. 2). *C.* sp. 1 macroscopically appears similar to *C. marginatum.* Similarly, *C.* sp. 2 is morphologically consistent with the description of *C. attenuatum* and was found in a known host of *C. attenuatum*. Metacercariae were recovered from the host species *Pimephales promelas*, *Ictalurus punctatus*, *Lithobates catesbeianus*, *Lithobates kauffeldi*, *Lepomis* sp., *Gambusia affinis*, *Menidia beryllina*, *Ameiurus natalis*, *Moxostoma erythrurum*, *Morone saxatilis*, *Hypentelium nigricans*, and *Perca flavescens*. A subset of the metacercariae were hologenophores with molecular data generated from the ribosomal internal transcribed spacer regions (ITS), cytochrome *c* oxidase subunit 1 (cox-1) and the large ribosomal subunit (28S). A total of 111 adult *Clinostomum* belonging to the species *C. album, C. caffare, C. marginatum,* and *C. poteae* and one of unknown identity (*C.* sp. 3) were measured. Adult *Clinostomum* were recovered from the host species *Ardea alba*, *Phalocrocorax auritus*, *Ardea herodias*, and *Pelecanus erythrorhynchos*. A subset of the adults were molecularly characterized using the markers ITS, cox-1, and 28S. Statistical analysis was performed using a modified linear discriminate analysis (LDA) to identify the most discriminatory morphological ratios for distinguishing between cogeners at each life stage analyzed. These results, coupled with molecular data, were used to quantify the efficacy of utilizing metacercariae and adults to distinguish *Clinostomum* species and clarify best practices for future work distinguishing and describing these and other taxa.

What's in the Water: Identifying *Planorbella trivolvis* and *Biomphalaria havanensis* DNA Using Real-Time qPCR

Celene M. Slifka[1], Thomas G. Rosser[1], Geoffrey C. Waldbieser[2], Charles C. Mischke[3], David J. Wise[3] and Matt J. Griffin[4]

[1]Department of Comparative Biomedical Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762; [2]USDA-ARS Warmwater Aquaculture Research Unit, Stoneville, Mississippi 38776; [3]Mississippi Agriculture and Forestry Experiment Station, Thad Cochran National Warmwater Aquaculture Center, Mississippi State University, Stoneville, Mississippi 38776; [4]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Thad Cochran National Warmwater Aquaculture Center, Mississippi State University, Stoneville, Mississippi 38776

Bolbophorus damnificus is a digenean trematode responsible for significant economic losses and farm closures in Mississippi catfish aquaculture. First recognized as a threat in the 1990's, the parasite causes significant reductions in feeding activity, which severely limits growth. In addition, severe outbreaks can cause direct losses due to mortality in the fingerling and stocker stage of production. The life cycle involves three hosts: the American White Pelican *(Pelecanus erythrorhynchos),* a snail intermediate host (*Planorbella trivolvis* or *Biomphalaria havanensis*) and an Ictalurid catfish. Previous work indicates that channel *(Ictalurus punctatus),* blue *(Ictalurus furcatus),* and channel x blue catfish hybrids are equally susceptible to infection by *Bolbophorus damnificus*, although the host response is more subdued in hybrid catfish. Control methods involving bird harassment are logistically challenging and labor intensive. As a result, current management practices focus on chemical eradication of the snail host through pondside applications of copper sulfate or hydrated lime; although both of these chemotherapeutants have limitations. Copper sulfate can be highly toxic to fish at high temperatures and the phytotoxicity of copper can kill beneficial algal blooms, leading to catastrophic oxygen depletions. Similarly, hydrated lime is limited in efficacy as it requires snail contact to be effective. Therefore, it has little effect on snail populations that are not present in the pond margins at the time of application. As a result, alternative molluscicides are currently being explored. Researchers at the Mississippi State University College of Veterinary Medicine, in cooperation with scientists at the USDA Warmwater Aquaculture Research Unit, have sequenced the mitochondrial genomes of these two problem snails (*P. trivolvis* and *B. havanensis*) and identified unique regions suitable for discriminatory quantitative PCR analysis (qPCR). Primers and probes have been developed to target the cytochrome oxidase subunit 1 region for each species. The end goal is to use an eDNA/qPCR approach to estimate snail densities in catfish ponds and identify optimal treatment regimens (dose/# of applications/etc.) for current therapeutics (copper sulfate and lime). This assay will also be employed to evaluate the efficacy and utility of copper and lime alternatives. Lastly, this assay also provides a molecular confirmatory test to support snail identification, which is an invaluable research tool. This assay will greatly aid current research developing best management practices to mitigate the negative impacts of *B. damnificus* and other digenetic trematodes on catfish production in the southeastern United States.

Overview and Future Research Directions of *Bolbophorus damnificus* Trematode Pathogenesis in Channel (*Ictalurus punctatus*) and Hybrid (Channel Catfish x Blue Catfish *Ictalurus furcatus*) Catfish

Ashley L. Powell[1], Matt J. Griffin[2], Alvin C. Camus[1]

[1]Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA; [2]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Thad Cochran National Warmwater Aquaculture Center, Mississippi State University, Stoneville, Mississippi, USA

First described in 1998, the trematode *Bolbophorus damnificus* causes significant mortalities and production losses in ictalurid catfish (channel, *Ictalurus punctatus*, and channel x blue, *I. furcatus*, hybrid) aquaculture ponds in the Southeastern United States. Catfish are infected by cercariae released from rams-horn snails, *Planorbella trivolvis,* or the exotic snail *Biomphalaria havanensis* and develop into metacercariae primarily within superficial skeletal muscle. The life cycle is completed when fish are consumed by the migratory American white pelican, *Pelecanus erythrorhynchus*, final host. Adult trematodes in the pelican's gastrointestinal tract release eggs excreted in the feces that hatch into miracidia infective to the snail first intermediate host. Infection rates in cultured catfish are highest during summer months, coinciding with the peak fish production period. Morbidity and mortality occur by unknown mechanisms primarily during encapsulation of metacercariae 7–10 days post-infection. Proposed mechanisms include toxin release by the parasite and homeostatic disruption including osmotic imbalance. A profound anemia is associated with encapsulation corresponding to transient angiogenesis and inflammation 4–13 days post-infection. Ultimately manifesting as decreased feed consumption and weight gain, parasitism results in insidious production losses difficult to detect until harvest and, potentially, carcass condemnation by processing plants. However, once metacercariae are encapsulated, fish can recover, and survival can be comparable to unaffected cohorts. In addition, co-infections with *Edwardsiella ictaluri*, *Flavobacterium columnare*, and the myxosporidian *Henneguya ictaluri* exacerbate mortality rates caused by the pathogens alone. Management involves controlling snail populations with chemical applications, such as copper sulfate, but must be approached cautiously to avoid damaging algal blooms and subsequent oxygen depletions. The American white pelican is protected under the Migratory Bird Treaty Act, although limited removal may be permitted. Proposed research will characterize and contrast microscopic findings during trematode encapsulation in both channel and hybrid catfish, as well as identify host or parasite-derived angiogenic factors through transcriptomic analysis. Hematologic, serologic, and histologic studies will characterize the anemia and investigate other homeostatic disturbances, such as osmotic imbalance. A metabolomic study will further evaluate impacts of parasitism on the host. In addition, the study will compare mortalities associated with co-infection between *B. damnificus* and the bacterial pathogens, *Aeromonas hydrophila*, *E. piscicida*, and *F. columnare.* Based on findings, potential management strategies will be further explored with the goal of expanding insight into *B. damnificus* pathogenesis and mitigating losses.

Estimation of Genetic Parameters and Genetic Co-variation Between *Piscirickettsia salmonis* Resistance and Sea Lice (*Caligus rogercresseyi*) Susceptibility in Atlantic Salmon (*Salmo salar*) Using Genomic Information

Baltasar F. Garcia[1], Paulina López[2], Daniela Cichero[2], Thomas Moen[3] and José M. Yañez[1,4]

[1]Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, 8820000, Chile; [2]AquaGen Chile, Puerto Varas, 5550513, Chile; [3]AquaGen AS, Trondheim, P.O. Box 1240, Norway; [4]Núcleo Milenio INVASAL, Concepción, Chile

Currently, the Atlantic salmon farming has faced several disease problems that may compromise the increase of production. The Salmon Rickettsial Syndrome (SRS) caused by the *Piscirickettsia salmonis* intra-cellular bacterium and the Caligdosis caused by the sealice parasite *Caligus rogercresseyi* are two of the main diseases present in the Chilean salmon production. One alternative to overcome the problems caused by these pathogens is to perform genetic selection increasing the resistance of animals against the abovementioned diseases. The aim of this study was to estimate heritability (h^2) and genetic correlation (r_g) for resistance to *P. salmonis* and susceptibility to *C. rogercresseyi* in Atlantic salmon. Data used in this study was provided by AquaGen breeding company from two controlled challenges performed in 2020. For the SRS challenge, 2014 animals were infected with *P. salmonis* and evaluated during 60 days to record day of death (DD) and binary survival (BS; 0=dead and 1=alive) of each fish. For the sealice challenge, 1128 animals were infected with 45 *C. rogercresseyi* copepodites prior to evaluation. Later, a disinfestation process was applied using gradual desalinization of tanks followed by a new reinfestation with the same number of parasites. Lice counting (LC) was performed by manual counting at two different time points: 15 days after first infection and 15 days after second infection. All challenged animals (3142) were identified with PIT-tags and genotyped using a 70K customized single nucleotide polymorphism (SNP) Affymetrix array. After quality control of genotypes (call-rate > 0.1, minor allele frequency > 0.01 and Hardy-Weinberg equilibrium p > 10^{-8}), 61,493 SNPs were retained for both groups. Single-trait GBLUP analysis were performed for SRS resistance (DD and BS) and sealice susceptibility (LC1 and LC2) to estimate each h^2. For the estimations of r_g, bi-trait models were applied using SRS and sealice phenotypes as information. Weight before challenge was used as fixed effect in all models. Results showed low to medium h^2 for SRS resistance (0.11 ± 0.05 and 0.15 ± 0.05, for DD and BS, respectively) and sealice susceptibility (0.17 ± 0.04 and 0.10 ± 0.04, for LC1 and LC2, respectively). These results reinforce the idea that both groups of traits may be improved through artificial selection as substantial additive genetic variance was found in the single-trait analysis. For the r_g, we found high and positive significant values between SRS resistance measured as DD and sealice susceptibility (0.74 ± 0.07 and 0.76 ± 0.09, for DD-LC1 and DD-LC2, respectively). However, for BS, the genetic correlations were lower and not significant (0.04 ± 0.20 and 0.22 ± 0.31, for BS-LC1 and BS-LC2, respectively). The positive r_g found between DD and sealice susceptibility suggests that selection for SRS resistance may favor the infection of sealice. For BS, the not significant estimations may be related to the use of a linear model to evaluate a binary trait which produces biased estimations. Although positive r_g for SRS resistance and sealice susceptibility were already reported in rainbow trout, it is important to mention that high h^2 values were estimated using the bi-trait models possibly implying in up-biased r_g estimations. Nevertheless, if this positive r_g is confirmed, a selection index may be applied in a breeding program that aims to improve both traits simultaneously. Finally, more studies would be necessary to disentangle the nature of this positive r_g and understand if it is caused by pleiotropy or linkage present in the population.

Wednesday, September 7th
Breakout Room B

Pathology of Fish and Shellfish: Paola Barato

9:30 AM	**Barato:** Introduction and Opening Remarks
9:45 AM	**Groman:** Histopathological Responses in Primary Organ Systems of Salmonids to Bacterial and Viral Disease Conditions
10:30 AM	Refreshments
10:45 AM	**Stilwell:** Infectious Disease Pathology of U.S. Catfish Aquaculture
12:00 PM	Lunch
1:30 PM	**Camus:** Pathology of Main Diseases of Elasmobranchs
2:15 PM	**Barato:** Select Diagnostic Cases of Tilapia
3:00 PM	Refreshments
3:15 PM	**Ildefonso**: Histoscore in Tilapia to Evaluate Substances and Vaccines
4:00 PM	**Ferguson:** Selected Diagnostic Cases of Shellfish
4:45 PM	**Sandoval:** Aluminum Intoxication in Atlantic Salmon Fingerlings

Histopathological Responses in Primary Organs Systems of Salmonids to Bacterial and Viral Disease Conditions

David B. Groman[1]

[1]Aquatic Diagnostic Services, Atlantic Veterinary College, University of Prince Edward Island, 550 University Ave., Charlottetown, Prince Edward Island, Canada. C1A 4P3

Tissue damage and inflammation linked to clinical bacterial and viral diseases in salmonids can show both similar and pathognomonically differing histopathologic changes. This presentation will target the more common bacterial and viral disease changes in several organs: e.g., brain / eye, heart, kidney, liver and spleen. Specifically, responses to the following bacteria diseases will be discussed: Furunculosis, Yersiniosis, Nocardiosis, Bacterial Kidney Disease and Vibriosis. These responses will be contrasted by discussing changes in these organs and tissues to the following viral diseases: Infectious Salmon Anemia, Viral Hemorrhagic Septicemia, Infectious Pancreatic Necrosis, Viral Nervous Necrosis and Piscine Myocarditis Virus. It is the intent of this presentation to provide histopathologist with some incite into how the immune system and target tissues of salmonids respond to these agents.

Infectious Disease Pathology of U.S. Catfish Aquaculture

Justin M. Stilwell[1]

[1]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762

Catfish production represents the largest sector of foodfish aquaculture in the United States and a significant economic driver in the southern states. Infectious disease outbreaks at all stages of production often result in either lost production efficiency, compromised filet quality, and/or fish mortality. Accurate, timely diagnosis, development of disease prevention and intervention strategies, and understanding the epidemiology and pathogenesis of these agents are crucial to mitigating impacts in the industry. This talk will summarize the pathologic lesions and processes associated with common, economically significant pathogens of US channel catfish and hybrid channel × blue catfish culture.

Histopathologic Review of Diseases in Captive Elasmobranchs

Alvin C. Camus[1]

[1]Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA

This presentation embodies a compilation of pathologic findings in captive elasmobranchs characterized by the Aquatic Diagnostic Service at the University of Georgia's College of Veterinary Medicine over the 16-year period from 2006 to 2022 and includes representatives from most groups of infectious disease agents. Elasmobranchii are a subclass of approximately 900 species of primarily marine cartilaginous fishes that include the sharks (superorder Selachii) and skates, rays, and sawfish (superorder Batoidea). Few well-documented major diseases are described in elasmobranchs compared to teleosts and literature resources are limited. However, as concern grows over wild elasmobranch populations and elasmobranchs become increasingly displayed in public aquaria, reports of infectious and non-infectious diseases are expected to increase. Diagnostic approaches to elasmobranchs are similar to those of teleosts, but pathologists should be familiar with the morphologic differences encountered in these species. In addition to their cartilaginous skeletons, with some species exceptions or variations, morphologic features distinguishing elasmobranchs from boney teleosts include the presence of five to seven external gill slits and gill filaments supported throughout much of their lengths by interbranchial septa. The skin contains small placoid scales (dermal denticles) possessing a pulp cavity, dentine, and enamel-like vitrodentine. Teeth (modified denticles) occur in rows or plates on articulated jaws, but the upper jaw is not fused with the cranium. The dorsal fin or fins and fin spines are rigid, and the anal fins of males are modified as claspers used in copulation. Internally, elasmobranchs lack a swim bladder and coelomic adipose. Livers are large and friable due to the presence of abundant hepatocellular lipid stores that aid buoyancy. Hematopoiesis occurs in the coelomic epigonal organ and in some species a Leydig organ present in the esophageal wall. Reproductive organs are embedded within the anterior portion of the epigonal organ. The pancreas is discrete and fused with the spleen in some species. Stomachs are typically large, distensible, and followed by a short intestinal segment leading to the morphologically variable spiral valve or intestine. The terminus of the gastrointestinal tract gives rise to the rectal gland.

Histopathological Responses of Tilapia to Infectious Disease Conditions

Paola Barato[1]

[1]Corporación Patologia Veterinaria/CORPAVET and MolecularVet,
Bogotá and Neiva, Huila, Colombia

Histopathological findings in tilapia will be presented for the more common disease with changes in several organs: e.g., brain/eye, gill, heart, kidney, liver, spleen, stomach, and intestine. Specifically, responses to the following diseases will be discussed: TiLV, ISKNV, Streptococcosis, Edwardsiellosis, Francisellosis, Flavobacteriosis, Aeromoniasis, and/or Parasitisms (coccidiosis, and external parasitism). This presentation provides histopathologists with some key characteristics of responding to these agents in tilapia.

Histoscore Model in Tilapia to Evaluate Vaccines and Diet Modifications

Ricardo Ildefonso[12], Victoria Jaramillo[1], Marco Rozas-Serri[12], Miguel Fernandez[2], Mateus Cardoso[2], Darling Coñuecar[1], Sandie Barbosa[2], Hugo Roa[2]

[1]Laboratorio Pathovet Chile, Puerto Montt, Región de Los Lagos, Chile; [2]Laboratorio Pathovet Brasil, Riberao Preto, Sao Paulo, Brasil

The grading of microscopic alterations in organs is a complementary tool to histological analysis that allows obtaining more objective results and a more accessible interpretation. In this case, we present the results of histoscore in Nile Tilapia (*Oreochromis niloticus*) organs: spleen in fish intraperitoneally vaccinated against *Streptococcus agalactiae* and/or an adjuvant, and liver in a second trial with different percentages of feed restriction. These organs were selected for their participation in the physiological processes of immunity and metabolism of nutrients, respectively. Lesions in these organs were grouped into circulatory disorders, regressive changes, progressive changes, inflammatory disorders, and presence of microorganisms. The histoscore is a quantitative expression of the histological changes in an organ, which considers the anatomical segments affected in an organ, the type of pathological alteration and the extent of these changes on the tissue surface analyzed. The numerical value obtained is divided into categories of mild, moderate or severe damage, with 0 being the absence of lesions, while 3 expresses a maximum degree of morphological alterations. In liver the histoscore values were higher or with greater degree of histological changes reaching levels classified as moderate in those fish subjected to high percentages of food restriction (80%), while in fish with lesser restrictions the values of histoscore were progressively smaller classifying within the range of mild damage. In the spleen, congestion, and hyperplasia of melanomacrophage centers were the most frequent morphological changes, but in a follow-up of up to 7 days post inoculation the histoscore remained within a range of mild grade. In liver samples the histoscore value was related to the degree of dietary restriction, therefore, it is a useful tool to assess histological changes in this organ. In the spleen, the changes were minimal, probably requiring a longer follow-up time to obtain greater histological changes.

This work was supported by CORFO, project 20CVID-128114.

Selected Diagnostic Cases of Shellfish

Jayde A. Ferguson[1], Theodore R. Meyers[1]

[1]Alaska Department of Fish and Game, Division of Commercial Fisheries, Fish Pathology Section, Anchorage and Juneau, Alaska, USA

Fishermen in Alaska have encountered weathervane scallops, *Patinopecten caurinus,* with abnormal adductor muscles, colloquially termed "weak meat", characterized by the retention of muscle when shucked, an obvious darkened discoloration, and/or an abnormal texture making the product unacceptable for marketing. Samples were submitted for gross examination and histopathology in 2015 where adductor muscles from all affected scallops had many large foci of an apicomplexan associated with necrosis, fibrosis, and muscular atrophy. Molecular analyses confirmed that this parasite was *M. kathae*, which is the same apicomplexan that caused the collapse of Icelandic scallops and a suspected cause for gray meat disease and mass mortality of Atlantic sea scallops in northeast North America. We conducted a statewide survey in Alaska and found a high overall prevalence and infection intensity. Scallops from the Bering Sea and Southwest Kodiak were most severely infected. This newly recognized parasite is an important cause for scallop mortality, morbidity, and unmarketable product quality in the northern hemisphere. Bitter Crab Disease is caused by the parasitic dinoflagellate, *Hematodinium* sp. that parasitizes the hemolymph causing systemic disease and mortality in tanner and snow crabs (*Chionoecetes bairdi* and *C. opilio*) in Alaska and Canada and rarely in Alaskan Dungeness crab, *Cancer magister*. Clinical signs include lethargy, increased handling mortality, an exaggerated reddening of the carapace, pale viscera, flaccid chalky texture of meat and white opaque hemolymph caused by parasite presence. Cooked meat is also unmarketable due to an astringent aspirin aftertaste resulting in the name 'bitter crab disease'. The key diagnostic feature of the parasite is a dinokaryon nuclear division producing condensed V-shaped pairs of chromosomes that can be seen in some trophonts. Mortality can be as high as 100% in captive tanner crabs and there is a higher prevalence in new shell crabs <60mm carapace width. *Briarosaccus callosus* is a cosmopolitan rhizocephalan barnacle that parasitizes several lithodid crab species, most notably from the southeast coast of North America, Antarctica, sub-Antarctica, southwest Indian Ocean, Bering Sea, coastal waters of Kamchatka, Gulf of Alaska, southeast Alaska, and British Columbia, Canada. In Alaska, this parasite is known to infect red (*Paralithodes camtschatica*), blue (*P. platypus*), and golden (*Lithodes aequispina*) king crabs. Clinical signs include a large orange/red externa containing ova and/or larvae attached by a stalk below the abdominal flap. The interna radiates into the crab viscera as a green dendritic mass that replaces the hepatopancreas and extends rootlets into all major organs and tissues in the visceral cavity, including nerves, gills, and muscle. Gonads of both crab sexes become atrophied or lost. Parasitized male crabs display feminized traits of an enlarged abdominal flap, enlarged coxal setae, atrophied gonads, and protective behavior for the attached externa. Histologically there is generally no inflammation, but rootlets may become encapsulated when inflammation is stimulated by concurrent rickettsial infections. Parasitized crabs can successfully molt with attached externa, but the odds of long-term survival are unknown once the externa is lost or senesces. Externa do not regenerate and interna rootlets become atrophied and necrotic causing an intense inflammatory response that may kill the crab. The parasite alters the endocrinology of the host crab causing castration in both sexes and suppressed growth that limits recruitment. This parasite can likely impact crab populations when it is highly prevalent which has varied in Alaska from <1% in red king crabs, 20% in golden king crabs, and up to 76% in blue king crabs.

Aluminum Intoxication in Atlantic Salmon Fingerling

Carlos Sandoval[1]

[1]Veterinary Histopathology Center (VEHICE), Veterinary Research, Libertad 590, Puerto Montt, Chile

Histopathological findings in Atlantic salmon fingerlings displaying aluminum intoxication, with a sudden mortality (up to 80%), will be presented. This presentation will mainly cover changes in kidney and gills, demonstrating the evolution of the pathology. Also, other analysis such as aluminum histochemistry will confirm the presence of this heavy metal in cartilage and bone. The aim of this presentation is to provide knowledge about the main findings that may suggest aluminum poisoning in freshwater stages.

Thursday, September 8th
Main Ballroom

Genomic Applications in Fish Health:
Phillip Dettleff/Sebastian Escobar

9:30 AM	**Dettleff**: Introduction and Welcome
9:45 AM	**Dettleff**: Transcriptomic Applied to Fish Health: Pathogen and Stress Response in Fish.
10:00 AM	**Yáñez**: On the Use of Ultra-dense Genome-wide Information to Boost Host Response Against Diseases in Aquaculture
10:15 AM	**Escobar**: CRISPR-Cas9: A Genetic Tool to Study Gene Functions in Fish.
10:30 AM	Refreshments
10:45 AM	**Valenzuela-Munoz**: Genomics Applied to Understand the *Caligus rogercresseyi*-Atlantic salmon Interaction.
11:00 AM	**Valdés**: Functional Genomics Applied to Aquaculture and Teleost Muscle Growth
11:15 AM	**Martinez**: Developing Genomic Resources of Non-model Species. The Case of the Diversification Program of *Seriola lalandi* for a Better Management of Fish Health.
11:30 AM	**Dettleff**: Evaluating High Temperature Effects on Red Cusk-eel (*Genypterus chilensis*) Trough Gill De Novo Transcriptome Assembly.
11:45 AM	**Roh**: Endogenous DNA Is Highly Dynamic Constituent of Skin Mucus in Atlantic Salmon
12:00 PM	Lunch
1:00 PM	**Piña-Elgueda**: Description of the Genetic Basis of Sea Lice (*Caligus rogercresseyi*) Counts Using a Repeated Measures Genome-Wide Association Study (GWAS) in Atlantic Salmon
1:15 PM	**Tapia**: Differential Gene Expression Patterns of Early Response Against Sea Lice Infestation in the Parasitized Skin of Coho and Atlantic Salmon
1:30 PM	**Vidal**: Unveiling the Role of Differential Alternative Splicing Between Resistant and Susceptible Atlantic Salmon to Sea Lice, *Caligus rogercresseyi*
1:45 PM	**Cáceres**: Meta-analysis of GWAS for Sea Lice Load in Atlantic salmon
2:00 PM	**Núñez-Acuña**: Duplicated Genome Regions in *Caligus rogercresseyi* Associated with Pharmacological Resistance and Evaluation at Sea Lice Populations
2:15 PM	**Marin-Nahuelpi**: Meta-analysis of GWAS for *Piscirickettsia salmonis* resistance in Atlantic salmon
2:30 PM	**Tekedar**: Tad Operon Contributes to Virulence of Epidemic Isolate *Aeromonas hydrophila* ML09-119
2:45 PM	**Chicoski**: Genomic Features of Fish Pathogens Edwardsiella spp. Isolated in Brazil Insight About Genomic, Antibiotic Resistance and Virulence Factors

Thursday, September 8th
Main Ballroom

Genomic Applications in Fish Health:
Phillip Dettleff/Sebastian Escobar

3:15 PM **Dubytska**: Edwardsiella ictaluri T3SS Effector EseN Modulates Expression of Host Genes Involved in the Immune Response

3:30 PM **Valdés**: Role of Mineralocorticoid and Glucocorticoid Receptors in Teleost Somatic Growth and Stress

3:45 PM **Gallardo-Hidalgo**: Genome-Wide Association Study for Growth Traits Under Upper and Lower Thermal Rearing Conditions Using Genome-wide Imputation, Multi-trait Analysis and Gene-Based Association Approach in Rainbow Trout (*Oncorhynchus mykiss*)

Transcriptomics Applied to Fish Health: Pathogen and Stress Response in Fish

Phillip Dettleff[1].

[1]School of Veterinary Medicine, Pontificia Universidad Catolica de Chile, Santiago, Chile;

The transcriptional regulation is a key step in the cell response to intra and extracellular signals, including infection with pathogens and stressor factors. The RNA-seq technology has allowed obtaining a high amount of information to understand how the cell regulates the different pathways in response to these types of stressors. In aquaculture, this technology as allows understanding in a better way how the different tissues respond against infection with viruses or bacteria, as well as the stress response against external factors, including temperature, acidification, or management stressors. Understanding how fish respond to this factor contribute to generating molecular biomarkers to evaluate stress status and immune response among others. Additionally, this technology has contributed to understanding how the disease resistance act in fish. In this workshop session, we will review the contribution of RNA-seq technology to fish health in aquaculture species, showing several studies in salmonids, cusk-eel, and other fish species oriented to understanding disease resistance, host-pathogen interaction, and environmental stress response. This talk will evidence how RNA-seq could be a valuable tool to improve fish health. Funding: CONICYT FONDECYT Postdoctorado 3180283.

On the use of Ultra-dense Genome-wise Information to Boost Host Response Against Diseases in Aquaculture

José M. Yáñez[1], Rodrigo Marín-Nahuelpi[1], Jousepth Gallardo-Hidalgo[1], Charles Sánchez[1], Pablo Cáceres[1], Baltasar Garcia[1]

[1]Faculty of Veterinary and Animal Sciences, University of Chile

Selective breeding represents an efficient and sustainable approach to improve health status of aquaculture species by enhancing host response against infectious and parasitic diseases. Genomic prediction approaches can be used to accelerate the genetic progress for traits which are difficult to measure on the selection candidates themselves (e.g., disease resistance). In fact, medium-density single nucleotide polymorphisms (SNP) genotyping panels are currently used to speed up genetic progress for disease resistance in several aquaculture species. Here we focused on the latest achievements on the application of ultra-dense genome-wide information, using whole-genome resequencing methods, to enhance selective breeding to boost host response against diseases in aquaculture. The application of whole-genome re-sequencing and meta-analysis for the identification of putative functional causative variants involved in host disease resistance in salmon, and their impact on accelerating selective breeding will be also addressed.

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CRISPR-Cas9: A Genetic Tool to Study Gene Functions in Fish

Sebastián Escobar-Aguirre[1]

[1]Laboratorio Acuicultura, Departamento Ciencias Animales, Facultad de Agronomía e Ingeniería Forestal Pontificia Universidad Católica de Chile, Santiago, Chile; [2]Cermaq-Chile.

The clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) system has been widely used in animals as an efficient genome editing tool. Species including zebrafish and medaka, but also in food species such as Tilapia and Salmo salar. In fish cells, the technique has been difficult to implement due to the lack of proper vectors that use active promoters to drive the expression of both small guide RNA (sgRNA) and Cas9 protein. Thus, the implementation of this tool to perform targeted gene knockout will allow for assessment of genes function. Thus, the present workshop is aimed to provide a framework and relevant information to perform CRISPR in both fish and cells, incorporating; design of sgRNA, discussion of suitable protocols, features from different fish cell lines, implementation and difficulties in egg´s microinjection.

Grant: FONDECYT #11190649.

Genomic Applied to Understand the *Caligus rogercresseyi*-Atlantic Salmon Interaction

Valentina Valenzuela-Muñoz[1,2], Cristian Gallardo-Escárate, Gustavo Núñez-Acuña, Diego Valenzuela-Miranda[2]

[1]Interdisciplinary Center for Aquaculture Research (INCAR), University of Concepción, P. O. Box 160-C, Concepción, Chile; [2]Laboratory of Biotechnology and Aquatic Genomics, Department of Oceanography, University of Concepción, Concepción, Chile

The sea lice *Caligus rogercresseyi* and *Lepeophtheirus salmonis* are marine ectoparasites, a primary concern for salmon aquaculture worldwide. The successful infestation process allows the parasite access to nutrients for reproduction and adult development. Trigger tissue damage and immunosuppression in infected fish. Genome research has been intensively used to understand the host-parasite interaction. Next-generation sequencing technologies currently allow the identification of genes and their expression profile in host and parasites during an infestation process. Furthermore, with the new technologies is possible to identify the genome proportion without coding potential, but that has high relevance in the gene expression regulation. We reported the main research advance using the genomic approach generated by our group over the last years around *C. rogercresseyi*-Atlantic salmon interaction. Since the host-recognition process, host response, and sea lice microbiota. In addition, the non-coding RNAs associated with this interaction process has widely studied. Finally, the application of sea lice genome to develop new sea lice controls strategies. The use of genomic tools allowed us to increase the knowledge of Atlantic salmon-sea lice interaction, a relevant tool to develop new strategies for sea lice control.

Funding: ANID-Chile through the grant FONDAP (#15110027), FONDECYT (#1210852), FONDECYT (11220307)

Role of Mineralocorticoid and Glucocorticoid Receptors in Teleost Somatic Growth and Stress

Rodrigo Zuloaga[1, 2], Jorge Aedo[1,2], Daniela Aravena[1,2], Alfredo Molina[1,2,3], Juan Antonio Valdés[1,2,3]

[1]Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile; [2]Interdisciplinary Center for Aquaculture Research (INCAR), Víctor Lamas 1290, PO Box 160-C, Concepción, Chile; [3]Centro de Investigación Marina Quintay (CIMARQ), Universidad Andrés Bello, Quintay, Chile

Corticosteroids have two major roles in vertebrates: a glucocorticoid function involved in stress response and growth, and a mineralocorticoid function involved in hydromineral and osmoregulation balance. In many vertebrates, these functions are associated with two hormones, cortisol and aldosterone. These hormones, cortisol and aldosterone, mediate their effects through activation of the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR), respectively. In teleost, it has long been held that a single hormone, cortisol, has both glucocorticoid and mineralocorticoid actions, since fish lack aldosterone. However, recent data suggests that 11-deoxycorticosterone (DOC) may act as an MR physiological ligand. The cumulative evidence has shown that osmoregulation in fish are mediated by cortisol and GR, ruling out the participation of MR. Thus, the main function of the mineralocorticoid system in teleost fish remains to be determined. Here we present evidence about the physiological role of MR in teleost, and to shed lights on how MR signaling is coordinated by two potential ligands, DOC and cortisol. To understand in a comprehensive and global manner how GR and MR modulates the skeletal muscle transcriptomic response, we performed an RNA-seq analysis. Juvenile rainbow trout (*Oncorhynchus mykiss*) were intraperitoneally injected with physiological doses of cortisol (1 mg/kg) or DOC (0,1 mg/kg). We also include a pre-treatment with mifepristone (GR antagonist) and spironolacton (MR antagonist). cDNA libraries were constructed from the skeletal muscle of rainbow trout groups. The expression analysis revealed that GR group mediated by cortisol, biological processes were significantly enriched in ubiquitin-dependent protein catabolic process, myofibril assembly, and autophagy. In MR group mediated by DOC, biological processes were significantly enriched in mitotic nuclear division, nuclear division, and striated muscle cell development. These results suggest that GR and MR have a differential participation in fish stress response and muscle growth. Funded by FONDAP 15110027 and FONDECYT 1201498

Developing Genomic Resources of Non Model Species: The Case of the Diversification Program of *Seriola lalandi* for a Better Management of Fish Health.

Victor Martinez[1]

[1]Favet-inbiogen, Faculty of Veterinary Sciences, University of Chile

The Carangidae family are a large superclade of finfish, consisting of many species inhabitng the tropical and sub-tropical marine waters. The seriola genus, in particular comprise around nine species, which are important species for fisheries and aquaculture, providing significant economic benefits to humans. Still genomic resources for the genus are scarce. As part of the Chilean diversification program, the production of Seriola lalandi is increasing steadily in Chile. Several countries have started their own production using larvae exported from the Chilean program. This species lacks genomic resources. We developed two sex specific reference genomes for the species based on individuals coming from a wild population from the south pacific. We further developed the first linkage map suing information from a SNP genotyping array of more than 90K variants that is able to capture the genetic variation from populations from New Zealand and Australia, but from Seriola dorsalis a sister species from the northern hemisphere. We integrate genomic information and linkage information in order to anchor scaffolds to chromosomes. The final reference comprises the 24 chromosomes and have a 91.3 BUSCO of 97% at the genome level. The annotation of the genome gave insights on the evolution of several immune response genes, including several genes involved in muscle development and sex determination. Several Kegg pathways including MAPK signalling, Regulation of actin cytoskeleton, focal adhesion: all related with host pathogen interaction. The activity of MAPKs plays a significant role on the immune system, this is done by targeting cytokine production upon signalling from activated TLR receptors. Moreover, MAPKs are involved in initiation of innate immunity and regulate binding of cytokines to receptor. Focal adhesion plays a critical role in immune response. We further perform a full sequence genome wide association analysis on sex determination. The results show that a single gene was found explaining more than 99% of the variation of sex. Using the information of the reference genome developed, we further develop a sequencing platform in order to perform paternity and sex determination at a low cost comprising more than 400 genes. This platform is now used in several programs worldwide in order to secure the genetic health of broodstock, due to the biology of spawnings, while preserving the genetic variation at the whole genome level. The genomic developments of this non-model species are essential for a better understanding of the biology of the species. We are incorporating all this information into the National breeding program of Seriola of genomic selection and for studying more in depth, for example parasite driven evolution and the biology of several important traits including spinal deformities, and sex determination using CRISPR. This will help a more sustainable production system in the short and long term.

Evaluating High Temperature Effects on Red Cusk-eel (*Genypterus chilensis*) Trough Gill *De Novo* Transcriptome Assembly

Phillip Dettleff[1], Rodrigo Zuloaga[2,3], Marcia Fuentes[2,3], Jorge Aedo[2,3], Juan Manuel Estrada[2,4], Alfredo Molina[2,3], Juan Antonio Valdés[2,3].

[1]School of Veterinary Medicine, Pontificia Universidad Catolica de Chile, Santiago, Chile; [2]Laboratory of Molecular Biotechnology, Faculty of Life Sciences, Andres Bello University, Santiago, Chile; [3]Interdisciplinary Center for Aquaculture Research (INCAR), Concepcion, Chile; [4]Marine Research Center Quintay (CIMARQ), Universidad Andres Bello, Quintay, Chile.

The *Genypterus* genus contains native species of economic relevance with high potential for aquaculture diversification, including the red cusk-eel (*Genypterus chilensis*). In climate change scenario, increase in temperature can generate stress in the native and commercial populations, affecting the performance of fish. The objective of this experimental work was to study the effect of heat stress in red cusk-eel, determining the effect in gills. Red cusk-eel were separated into control and stress groups. The groups were maintained at control temperature (14°C) or subjected to high-temperature stress (19°C) for five days. At the end of the experiment, fish were euthanized, sampling gills for cortisol level, oxidative damage evaluation (lipid peroxidation, protein carbonylation, and DNA damage), and *de novo* assembly and gene expression evaluation through RNA-seq. High temperature produces a significant increase in cortisol levels in the stress group, generating oxidative damage in gills, observed by increased protein carbonilation and lipoperoxidation. For transcriptomic analysis, a first *de novo* transcriptome assembly of gills was generated, with more than 24,000 annotated transcripts. A relevant modulation of gene expression was observed in gills in response to thermal stress, with 2,753 differentially expressed genes, with enriched terms related to unfolded proteins, DNA replication process and immune response, among others. These results showed that thermal stress can affect several molecular pathways in gills, generating damage and modulating the transcriptional regulation of processes that can affect the performance of red cusk-eel, information that should be considered in a climate change world scenario. Funding: CONICYT FONDECYT Postdoctorado 3180283.

Endogenous DNA is Highly Dynamic Constituent of Skin Mucus in Atlantic salmon

HyeongJin Roh[1], Lars Eirik Myklatun[1], Per Gunnar Fjelldal[1], Antony J. Parbhu Philip [1], Tomasz Furmanek[1], Dhamotharan Kannimuthu[1], Tore Kristiansen[1], Craig Morton[1], Søren Grove[1,2]

[1]Institute of Marine Research, P.O. Box 1870, Nordnes, N-5817 Bergen, Norway; [2]Faculty of Veterinary Medicine, Norwegian University of Life Sciences, 1433, Ås, Norway

Mucus is a diverse, complex and multifunctional matrix that covers mucosal surfaces in fish, including the skin. The liquid part of skin mucus can conveniently be sampled by use of a simple and minimal-invasive absorption method, and has been shown to contain a rich collection of proteins and metabolites. The specific finding that salmon DNase I is highly abundant in skin mucus prompted an investigation of DNA in skin mucus which we report here. The present study is based on a smoltification experiment. At intervals, spanning 50 - 725 day degrees (dd°C), 12 fish from each smoltification regime were sacrificed, and 12 others subjected to a seawater challenge (SWC) procedure consisting of a 24 hour stay in seawater before being sacrificed. Skin mucus was sampled from all sacrificed fish and analyzed by quantitative and qualitative approaches. The quantity of total DNA was modest (237 ± 333 ng mL^{-1}) in mucus from fish sampled from freshwater irrespective of smoltification regime, and remained so during the experiment. In contrast, total DNA was up to approximately 30 times elevated in mucus from SWC fish, and differences were observed between smoltification regimes. Importantly, the increase in total DNA in SWC fish developed from low at 50 dd°C to a plateau reached at 350 dd°C, the latter likely coinciding with fish being fully smoltified. Total DNA remained elevated in mucus at least 120 days after fish were permanently transferred to seawater. By qPCR, the level of salmon 18S rRNA DNA was 56 times increased in mucus from SWC fish at 425 dd°C, whereas no difference was seen for bacterial 16S rRNA DNA, suggesting that the increase in total DNA after SWC was of endogenous/salmon origin. The levels of both salmon mitochondrial genome and nuclear genome DNA were increased in SWC fish, mirroring the kinetics of total DNA. However, while levels of nuclear DNA remained high 120 days after fish transfer to seawater, levels of mitochondrial DNA had fallen to very low.

Description of the Genetic Basis of Sea Lice (*Caligus rogercresseyi*) Counts Using a Repeated Measures Genome-Wide Association Study (GWAS) in Atlantic salmon

Agustin Piña-Elgueda[1], Rodrigo Marín-Nahuelpi[1], F. Baltazar Garcia[1], Pablo Cáceres[1], Paulina Lopéz[2], Daniela Cichero[2], Thomas Moen[3] José Manuel Yañez[1, 4]

[1]Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, 8820000, Chile; [2]AquaGen Chile, Puerto Varas, 5550513, Chile; [3]AquaGen AS, Trondheim, P.O. Box 1240, Norway; [4] Núcleo Milenio INVASAL, Concepción, Chile

Caligus rogercresseyi is the primary parasitic disease affecting the Chilean salmon farming industry. Genomic studies have showed sufficient additive genetic variation for resistance to this parasite. With the use of Genome-Wide Association Studies (GWAS), a group of genotyped individuals with phenotype records can be tested for an association between a SNP marker and a particular trait of interest. The aim of this study was to perform a GWAS in Atlantic salmon to evaluate sea lice count using repeated measures. A population of 1,245 Atlantic salmon smolts were challenged twice with *C. rogercresseyi* at a 45 copepods/fish rate. PIT-Tags, weight, length and total body lice count were measured at three timepoints for each challenge. All challenged animals identified with PIT-tags were genotyped with a 70K custom SNP Affymetrix array. After quality control 59,477 SNPs were retained. Genetic parameters were estimated using BLUPF90 family programs using a repeatability model, including challenge as fixed effect, weight at each challenge as covariate, and the individual effect as a permanent environment effect. The estimated heritability and repeatability were 0.1 ± 0.02 and 0.33 ± 0.02, respectively. The results of GWAS analysis showed only one SNP at chromosome-wide significance threshold, in chromosome 21 (p-value$<10^{-5}$). Repeatability value in this study was of moderate magnitude for lice count within different measures, meaning that most of the variability for the trait is attributable to temporary environmental effects. The use of repeated measures did not improve GWAS, but more studies are necessary to evaluate if an increase in samples/measures may estimate significant associations.

Differential Gene Expression Patterns of Early Response Against Sea Lice Infestation in the Parasitized Skin of Coho and Atlantic salmon

Paulina López[1], Fabian Grammes[2], Lucas Venegas[2], Daniela Cichero[3,4], José L. Bartheld[3], Robert Deerenberg[4], David Tapia[1], Matias Medina[2,3], Jacob S. Torgersen[2] and José M. Yáñez[1].

[1]Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, Chile; [2] AquaGen AS, Trondheim, Norway; [3] Blue Genomics Chile, Puerto Varas, Chile.[4] AquaGen, Puerto Varas, Chile.

Salmon lice infestation is a major concern for salmon aquaculture worldwide, affecting animal welfare and causing production losses. Susceptibility to sea lice differs considerably among salmonids, with Atlantic salmon (*Salmo salar*) being susceptible and Coho salmon (*Oncorhynchus kisutch*) being resistant. The underlying mechanisms of louse resistance have been studied through comparative gene expression patterns analysis between resistant and susceptible salmon species. However, no high-throughput comparative transcriptomic analysis has focused on the early skin response at the parasite attachment site. Thus, the objective of this work was to evaluate the gene expression patterns of early response against sea lice infestation in parasitized skin of Coho salmon and Atlantic salmon. We conducted a cohabitation trial with Atlantic and Coho salmon under experimental challenge with sea lice (*Caligus rogercresseyi*) to compare their early (2 dpi) and late (6 dpi) transcriptome responses in the skin trough RNAseq. The analysis revealed a differential gene expression pattern between the two species when comparing skin lice attachment sites versus healthy skin. In Atlantic salmon, downregulation of immune-related pathways was observed at early and late time post-infestation, with a more marked response at late time, especially in cytokine-mediated related pathways. On the other hand, in Coho Salmon, a more marked transcriptomic response was observed early, with a deregulation of pathways associated with extracellular matrix organization, oxidative stress and ion transport. Furthermore, pathways related to epidermal growth were downregulated in the parasitized skin of Coho salmon but upregulated in Atlantic salmon. These differences in the skin transcriptomic response of resistant Coho salmon and susceptible Atlantic salmon may provide insight into genes and pathways related to sea lice resistance.

Unveiling the Role of Differential Alternative Splicing Between Resistant and Susceptible Atlantic Salmon to Sea Lice, *Caligus rogercresseyi*

Rodrigo Vidal[1], Scarleth Bravo[1], Francisco Leiva[1], Javier Moya[2] and Osiel Guzman[3]

[1]Laboratory of Genomics, Molecular Ecology and Evolutionary Studies, Department of Biology, Universidad de Santiago de Chile, Santiago, Chile; [2]Benchmark Animal Health Chile, Santa Rosa 560 Of.26, Puerto Varas, Chile; [3]IDEVAC SpA, Francisco Bilbao 1129 Of. 306, Osorno, Chile.

Sea lice, correspond to pathogenic marine ectoparasite copepods, belonging to the Caligidae family, which represent one of the most serious threats to the worldwide aquaculture industry. In the Southern and Northern hemispheres salmon farming industries, the sea lice species *Caligus rogercresseyi* and *Lepeophtheirus salmonis*, represent the principal concerns, respectively. In the last years, several studies have used high throughput RNA- sequencing (RNA-seq)/microarray platforms, to evaluated and characterize the transcriptomic response-at the gene level- of *Salmo salar* (Atlantic salmon) to sea lice infestation. However, no methodical genome-wide researches of alternative splicing (AS) process in response to ectoparasites is available for teleost, by which whether differential splicing is a relevant response of salmonids to sea lice infestation is unknown. AS is a common regulated cellular process that increased cell protein and RNA heterogeneity. In the present study, we realized a genome-wide analysis of differential splicing from healthy and infested (ectoparisite attachment site) Atlantic salmon skin tissue from RNA-seq datasets, and then identified isoforms associated to susceptible and resistant phenotypes, to determine the role and contribution of AS in the Atlantic salmon response to *C. rogercresseyi* infestation. Ours results suggested a relevant presence of differential splicing in Atlantic salmon upon *C. rogercresseyi* infestation and key isoforms associated to the developed of resistant Atlantic salmon phenotypes. Furthermore, our results advance our knowledge of the biological importance of AS in the immune response of salmonids.

Meta-analysis of GWAS for Sea Lice Load in Atlantic salmon

Cáceres P.[1], Lopéz P.[1,2], Garcia B.[1], Cichero D.b, Ødegård J.[3], Moen T.[3], Yáñez J.M.[1,4]

[1]Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, 8820000, Chile; [2]Blue Genomics Chile, Puerto Varas, 5550000, Chile; [3]AquaGen AS, Trondheim, P.O. Box 1240, Norway; [4]Millennium Nucleus of Austral Invasive Salmonids (INVASAL), Concepción, Chile

Sea lice (Caligus rogercresseyi) is an ectoparasite that causes major production losses in the salmon aquaculture industry in the southern hemisphere. Atlantic salmon (Salmo salar) is an important salmonid for the aquaculture industry and a highly susceptible species to sea lice infestation. Genetic variation for host resistance to sea lice, defined as parasite load, has been found in Atlantic salmon. In addition, sea lice load has been shown to be a polygenic trait, controlled by several quantitative trait loci (QTL) of small to medium effect, which makes difficult to map them with sufficient statistical power when the sample size is limited. The use of medium density single nucleotide polymorphisms (SNP) can also affect the success in identifying genetic variants significantly associated to sea lice load with high accuracy. In order to improve the ability to detect QTL significantly associated to sea lice burden, here we combined genotype imputation from medium- to high-density SNP and meta-analysis of genome-wide association studies (GWAS) in different populations of Atlantic salmon. The imputation of genotypes of 6,144 fish challenged against sea lice from four year-classes was performed to increase density from 70K SNP to 600K SNP. A meta-analysis of GWAS was then carried out for three different traits: lice count, lice density and log-lice density. Using this approach, we detected a genomic region highly associated to sea lice load on ssa03, ss12 with a great peak and several other regions surpassing the significance threshold across almost all the chromosomes. We also identified important genes harboring this significant region, which are mainly related to tissue reparation, such as Mucin-16-like isoform X2 and Filamentous growth regulator 23-like isoform X1. In addition, on ssa03, cytoskeletal modification and immune response related genes such as, Coronin 1A and Claudin, were also found close to significant single-nucleotide polymorphisms (SNPs). Our results confirm the highly polygenic architecture of sea lice burden and provide novel insights into the functional candidate genes associated to sea lice load variation in Atlantic salmon.

Duplicated Genome Regions in *Caligus rogercresseyi* Associated with Pharmacological Resistance and Evaluation at Sea Lice Populations

Gustavo Núñez-Acuña[1], Constanza Sáez-Vera[1], Valentina Valenzuela-Muñoz[1], Diego Valenzuela-Miranda, Cristian Gallardo-Escárate[1]

[1]Laboratory of Biotechnology and Aquatic Genomics, Interdisciplinary Center for Aquaculture Research (INCAR). University of Concepción, Concepción, Chile.

Undoubtedly, the pharmacological resistance against delousing drugs on the sea lice *Caligus rogecresseyi* is an increasing concern for salmon farms sustainability. In Chile, the second largest farmed salmon producer country, monitoring programs for drug sensitivity of sea lice based on molecular markers have been designed by local authorities. However, the increasing knowledge on the sea louse genome have led to the discovery of novel molecular markers based on Copy Number Variants (CNVs) of coding and non-coding regions. This study aimed to evaluate the presence of gene duplications, associated to drug sensitivity in *C. rogercresseyi*, on lice populations obtained from salmon farms located in different regions of Chile. The full genome of *C. rogercresseyi* was used as a reference to map a combination of long-read and short-read sequences obtained through Nanopore and Illumina technologies. CNVs were identified through comparative coverage, sequence homology and collinearity analyses. Genome duplications or CNVs in functional genes were identified with differential p-value among populations, but most of the differential variants were found in transposable elements and in genes related to drugs detoxification system. The potential impact of this study for salmon aquaculture is the identification of novel molecular markers based on CNVs, complementing the monitoring programs for *C. rogercresseyi* resistance to delousing drugs.

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Meta-analysis of GWAS for *Piscirickettsia salmonis* Resistance in Atlantic salmon

Rodrigo Marín-Nahuelpi[1], Baltasar F. Garcia[1], Paulina López[2], Daniela Cichero[2], Thomas Moen[3], José M. Yañéz[1,4]

[1]Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, 8820000, Chile; [2]AquaGen Chile, Puerto Varas, 5550513, Chile; [3]AquaGen AS, Trondheim, P.O. Box 1240, Norway; [4]Núcleo Milenio INVASAL, Concepción, Chile

The Salmon Rickettsial Syndrome (SRS) caused by the *Piscirickettsia salmonis* intra-cellular bacterium that causes major production losses in the salmon aquaculture industry in the southern hemisphere. Atlantic salmon (Salmo salar) is an important salmonid for the aquaculture industry and a highly susceptible species SRS infection. Genetic variation for host resistance SRS, defined as binary survival (BS) and day of death (DD), has been found in Atlantic salmon. Although quantitative effect loci (QTL) of low to medium effect have been discovered, these may vary depending on the study population and due to factors, such as individuals sample size, genetic background and single nucleotide polymorphisms (SNP) panel density. Available tools such as high-density genotype imputation and meta-analyses allow for improved statistical power in detecting QTL associated with resistance to SRS infections. Herein, we used imputation methods to drive marker density from 50K to 600K across 4 Atlantic salmon populations to perform independent genome-wide association analyses (GWAS) and then combine summary statistics in a meta-analysis (METAL) in order to increase the statistical power of detecting genomic variants associated with SRS resistance. We found significant heritabilities (h^2) across all 4 populations for both traits varying from 0.17 to 0.30. In addition, we were able to identify a genomic region highly associated with both definitions of SRS resistance on chromosome ssa02. We also found some significant non-overlapping peaks for BS on chromosomes ssa11, ssa24 and ssa26 and for DD on chromosomes ssa13, ssa17 and ssa29. In the overlapping region between the two traits, we found *Ribosome biogenesis protein BMS1 homolog (BMS1)*, a gene that regulates early rRNA processing and it is involved in ribosome biogenesis (which determines translation capacity) and protein folding. Infection with SRS can interfere with host protein synthesis and suppress the innate immune response via CsrA superfamily virulence-related proteins. These are RNA-binding protein and global regulators of carbohydrate metabolism genes facilitating mRNA decay. Rapid responsiveness at mRNA level is imperative to establish an immune response to infection. These findings highlight potential new genes involved into SRS resistance in Atlantic Salmon.

Tad Operon Contributes to Virulence of Epidemic Isolate *Aeromonas hydrophila* ML09-119

Hasan C. Tekedar[1], Lary Hanson[1], Matt J. Griffin[2,3], Geoffrey C. Waldbieser[3,4], Salih Kumru[1,5], Jochen Blom[6], Hossam Abdelhamed[1] and Mark L. Lawrence[1]

[1]Department of Comparative Biomedical Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762, USA; [2]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Stoneville, MS 38776, USA; [3]Thad Cochran National Warmwater Aquaculture Center, Delta Research and Extension Center, Mississippi State University, Stoneville, MS 38776, USA; [4]U.S. Department of Agriculture, Agriculture Research Service, Stoneville, MS 38776, USA; [5]Faculty of Fisheries, Recep Tayyip Erdogan University, Rize 53100, Turkey; [6]Bioinformatics & SystemsBiology, Justus-Liebig-University Giessen, Hesse, Germany

Aeromonas hydrophila is a Gram negative motile, polar flagellated, short, rod shaped, mesophilic species that is ubiquitous in aquatic environments and capable of causing severe infectious in several host species such as fish, invertebrates, amphibians, and reptiles. Since 2009, an emergent clonal group of virulent *A. hydrophila* (vAh) has been causing outbreaks that resulted in significant mortalities and economic losses to catfish farmers in US. Significant progress has been made to understand molecular mechanisms of *A. hydrophila* infection, but effective control of vAh has not been achieved. Our goal is to develop live attenuate vaccine or effective control method to reduce vAh outbreaks in catfish industry. Intriguingly, our previous comparative genomics research results showed that vAh strains encode a complete Tad (tight adherence) operon that consist of 13 genes, whereas Tad system is absent in the the majority of the other evaluated *A. hydrophila* genomes except couple strains, one of which is human clinical isolate. The Tad system contributes to biofilm formation, colonization, and virulence for large number of pathogens from different genus members. Using an in-frame deletion mutagenesis method, we successfully knocked out the entire Tad operon (13 genes were mutated) from epidemic isolate *Aeromonas hydrophila* ML09-119, which resulted in deletion of 10827 bp from the wild type strain ML-09-119 genome. Our immersion challenge in catfish fingerlings showed that the mortality rate was significantly lower ($p < 0.05$) in the catfish fingerlings infected with vAh*Δtad* mutant than parent vAh strain ML09-119 (74.36% mortality vs. 14.65% mortality respectively). Results from this study will enhance our understanding of molecular pathogenesis of *A. hydrophila*, and results could yield novel control measures for this disease that is having a major impact on catfish aquaculture.

Genomic Features of the Emergent Fish Pathogens *Edwardsiella* spp. Isolated from Brazil: Insight About Genetic Diversity, Antibiotic Resistance and Virulence Factors

Larissa M. Chicoski[1], Natalia A. Ferrari[1], Arthur R. da Costa[1], Ulisses de P. Pereira[1]

[1]Fish Bacteriology Laboratory. Department of Preventive Veterinary Medicine. College of Veterinary Medicine. State University of Londrina, Paraná State, Brazil. 89057-970.

Edwardsiella is a globally distributed Gram-negative facultative intracellular bacteria responsible for gastroenteric and septicemic disease in a broad range of animals, including fish. Differentiation between the five species of this genus is difficult due to the high homology of the 16S rRNA loci (>99%). The main objectives of this study were to perform the genomic sequencing of *E. anguillarum* and *E. tarda* isolated from outbreaks in Brazilian fish farms, to characterize *in vitro* antibiotic resistance and genomic diversity, as well as an *in-silico* analysis of virulence. Fragments of the brain, kidney, liver, and abdominal cavity liquid were collected aseptically in nine tilapia (*Oreochromis niloticus*) farms from São Paulo and Paraná states, striated on Blood Agar plates, and incubated for up to 96 hours at 28°C. The morphology of the colonies was observed, followed by staining (Gram) and phenotypic tests of catalase, oxidase, mobility, production of H_2S, urease, esculin, citrate, indole, and triple sugar iron for the initial classification of the pathogen. To determine the susceptibility of the microorganism to antibiotics, disk diffusion tests in Mueller-Hinton plates were performed using 27 antimicrobials from the aminoglycosides, amphenicols, beta-lactams, lincosamides, quinolones, polymyxins, sulfonamides, and tetracyclines classes. The genetic material of the colonies was extracted (Puregene DNA Isolation Kit - Qiagen®), and the samples were submitted to multiplex Polymerase Chain Reactions (mPCR) followed by 16S and *gyrB* gene sequencing. To establish an identity matrix between the isolates were used the programs Gegenees v3.1, a software for fragment analysis using an all-against-all BLAST comparison, SplitsTree v4.16.1 for construction of a neighbor-joining tree, PGAdb-builder and wgMLST for pan-genome assembly and phylogenomic analysis for each species. Genes of antibacterial resistance were characterized through ResFinder 4.1, and the pathogenicity islands were detected by the GIPSy Software version 1.1.2. All the isolated strains showed phenotypic resistance to bacitracin, clindamycin, lincomycin, oxacillin, and penicillin, and high levels of resistance to tetracycline (8/9), amoxicillin (5/9), cephalothin (5/9), chloramphenicol (5/9), neomycin (5/9), and polymyxin (5/9). The samples VSF479, VFS488, VSF489, and VSF480 were compatible with *E. tarda* with near 80% similarity, while the samples VSF481, VFS485, VSF482, VFS483, and VFS97 showed 90% similarity with *E. anguillarum* and 100% similarity between them. This may indicate that the same strain/variation is possibly circulating in these regions. Two-thirds of the samples (6/9) (3 of *E. tarda* and 3 of *E. anguillarum*) possess the *tet(A)* gene, which confers resistance to tetracycline, and 55,5% (5/9) the *floR*, which is gives the characteristic of resistance against amphenicols, phenotypically expressed trait. Strains of *E. tarda* still presented the genes *aph(3'')-Ib* e *aph(6)-Id* (that confers resistance to aminoglycoside), *dfrA8* (trimethoprim), *qnrS1*, *qnrB19* (quinolone), *sul2* (sulfamethoxazole), *bla TEM-1A* and *bla TEM-1B* (beta-lactam). *E. tarda* strains presented an average of 14 pathogenic islands while *E. anguillarum* has shown 23 when analyzed throughout the genome. The presence of virulence factors, organized in the pathogenic islands in both species, mainly in *E. anguillarum,* may be related to the increased frequency of identification and the severity of outbreaks caused by this pathogen which happened last years in Brazil (studies still not published). The in-depth genetic analysis allowed the identification of genetic variation between strains and the diversity present among them and betwixt species. The detection of resistant strains, as reported in this study, beyond being a One Health issue, impacts all the productive chain by mistreating the specific diseases, and the presence pathogenicity loci indicates genomic advantage to function of important virulence factors. It is required more studies regarding pathophysiology, antimicrobial resistance, and virulence factors with the approach of genomic analysis to identification of sites that can be used to reduce the population of microorganisms and their damage in fish farming.

Edwardsiella ictaluri T3SS Effector EseN Modulates Expression of Host Genes Involved in the Immune Response

Lidiya Dubytska[1], Ranjan Koirala[1], Azhia Sanchez[1], Ronald Thune[2, 3]

[1]Department of Biology and Chemistry, Southern University and A & M College, Baton Rouge, LA 70813; [2]Department of Pathobiological Sciences, Louisiana State University School of Veterinary Medicine, Baton Rouge, LA 70803; [3] School of Animal Science, Louisiana State University Agricultural Experiment Station, Baton Rouge, LA

Edwardsiella ictaluri is a Gram-negative bacterial pathogen that causes enteric septicemia of catfish (ESC), a major disease of farm-raised channel catfish, *Ictalurus punctatus*. *E. ictaluri* is able to invade and replicate in phagocytic and non-phagocytic cells. The T3SS plays an important role in virulence in many Gram-negative bacterial pathogens. EseN is a type III secretion system (T3SS) effector that is encoded on the *Edwardsiella ictaluri* chromosome and is homologous to a family of T3SS effector proteins with phosphothreonine lyase activity. Previously we demonstrated that *E. ictaluri* invasion activates extracellular signal-regulated kinases 1 and 2 (ERK1/2) early in the infection, which are subsequently inactivated by EseN. Comparative transcriptomic analysis showed a total of 753 significant differentially expressed genes in head-kidney-derived macrophages (HKDM) infected with an EseN mutant (ΔEseN) compared to HKDM infected with wild type (WT) strain. This data demonstrates strong indications for the M1 phenotype of HKDM in response to *E. ictaluri* infection and a significant role of EseN in manipulation of this process. Our data also indicates that *E. ictaluri* EseN is involved in modulation of pathways involved in the immune response to infection and expression of several transcription factors, including NF-κβ (c-*rel* and *relB*), *creb3L4*, *socs6* and *foxo3a*. Regulation of transcription factors leads to regulation of proinflammatory interleukins (IL-8, IL-12, IL-15, IL-6) and cyclooxygenase-2 (COX-2) expression. Upregulation of COX-2 mRNA leads to increased production of prostaglandin E2 (PGE2), which is the product of COX-2 activity. Overall, the modulation of several cytokines (IL-8. Il-15, Il-6, Il-12) expression, COX-2 expression and activity, ERK1/2 activation and NF-κβ expression by *E. ictalure* EseN in infected HKDM indicate that EseN is essential for successful systemic infection by this bacterium. Importantly, EseN is required for efficient replication in the catfish head kidney and for maximum virulence in the catfish host.

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Functional Genomics Applied to Aquaculture and Teleost Muscle Growth

Juan Antonio Valdés[1]

[1]Laboratorio de Biotecnología Molecular, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Chile. Centro de Investigación Marina Quintay (CIMARQ), Universidad Andrés Bello, Chile. Interdisciplinary Center for Aquaculture Research (INCAR), Concepción, Chile

In the last years next generation sequencing technologies (NGS) and RNA-seq have revolutionized the fields of transcriptomic and functional genomics, providing the possibility to investigate gene expression and pathways involved in countless biological processes of non-model aquatic animals. Stress is a major concern in aquaculture as many stressors are present which can prompt the fish to compromised growth and health. Although the concept of stress has a bad reputation, physiological stress response helps to promote survival of an organism during adverse situations. The biochemical response that accompanies stress plays an important role in the metabolic adjustments that are critical for meeting the increased energy demand. In this work we studied the effect of several stressors on the metabolic and growth response of marine and freshwater species relevant for aquaculture. Using Illumina RNA-seq technology, skeletal muscle transcriptomes were analyzed, revealing dynamic gene expression profiles associated with the stress response phase and physiological status of the organism. This work will allow us, from a functional genomics perspective, to holistically understand the molecular processes associated to response to stress in fish in the context of aquaculture activity. Funded by FONDAP 15110027 and FONDECYT 1201498

Genome-Wide Association Study for Growth Traits Under Upper and Lower Thermal Rearing Conditions Using Genome-Wide Imputation, Multi-trait Analysis and Gene-Based Association Approach in Rainbow Trout (*Oncorhynchus mykiss*).

Jousepth A. Gallardo-Hidalgo[1], David A. Tapia[1], Baltasar Fernandes[1,2], Rodrigo I. Marin-Nahuelpi[1], Pablo A. Cáceres[1], José M. Yáñez[1,3,*]

[1]Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago 8820000, Chile; [2]School of Agricultural and Veterinary Sciences, UNESP - São Paulo State University, Jaboticabal 14884-900, Brazil; [3]Núcleo Milenio INVASAL, Concepción, Chile

Aquaculture faces important challenges due to climate changes that affect its sustainability. The increase in water temperature is an important factor that affects several physiological processes, causing stress and impacting the health of fish species, such as rainbow trout. Therefore, breeding programs that currently select individuals with superior performance in a specific range of temperature could be suboptimal in the near future, as a result of genotype-environment interactions (GEI) in environmental conditions with higher temperature. Growth rate is one of the traits with larger influence on aquaculture profitability, so determining its genomic architecture under elevated temperatures is crucial to understand the molecular basis of the phenotype expression in a climate change scenario. In this study, we challenged a rainbow trout population to grow under chronically high (~22 °C at 77 weeks) and low (~7 °C at 67 weeks) non-lethal temperature conditions. The objectives of this study were i) describe the genetic architecture of growth under high and low rearing temperature conditions using whole genome sequencing, imputation and multi-trait association, and ii) identify putative causal genes related with growth under these contrasting conditions using a gene-based association approach. The results showed the presence of genetic variance for growth under high (BW77HT) and low (BW67LT) temperature, with genomic heritabilities of h^2_{snp}= 0.35 ± 0.1 and h^2_{snp}= 0.41 ± 0.08, respectively; ii) the genetic architecture and putative genes associated with growth under high temperature is different from growth under lower temperature conditions with lower weight, with a low correlations between the effects of the markers of r= 0.15-0.18; iii) candidate genes that could explain the response of fish to heat stress are involved in protein degradation, maintenance of homeostasis and maintenance of cardiovascular functions under heat stress were identified as the main related functions.

Thursday, September 8th
Breakout Room A

Myxozoa: Jerri Bartholomew/Ethan Woodyard

9:30 AM **Bartholomew**: *Ceratonova shasta*: Biological and Artistic Insights on What Drives Host-Myxozoan Interactions in Large River Systems

9:45 AM **Americus**: The Myxozoan Parasite *Ceratonova shasta* Uses a Minimal Genetic Repertoire to Infect Its Fish and Invertebrate Hosts

10:00 AM **Ghai**: Morphological and Molecular Identification of Myxozoan Parasites and Its Effect on Cultured Indian Major Carps in Panjab, India

10:15 AM **Kaur**: Diversity of Myxozoan Parasites Associated with Diseases in Aquaculture and Wild Fish Stocks in India

10:30 AM Refreshments

10:45 AM **Gorgoglione**: Discovery of *Tetracapsuloides bryosalmonae* Infecting Salmon in the Great Lakes

11:00 AM **Stilwell**: Massive Branchial Henneguyosis: A Distinctive Myxozoan-Induced Gill Disease of Catfish Caused by Massive Interlamellar Infection of *Henneguya exilis*

11:15 AM **Woodyard**: *Myxidium mollismum* n, Sp., a Novel Myxozoan from the Common Elder *Samoteria moillissima*

11:30 AM **Ferguson**: Proliferative Kidney Disease and Surveilance in Wild Alaska Salmon

Ceratonova shasta: Biological and Artistic Insights on What Drives Host-Myxozoan Interactions in a Large River System

Jerri L. Bartholomew, Stephen D. Atkinson, Julie D. Alexander, and Sascha L. Hallett

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

For the past 20 years our laboratory has researched the salmon parasite *Ceratonova shasta* in the Klamath River, Oregon. This river, positioned for the largest dam removal project in history, has suffered precipitous declines in its salmon populations, in large part because of infection by this myxozoan in out-migrating juveniles. Our long-term research has explored many different aspects of this host-parasite interaction with the aim of providing guidance for river management. Establishment of index sites for water sampling has provided insights into how parasite distribution and abundance varies among water years, leading to management strategies for reducing disease through dilution flows. Monitoring density and infection prevalence of the annelid invertebrate host, *Manayunkia occidentalis*, provides insights on risk prediction and the effects of scouring flows. However, complicating our predictions is the discovery that the parasite is actually a species complex, with different genotypes affecting different salmonid species: genotypes 0, I and II associated with disease in Steelhead/Redband trout, Chinook salmon and Coho salmon, respectively. Thus, the distribution of the different parasite genotypes is affected by the dams that serve as barriers to host migration, and will now be affected by the removal of these barriers. This talk will present an overview of our research, not only from the biological and management perspectives, but also using art and music to explore relationships.

The Myxozoan Parasite *Ceratonova shasta* Uses a Minimal Genetic Repertoire to Infect its Fish and Invertebrate Hosts

Benjamin Americus[1], Tamar Lotan[2], Jerri L. Bartholomew[1], Stephen D. Atkinson[1]

[1]Department of Microbiology, 226 Nash Hall, Oregon State University, Corvallis, OR 97331 USA; [2]Department of Marine Biology Room 281, Multipurpose Building University of Haifa 199 Aba Khoushy Ave. Mount Carmel, Haifa 3498838, Israel

The myxozoans are an anciently derived clade of Cnidaria, related to jellyfish and sea anemones. They are microscopic parasites that typically utilize vertebrate (fish) and invertebrate (annelid) hosts, and alternate between two life stages: a fish-infective actinospore and an invertebrate-infective myxospore. Myxozoans are the causative agents for fish diseases including proliferative kidney disease, enteromyxosis and "Hamburger Gill" disease, and therein cause ecological impacts and economic losses in freshwater and marine fish stocks worldwide. Myxozoans have reduced genomes and simplified morphologies relative to free-living cnidarians. To better understand the extent of genetic streamlining that has occurred in Myxozoa, we extracted RNA from *Ceratonova shasta* infections in both its fish and invertebrate hosts, and assembled transcriptomes using our new *C. shasta* long-read reference genome. These genome-guided transcriptomes are free of the host contamination that plagued past analyses and allow comparisons to free-living model organisms and between myxozoan lifestages. We have compared the full transcriptomes and specific genes families related to host sensing and venom. We found that the transcriptome of developing *C. shasta* myospores is larger than that of developing actinospores (25758 vs. 15021 genes), with a core set of 7815 genes shared between life stages, which includes many enzymes for metabolism. Life stage specific genes include structural proteins like vinculin and reticulon. For sensing and responding to their hosts, myxozoans lack the nematocyte-specific chemosensor and voltage-gated calcium channel of free-living cnidarians. Myxozoans have retained nematocysts with nematogalectin tubules and minicollagen capsules, which are identical between life stages. Both *C. shasta* life stages have retained only ~10% of the venom-like compound diversity of free-living cnidarians. Time series expression data suggests *C. shasta* uses some venom-like compounds for proliferation within its hosts rather than solely during initial attachment. Altogether, these findings suggest *C. shasta* lacks many features found in free-living Cnidaria and expresses similar transcriptomes in both its vertebrate and invertebrate hosts. Targeting of proteins specific to each life stage may aid in improved assay design for specific parasite detection.

Morphological and Molecular Identification of Myxozoan Parasites and Its Effect on Cultured Indian Major Carps in Panjab, India

Nitin Ghai, Harpreet Kaur* Kharoon nissa

Parasitology Laboratory, Department of Zoology, Panjab University, Chandigarh 160014, India

Since India ranks second in aquaculture, the importance of diseases especially of Myxozoan parasites are of high concern. The present study aims at the identification of species and to explore the pathogenicity of the parasite on the host system. Fresh spores were used for morphology and morphometric characterisation using a Phase contrast microscope with permanent staining. A Qiagen kit was used to extract genomic DNA, which was then used to undertake phylogenetic analysis using the 18s ribosomal sequence. In both infected and healthy fish blood, many haematological parameters were examined. On the ventricle of the heart, creamish white plasmodia (Type B plasmodia seen under stereozoom microscope). The impact and severity of parasite load were demonstrated by significant variations in blood parameters. The tissue specificity of infection is quite unusual, however it has been detected in the heart of *Labeo rohita* and is fatal to the host. Its presence in the heart indicates the extent of the host's loss.

Diversity of Myxozoan Parasites Associated with Diseases in Aquaculture and Wild Fish Stocks in India

Harpreet Kaur[1], Ranjeet Singh[2], Anu Katoch[3], Rajni Kumari[4], Shoib Ail Dar[5], Ishtiyaq Ahmad[6], Aditya Gupta[7], Kharoon Nissa[8] and Nitin Ghai[9]

[1]Department of Zoology, Panjab University, Chandigarh, India; [2]Scientist B, Ministry of Environment and Climate Change, Government of India, New Delhi; [4]Sri Guru Gobind Singh College, Sector-26, Chandigarh, 160019; [5]Centre of Research for Development, University of Kashmir, Sri Nagar, India; [6]DST Sponsored Fish Nutrition Research Laboratory, Department of Zoology, University of Kashmir, Srinagar, India; [7]School of Zoology, George S. Wise, Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel; [8]Department of Zoology, Panjab University, Chandigarh, India; [9]Department of Zoology, Panjab University, Chandigarh, India

Myxozoans are highly diverse and vastly distributed cnidarian endoparasites in freshwater and marine habitats all over the world. They haveheteroxenous life cycle, including invertebrate and fish hosts, and have been regarded as one of the emerging parasite group infecting cultured fish. Most myxozoans are not overtly pathogenic to fish hosts, but some are responsible for considerable economic losses in fisheries and aquaculture. Fish parasitology has a long tradition in Indian subcontinent and numerous parasitologists have contributed considerably to the current knowledge of the diversity and biology of metazoan parasites of freshwater fishes. The importance of fish parasites has decreased during the last few decades in Indian subcontinent, which is reflected in the considerable decline of funding and corresponding decrease of attention paid to these parasites. However, there is exception of Myxozoa, to which significant focus has been given in the recent past and much of the data has been published in good journals. This was only possible because of the systematic use of morphological and molecular data along with other features, such as the site and type of infection, offering a higher degree of congruence with molecular data.

The data provided in this paper exhibit an overview of the diversity in Indian fishes. As many as 70 species infecting vast range of hosts have been reported by the author and her coworkers in the state of Punjab, India. Punjab is a land of five rivers and has a total of six wetlands which have been designated as wetlands of international importance. These are - Harike Wetland, Ropar Wetland, Kanjli Wetland, Keshopur - Miani Community Reserve, Beas Conservation Reserve and Nangal Wildlife Sanctuary.In Punjab, there are around seven species of fish that are farmed. While the famous singhara and sole varieties are found in natural water, breeds such as the grass carp, silver carp, common carp, Indian Rohu, Katla and Mrigal are grown in village ponds.The organs infected were gills, scales, fins, opercular cavity, nasal cavity, eye ball, muscles, liver, gall bladder,kidney, heart etc. The species complex among the member species of *Thelohanellus* genus from the Indian subcontinent infecting gills, fins and muscles of cyprinid carps has been also studied. The species forming species complex are *T. rohitae, T. catlae, T. jiroveci, T. seni, T. bifurcata, T. dykovae, T. neocyprini, T. filli, T. muscularis* and *T. theinensis*. The phylogenetic analysis was done on the basis of 18S rDNA which showed close similarity between the species. The homogeneity was found to be between 90 to 99%. The factors responsible for the species complex could be phylogeography, host reluctant, organ and tissue specificity of these myxozoan parasites. Study of more genetic markers facilitated with morphotaxonomy can be used to sort out the occurrence of species complexes among the morphologically different species having similar genetic makeup and vice-versa.

Discovery of *Tetracapsuloides bryosalmonae* Infecting Salmon in the Great Lakes

Cahya Kurnia Fusianto[1], Gary Whelan[2], Luke VanBlois[1], Bartolomeo Gorgoglione[1]

[1] Fish Pathobiology and Immunology Laboratory, Dept. Pathobiology and Diagnostic Investigation/Dept. Fisheries and Wildlife, Michigan State University, East Lansing, MI 48824;
[2] Fisheries Division, Michigan Department of Natural Resources, Lansing, MI 48909

Changing ecosystems expose fishes to emerging pathogens, threatening wild and enhanced populations. The myxozoan parasite *Tetracapsuloides bryosalmonae* causes Proliferative Kidney Disease (PKD), a severe immunosuppressant pathology that impacts salmonid management across Europe and North America. Infection prevalence and clinicopathology in susceptible species are worsened by seasonal water temperature rises linked to climate change, which allow this parasite to move northward, spreading to new water bodies. In North America, *T. bryosalmonae* infections are known in Trout and Pacific Salmon species across Western States, up to Alaska. Currently *T. bryosalmonae* and PKD have not been described in the Great Lakes. In coordination with MI-DNR, we opportunistically sampled Chinook (*Oncorhynchus tshawytscha*) and Coho Salmon (*O. kisutch*) at river weirs during their spawning migrations from the lakes. During the Fall of 2020 and 2021, 167 kidney samples were collected from large adults (sexually mature) and jacks (precocious males), returning from Lake Michigan (at Boardman River weir, Little Manistee River weir, and Platte River weir), and from Lake Huron (Swan River weir). To retrieve preliminary data on *T. bryosalmonae* presence in these fish populations and water bodies, DNA specimens were screened using PCR, targeting the malacosporean 18S small subunit ribosomal RNA gene. Both adults and jacks of Chinook and Coho Salmon were found to be positive to *T. bryosalmonae*. Parasite prevalence varied across sites and species (ranging from 7 to 52%). At one sampling location, parasite prevalence in adult Coho Salmon was higher in 2021 (52%) than in 2020 (23%). Sequence BLAST analysis unequivocally confirmed (100% sequence similarity to other *T. bryosalmonae* reference sequences available) the first detection of *T. bryosalmonae* from fish hosts in the Great Lakes. Further studies are ongoing to better assess *T. bryosalmonae* prevalence and distribution across the Great Lakes, and to confirm the occurrence of coelozoic/histozoic parasite stages and of PKD in each species. Prophylactic and therapeutic treatments against PKD are still not available, hence predicting outcomes of *T. bryosalmonae* infections and PKD outbreaks may guide actionable management solutions to mitigate the economic and ecological damages in the larger freshwater system in the world.

Massive Branchial Henneguyosis: A Distinctive Myxozoan-Induced Gill Disease of Catfish Caused by Massive Interlamellar Infections of *Henneguya exilis*

Justin M. Stilwell[1,2], Matt J. Griffin[3], John H. Leary[1], Lester H. Khoo[3], Alvin C. Camus[1]

[1]Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602; [2]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762; [3]Thad Cochran National Warmwater Aquaculture Center, Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Delta Research and Extension Center, Stoneville, MS 38776

Proliferative gill disease (PGD), caused by the myxozoan parasite *Henneguya ictaluri*, is the most prevalent parasitic disease of United States catfish aquaculture. Recently, an unusual myxozoan-induced gill disease caused by massive burdens of *Henneguya exilis* has been diagnosed within channel (*Ictalurus punctatus*) x blue (*Ictalurus furcatus*) hybrid catfish monoculture. Targeted metagenomic sequencing and *in situ* hybridization (ISH) were used to examine myxozoan community composition between massive branchial *Henneguya exilis* infections and clinical PGD cases to identify myxozoan species contributing to pathology. Thirty ethanol-fixed gills from seven hybrid catfish massive branchial *Henneguya* cases were subjected to targeted amplicon sequencing of the 18S rDNA gene (Illumina MiSeq) and compared to a metagenomic dataset generated from clinical PGD cases. Further, serial sections of 21 formalin-fixed gills (3 per case) were analyzed by RNAscope® duplex chromogen ISH assays targeting 8 different myxozoan species. Metagenomic and ISH data were in agreement, indicating myxozoan community composition significantly differs between PGD and branchial henneguyosis cases, with different myxozoan communities contributing to disease pathogenesis. Findings indicate PGD in farm-raised catfish can consist of mixed species infections, while branchial henneguyosis was attributed to nearly pure infections of *H. exilis*. Other *Henneguya* spp. were rare in branchial henneguyosis, although *H. ictaluri* was identified by ISH in infrequent PGD-like lesions, supporting previous work evincing hybrids are susceptible to acute stages of *H. ictaluri*. Building upon previous pathologic descriptions and molecular characterization, this work provides the case definition for a distinct, potentially emerging, myxozoan-induced gill disease of farm-raised catfish, tentatively termed massive branchial henneguyosis.

Myxidium mollisimum n. sp., a Novel Myxozoan from the Common Eider *Somateria moillissima*

Ethan T. Woodyard[1], Thomas G. Rosser[2], Justin M. Stilwell[1], Celene Slifka[2], Divya Rose[2], Matt J. Griffin[3]

[1]Thad Cochran National Warmwater Aquaculture Center, Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Delta Research and Extension Center, Mississippi State University, Stoneville, MS 38776; [2]Department of Comparative Biomedical Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762; [3]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762

Myxozoans are an important subphylum of cnidarian parasites, mostly known for the diseases they cause in commercially important freshwater and marine fish. With more than 2200 described species, myxozoans are an extremely diverse group, with reports from a variety of aquatic and terrestrial hosts. Herein, a novel *Myxidium* species is morphologically and histologically described from the common eider *Somateria mollissima*, a large circumpolar sea-duck. Fresh myxospores were collected from the gallbladder of *S. mollissima* from Louisiana, USA. Based on 50 fresh myxospores, the body is ellipsoidal in valvular view and very slightly sigmoidal in sutural view, bearing 9–11 striations along the surface. The spore bodies measure 18.0–20.5µm long, 8.0–10.4µm wide, and 7.8–9.6 µm thick. The two polar capsules are 4.9–7.3 µm apart and bear 4–5 turns in the polar tubules. Larger and smaller capsules measure 5.2–6.2 × 3.6–4.9 µm and 4.1–6.0 × 3.6–5.4 µm, respectively. Histologically, the liver exhibited severe plasmacytic cholangiohepatitis with extramedullary hematopoiesis, cholestasis, and rare myxospores consistent with those identified in the gallbladder. While molecular and scanning electron microscopy characterization are ongoing, the present morphological characters distinguish the novel *Myxidium* sp. from the larger *M. anatidum.* The name *Myxidium mollisimum* n. sp. is provisionally ascribed after the specific epithet of the host. While hepatobiliary lesions are attributed to infection with *Myxidium mollisimum* n. sp., the gross and histological examinations suggest the host died from aspergilloses, with the contribution of infection with *Myxidium mollisimum* n. sp. being unknown. This is only the second myxozoan described from an avian host.

Proliferative Kidney Disease and Surveillance in Wild Alaska Salmon

Jayde A. Ferguson[1], Davis J. Stewart[1], Franklin R. Woitel[1], Theodore R. Meyers[1], Cahya Fusianto[2], Bartolomeo Gorgoglione[2]

[1]Alaska Depart. of Fish and Game, Division of Commercial Fisheries, Fish Pathology Section, Anchorage, Alaska, USA; [2]Fish Pathobiology & Immunology Laboratory, Dept. Pathobiology & Diagnostic Investigation Michigan State University, East Lansing, Michigan, USA

Proliferative Kidney Disease (PKD) is an emerging health issue for fishery management as global warming progresses. The myxozoan parasite *Tetracapsuloides bryosalmonae* has a two-host lifecycle, with bryozoans releasing waterborne malacospores infectious to susceptible salmonids. The infection may cause a severe immunopathology with seasonal mortality, or immunocompromise the host predisposing them to polymicrobial infections. Climate change events can enhance the parasite's ability to reproduce and its virulence to fish that also become more susceptible from thermal stress. We recently confirmed that *T. bryosalmonae* has been circulating as far north as Alaska. To better understand the impact of *T. bryosalmonae* and occurrence of PKD in Pacific salmon populations, we initiated a broodstock surveillance program using qPCR. The parasite was found in one Chinook salmon (*Oncorhynchus tshawytscha*) stock, although at low prevalence, from Crooked Creek on the Kenai Peninsula. This site is relatively close to an older case documented previously, in which PKD was found in sockeye salmon (*O. nerka*) pre-smolts in freshwater netpens. Another location of interest is the Yukon River, the third longest river in North America, where subsistence fishers have harvested salmon for many generations. Chinook salmon have been declining in this river system for many years and some of the worst returns on record have occurred since 2020. Large discrepancies in Canadian-origin fish abundance between sonar projects at the mouth and the Canadian border has indicated high en route mortality. As part of a larger project of assessing the causes for this decline, we are performing targeted pathogen screening at separate locations on the Yukon River to study parasite-associated mortality. *T. bryosalmonae* was selected because 5 adult chum salmon (*O. keta*) returning to this river in 2011 had clinical PKD. In 2020 we documented an unusual case of a Dolly Varden (*Salvelinus malma*) returning to a nearby river system with severe/resolving PKD and severe *Ichthyophonus* sp. infection, that is also a targeted parasite in this study. qPCR was used to screen fish for both pathogens including histology to assess any tissue pathology associated with these infections. Both *T. bryosalmonae* and *Ichthyophonus* sp. are likely important drivers of adult Pacific salmon pre-spawning mortality in Alaskan river systems, with the potential to become increasingly more impactful under climate change conditions in Alaska and elsewhere.

Thursday, September 8th
Breakout Room A

Immunology/Vaccinology:
Lora Petrie-Hanson/Beth Peterman

1:00 PM	**Adamek**: Vaccination Protects the Skin Barrier and Gill Function from Disruption Caused by Cyprinid Herpesvirus 3
1:15 PM	**Soto-Davila**: Effect of Feeding Strategy of Jameison® Probiotic on Growth Performance and Immune Response of Chinook Salmon (*Oncorhynchus tshawytscha*) Challenged with *Vibrio anguillarum*
1:30 PM	**Liu**: Evaluating the Efficacy of New Oral Vaccine Feeds Against Salmonid Novirhabdovirus in Rainbow Trout (*Oncorhynchus mykiss)*
1:45 PM	**Jones**: Evaluating a Novel Oral Vaccine Delivery Platform in Rainbow Trout *Onchorhynchus mykiss*
2:00 PM	**Thorarinsson**: Effect of Vaccines Against Pancreas Disease in Atlantic Salmon Challenged with Salmonid Alphavirus, Subtype 2
2:15 PM	**Thorarinsson**: Effect of Vaccines Against Pancreas Disease on Viral Shedding and Disease Transmission from Atlantic Salmon Challenged with Salmonid Alphavirus, Subtype 2
2:30 PM	**Giudicelli**: Beneficial Effects of Marine Bacillus Multi-Strains Consortium Encapsulated in Algae on Growth Performance, Mucosal Microbiota Modulation, Density Stress Resistance and Immunity Gene Expressions of Atlantic Salmon *Salmo salar*
2:45 PM	**Cortes**: The Phagosome–Lysosome Fusion Is the Target of a Purified Quillaja saponin Extract (PAQ-Xtract) in Reducing Infection of Fish salmon Macrophages by the Bacterial Pathogen *Piscirickettsia salmonis*
3:00 PM	Refreshments
3:15 PM	**Aedo**: Early Regulation of Immune-Related Genes Mediated by Cortisol in Rainbow Trout (*Oncorhynchus mykiss*) Gills
3:30 PM	**Petrie-Hanson**: Epigenetic Changes Associated with Increased Phagocyte Functions Demonstrate Trained Immunity in Catfish Leukocytes
3:45 PM	**Saez**: Evaluation of Iron Metabolism-Related Genes Post-Vaccination of Atlantic Salmon.
4:00 PM	**Casadei**: Antimicrobial Peptide Modulation in Rainbow Trout During Acute Stress

Vaccination Protects the Skin Barrier and Gill Function from Disruption Caused by *Cyprinid herpesvirus 3*.

Mikolaj Adamek[1], Marek Matras[2], Alexander Rebl[3], Magdalena Stachnik[2], Verena Jung-Schroers[1], Lars Schröder[4], Walter Fuchs[4], Michal Reichert[2], Dieter Steinhagen[1]

[1]Fish Disease Research Unit, Institute for Parasitology, University Of Veterinary Medicine Hannover, Hannover, Germany; [2]Laboratory of Fish Diseases, National Veterinary Research Institute, Pulawy, Poland; [3]Fish Genetics Unit, Institute of Genome Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany; [4]Institute of Molecular Virology and Cell Biology, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany

Epitheliotropic viruses can be particularly dangerous in the aquatic environment, which is osmotically and microbiologically hostile for fish. If the pathogen can disrupt the mucosal barriers the breach can lead to severe secondary consequences i.e. disruption of the osmotic balance or induction of secondary infections. We used a live attenuated virus vaccine against *cyprinid herpesvirus 3* (CyHV-3) to study, which aspects of non-direct protection are important in the water environment and to fill in the still existing knowledge gaps on how these vaccines effectively protect fish from the deadly disease caused by CyHV-3. Common carp were vaccinated against CyHV-3 using a double deletion vaccine virus KHV-TΔDUT/TK in absence or presence of a mix of common carp beta-defensins 1, 2 and 3. The fish were challenged 2.5 months post vaccination with a hyper-virulent Polish isolate of CyHV-3. Blood, skin, gill, and kidney were collected 2, 7, 14, 28 days post vaccination and post challenge for monitoring immune responses by SNT, RT-qPCR using Fluidigm, and pathology related to the disease. Vaccination induced marginal clinical signs, low virus load and minor upregulation of *cd8* and *igm* gene expression in vaccinated fish while the neutralisation activity of blood was rising from 14 days post vaccination. A challenge infection with CyHV-3 induced a severe disease with 80-100% mortality in non-vaccinated fish while in vaccinated fish no mortality was recorded and the virus load was >1000-fold lower. Histological analysis showed that vaccination protected from pathological changes in skin and gills. In the skin of non-vaccinated fish, T and B cells responses were severely downregulated, inflammation and stress responses were increased upon challenge, while vaccinated fish had boosted neutrophil, T and B cell responses. A disruption of skin and gill barrier elements (tight, and adherence junction, desmosomes, mucins) led to a severe osmotic disbalance and an uncontrolled increase of skin and gill bacteria loads which most likely exacerbated the pathology. Using a live attenuated virus vaccine, we show that increased neutrophil, T and B cell responses provide protection from CyHV-3 infection and preserved skin integrity and gill function, which supports a successful protection from secondary bacterial pathogens and osmotic disruption allowing vaccinated carp to cope with the hostile aquatic environment.

Effect of Feeding Strategy of Jamieson® Probiotic on Growth Performance and Immune Response of Chinook Salmon (*Oncorhynchus tshawytscha*) Challenged with *Vibrio anguillarum*

Manuel Soto-Dávila[1], Rory A. Webb[1], Tania Rodríguez-Ramos[1], Gillian McDonald[1], Brian Dixon

[1]Department of Biology, University of Waterloo, Waterloo, Canada

Chinook salmon is a native species of the Canadian Pacific coast with potential economic and environmental benefits for aquaculture. Chinook salmon farming is jeopardized by the risk of escapees diluting the genetic diversity of wild populations. Sterile triploid salmon offer a solution however, they have an a 10-30% higher disease mortality rate compared to diploid fish. An important non-toxic and non-polluting tool in aquaculture to improve fish growth, stress tolerance and non-specific defense is probiotic treatment. In finfish aquaculture, many probiotics have been tested, including lactic acid bacteria (LAB; Lactobacillus and Carnobacterium) and Bacillus spp. Commercially available, low-cost Jamieson® probiotic contains 14 different strains and has shown beneficial effects in humans. There is currently no evidence of its effects in Chinook salmon. To determine its impact on Chinook salmon growth and immune response, fish were randomly assigned to four net pens and given either regular feed control or probiotic treatment for a year. After this, fish were transferred to troughs and challenged with Vibrio anguillarum. There were no statistically significant differences in weight (g) and length (cm) among treatments. In head kidney tissues, probiotic supplementation modulated the transcript levels of il10, camp, and hamp genes compared to the control group. In contrast, probiotic treatment did not significantly modify the expression of il1b, il8, and tnfα. In hindgut samples, a significant modulation in the transcript expression of il8, cldn1, and ocln was observed in fish supplemented with Jamieson® probiotic compared to the control group. Additionally, il1b, camp, hamp, and transferrin expression was modulated among treatments at different time-points. On the contrary, no significant differences were observed in il10, tnfa, cldn3, cldn12, and zo-1 in hindgut samples. Finally, the relative expression of the transcripts encoding il1b, il10, tnfa, and camp in spleen samples were modulated after probiotic supplementation, while no significant differences were observed in the expression of il8, hamp, and transferrin of fish supplemented compared to the control. Collectively, our results suggest that Jamieson® probiotic supplementation can positively modulate the early inflammatory response against V. anguillarum infection and help improve Chinook salmon aquaculture in an environmentally sustainable manner.

Evaluating the Efficacy of New Oral Vaccine Feeds Against *Salmonid Novirhabdovirus* in Rainbow Trout (*Oncorhynchus mykiss*)

Bartolomeo Gorgoglione[1], Jie Li[2], Juan-Ting Liu[1], Vikram N. Vakharia[2]

[1]Fish Pathobiology and Immunology Laboratory, Dept. Pathobiology and Diagnostic Investigation/Dept. Fisheries and Wildlife, Michigan State University, East Lansing, MI 48824;

[2]Institute of Marine & Environmental Technology, Dept. of Marine Biotechnology, University of Maryland Baltimore Country, Baltimore, MD 21202

Salmonid novirhabdovirus (IHNV) is a pathogen of major economic concern to the aquaculture industry, causing infectious haematopoietic necrosis (IHN) in trout and salmon species. IHNV infection is OIE-notifiable, as causing the currently most important viral disease affecting salmonids in the Northern hemisphere. Although an injectable plasmid-based DNA vaccine of glycoprotein (G) gene is very effective, there are no oral vaccines available that could be administered for mass vaccination of fry. In this study, two recombinant baculoviruses were generated to produce IHNV-G and IHNV-G-C5a proteins in insect larvae. In the latter construct, C5a was coupled with IHNV-G protein to enhance immune response in rainbow trout (*Oncorhynchus mykiss*). The expression of recombinant IHNV-G and IHNV-G-C5a proteins in cabbage looper (*Trichoplusia ni*) insect larvae was confirmed by Western blotting and chemiluminescence assays. To vaccinate trout orally, the insect larvae expressing recombinant IHNV-G protein(s) were converted into a powder form after freeze-drying, added to artificial feed at 3%, top-coated with gelatin binder and oil. Commercial trout were fed with these feedings containing IHNV-G and IHNV-G-C5a protein for 2 weeks and boosted 4 weeks post primary immunization with the same feeding. Fish were then challenged with IHNV by immersion 4 weeks post booster. Fish survival was evaluated, and blood and spleen samples were collected at 7 and 14 days post viral challenge (dpc). Non-vaccinated and IHNV-G fed trout succumbed to death and had mortality ranging from 91.7 to 97.6% and from 70.9 to 88.4% on day 8 and 15 dpc, respectively. However, IHNV-G-C5a fed group exhibited a reduced mortality of 51.2% at 8 dpc and increased to 81.7% at 15 dpc (at experiment termination), suggesting some resistance against virulent IHNV challenge. Individual viral load was measured by qPCR detection of IHNV nucleoprotein (N) from cDNA, showing no significant difference across experimental groups. The expression of selected trout immune response markers was evaluated across experimental groups, including the transcription of Type I IFN-a, MX-1, CD4, and IgM genes. Further study is needed to assess how the new oral vaccine may be effective to mitigate IHNV infection and how it could modulate the host immune response towards IHNV exposure.

Evaluating a Novel Oral Vaccine Delivery Platform in Rainbow Trout *Oncorhynchus mykiss*

Evan M. Jones[1], Jie Ma[1], Matt Hawkyard[2], Mary R. Arkoosh[3], Joseph P. Dietrich[3], Cameron M. Schuster[3], Luke P. Oliver[1], Veronica L. Myrsell[1], and Kenneth D. Cain[1]

[1]Department of Fish and Wildlife Sciences, University of Idaho, Moscow, ID 83844; [2]Aquaculture Research Institute, University of Maine, Orono, ME 04469; [3]NOAA Northwest Fisheries Science Center, Newport Research Station, Newport, OR 97365

As commercial aquaculture operations grow and intensify, fish farmers continue to adopt vaccination programs to prevent losses from disease. Oral vaccination can significantly reduce costs of these programs while allowing for low stress vaccine delivery to small fish. This study evaluates the ability of a novel alginate-based oral vaccine particle to stimulate immunity and provide protection against the causative agent of furunculosis, *Aeromonas salmonicida*. A formalin-killed, whole cell *A. salmonicida* vaccine was produced and used to vaccinate ~ 2.5 g juvenile rainbow trout (*Oncorhynchus mykiss*) via four routes: intraperitoneal injection (IP), anal intubation (AL), immersion (BA), and oral vaccination by alginate-particle ingestion (OV). Control groups included a particle containing no vaccine (CP) and a sterile PBS intraperitoneal injection (PB). All treatment groups received a booster 2 weeks after their first dose. Two trials were performed, with some differences in vaccine formulations. Specific antibodies in serum were measured at 0, 2, 4, 6, 8, and 13 weeks post vaccination (wpv). Fish were then challenged in triplicate tanks for each treatment by a 24 hour static immersion with 1×10^7 cfu/mL of *A. salmonicida*. Fish were challenged at 13 and 4 wpv for trials 1 and 2, respectively. In trial 1, log antibody titers were significantly elevated in the OV group (1.57) relative to both CP and PB groups (0,0) at 4 wpv, before declining. For trial 2, the OV group had a slight, but not statistically significant, elevation of antibody titers at 6 wpv (0.86) before declining to baseline levels by 8 wpv. The IP group had the highest titers for all timepoints throughout both trials, ranging from 1.89 to 4.16. This was followed by the BA and AL groups, whose titers throughout the trial ranged from 0.34 to 2.06. For the pathogen challenge in trial 1, survival in the OV group (51.1%) was not significantly different from other treatment groups where survival ranged from 17.8% to 70.0%. However, the CP group had significantly higher survival (64.4%) relative to the PB group (17.8%), as did the AL (68.9%), BA (64.4%), and IP (70.0%) groups. In trial 2, the OV group (68.3%) had significantly higher survival relative to the PB group (36.7%), but not CP fish (51.7%). The IP group, which incorporated an adjuvant in trial 2, had significantly higher survival (96.7%) relative to all other treatments including AL (63.3% survival) and BA (65.0% survival). The oral vaccine particle successfully stimulated an antibody response and both groups fed the alginate-particle showed resistance to *A. salmonicida* in a pathogen challenge. The anal vaccination demonstrated the ability of killed vaccines to stimulate gut associated lymphoid tissue (GALT) which can provide protection against pathogens. Interestingly, in trial 1 the control particle without vaccine provided protection equal to other vaccine treatments, and even some protection relative to the PBS control group in trial 2. The reason for this is not clear but may be due to an adjuvant effect of the alginate-particle. Importantly, the oral vaccine stimulated a specific antibody response, which provides evidence for successful delivery of oral vaccines using this alginate-particle platform. However, adjustments to particle formulation, dose, and delivery strategy requires further research.

Effect of Vaccines Against Pancreas Disease in Atlantic Salmon Challenged with Salmonid Alphavirus, Subtype 2

Ragnar Thorarinsson[1], Hilde Sindre[2], Jeffrey C. Wolf[3], Eystein Skjerve[4] and Anne Ramstad[5]

[1]Elanco Animal Health, Bergen, N-5058 Norway; [2]Norwegian Veterinary Institute, Ås, N-1433 Norway; [3]Experimental Pathology Laboratories Inc., Sterling, Virginia 20166, USA; [4]Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Ås, N-1433 Norway; [5]VESO Vikan, Namsos, N-7810, Norway.

PIT-tag marked parr were immunized with two different pancreas disease (PD) vaccines and a hexavalent oil-adjuvanted vaccine (OAV). The control groups were injected with the same hexavalent OAV lacking salmonid alphavirus (SAV) antigen, or with saline. All groups except the saline control were simultaneously immunized against enteric redmouth disease to reflect the vaccination strategy commonly used in Mid-Norway. After an immunization period of ~1500 degree days at 12-13 °C, the fish were exposed to SAV, subtype 2 (SAV2) using a cohabitation challenge in seawater. Samples were taken before challenge and at 19, 54 and 84 days post challenge. Results including growth, side effects and levels of SAV2 neutralizing antibodies prior to challenge will be presented. Post challenge data including mortality, viremia, levels of neutralizing antibodies, levels of pathological changes in the heart and pancreas and loss of growth caused by PD through the challenge period will, in addition, be presented and discussed.

Effect of Vaccines Against Pancreas Disease on Viral Shedding and Disease Transmission from Atlantic Salmon Challenged with Salmonid Alphavirus, Subtype 2

Ragnar Thorarinsson[1], Hilde Sindre[2], Jeffrey C. Wolf[3], Eystein Skjerve[4] and Anne Ramstad[5]

[1]Elanco Animal Health, Bergen, N-5058 Norway; [2]Norwegian Veterinary Institute, Ås, N-1433 Norway; [3]Experimental Pathology Laboratories Inc., Sterling, Virginia 20166, USA; [4]Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Ås, N-1433 Norway; [5]VESO Vikan, Namsos, N-7810, Norway.

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Beneficial Effects of a Marine *Bacillus* Multi-strain Consortium Encapsulated in Algae on Growth Performance, Mucosal Microbiota Modulation, Density Stress Resistance and Immunity Gene Expressions of Atlantic Salmon *Salmo salar*

Carine Le Ker PhD[1], Fanny Giudicelli PhD[1]

[1]Marine Akwa

Aquaculture production is increasingly looking towards innovative probiotic solutions to further improve gut health and production sustainability. A 56-day feeding trial was performed in recirculating aquaculture system on 124g initial body weight healthy *Salmon salar* (n=80 fishes/350L tank) to evaluate the effects of a marine probiotics consortium (MPC) constituted of four marine *Bacillus* strains encapsulated in algae. The treatment with MPC at 0.5% improved weight gain, final body weight, specific growth rate and yield by respectively 6, 3.2, 1.9 and 3.87% and decrease FCR from 1.01 to 0.95 (not significant). On the other hand, the MPC's use significantly increased fish size by 3.2% (p= 0.052). Dietary inclusion of MPC enhanced intestinal villi size by 6.54% and decreased deformations frequency. The results of high-throughput sequencing showed an improvement of salmon intestinal bacterial communities with MPC diet supplementation. The colonisation of intestinal mucus by *Bacillus* probiotics has been demonstrated, and it was associated with the emergence of other beneficial bacteria as well as a depletion of pathogens species like *Vibrio splendicus*. Those results illustrate perfectly the exclusion competition mechanisms of probiotics in fish tract. In response to a density stress applied at day 50, MPC's use decreased significantly the production of cortisol and glucose by respectively 23.6 and 10.5% compared to control stressed fish. In addition, impact on several RNA markers related to immunity has been investigated. In conclusion, this study demonstrated the ability of the MPC encapsulated in algae to enhance Atlantic salmon growth performances, immune status, stress resistance and intestinal microbiota modulation during smolt phase.

The Phagosome–Lysosome Fusion Is the Target of a Purified *Quillaja saponin* Extract (PAQ-Xtract) in Reducing Infection of Fish salmon Macrophages by the Bacterial Pathogen *Piscirickettsia salmonis*

Hernán D. Cortés[1], Fernando A. Gómez[2] and Sergio H. Marshall[2]

[1]Desert King Chile; [2]Laboratorio de Genética e Inmunología Molecular, Instituto de Biología, Pontificia Universidad Católica de Valparaíso, Valparaíso 2340000, Chile

Piscirickettsia salmonis, the etiological agent of Piscirickettsiosis, is a Gram-negative and facultative intracellular pathogen that has affected the Chilean salmon industry since 1989. The bacterium is highly aggressive and can survive and replicate within fish macrophages using the Dot/Icm secretion system to evade the host's immune response and spread systemically. To date, no efficient control measures have been developed for this disease; therefore, the producers use large amounts of antibiotics to control this pathogen. In this frame, this work has focused on evaluating the use of saponins from *Quillaja saponaria* as a new alternative to control the Piscirickettsiosis. It has been previously reported that purified extract of *Q. saponaria* (PAQ-Xtract) displays both antimicrobial activity against pathogenic bacteria and viruses and adjuvant properties. Our results show that PAQ- Xtract does not present antimicrobial activity against *P. salmonis*, although it reduces *P. salmonis* infection in an in vitro model, promoting the phagosome–lysosome fusion. Additionally, we demonstrate that PAQ-Xtract modulates the expression of *IL-12* and *IL-10* in infected cells, promoting the immune response against the pathogen and reducing the expression of pathogen virulence genes. These results together strongly argue for specific anti-invasion and anti-intracellular replication effects induced by the PAQ-Xtract in macrophages.

Early Regulation of Immune-Related Genes Mediated by Cortisol in Rainbow Trout (*Oncorhynchus mykiss*) Gills

Jorge E. Aedo [1,2], Daniela Aravena-Canales [1,2], Rodrigo Zuloaga[1,2], Juan A. Valdés [1, 2] and Alfredo Molina [1,2,]

[1]Universidad Andres Bello, Departamento Ciencias Biológicas, Facultad de Ciencias de la Vida, 8370146 Santiago, Chile; [2]Interdisciplinary Center for Aquaculture Research (INCAR), 4030000 Concepción, Chile.

Different stressful events such changes in salinity environment and hypoxia, regulate the expression of immune-related genes in fish gills. Cortisol in the main hormone mediating stress response in fish, however, its participation during early immune responses in gills is poorly understood. Cortisol exerts its effects through two different pathways, genomic/classic (which has been extensively studied) and membrane-initiated actions (which recently has begun to be explored). In this work, we sought to evaluate the impact of both cortisol pathways on the expression of key immune-related genes in gills. Therefore, juvenile rainbow trout were exogenous administered with physiological doses of cortisol or cortisol-BSA (analog of cortisol exclusive inductor of membrane-initiated effects) and the expression of selected immune related genes (*il-1β*, *tnfa*, *il-8, tgfβ*, *il-10*, among others) was evaluated by real time PCR. We observe that both cortisol and cortisol-BSA promotes changes in the expression of immune-related genes in rainbow trout gills after three hours of treatment compared with vehicle group. Overall, we suggest that cortisol, potentially through membrane-initiated actions, modulates the early expression of immune-related genes in gills.

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Epigenetic Changes Associated with Increased Phagocyte Functions Demonstrate Trained Immunity in Catfish Leukocytes

Lora Petrie-Hanson[1] and Ann E. (Beth) Peterman[1]

[1]Department of Comparative Biomedical Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762

Trained Immunity (TI) is the immunomodulation of innate immune cells that provides non-target protection following stimulation. It is determined by epigenetic reprogramming. TI is characterized by metabolic changes that modify immune cell functions. Our study was performed to determine if TI occurs in leukocytes from catfish exposed to beta glucan (Bg), a known inducer of TI. We evaluated this using functional assays, differential transcriptome analysis and differential chromosome modification analysis. In the first set of experiments, channel catfish were intra-peritoneally (IP) injected with 0, 5, 20, or 35 micrograms Bg/g fish. Fourteen days later, anterior kidney (ak) leukocytes were isolated. Flow cytometry analyzed phagocytosis of *Edwardsiella ictaluri*-FITC and *E. piscicida*-FITC. Reactive oxygen species bursts (ROS) and lactate dehydrogenase (LDH) assays were performed. In another experiment, catfish were IP injected or gastric gavaged with 5 micrograms Bg/g. Cells from fish that received Bg phagocytosed *E. ictaluri* 1.2 times greater than control cells. Cells from fish that received Bg by gastric gavage phagocytosed *E. ictaluri* 1.5 times greater than control cells. Furthermore, cells from fish that received Bg by IP injection or gastric gavage phagocytosed *E. piscicida* 2 times greater than control cells. Channel catfish ak cells demonstrated dose-correlated ROS and increased LDH conversions. The next set of experiments defined how Bg effects different kinds of leukocytes. Channel catfish were intra-peritoneally (IP) injected with PBS or 50 micrograms of Bg/gm of fish. Fourteen days later, anterior kidney (ak) leukocytes were isolated. Flow cytometry analyzed phagocytosis or binding of mcherry:*E. ictaluri* and mcherry:*E. piscicida* by cells labeled with monoclonal antibodies L/CD207, mpeg-1, 51a, nccrp-1, 9E1, or C24a for dendritic cells, macrophages, neutrophils, non-specific cytotoxic cells, B-cells or T-cells, respectively. Dendritic cells, macrophages, neutrophils and B cells from fish exposed to Bg phagocytosed significantly more mcherry:*E. ictaluri* than those cells from control fish. Exposure to Bg also enhanced bacterial binding by catfish NCCs. Differential transcriptome analysis of untrained and trained, non-stimulated anterior kidney tissue using RNA Seq demonstrated the BGAK vs PBSAK volcano plot showed 781 significantly differentially expressed genes. 321 were upregulated and 397 were downregulated, with 20167 genes not significantly changed. The DEGs were enriched in the KEGG pathway, and initial results show the ribosome pathway and the taurine and hyptotaurine metabolism pathway were significantly affected. Chromosome immunoprecipitation sequencing (ChIP Seq) on ak tissues using antibodies specific for H3K4me1, H3K4me3, H3K27ac and H3K27Me3 modifications elucidated the genomic location of histone modifications that occurred during training. Following exposure to Bg, H3K4me3 differential peaks were associated with the promoter regions of 5 genes in the toll like receptor signaling pathway which was highly significant in the KEGG analysis. H3K27me3 differential peaks (polychrome repression) were associated with the genes of 4 components of the Wnt signaling pathway which was highly significant in the KEGG analysis. H3k27ac differential peaks were associated with the enhancers of genes as members of the Toll like receptor, RIG-1 receptor signaling, regulation of actin cytoskeleton, calcium signaling, and apoptosis pathways. H3K4Me1 had pathways that were associated with developmental and cell differentiation but included genes with roles in immune functions.

Evaluation of Iron Metabolism-Related Genes Post-vaccination of Atlantic salmon

Alejandro Sáez[1], Amanda Escorza[1], Esperanza Anguita[1], Berta Contreras[2], Amadeo Guzmán[2], Sebastián Escobar-Aguirre[1].

[1]Laboratorio Acuicultura, Departamento Ciencias Animales, Facultad de Agronomía e Ingeniería Forestal Pontificia Universidad Católica de Chile, Santiago, Chile; [2]Cermaq-Chile.

Pathogens such as *Piscirickettsia salmonis*, the causative agent of Salmonid Rickettsial Septicaemia (SRS), are a crucial production dilemma for aquaculture in Chile due to their enormous impact only in economic terms but also on the sustainability and welfare of salmonids. Previous work on this subject has identified, through genomic analysis, a set of candidate genes in families of natural SRS-resistant salmonids, which could be involved in the modulation of biological processes associated with reduced susceptibility to SRS. Among these processes, regulation of iron metabolism stands out as limiting *P. salmonis* replication. However, most of these studies have been performed in controlled conditions in the laboratory. Thus, our work aimed to evaluate the protective mechanism caused by SRS vaccines and iron metabolism of salmonid fishes reared in natural conditions in seawater (cages). The methodology included two groups of salmo salar (SRS vaccine and SRS sham) reared in sea cages located at Colaco, Chile. 5 animals per group were sampled in different periods from April to June. Head kidney was removed from all animals, and RT-PCR was performed using *Transferrin*, *Transferrin receptor*, and *Ferritin* primers. Our result showed that the *Transferrin receptor* exhibits a significant increase in mRNA level expression compared to control during a critical infestation process, with a decreased level of mortality throughout the experiment in the same group. We corroborate that SRS vaccines protect against SRS, but also iron metabolism-related genes present a good correlation in this defensive performance in salmonids fishes cultivated in seawater.

Antimicrobial Peptides Modulation in Rainbow trout During Acute Stress

Yago Vinicius Serra dos Santos[1], Elisa Casadei[1]

[1]University of New Mexico, Biology Department, Albuquerque New Mexico, NM87131-0001, USA

Intensive aquaculture affects fish health and well-being. Fish are highly susceptible to stress including over-crowding, transportation and manual handling which are often associated with increased disease susceptibility. Stressors can also perturb microbiota, via direct and indirect mechanism. The innate immune system is the first line of defense against infection providing a fast and continuous protection from pathogens including bacteria, viruses, parasites and fungi. In fish, the skin is a very dynamic tissue that constantly senses environmental danger and protects the fish host from pathogen invasion while maintaining a healthy microbiome. The fish epidermis secretes large amounts of mucus that covers and protects the entire body surface. Mucus secretions are rich in antimicrobial peptides (AMPs) which can directly recognize and kill invading pathogens and/or recruit immune cells necessary to eliminate microbes. AMPs are considered natural antibiotics due to their ability to kill pathogens very rapidly and efficiently. Yet, the biology of AMPs such as beta defensins in fish is not well understood. The goal of this study is to determine the role of AMPs in trout skin during homeostasis and stress responses. Our data shows that trout beta defensins have microbicidal activity against several bacterial pathogens and they are expressed in the skin and modulated by transport stress. Moreover, whereas acute transport stress increases the numbers of skin-associated bacteria, chronic stress significantly decreases skin-associated bacterial numbers. *In silico* analysis of the regulatory region for trout beta defensin genes shows robust presence of heat shock binding sites and other stress-related transcription factors, suggesting that imbalanced microbiota may be a result of stress-induced changes in beta defensin expression. Combined, our results indicate that stress regulates different trout beta defensins in a molecule-specific manner resulting in impaired responses to pathogens and microbiota. Given the impact that stress has on the sustainability of the fish farming industry, our results underscore the value of targeting antimicrobial peptides prior to or during stress procedures to enhance the productivity and sustainability of the salmonid farming industry in the US.

9th International Symposium on Aquatic Animal Health

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Bacteriology

Abraham	Detection of *Lactococcus garvieae* and *Lactococcus petauri* in Environmental Samples and Wild Fish from Four Lakes in Southern California
Araya-León	Development and Validation of a PCR for the Specific Identification of *Tenacibaculum piscium*
Armwood	Comparative Pathogenicity and Cross-Protective Potential of *Edwardsiella tarda*, *Edwardsiella piscicida*, and *Edwardsiella anguillarum* in Channel, Blue, and Channel ♀ X Blue ♂ Hybrid Catfish
Armwood	Intraspecific Variation of *Edwardsiella anguillarum* from Non-Anguillid Fish from Varied Geographic Origins
Díaz-Riquelme	DVAqua®, a postbiotic for aquaculture.
Echeverría-Bugueño	Skin-Mucus Response of Healthy and Diseased Atlantic Salmon (*Salmo salazar*) to *Tenacibaculum dicentrarchi* Isolates Under In Vitro Conditions
Echeverría-Bugueño	Proteomic Analysis of Outer Membrane from the Fish Pathogen *Vibrio ordalii*
Fernandez-Alarcon	Antimicrobial Susceptibility Profile of Bacterial Isolates from Nile Tilapia from Different Regions of Brazil
Hawke	*Streptococcus dysgalactiae*: A Pathogen of Feral Populations of Silver Carp *Hypophthalmichthys malitrix*
Jung-Schroers	Friends or Foes? Relevance of Bacteria from the Aquatic Environment for Fish and Crustaceans
LaFrentz	Columnaris disease is caused by *Flavobacterium columnare* and three newly described *Flavobacterium* spp.
Moreno	Antimicrobial In Vitro Activity of Organic Acids: Citric Acid, Sorbic Acid and Phytoextracts *Cinnamomun verum*, *Thymus vulgaris* and *Cinnamomun verum*, *Rosmarinus officinalis* Against *Streptococcus agalactiae* Isolated from Tilapia (*Oreochromis* sp.) With Streptococcosis
Pontigo	Genetic and In Vitro Susceptibility of an Endemic Pathogen, *Renibacterium salmonarum*, in Salmon Farming in Chile
Suarez	Nanoemulsion *Cymbopogon flexuosus* effects´ on the silver catfish *Rhamdia quelen* with natural infection of *Aeromonas hydrophila*

General Session

Adamek	Straight to the Heart – Use of Heart Cell Cultures for Measuring the Impact of Pollutants In Vitro
Atencio-García	Strange yellow coloration in Fishes from the Cauca River, Colombia: Possible Causes
Atencio-García	Fish Health in the Cauca River (Colombia)
Dettleff	Determination of Microplastics prevalence in the Commercial Fish Chilean Hake (*Merluccius gayi*) from Maule Region in Chile.
Giralt	Systematic Review: Causes associated with melanosis in fillet of Atlantic Salmon.
Godoy	Heart and Skeletal Muscle Inflammation (HSMI) in Coho Salmon (*Onchorhynchus kisutch*): Clinical and Experimental Evidence
Jensen	A Comparison of Two Fish Health Indices Applied to Freshwater Species of the Chesapeake Watershed
Jung-Schroers	Spinal Damages in Eels After a Possible Passage Through Hydroelectric Power Plants
Mayes	A Non-traditional Framework for Assessing the Abundance and Environmental Health of Data-Poor Fish Stocks
McEachran	Assessing the Risk of Fish Pathogen Induction via Illegal Release of Live Baitfish by Recreational Anglers
Oke	The Effects of Composite Algal Supplementation (*Spirulina platensis* and *Phaeodactylum tricornutum*) on the Growth Performance and Health of African Catfish
Ortega	Case Report: Strawberry Disease in Rainbow Trout (*Onchorynchus mykiss*) in Puno, Peru
Parker-Graham	Influence of Formalin Treatment Reduction on Survival and Egg Stress Response in *Oncorhynchus mykiss* During Incubation
Richardson	A Stochastic Model to Investigate Atypical *Aeromonas hydrophila* Disease Dynamics in Catfish Aquaculture Ponds
Saéz	Evaluation of a new biodegradable technology packaging for the jumbo squid *Dosidicus gigas*
Sharpton	Using Zebrafish to Disentangle the Impact of Environmental Exposure on Host-Microbiome Interactions
Smith	Using Genomic Applications to Understand Wild Smallmouth Bass Immune Function
Smolarz	Bivalves transmissible neoplasia (BTN) in *Macoma balthica* from the Baltic Sea

Genomics

Muñoz-Cerro	Front-Loading of Immune Genes Contributes to the Resistance of *Argopecten purpuratus* Scallop Larvae to *Vibrio bivalvicida* Infection
Sánchez	Use of Whole Genome Sequence Level Imputed Genotypes for Resistance to Salmon Rickettsiosis Syndrome (SRS) In Rainbow Trout (*Oncorhynchus Mykiss*).
Ulloa	RNA Sequencing Study Reveals the Genetic Variation (genes?SNPs) That Confer Dietary Tolerance and Favor Zebrafish (*Danio rerio*) Growth
Zhang	Genome-wide Analysis of Microsporidian ADP/ATP Carrier Proteins, with 2 Copies in *Ameson portunus* Infecting the Swimming Crab *Portunus trituberculatus*

Microbiome Applications in Fish Health

Divya	Diversity of Microbial Community Between Medicated and Non-Medicated Catfish Ponds in the Mississippi Delta
Koepper	The Shell Microbiome of American Lobster *Homerus americanus* in Atlantic Canada
Older	Nanopore Sequencing for Aquaculture Bacterial Microbiota Profiling
Venegas	Effect of Rearing Conditions on the Recruitment and Resilience of Atlantic Salmon's Microbiota (*Salmo salar*) Throughout its Different Developmental Stages
Yamamoto	The Intestinal Microbiota of Channel Catfish (*Ictalurus punctatus*) After Florfenicol Treatment Followed by Dietary Prebiotic or Probiotic Supplementation

Immunology/Vaccinology

Aceituno	A Novel VHSV Subunit Vaccine: A New Prophylactic Tool with Oral Delivery Potential
Balami	Effect of *Lactobacillus plantarum* on growth performance, immune responses, and disease resistance of striped catfish (*Pangasianodon hypophthalmus*)
Gomaa	Developing a Dual Live Attenuated Vaccine to Prevent Motile Aeromonas Septicemia and Enteric Septicemia of Catfish
Nguyen	Glucan Immunostimulation Against Columnaris and Streptococcosis in a White Sturgeon (*Acipenser transmontanus*) Model
Tattiyapong	Development an Indirect ELISA to Measure Anti-Tilapia Lake Virus Antibodies in Tilapia Serum
Wolter	Characterization of the Innate and Acquired Immune Response Associated with Use of a Heterologous Vaccine with Immunomodulatory Activity in Atlantic Salmon

Parasitology

Boettiger	Field Performance of Lufenuron (Imvixatm) in Chilean Atlantic Salmon From 2016-2021
Georges	Epidemiology of Myxosporean Infections in Economically Important and Dietary Freshwater Fishes in the Sudano-Guinean Zone of Cameroon
Gupta	Myxozoan Diversity Infecting the Freshwater Fishes in the Sea of Galilee, Israel
Jung-Schroers	Development of Alternative, Ecologically Safe, Effective, and Well Tolerated Control Strategies Against *Ichtyophthirius multifilis*
Nguyen	Morphological, Molecular and Histopathological Characterization of *Dermocystidium* sp. in Redspot Darters *Etheostoma artesiae* in Mississippi, United States
Tomamichel	The Effect of Temperature, Host, and Parasite Traits on Parasite-Induced Mortality in Fisheries: A Meta-Analysis
Vieira	Report on the Occurrence of a New Putative *Henneguya* Species, a Gill Parasite of the *Astyanax lacustris*, Based on Morphological and Molecular Evidence
Walsh	The Use of Histopathology and Laser Capture Microdissection (LCM) for Myxozoan Identification from Multiple Fishes in the Eastern United States
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Jung-Schroers	Friends or Foes? Relevance of Bacteria from the Aquatic Environment for Fish and Crustaceans
LaFrentz	Columnaris disease is caused by *Flavobacterium columnare* and three newly described *Flavobacterium* spp.
Moreno	Antimicrobial In Vitro Activity of Organic Acids: Citric Acid, Sorbic Acid and Phytoextracts *Cinnamomun verum*, *Thymus vulgaris* and *Cinnamomun verum*, *Rosmarinus officinalis* Against *Streptococcus agalactiae* Isolated from Tilapia (*Oreochromis* sp.) With Streptococcosis
Pontigo	Genetic and In Vitro Susceptibility of an Endemic Pathogen, *Renibacterium salmonarum*, in Salmon Farming in Chile
Suarez	Nanoemulsion *Cymbopogon flexuosus* effects´ on the silver catfish *Rhamdia quelen* with natural infection of *Aeromonas hydrophila*

Detection of *Lactococcus garvieae* and *Lactococcus petauri* in Environmental Samples and Wild Fish from Four Lakes in Southern California

Taylor Abraham[1]; Zeinab Yazdi[1], Eric Littman[1], Khalid Shahin[1,2,], Taylor Heckman[1], Eva Marie Quijano Carde[1], Diem Thu Nguyen[1], Mark Adkison[3], Tresa Veek[3], Kavery Mukkatira[3], Christine Richey[3], Kevin Kwak[3], Ian Gardner[4], Timothy J. Welch[5]; Esteban Soto[1]

[1]School of Veterinary Medicine - University of California Davis, Davis, CA, 95616; [2]National Institute of Oceanography and Fisheries, P.O. Box 43511, Suez, Egypt, [3]California Department of Fish and Wildlife, Rancho Cordova, CA, 95670, USA; [4]Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, Canada; [5]National Center for Cool and Coldwater Aquaculture, USDA-ARS, Leetown, WV, 25430

Lactococcus garvieae and *Lactococcus petauri* are the etiological agents of piscine lactococcosis, an important emerging disease of fish in North America. To gain a better understanding of the presence of *L. garvieae* and *L. petauri* in California, 359 fish (of 8 species) and 161 environmental samples (soil and water) were collected from four lakes near two affected fish farms during an outbreak of piscine lactococcosis in California in 2020. *Lactococcus garvieae* was isolated from brains of two largemouth bass *(Micropterus salmoides)* in one of the lakes by standard microbiological methods. Additionally, *L. garvieae* / *L. petauri* were detected in 14 fish (8 bluegills and 6 largemouth bass) from 3 out of the 4 lakes using a recently developed quantitative PCR (qPCR) assay. Of the collected environmental samples, all 4 lakes tested positive for *L. garvieae* / *L. petauri* in the soil samples, while 2 of the 4 lakes tested positive in the water samples through qPCR. The second objective of the study was to compare the virulence of *L. garvieae* and *L. petauri* in rainbow trout (*Oncorhynchus mykiss*) and largemouth bass. Treatment groups were intracelomically injected with representative isolates of *L. petauri* (n=17) or *L. garvieae* (n=6) previously recovered from cultured or wild fish in North America and monitored for 14 days post-challenge (dpc). Challenged largemouth bass did not show any signs of infection or morbidity post-injection throughout the challenge period. Rainbow trout infected with *L. petauri* showed clinical signs within 3 dpc and presented a significantly higher cumulative mortality (62.2%; $p<0.0001$) at 14 dpc when compared to *L. garvieae* infected treatments (5%), suggesting that North American isolates of *L. petauri* are more virulent to rainbow trout than North American isolates of *L. garvieae.* The study demonstrates the potential use of qPCR to detect *L. garvieae* and *L. petauri* in the environment, and its usefulness as a surveillance tool in natural and commercial settings. Additionally, the study confirms *L. petauri* as a highly pathogenic emerging pathogen of rainbow trout in the USA.

Development and Validation of a PCR for the Specific Identification of *Tenacibaculum piscium*

Henry Araya-León[1,2], Rute Irgang[1,2], Mónica Saldarriaga-Córdoba[2,3] and Ruben Avendaño-Herrera[1,2,4]

[1]Laboratorio de Patología de Organismos Acuáticos y Biotecnología Acuícola, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Viña del Mar, Chile; [2]Centro FONDAP, Interdisciplinary Center for Aquaculture Research (INCAR), Universidad Andrés Bello, Viña del Mar, Chile; [3]Centro de Investigación en Recursos Naturales y Sustentabilidad, Universidad Bernardo O'Higgins, Santiago, Chile; [4]Centro de Investigación Marina Quintay (CIMARQ), Universidad Andrés Bello, Quintay, Chile.

Tenacibaculosis is an emerging disease that severely affects salmonid farming in Chile, producing high mortalities and causing great economic losses. Recently, the distribution of salmonid host species and the diversity of new bacterial species of the genus *Tenacibaculum* have increased worldwide. This work describes a new polymerase chain reaction (PCR) assay for the specific and rapid detection of *Tenacibaculum piscium*, a species recently described and identified in tenacibaculosis outbreaks in Norway and Chile. The designed primers amplified a 678 bp fragment of the peptidase gene from *T. piscium*. This method was rapid and specific for *T. piscium* since no other chromosomal DNA amplification products were obtained from the analyzed bacterial culture, including several *Tenacibaculum* spp. The PCR detected up to 500 pg of DNA, with a pure culture detection limit of 600 cells per PCR tube. The PCR approach described in this work allowed for the detection of *T. piscium* in mixed plate cultures obtained from challenged fish, an important outcome considering that the identification of this bacterium is difficult. Furthermore, when the protocol was used on seeded fish samples (i.e., gills, liver, kidney, and mucus), the sensitivity limit was suitable for diagnosis. Our results indicated that the designed specific primers and PCR method provide a rapid and specific diagnosis of *T. piscium.*

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Comparative Pathogenicity and Cross-protective Potential of *Edwardsiella tarda, Edwardsiella piscicida,* and *Edwardsiella anguillarum* in Channel, Blue, and Channel ♀ x Blue ♂ Hybrid Catfish

Abigail R. Armwood[1], Matt J. Griffin[2,3], Bradley M. Richardson[4], David J. Wise[2,5], Cyndi Ware[2], and Alvin C. Camus[1]

[1]College of Veterinary Medicine, University of Georgia, Athens, GA; [2]Thad Cochran National Warmwater Aquaculture Center, Mississippi State University, Stoneville, MS; [3]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Stoneville, MS; [4]Thad Cochran National Warmwater Aquaculture Center, USDA-ARS, Stoneville, MS; [5]Mississippi Agriculture and Forestry Experiment Station, College of Forest Resources, Mississippi State University, Stoneville, MS

Edwardsiella tarda has historically been a bacterial pathogen of nominal concern in farmed catfish in the USA. In the 2010s, *E. tarda* was reaffiliated into three species: *E. tarda, E. piscicida,* and *E. anguillarum*, of which *E. piscicida* is currently recognized as a significant, emergent pathogen within the industry. This reorganization has obscured previously documented disease pathologies of these bacteria in catfish. This study clarifies discrepancies in pathologic changes and virulence of *E. tarda, E, piscicida,* and *E. anguillarum* in channel (*Ictalurus punctatus*), blue (*I. furcatus*), and channel x blue hybrid catfish and assesses potential cross-protection of *Edwardsiella* congeners against subsequent *E. ictaluri* and *E. piscicida* challenge. Channel, blue, and hybrid catfish (20 fish/tank; 3 tanks/treatment) were intraperitoneally challenged with varying doses of *E. tarda, E. piscicida,* and *E. anguillarum.* Bacterial and histopathology samples were collected at 1, 2, 3, 5, 10, and 14-days post challenge (dpc). Cumulative mortality was recorded for 14-dpc in separate non-sampled tanks (20 fish/tank). Cumulative mortality associated with *E. piscicida* challenge ranged from 25-95%, 20-55%, and 80-100% in hybrids, channels, and blues, respectively. Conversely, *E. anguillarum* and *E. tarda* produced ≤5% mortality, regardless of catfish species, at comparable doses. Gross changes included poor body condition, mild ascites, transmural body wall ulcerations, and, rarely, dorsocranial ulceration (hole-in-the-head). Histologic lesions were characteristic of acute gram-negative sepsis, with more severe lesions in fish infected with *E. piscicida*, regardless of dose. It required 100X greater doses of *E. anguillarum* and *E. tarda* to produce similar pathology. Predominant early lesions included disseminated hemorrhage and necrosis, often with numerous bacteria accompanied by gastric submucosal edema. Transition to a granulomatous response with reduced bacterial numbers began around 3-dpc and was the dominant lesion observed by 5-dpc. Lesions were uncommon in all fish surviving 14-dpc. At 100 days post-challenge, surviving fish were subsequently exposed to *E. piscicida* via intraperitoneal injection or *E. ictaluri* via 30-minute immersion bath. Hybrids previously challenged with *E. piscicida* or *E. anguillarum* were more likely to survive *E. piscicida* challenge than naïve controls ($p < 0.01$), while channels were more likely to survive an *E. ictaluri* challenge after *E. piscicida* challenge ($p < 0.05$). Results substantiate previous reports indicating *E. piscicida* is more pathogenic in catfish compared to *E. anguillarum* and *E. tarda*. Cumulative mortality varied between catfish species, with blue and hybrid catfish more severely affected, supporting previous work pointing to increased susceptibility of hybrid and blue catfish to these agents. Lastly, this work corroborates research evincing a cross-protective effect among some *Edwardsiella* congeners. Further work is warranted to elucidate the cross-protective nature of the immune response induced by *E. anguillarum* and *E. piscicida* against subsequent *E. ictaluri* and *E. piscicida* challenge.

Intraspecific Variation of *Edwardsiella anguillarum* from Non-Anguillid Fish from Varied Geographic Origins

Abigail R. Armwood[1], Ethan Woodyard[2], Divya Divya[2], Geoffrey C. Waldbieser[3,4], Bradley M. Richardson[3,4], Esteban Soto[5], Cindy Stein[6], Michal Ucko[7], Cynthia C. Ware[2,3], Alvin C. Camus[1], Matt J. Griffin[2,3]

[1]Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA, USA; [2]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Stoneville, MS, USA; [3]Thad Cochran National Warmwater Aquaculture Center, Mississippi State University, Stoneville, MS, USA; [4]Warmwater Aquaculture Research Unit, Agricultural Research Service, United States Department of Agriculture, Stoneville, MS, USA; [5]Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California Davis, Davis, CA, USA; [6]Center for Veterinary Medicine, U.S. Food and Drug Administration, Laurel, Maryland, USA; [7]Israel Oceanographic and Limnological Research, National Center for Mariculture, Eilat, Israel

Edwardsiella anguillarum is a gram-negative, facultative intracellular bacterium, synonymous with previous descriptions of atypical, fish-pathogenic *E. tarda.* Originally described from eels *(Anguilla* spp.) in 2015, *E. anguillarum* has become an increasingly important global fish pathogen, particularly in tilapia aquaculture. This study describes intraspecific phenotypic and genotypic variability among seventeen *E. anguillarum* isolates from non-anguillid fish hosts and varied geographic origins. Phenotypic characterization included tests for motility, triple sugar iron tests for sugar fermentation and hydrogen sulfide production, disk diffusion assays testing sensitivity to florfenicol, sulfadimethoxine/ormetoprim, and oxytetracycline, as well as broth microdilution tests (Sensititre™ Vet Avian AVIAN1F) to establish minimal inhibitory concentrations against florfenicol, oxytetracycline, and 16 other antimicrobials. In addition, various phenobiochemical tests were conducted using the BBL™ Crystal™ Enteric/Nonfermenter Identification system. Genomic DNA was isolated for *Edwardsiella* species-specific multiplex PCR, repetitive extragenic palindromic sequence-based PCR (rep-PCR), and Oxford Nanopore and Illumina sequencing. Assembled genomes were analyzed using select concatenated genes from three previously published *Edwardsiella* multilocus sequence analysis (MLSA) schemes. Estimates of average evolutionary divergence among *E. anguillarum* isolates were calculated in MEGA11 using the Maximum Composite Likelihood model. From these three published MLSA strategies, eight gene targets with the highest evolutionary divergence were identified to produce an optimal MLSA scheme to delineate intraspecific variation among *E. anguillarum* isolates. Isolates demonstrated similar biochemical characteristics, with slight variation in motility and hydrogen sulfide production. Rep-PCR with the ERIC II primer set revealed two distinct genetic clusters, with no discrete clusters utilizing the GTG_5, BOX or ERIC I&II amplification strategies. While rep-PCR deemed the isolates largely clonal, MLSA schemes using reference genes from previous genetic studies revealed *E. anguillarum* isolates form five discrete phylogroups. The optimized MLSA scheme included *dnaK, gyrB, metG, pyrG, gyrA, adk, atpD*, and *phoR* genes. Isolates were susceptible *in vitro* to many antimicrobials, including florfenicol and oxytetracycline. Antimicrobial susceptibility profiles varied slightly between isolates and MLSA phylogroups, with no discriminatory agent identified to differentiate the five clades. Clindamycin and penicillin susceptibilities revealed some differences associated with geographic origin. Large, approximately 91.4 kB plasmids were identified in Costa Rican and Colombian analyzed isolates (9/17). This study provides a foundation for delineating drivers of intraspecific variation among *E. anguillarum* isolates from different hosts and geographic regions. Extension of this work is warranted as *E. anguillarum* isolates continue to be reported from new hosts and provenances.

DVAqua®, a Postbiotic for Aquaculture

Katherine R. Díaz Riquelme[1]

[1]Institute of Nutrition and Food Technology, Universidad de Chile.

DVAqua® is a natural product obtained after fermentation of *Saccharomyces cerevisiae*. It is composed of cell walls and soluble cell materials, and its administration in diets has been shown to provide health benefits, meeting the main requirements set forth by The International Scientific Association of Probiotics and Prebiotics (ISAPP) to classify as postbiotic. Among its most characteristics components are mannan-oligosaccharides and β-glucans, both known for their ability to modulate the expression of immune genes. Similarly, they have been studied for modulating the intestinal microbiota and reducing colonization by pathogenic bacteria: mannan-oligosaccharides are capable of binding to the carbohydrate binding sites of bacterial lectins, and β-glucans – being non-digestible polysaccharides – are fermented producing, for example, short chain fatty acids that reduce the intestinal pH. Research developed in different aquatic organisms of productive interest show that the supplementation of diets with DVAqua® has benefited, for example: the presence of beneficial bacteria, the increase in intestinal microvilli, the decrease in total *Vibrios*, the increase in survival, the feed conversion, growth and weight gain, resistance to pathogens and immune response. In this way, the present bibliographic review aims to present, in a summarized and informative way, the main health benefits provided by the administration of DVAqua®, with emphasis on survival, intestinal microbiota and immune response. The species documented in scientific articles that report the use of this postbiotic additive and its effects on any of these three topics are: hybrid tilapia (*Oreochromis niloticus* ♀× *O. aureus* ♂), common carp (*Cyprinus carpio*), Japanese halibut (*Paralichthys olivaceus*), McConaughy strain rainbow trout (*Oncorhynchus mykiss*), Shasta strain rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), Chinook salmon (*Oncorhynchus tshawytscha*), lake trout (*Salvelinus namaycush*), and whiteleg shrimp (*Litopenaeus vannamei*). With this literature review it is expected to contextualize the use of commercial product DVAqua® within the approach of animal health and welfare, which can be used in aquaculture of different species. In additions, this work seeks to bring the term postbiotic closer to the community, since we will begin and hear more frequently from now on.

Skin-mucus Response of Healthy and Diseased Atlantic salmon (*Salmo salar)* to *Tenacibaculum dicentrarchi* Isolates Under *in vitro* Conditions

Macarena Echeverría-Bugueño[1,2], Rute Irgang[1,2] and Ruben Avendaño-Herrera[1,2,3]

[1]Universidad Andrés Bello, Laboratorio de Patología de Organismos Acuáticos y Biotecnología Acuícola, Viña del Mar, Chile; [2]Interdisciplinary Center for Aquaculture Research (INCAR), Universidad Andrés Bello, Viña del Mar, Chile; [3]Centro de Investigación Marina Quintay (CIMARQ), Universidad Andrés Bello, Quintay, Chile.

An important physical and chemical barrier against pathogens for fish is the skin mucus, the composition, and traits of which vary among fish species. A commonality is a uniform, viscous structure that envelops the fish's body. Tenacibaculosis caused by *Tenacibaculum dicentrarchi* results in external skin injuries, including ulcers and hemorrhages, for Atlantic salmon (*Salmo salar*). In other words, *T. dicentrarchi* can overcome the first line of defense – the skin mucus barrier. This study assessed the antibacterial potential of mucus against different *T. dicentrarchi* isolates, in addition to considering the health status of fish by assessing mucus collected from healthy and sick *S. salar.* In all cases, esterase, protease, antiprotease, peroxidase, and lysozyme activities were detected, as well as pathogen binding to the mucus through the glycosylation pattern and quantity of proteins present in each mucus sample. Our results demonstrated that the response was limited by origin of the mucus sample, with a lower response found for mucus from sick fish. As such, the initial health status of fish defines, in part, the ability to respond to *T. dicentrarchi.* When evaluating bactericide activity and the growth potential of different *T. dicentrarchi* isolates in the mucus, no significant differences were found between the mucus from healthy and sick fish. Differential responses, however, were related to the protein concentration of the mucus, showing that as the number of proteins in the mucus decreases after infection, the response to pathogens likewise becomes deficient.

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Proteomic Analysis of Outer Membrane Vesicles from the Fish Pathogen *Vibrio ordalii*

Macarena Echeverría-Bugueño[1,2], Mauricio Hernández[3], Rute Irgang[1,2] and Ruben Avendaño-Herrera[1,2,4]*

[1]Laboratorio de Patología de Organismos Acuáticos y Biotecnología Acuícola, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Viña del Mar, Chile; [2]Centro FONDAP, Interdisciplinary Center for Aquaculture Research (INCAR), Universidad Andrés Bello, Viña del Mar, Chile; [3]Division of Biotechnology, MELISA Institute, San Pedro de la Paz, Chile; [4]Centro de Investigación Marina Quintay (CIMARQ), Universidad Andrés Bello, Quintay, Chile.

Vibrio ordalii, formerly *Vibrio anguillarum* biovar II, is the causative agent of atypical vibriosis, a hemorrhagic septicemia in several fish species, particularly salmonids. Most research on *V. ordalii* has focused on the phenotypic, serotypic, and genetic differences among isolates, but pathogenesis is still not fully understood. Our group recently investigated traits potentially involved in the virulence mechanisms of *V. ordalii*, with *in vitro* findings supporting the facultative intracellular behavior of this pathogen and the production and release of outer membrane vesicles (OMVs) under normal growth conditions. The present study aimed to further advance the proteomic knowledge available for the Chilean *V. ordalii* Vo-LM-18 strain and respective OMVs. Greater information on these points would mean a step towards clarifying protein compositions and, perhaps, in determining protein candidates for vaccine development. The conducted proteomic analysis detected more than 1,500 proteins in the vesicles of the Vo-LM-18 strain, with numerous differentially expressed proteins between the OMVs and within the bacterium. Furthermore, the OMVs evidenced virulence factors associated with hemolysis, nutrient uptake, host colonization, and iron uptake. These factors evidenced greater expression in the OMVs than within the Vo-LM-18 strain, suggesting a primary role of vesicles in the infection process.

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Antimicrobial Susceptibility Profile of Bacterial Isolates From Nile Tilapia From Different Regions of Brazil

Miguel F. Fernandez-Alarcon[1], Renata Galetti[1], Mariene M. Natori[1], Sandie B. B. Santos[1], Mateus C. Guimarães[1], Hugo M. Roa-Molina[1], Marco Rozas-Serri[2]

[1]Laboratório Pathovet Limitada, Ribeirão Preto, São Paulo, Brazil; [2]Pathovet Labs, Puerto Montt, Chile.

Global tilapia production had a slight growth of 2% in 2021, reaching around 6.25 million tons. Brazil is the fourth largest producer in the world, producing 534 thousand tons in 2021. Nowadays, bacteria, viruses, and fungi pathogens are among the most important causes of economic loss in fish farms. Furthermore, the bacterial pathogens most related to damage in Nile tilapia culture are *Aeromonas* sp., *Edwardsiella* sp., *Flavobacterium columnare*, *Francisella orientalis* (Gram-negative), and *Streptococcus agalactiae* (Gram-positive). The use of antimicrobials is an acute strategy to reduce the high mortality during an outbreak, and this practice may contribute to the emergence of resistant bacteria, especially when the treatment is not well prescribed. In that context, to understand the antimicrobial susceptibility profile of bacterial isolates from Nile tilapia we have characterized 39 isolates from our Laboratory strain database from three different Brazilian States and nine different fish farms, isolated between April 2021 and June 2022. Our database is composed of seven *Aeromonas hydrophila*, five *Edwardsiella anguillarum*, twenty-two *Streptococcus agalactiae*, three *F. columnare,* and two *F. orientalis.* Among all bacteria studied *E. anguillarum* presented the worrisome antimicrobial susceptibility profile being 100% of isolates resistant to florfenicol and oxytetracycline, 100% presenting intermediary susceptibility to amoxicillin, and 100% were susceptible to enrofloxacin. Besides, *A. hydrophila* presented 14.3% of resistance and 14.3% intermediary resistance to florfenicol, and 100% of isolates were susceptible to enrofloxacin and 100 % resistant to amoxicillin. Only 28.6% of isolates showed resistance to oxytetracycline. *S Streptococcus agalactiae* showed the most diversity in the antimicrobial susceptibility profile, being 100% susceptible to florfenicol, 86.4% presented susceptibility to oxytetracycline, and 9.1% of the isolates showed susceptibility to enrofloxacin, and 95.5% were susceptible to amoxicillin. *F. columnare* and *F. orientalis* presented 100% of susceptibility to all antimicrobial tested. Furthermore, two *E. anguillarum* were selected for the determination of minimal inhibitory concentration (MIC) for florfenicol and oxytetracycline and both isolates presented MIC of 64 µg/ml for florfenicol and 128 µg/ml for oxytetracycline. To understand the impact of these MIC values on the Nile tilapia culture the concentration of both antimicrobials was quantified in adult Nile tilapia plasma using the ELISA methodology. In the plasma samples analyzed we have found 2 µg/ml of florfenicol and 0.135 µg/ml of oxytetracycline (maximum concentration found). The results infer that the concentration of florfenicol and oxytetracycline in fish plasma was not enough to inhibit antimicrobial-resistant bacteria causing diseases in Nile tilapia. The data presented suggest that the evaluation of the antimicrobial resistance profile of bacteria should be monitored in fish farms to define adequate and sustainable therapeutic strategies.

Streptococcus dysgalactiae: A Pathogen of Feral Populations of Silver Carp *Hypophthalmichthys molitrix*

John P. Hawke[1], Ryan Daniel [2], Yuliya Sokolova[3], Jacqueline Elliott[1], Ingeborg Langohr[1,4], Fabio Del Piero[1,4], Esteban Soto[5] and Matt J. Griffin[6]

[1]Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge LA 70803; [2]Louisiana Department of Wildlife and Fisheries, District 2, Monroe, LA, 71203; [3]Department of Microbiology, Immunology, and Tropical Medicine, George Washington University, Washington, D.C., 20052; [4]Louisiana Animal Disease Diagnostic Laboratory, Louisiana State University, Baton Rouge, LA 70803; [5] Aquatic Animal Health Laboratory, Department of Medicine & Epidemiology, School of Veterinary Medicine, University of California, Davis, CA, 95616; [6]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Stoneville, MS 39762

In August of 2018, a series of large fish kills occurred on the Mississippi River in North Louisiana involving only silver carp *Hypophthalmichthys molitrix*. Clinical signs observed in moribund animals included: erratic swimming behavior, such as spiraling and spinning at the surface. A moribund specimen was captured by dip net near the surface at Lake Providence Landing in East Carroll Parish in North Louisiana and submitted for analysis. An aseptic necropsy was performed and diagnostic procedures including bacteriology, parasitology, histopathology, virology, and electron microscopy revealed that a gram-positive coccus was the primary pathogen. Pure cultures of the organism were obtained from the brain, and it was the predominant colony type isolated from the spleen, kidney, and liver. Bacterial sepsis, caused by the gram-positive coccus involving multiple organ systems, was diagnosed histologically. Bacterial colonization and necrotic lesions were seen in the spleen, liver, kidney, heart, eye, and brain. Numerous cocci were observed dividing intracellularly in phagocytic cells of the kidney and brain by transmission electron microscopy. The organism was identified as *Streptococcus dysgalactiae* subsp. *dysgalactiae* by conventional biochemical methods and later by MALDI-TOF mass spectrometry and sequencing the 16S rRNA gene. Multi-locus sequence analysis clusters this isolate along with two other *S. dysgalactiae* isolates from fish in a divergent phyletic group, separate from other *S. dysgalactiae* subsp. *dysgalactiae* isolates from terrestrial animals, implying a possible novel clade pathogenic for fish.

Friends or foes?
Relevance of Bacteria from the Aquatic Environment for Fish and Crustaceans

Verena Jung-Schroers[1], Felix Teitge[1], Julia Bauer[1], Mikolaj Adamek[1], Angela Boley[2], Christina Peppler[3], Dieter Steinhagen[1]

[1]Fish Disease Research Unit, Institute for Parasitology, University of Veterinary Medicine Hannover, Germany, [2]Institute for Sanitary Engineering, Water Quality and Solid Waste Management (ISWA), University of Stuttgart, Stuttgart, Germany, [3]Polyplan-Kreikenbaum GmbH, Bremen, Deutschland

Both autotrophic and heterotrophic bacteria are found in recirculating aquaculture systems. Autotrophic bacteria are essential for maintaining optimal water chemistry; heterotrophic bacteria break down organic substances, but as facultative pathogens they can also lead to outbreaks of disease in the animals kept. An important goal in aquaculture is to prevent outbreaks of clinical bacterial infections. Classical management measures are intended to reduce the total amount of bacteria in the systems and in particular the number of heterotrophic bacteria. The influence of different measures on the bacterial populations in aquaculture systems was examined in several projects. The ultrafiltration of circulating water through a membrane denitrification reactor, the use of UV light and the application of ozone and peracetic acid in different concentrations to the water were examined in detail. All treatment methods can affect the composition of the bacterial microbiome in the housing facilities but also on the surfaces of the animals kept. The use of UV light and the addition of high concentrations of peracetic acid led to a selection for specific bacteria that were apparently less sensitive to the applied measures. The focus of bacterial population management should be on maintaining a stable and diverse microbiome, which is why selective methods do not seem advisable. Ultrafiltration through the membrane denitrification reactor and the addition of ozone to the water proved to be the most suitable methods for stabilizing the microbiome. The results showed that each method can only be successful in stable systems without additional external influences. Even promising approaches failed as soon as the amount of organic material in the system increased significantly. For future studies, it is important to improve knowledge about the relationships between bacterial species in aquaculture systems and to look at the interactions of the individual species. The pathogen-based approach should therefore be changed to a more pathobiome-based approach and the main goal should become the support of naturally occurring microbiomes in aquaculture systems by stabilizing bacterial diversity.

Columnaris Disease Is Caused by *Flavobacterium columnare* and Three Newly Described *Flavobacterium* spp.

Benjamin R. LaFrentz[1], Stanislava Králová[2,3], Claire R. Burbick[4,5], Trevor L. Alexander[4], Conner W. Phillips[4], Matt J. Griffin[6], Geoffrey C. Waldbieser[7], Julio C. García[1], Fernanda de Alexandre Sebastião[8], Esteban Soto[8], Thomas P. Loch[9], Mark R. Liles[3], Kevin R. Snekvik[4,5]

[1] Aquatic Animal Health Research Unit, USDA-ARS, Auburn, AL; [2] Department of Experimental Biology, Czech Collection of Microorganisms, Masaryk University, Czech Republic; [3] Department of Biological Sciences, Auburn University, Auburn, AL; [4] Washington Animal Disease Diagnostic Laboratory, Pullman, WA; [5] Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA; [6] Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Stoneville, MS; [7] Warmwater Aquaculture Research Unit, USDA-ARS, Thad Cochran National Warmwater Aquaculture Center, Stoneville, MS; [8] Department of Medicine & Epidemiology, School of Veterinary Medicine, University of California, Davis, CA; [9] Department of Fisheries and Wildlife, College of Agriculture and Natural Resources, Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University, East Lansing, MI

Flavobacterium columnare is the causative agent of columnaris disease in freshwater fish. Four discrete genetic groups exist within the species and research has demonstrated associated host and virulence differences. The present study determined the taxonomic status of the four genetic groups of *F. columnare* using polyphasic and phylogenomic approaches. Phylogenetic analyses of 16S rRNA and *gyrB* genes using different methodologies demonstrated the four genetic groups formed well-supported and distinct clades within the genus *Flavobacterium*. The average nucleotide identity (ANI) and digital DNA-DNA hybridization (GGDC) values between *F. columnare* ATCC 23463^T, genetic group 2 isolate AL-02-36^T, genetic group 3 isolate 90-106^T, and genetic group 4 isolate Costa Rica 04-02-TN^T were less than 90.84% and 42.7%, respectively. Chemotaxonomic, MALDI-TOF characterization and ANI/GGDC calculations afforded differentiation between the genetic groups, indicating each group is a discrete species. Herein, the names *F. covae* sp. nov., *F. davisii* sp. nov., and *F. oreochromis* sp. nov. are proposed to represent genetic groups 2, 3, and 4, respectively. Since these pathogens are globally distributed and have significant impacts on wild and cultured fish species, recognition of the four species will advance and improve research to define host-pathogen-environment relationships, epidemiology, and develop effective control and prevention measures in aquaculture. Such research needs to target the correct bacterial species and research findings can be properly interpreted by correct and consistent taxonomic assignment.

Antimicrobial *in vitro* Activity of Organic Acids: Citric Acid, Sorbic Acid and Phytoextracts *Cinnamomun verum*, *Thymus vulgaris* and *Cinnamomun verum*, *Rosmarinus officinalis* Against *Streptococcus agalactiae* Ib Isolated from Tilapia (*Oreochromis* sp.) with Streptococcosis

July A. Moreno[1], Paola A. Barato[1], Gustavo A. Bermúdez[2]

[1]Corporación Patológica Veterinaria, Corpavet y MolecularVet SAS Bogotá, Colombia;
[2]PHARVET, Bogotá, Colombia

Streptococcosis is the most important bacterial disease in tilapia (*Oreochromis s*p.) production worldwide. It is caused mainly by *Streptococcus agalactiae* (GBS) serotypes Ia, Ib, III, IV, and IX in tilapia. This pathogen is frequently associated with meningoencephalitis, epicarditis, and septicemia. Antibiotic resistance has been described for GBS in aquaculture, for this reason, it has an urgent need to develop new alternative therapies to control bacterial diseases. Phytoextracts have shown efficient activity against the most important bacterial pathogens in aquaculture. Therefore, it is necessary to know the antibacterial properties of these products in pathogenic strain in order to explore its potential application in in vivo models. We evaluated in vitro different doses of product 1 with citric acid, sorbic acid and Phytoextracts *Cinnamomun verum* and *Thymus vulgaris* and product 2 with *Cinnamonium verum* and *Rosmarinus officinalis* against *S. agalactiae* Ib. *Streptococcus agalactiae* (GBS) Ib was aerobically cultured for 24 h at 30 °C until the exponential growth phase was reached at 5,7 x 10^4 ufc/ml. Four doses of each product were incubated with GBS at 30°C for 24 to 48h. All were tested in triplicate. Product 1 completely inhibited the growth of *S. agalactiae* Ib in the different doses evaluated. Product 2 did not show an inhibitory effect on *Streptococcus agalactiae* Ib growing at any concentration tested. The results from this study showed that dietary organic acids can exert strong antimicrobial effects and have the potential to impart beneficial effects on growth, nutrient utilization and disease resistance in tilapia. This can potentiate the effect of *Cinnamonium verum.* It has a protective effect on experimental *S. iniae* infection in tilapia. There have been a number of reports validating the *in vitro* antibacterial and antifungal activities of *Thymus vulgaris* this essential oil on some human pathogens, including *Staphylococcus, Pseudomonas, Escherichia coli, Candida,* and *Streptococcus,* and other species. It is interesting to note there is a synergism in the action of phytoextracts with organic acids. Citric acid, sorbic acid and Phytoextracts *Cinnamomun verum*, *Thymus vulgaris* inhibited the growing of GBS Ib and further studies are necessary to evaluate its effect in natural outbreaks.

Genetic and *in vitro* Susceptibility of an Endemic Pathogen, *Renibacterium salmoninarum*, in Salmon Farming in Chile

Juan Pablo Pontigo[1,4]; Diego Caro[2]; Karina Kush[2], Raúl Arias[5], Vinicius Maracaja[5], Paola Olmos[4], Yoandy Coca Rives[2,3], Marcos Godoy[1,2]

[1]Laboratorio de Biotecnología Aplicada, Facultad de Medicina Veterinaria, Universidad San Sebastián, Sede de la Patagonia, Lago Panguipulli 1390, Puerto Montt, Chile.[2]Centro de Investigaciones Biológicas Aplicadas (CIBA), Lago Panguipulli 1390, Puerto Montt, Chile. [3]Laboratorio de Energía Renovables y Residuos (LERR-UC). Escuela de Ingeniería Pontificia Universidad Católica de Chile. Avenida Vicuña Mackenna 4860, Macul, Santiago de Chile, 7820436, Chile. [4]Laboratorio Institucional, Universidad San Sebastián, Sede de la Patagonia, Lago Panguipulli 1390, Puerto Montt, Chile. [5]Beagle Bioinformatics, Santiago, Chile

Aquaculture is one of the industries with the highest growth rates in the world, and it is in Chile that it has experienced remarkably rapid growth, especially in the farming of salmonids. The sustained increase in production has been associated with the appearance of new diseases and the spread of pathogens in different geographical areas. One of the infectious-contagious diseases of productive importance characterized by its high morbidity is bacterial kidney disease (Bacterial Kidney Disease, BKD), usually of a chronic nature, whose causal agent is a Gram-positive bacterium called *Renibacterium salmoninarum*. The disease is clinically insidious and characterized by the presentation of granulomatous systemic inflammation. Natural shoots occur exclusively in salmonid fish. These diseases are most frequently controlled using antibiotic treatments, contributing to the development of antibiotic resistance and the consequences for human health and ecosystems. For this reason, new options to prevent and treat the most common diseases that affect salmon farming are necessary. In this work, Whole-genome sequencing (WGS) was carried out together with a minimum inhibitory concentration (MIC) analysis to carry out an associative search for antibiotic resistance genes, which allows us to observe and reconstruct the variations in genomic variables associated with the different concentrations of antibiotics exposed. For this, five strains of *R. salmoninarum* isolated from Atlantic Salmon (*Salmo salar*) and Salmon coho (*Oncorhynchus kisutch*) were isolated in KDM2 medium in 2021, subsequently their genetic material (DNA) was purified to proceed to sequence it employing Illumina platform (miSeq). Altogether, 20 strains were analyzed by MIC, presenting different ranges of antibiotic resistance. This study shows that the MICs established from *R. salmoniarum* can associate and know antibiotic resistance genes established for each lineage. All this information will provide a basis for developing better disease management strategies to prevent and control these industry pathogens, thus increasing the sustainability of national salmon farming.

Nanoemulsion *Cymbopogon flexuosus* Effects on the Silver Catfish *Rhamdia quelen* with Wild Infection of *Aeromonas hydrophila*

Cristian C. Suarez[1], Jose F. Valerio[1], Isabela Marashin[2], Samantha N. Godoi[3], Carla C. Zeppenfeld[4], Silvio T. da Costa[2], Aline Ourique[3], Bernardo Baldisserotto[4].

[1]Post-graduate program in Animal Husbandry; [2]Department of Morphology; [4]Department of Physiology and Pharmacology, Universidad Federal de Santa Maria; [3]Universidad Franciscana.

Silver catfish (*Rhamdia quelen*) is an important fish for aquaculture production in South Brazil. These fish are affected by the gram-negative bacteria *A. hydrophila*, which caused important losses as a result of infection. Previous authors found that the use of essential oils (EO) in the diet of this fish has protective effects against these kinds of diseases. The goal of this research was to determine the impacts of essential oils of *C. flexuosus (*EOCF*)* on the health and development of *R. quelem.* A 25-day feeding trial was conducted in a recirculating system with juvenile (mean individual weight of 12 g) silver catfish, stocked in aquariums of 70 L (17 fish by tank). Four concentrations of EOCF were tested (0.0, 0.25, 0.5, 1.0, and 2.0 g/kg of feed), with four replicates each treatment. Water parameters were: pH 6.5, temperature 21-24°C, dissolved oxygen levels 4.5-5 ppm, and a cycle of 12:12 light/dark. Other variables such as ammonia and nitrite were determined 3 times a week. The effects of EOCF dietary supplementation were evaluated with the measurement of mortality, initial and final weight, initial and final size, condition factor (K), Specifical Growth Rate (SGR), and hepatosomatic index. The results concerning this study were expressed as means ± standard deviation (SD) and were analyzed by one-way analysis of variance (ANOVA) using R software (version 4.1.0). A significant difference was expressed at the $p < 0.05$ level. As a preliminary result the animals, that were exposed to EO had less mortality compared with the control treatment and in some cases had a significant difference. On the other hand, the hepatosomatic index didn't have a significant difference between treatments. These initial results conclude that the EOCF has a protective level against *A. hydrophila* infections, and this new ingredient didn't produce hepatic damage. The final prospect is to find the effects of nanoemulsion of EOCF and compare them with other formulations which used the same EO in another species and other lining systems.

General Session (Virtual)

Adamek	Straight to the Heart – Use of Heart Cell Cultures for Measuring the Impact of Pollutants In Vitro
Atencio-García	Strange Yellow Coloration in Fishes from the Cauca River, Colombia: Possible Causes
Atencio-García	Fish Health in the Cauca River (Colombia)
Dettleff	Determination of Microplastics Prevalence in the Commercial Fish Chilean Hake (*Merluccius gayi*) from Maule Region in Chile.
Giralt	Systematic Review: Causes Associated with Melanosis in Fillet of Atlantic Salmon.
Godoy	Heart and Skeletal Muscle Inflammation (HSMI) in Coho Salmon (*Onchorhynchus kisutch*): Clinical and Experimental Evidence
Jensen	A Comparison of Two Fish Health Indices Applied to Freshwater Species of the Chesapeake Watershed
Jung-Schroers	Spinal Damages in Eels After a Possible Passage Through Hydroelectric Power Plants
Mayes	A Non-traditional Framework for Assessing the Abundance and Environmental Health of Data-Poor Fish Stocks
McEachran	Assessing the Risk of Fish Pathogen Induction via Illegal Release of Live Baitfish by Recreational Anglers
Oke	The Effects of Composite Algal Supplementation (*Spirulina platensis* and *Phaeodactylum tricornutum*) on the Growth Performance and Health of African Catfish
Ortega	Case Report: Strawberry Disease in Rainbow Trout (*Onchorynchus mykiss*) in Puno, Peru
Parker-Graham	Influence of Formalin Treatment Reduction on Survival and Egg Stress Response in Oncorhynchus mykiss During Incubation
Richardson	A Stochastic Model to Investigate Atypical *Aeromonas hydrophila* Disease Dynamics in Catfish Aquaculture Ponds
Saéz	Evaluation of a New Biodegradable Technology Packaging for the Jumbo Squid *Dosidicus gigas*
Sharpton	Using Zebrafish to Disentangle the Impact of Environmental Exposure on Host-Microbiome Interactions
Smith	Using Genomic Applications to Understand Wild Smallmouth Bass Immune Function
Smolarz	Bivalves Transmissible Neoplasia (BTN) in *Macoma balthica* from the Baltic Sea

Straight to the Heart - Use of Heart Cell Cultures for Measuring the Impact of Pollutants *in vitro*.

Torben Krebs[1], Julia Bauer[1], Sarah Graff[2], Marina Gebert[2], Sebastian Rakers[3], Dieter Steinhagen[1], Mikolaj Adamek[1]

[1]Fish Disease Research Unit, Institute for Parasitology, University Of Veterinary Medicine Hannover, Hannover, Germany; [2]Fraunhofer Research Institution for Individualized and Cell-Based Medical Engineering, Lübeck Germany; [3]Bluu Seafood, Bluu GmbH, Berlin, Germany

Circulatory disorders affecting heart and/or erythrocytes are a rising challenge to the sustainable development of salmonid aquaculture worldwide. In addition to pathogenic stressors, oil spills pose a significant risk to aquatic life, particularly in terms of acute and chronic toxicity to aquatic organisms such as fish. The same issue occurs with plastics in nano- and micrometer sizes, which are increasingly detected in aquatic ecosystems due to an increased global production of plastics over the past. The most common polymer types are polyethylene (PE), polypropylene (PP) and polystyrene (PS). These microplastics are released among other things from detergents and enter the oceans through sewers. More than half of the marine litter consists of plastics, which are degraded to secondary microplastics by saltwater and/or UV radiation. Despite the increasing number of fish in aquaculture and the associated growing interest in fish disease research, in vitro fish models for fish health issues are still in their infancy. Fish cell lines have great potential to address many aquaculture and food safety issues. In particular, heart cell cultures are under-researched and could be a breakthrough in several areas of sustainability in marine fish production. They could also help to solve some of the major diseases in salmonids. The aim of the work was to develop new and improve existing heart cell cultures from salmonids (Atlantic salmon, rainbow trout and brown trout) as tools to explore the effects of environmental stressors like nanoplastic particles and crude oil. Obtained results were compared with effects of induced by the exposure of larvae. The cultivation of heart cell cultures occurred to be challenging. Among other things, heart cells were very fragile, especially at the beginning, and it was difficult to maintain beating heart cells over a longer period of time. After extensive medium optimization heart cell cultures with prolonged beating capacity were obtained. The results from a comparison of the effect of different concentrations of crude oil and nanoplastic particles obtained *in vivo* in salmonid larvae and *in vitro* in cardiac cell cultures of different developmental stages will be presented. Additional consideration will be given whether the heart is capable of absorbing and depositing nanoplastics or crude oil.

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Fish Health in the Cauca River (Colombia)

Víctor J. Atencio-García[1], Diana L. Madariaga-Mendoza[1], Adriana Ortiz-Bedoya[2], Hernando Guerrero-Durango[1], José L. Marrugo-Negrete[3]

[1]Fish Research Institute-CINPIC/FMVZ/DCA, University of Córdoba, Montería, Col; [2]FMV, University of Santander, Valledupar, Col; [3]FCB/ Environmental Toxicology Laboratory, University of Córdoba, Montería, Col.

In 2014, the Cauca River (Colombia), the construction of the Ituango hydroelectric plant began, and the dam filling occurred in an emergency in 2019. Among the essential activities in this river, gold mining stands out. In late 2020, fish with strange behavior and coloration began to be observed, as well as some deaths that caught the attention of fishermen. The study aimed to assess the health condition of fish in the Cauca River. Considering the clinical signs of the fish were classified into four groups: yellow fish (yellowish stains on the skin); lacerated fish (lacerations, scaleless, and petechiae); dam fish (erratic swimming and petechiae), and apparently healthy fish (control). All groups were analyzed for potentially toxic elements (PTEs) such as As, Cd, Ni, Mn, Hg, Fe, and Hg-methyl (Hg-Me) on samples of muscle, liver, and gills (n=56). These analyzes were also realized on water (n=96) and sediments (n=96), as well as some physicochemical parameters (pH, temperature, redox potential). The analysis of PTEs was realized using spectrophotometric and spectrometric techniques. Also, samples of organs (liver, spleen, kidney) for histopathology were taken. The results show organic and inorganic contamination in river water and sediment and high concentrations of heavy metals such as As, Cd, Ni, Mn, Fe, and Hg-Me in the fish's liver, gills, and muscles. In general, the fish that were found dead were characterized by chronic poisoning by PTEs. In the group of yellow fish, this coloration was related to Hg-Me and Fe levels. In the lacerated fish, it is suggested that the lacerations were caused by contact with caustic and/or corrosive substances that subsequently caused an opportunistic bacterial and/or fungal infection. Fishes in the dam have a pathology compatible with possible cyanotoxin poisoning. Apparently healthy fish also exhibit PTEs poisoning. All groups of fish analyzed showed histopathological alterations, mainly in the liver, spleen, and gills, such as activation of melanomacrophage centers, lymphoid depletion, and inflammation associated with immunosuppression and epithelial cell death. In the Cauca River, organic pollution and PTEs are factors that affect the health of fish.

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Strange Yellow Coloration in Fishes from the Cauca River, Colombia: Possible Causes

Víctor J. Atencio-García[1], Diana L. Madariaga-Mendoza[1], Adriana Ortiz-Bedoya[2], Jurandy M. Penitente-Filho[3]; José L. Marrugo-Negrete[4]

[1]Fish Research Institute-CINPIC/FMVZ/DCA, University of Córdoba, Montería, Columbia; [2]FMV, University of Santander, Valledupar, Col; [3]Veterinarian, Valledupar, Columbia; [4]FCB/Environmental Toxicology Laboratory, University of Córdoba, Montería, Columbia

In late 2020, specimens of *Pseudoplatystoma magdaleniatum* were captured in the Cauca River (Colombia) with a strange yellow coloration in the mucosa, skin, muscle, digestive tract, and cavity fat. This coloration was also observed in pimelodids such as *Pimelodus grosskopfii* and *Sorubim cuspicaudus* and characiformids such as *Prochilodus magdalenae* and *Megaleporinus muyscorum*. The study aimed to determine the causes of the strange yellow coloration in fish from the Cauca river. In the gills, liver, and muscle of specimens that showed this odd coloration (n=20) and of fish with normal coloration (control, n=20) were analyzed heavy metals (Hg, As, Cd, Mn, Ni), Hg-methyl (Hg-Me), and Fe. Heavy metals were analyzed by spectrometric and spectrophotometric analysis. Histological sections were made in organs (liver, spleen, kidney, brain, heart, muscle, and gills); Prussian Blue staining was also performed to confirm Fe's presence and intensity. Histopathological lesions were classified into circulatory, inflammatory, degenerative, proliferative, and parasitic/bacterial disorders; while the intensity of the lesions was ranked by their extension on a scale of 0 to 3: (0) no lesion; (1) light, up to 25%; (2) moderate, from 25 to 50% and (3) severe, more than 50% of the tissue involved. The fish with strange yellow coloration registered values of Hg (22.4-758.3 µg/kg), Hg-Me (75.6-1194.6 µg/kg), Cd (1.1-7.3 µg/kg), and As (7.7-33.2 µg/kg) that are affecting their health and survival. The liver was more affected in the yellow fish than the control group, presenting hydropic degeneration, necrosis, activation of melanomacrophage centers, and intracytoplasmic pigments. The extension of the lesions in the different organs was recorded between 1 and 2, except in the liver, which reached grade 3. The results suggest that the strange yellow coloration in the skin, muscle, cavity fat, and digestive tract is associated with high concentrations of Hg-Me, which cause damage to hepatocytes that prevent the elimination of bile fluids, producing an accumulation of these in muscle tissues and viscera, which generates apparent icterus, due to the excess of bile fluids in the blood and lymph, manifesting itself in the muscle tissue. Besides, the yellow coloration in the mucosa may be related to Fe concentrations since this metal becomes available in the water column with the removal of soils for gold extraction and adheres to the mucosa of the fish.

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Determination of Microplastics Prevalence in the Commercial Fish Chilean Hake (*Merluccius gayi*) from Maule Region in Chile.

Nicolas Valderrama[1], Alvaro Machuca[2], Jaime Palomino[3], Marcia Arriagada[3], Phillip Dettleff[4].

[1]Faculty of Veterinary Medicine and Agronomy, Universidad de las Americas, Santiago, Chile; [2]Faculty of Natural Resources and Veterinary Medicine, Universidad Santo Tomas, Talca, Chile; [3]School of Veterinary Medicine, Universidad Bernardo O'Higgins, Santiago, Chile; [4]School of Veterinary Medicine, Pontificia Universidad Catolica de Chile, Santiago, Chile.

The Chilean hake (*Merluccius gayi*) is a demersal marine fish found in cold ecosystems along the Pacific Ocean coast of South America, representing one of the most consumed fish species in Chile, due to its low price and easy access. Due to its wide geographical location, this species could be exposed to marine pollution, specially microplastics. However, the knowledge related to microplastics prevalence on this species is scarce. The objective of this study was to evaluate the prevalence of microplastic at digestive level in Chilean hake of Maule region at Chile. Chilean hake were sampled from Duao coast, in Maule region, registering length, weight and sampling digestive tract. The digestive tract was digested in KOH and then filtered using Whatman glass microfiber and analyzed using a high-resolution optical microscope. An average of 28% of the fish sampled presented microplastics in the digestive tract, showing a superior percentage compared to previous data for Bio Bio region. More than 40% of microplastics found were fibers, followed by rectangular and irregular shapes, with red and blue colors as the most represented. The condition factor K (Htun-Han) of the sampled population was 0.68, a relatively low value compared with other references values for Chilean populations. This could be influenced by the high level of parasitosis of the sampled fish, with a 100% presence of anisakis in digestive tract. However, the condition factor in this population of Chilean hake was not influenced by the microplastic presence, presenting a low R^2 between microplastic number and K. This information evidence that microplastic pollution level is a relevant issue for Chilean hake, and that could be influenced by geographical location, requiring more studies to determine its effect at the physiological level on Chilean hake. Funding: Concurso Interno de Investigación, Creación e Innovación Tecnologica UST 11310013.

Systematic Review: Causes Associated with Melanosis in Fillet of Atlantic Salmon, *Salmo salar* (L.)

Erik S. Giralt[1], Rodrigo E. Pulgar[2], Jurij M. Wacyk[3].

[1]MSc Aquaculture student, Universidad of Chile, Fish Health Veterinarian, Multi-X Salmon Farming, Puerto Montt, Chile; [2]Genetics and Genetics of Biological Interactions Laboratory. Institute of Nutrition and Food Technology (INTA)-University of Chile, Santiago, Chile; [3]Animal Nutrition Laboratory, University of Chile, Santiago, Chile.

In economic terms, the Atlantic Salmon, *Salmo salar*, is the most important species farmed in Chile. The main final product of the cultivation of *Salmo salar* is the fillet, so its organoleptic characteristics are of primary importance, and among these, the color and visual appearance are a critical point. In this regard, the presence of melanosis (dark melanin spots) in the fillet of *Salmo salar* represents one of the main causes of rejection, currently arising in up to 20% of the fillets produced, with the consequent economic impact for the production. industry. Various studies and authors have proposed various theories as the cause of melanosis, but without reaching a consensus regarding the origin of the problem. The objective of the present study is to carry out a qualitative Systematic Review about the causes of melanosis in *Salmo salar* fillet. Databases available on the web will be used, from which the investigations that will be obtained, selected and reviewed will be used. They try to approach and/or answer which are the causes of melanosis in fillet of Salmo salar (L.), to subsequently carry out a synthesis and qualitative analysis of the information obtained.

Heart and Skeletal Muscle Inflammation (HSMI) in Coho Salmon (*Oncorhynchus kisutch*): Clinical and Experimental Evidence

Marcos Godoy[1,2], Jaime Romero[3], Juan Pablo Pontigo[1], Molly Kibenge[4], Rudy Suarez[5], Yoandy Coca Rives[6,7], Mauricio Labraña[5], Fred Kibenge[4]

[1]Laboratorio de Biotecnología Aplicadas, Facultad de Medicina Veterinaria, Universidad San Sebastián, Sede De la Patagonia, Lago Panguipulli 1390, Puerto Montt 5480000, Chile. [2]Centro de Investigaciones Biológicas Aplicadas (CIBA), Lago Panguipulli 1390, Puerto Montt 5480000, Chile. [3]Laboratorio de Biotecnología, Instituto de Nutrición y Tecnología de los Alimentos, Universidad de Chile, Santiago 7800024, Chile. [4]Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE C1A4P3, Canada. [5]Aquachile, Cardonal S/N, lote P.O BPX 30D, Puerto Montt, Chile. [6]Doctorado en Ciencias de la Ingeniería. Área Ingeniería Química y Bioprocesos. Escuela de Ingeniería. Pontificia Universidad Católica de Chile. Avenida Vicuña Mackenna 4860, Macul, Santiago de Chile, 7820436, Chile. [7] Laboratorio de Energía Renovables y Residuos (LERR-UC). Escuela de Ingeniería Pontificia Universidad Católica de Chile. Avenida Vicuña Mackenna 4860, Macul, Santiago de Chile, 7820436, Chile.

Heart and skeletal muscle inflammation (HSMI) is an infectious disease caused by Piscine orthoreovirus (PRV), a virus belonging to the family Reoviridae, genus Orthoreovirus. Subclinical infections have been reported in coho salmon (*Oncorhynchus kisutch*) in wild fish in Canada and associated with erythrocytic inclusion body syndrome (EIBS) in Japan. Based on the analysis of 20 cases of coho salmon (*O. kisutch*) from 15 farms, a clinical presentation is characterized by hemopericardium, clots in the abdominal cavity, pale liver, petechial hemorrhages in the peripyloric fat. The main histopathological findings were epicarditis, myocarditis, myositis, and hepatic necrosis, associated with the presence of PRV, these findings being similar to those described for cardiac and skeletal muscle inflammation (HSMI). Additionally, intraperitoneal (ip) inoculation under controlled conditions of coho salmon smolt (O. kisutch), with tissues infected with PRV genotype Ia, showed the presence of myositis and myocarditis with a higher frequency and severity in the range of time 7 and 8 weeks after inoculation. These findings are consistent with the classic clinical presentation of HSMI previously described in other species of salmonids. Additionally, results on viral kinetics are presented. These results constitute clinical and experimental evidence of the presence of heart and skeletal muscle inflammation (HSMI) in coho salmon (*O. kisutch*), farmed in Chile.

A Comparison of Two Fish Health Indices Applied to Freshwater Species of the Chesapeake Watershed

Josiah B. Jensen[1], Vicki S. Blazer[2], Patricia M. Mazik[3]

[1]West Virginia University, Division of Forestry and Natural Resources; [2]U.S. Geological Survey, Eastern Ecological Science Center, Leetown Research Laboratory; [3]U.S. Geological Survey, West Virginia Cooperative Fish and Wildlife Research Unit

Fish health indices calculated in the field such as the health assessment index (HAI) and deformity, erosion, lesion, and tumor counts (DELTs) are popular because they provide a numeric proxy for the general health of a population and can be performed with minimal training. These two attributes make them readily accessible options for monitoring fish health. These indices have been correlated with various sources of pollution, but there has been limited research comparing the utility of the two indices when applied at a watershed scale. This study looks at the potential application of these indices for monitoring fish health in the Chesapeake watershed of the Eastern United States. Freshwater fish of the Chesapeake have suffered several mortality events in the last 20 years affecting Smallmouth Bass *Micropterus dolomieu,* and a few other species. No single cause has been determined to trigger the mortalities. Due to this, multiple stressors are most likely the cause. Multiple stressors can negatively affect the health of sensitive species and result in disease and mortality. The health of these sensitive fish must be monitored to identify at risk populations. Field based indices may provide a health screening tool that could be widely applied with limited cost. To test their potential application, modified HAIs were created for Smallmouth Bass, White Sucker *Catostomus commersonii,* and Fantail Darter *Etheostoma flabellare*. Likewise, DELTs were calculated for these three species and fish community samples. Smallmouth Bass were collected in the spring and fall at 5 sites over 7 years (2013-2020) yielding a total 889 fish. These sites were spread throughout the watershed with varying degrees of upstream forested and agricultural land-use – two prominent landcover classes in the Chesapeake. Fish community DELTs were recorded from 30 small streams within one tributary of the watershed (Shenandoah River) and were also spread over differing upstream forest and agricultural land-use percentages. HAIs were calculated for White Sucker and Fantail Darter at a subset of those 30 sites yielding 248 and 400 fish respectively. Preliminary analysis of these indices showed some correlation with suspected pollution sources, such as poor HAI values being associated with the highest agricultural land-use. However, there were also significant correlations with age, sex, and season of sampling. These sample dependent correlations must be considered when designing a monitoring program. Certain cellular changes that could have significant impacts on fish health were also documented histologically which would have been missed if only the HAI and DELTs indices were used. Indices have their place in fish health surveillance, but their limitations must be acknowledged in a successful fish health monitoring program.

Spinal Damages in Eels After a Possible Passage through Hydroelectric Power Plants

Verena Jung-Schroers[1], Felix Teitge[1], Julia Bauer[1], Vanessa Guddorf[2], Dieter Steinhagen[1]

[1]Fish Disease Research Unit, Institute for Parasitology, University of Veterinary Medicine Hannover, Germany, [2]Clinic for small mammals, reptiles and birds, University of Veterinary Medicine Hannover, Germany

Eels are katadrome migratory fish that are migrating downstream the rivers to spawning areas in the sea. During their way, they are exposed to numerous risks, like passages through hydroelectric power plants. Because of their body shape and length as well as their swimming behaviour and their preference to swim with the mainstream, eels are more prone to be injured by a turbine passage than other fish species. Injuries might result in higher mortalities or in reduced swimming abilities. For the preservation of the European eel, it is existential to migrate into the sea for reproduction since artificial propagation of the species is still not possible. Therefore, examinations of the impact of hydroelectric power plants as a migratory obstacle are important. The damages must be evaluated, and the used turbines should be adapted if necessary. 77 eels were caught downstream of a hydroelectric power plant. The animals were euthanized and examined for external and internal injuries. Additionally, all eels were x-rayed, and the spinal damages were evaluated. Mainly mild external damages were seen in the fish. In 61 examined eels skin abrasions were detected. 39 eels showed bleedings in the skin and in 32 fish tearing of the skin occurred. In 27 eels internal bleeding could be detected. By x-raying, changes of the spinal column could be observed in 36 eels. The detected injuries of the spinal columns were mostly severe, and the defects ranged from compression and fractures of the vertebral bodies, displacements of the vertebral bodies against each other and tears of the spinous processes. These defects occurred mainly in the second third of the body and more often in larger animals. By an external examination the existence of internal damages and especially of damages of the spinal column could not be evaluated, as about half of the externally intact animals and the animals showing only very mild external alterations had damages of the spinal column. For examination of eels for damages due to a passage through a hydroelectric power plant taking x-rays is necessary to detect possible damages.

A Non-traditional Framework for Assessing the Abundance and Environmental Health of Data-Poor Fish Stocks

Cristin M. Mayes[1], Erin, Walters[2], Berlyanna Heres[2]

[1]University of Miami Rosenstiel School of Marine and Atmospheric Science; [2]Florida Fish and Wildlife Conservation Commission

Stock assessments are the primary source of all fishery management decisions made in the US and abroad; yet 80% of global catches occur in fisheries that lack the necessary data and resources required to achieve a quantitative model-based stock assessment, also known as data-poor fisheries. Methods typically used to assess such fisheries are inconsistent, labor-intensive, and provide high levels of uncertainty. In many cases, the status of data-poor fisheries remains unknown despite commercial importance and large impacts on livelihoods. The linkages between fish abundance, health, and environmental factors are typically poor or non-existent as well. This project has been designed to develop a quantitative method for assessing data poor fishery stocks using environmental parameters such as dissolved oxygen, relative sea level change, rainfall, wind direction, and the presence or absence of other sea-life. Such parameters provide a measure of health for both the environment and the fish stock. To accomplish this, an assessment of an ecologically and economically important species has been conducted and used to determine which available ecosystem parameters has the largest effect on the overall stock to develop a baseline for this non-traditional data-poor assessment method. A second assessment of the same species has then been conducted using only environmental parameters to compare the results of both assessments. This comparative analysis will determine the efficacy of the data-poor assessment approach. This project therefore explores environmental health, fish health, population dynamics, assessment methodologies and models by using environmental factors. It also provides an innovative ecosystem-based management approach for data-poor fisheries while providing a framework to help identify healthy aquatic animals and their overall environment.

Assessing the Risk of Fish Pathogen Introduction Via Illegal Release of Live Baitfish by Recreational Anglers

Margaret C. McEachran[1,2], Catalina Picasso[3], Janice M. Mladonicky[4], D. Andrew R. Drake[5], Dominic A. Travis[6], David C. Fulton[7,2], Nicholas B. D. Phelps[1,2]

[1]Minnesota Aquatic Invasive Species Research Center, University of Minnesota, St. Paul, MN 55108; [2] Department of Fisheries, Wildlife and Conservation Biology, University of Minnesota; [3] Center for Animal Health and Food Safety, Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN 55108; [4] Gabbert Raptor Center, University of Minnesota, St. Paul, MN 55108; [5] Great Lakes Laboratory for Fisheries and Aquatic Sciences, Fisheries and Oceans Canada, Burlington, ON, CA; [6] The Marine Mammal Center, Sausalito, CA 94965; [7] Minnesota Cooperative Fish and Wildlife Research Unit, U.S. Geological Survey, St. Paul, MN 55108

As the COVID-19 pandemic has well demonstrated, today's interconnected society provides ample opportunity for the inadvertent spread of dangerous diseases. Despite heightened awareness of disease spread among humans, however, the high-volume and wide-ranging trade in live animals remains a potential vector for the spread of pathogens that can hitch a ride with apparently healthy animals. In inland fisheries, the use and release of live baitfish by recreational anglers has been identified as a particularly important pathway for the spread of pathogens. Despite regulations prohibiting it, baitfish release is widespread and common among anglers, providing substantial opportunity for infectious disease spread via this pathway. To address key knowledge gaps and understand the social and ecological dimensions of risk of pathogen spread, we developed a stochastic risk assessment model of the angler-mediated movement of live baitfish. Using angler-provided data from a survey of adult anglers, we parameterized the model and applied it to a case study assessing the risk of pathogen introduction in Minnesota, USA, a state with a significant live bait fishing industry. We modelled pathogen introduction risk for three important pathogens (viral hemorrhagic septicemia virus (VHSV), Asian fish tapeworm *Schizocotyle acheilognathi*, and *Ovipleistophora ovariae*) across a variety of scenarios representing baseline, outbreak, and source-control scenarios. We found that the average number of angling trips resulting in pathogen release was high across all modeled scenarios, ranging from fewer than 10,000 in a small, localized outbreak of VHSV, to 1.2 million in a statewide outbreak of Asian fish tapeworm in multiple live baitfish species. Additionally, we found reducing the rate of illegal release could offer meaningful risk reduction in some scenarios with high pathogen prevalence and/or broad pathogen distribution, but this effect was less pronounced in scenarios where the outbreak was geospatially or otherwise limited. We used the Theory of Planned Behavior to identify the social and psychological determinants of baitfish release behavior and found that knowledge of the existing regulatory framework and subjective norms around live baitfish disposal play an important role in deterring illegal release. We identified potential management strategies that could meaningfully reduce pathogen introduction risk via the live baitfish pathway while supporting the important bait and fishing industries.

The Effects of Composite Algal Supplementation (*Spirulina platensis and Phaeodactylum tricornutum)* on the Growth Performance and Health of African Catfish (*Clarias gariepinus).*

Oluyemi K. Gbadamosi , Ojo P. Oke , Ajogunmaru B.

Department of Fisheries and Aquaculture Technology, Federal University of Technology Akure, PMB 704, Akure, Ondo State, Nigeria.

This study explored the supplementation of two microalgae: *Spirulina platensis* and *Phaeodactylum tricornutum* in African catfish (*Clarias gariepinus*) diets. The effect of the inclusion of the two microalgae on growth performance, nutrient utilization, hematological parameters and organosomatic index were investigated. Fish were fed five isonitrogenous and isocaloric diets containing 0mg/g of control diet, 10mg/g of *S. plantesis* and *P. tricornutum* (10SP; 30PT), and 20mg/g *S. platensis* and *P. tricornutum* (20SP; 20PT), *P. tricornutum* (30SP ; 30PT) and 40mg/g *S. platensis* and *P. tricornutum* (40SP; 40 PT). The result showed that diets supplemented with the composite algae mix significantly ($P<0.05$) boosted the growth performance and nutrient utilization compared to the control diet. The hematological analyses of the fish showed a significant increase ($P<0.05$) in the value of red blood cells (RBC) and haemoglobin (Hb) up to the supplementation level of 20mg/g of algae mix and decreased slightly when the supplementation level was 30mg/g. Furthermore, the value of white blood cells (WBC) and packed cell volume significantly ($P<0.05$) also increased with increase in supplementation level of the algae mix in all the treatments. Organosomatic I dex also increased with increase of the algae mix supplementation. This study showed that *S. platensis* and *P. tricornutum* can be supplemented in fish diets for improved growth performance, nutrient utilization and health of African Catfish, *Clarias gariepinus*.

Case Report: Strawberry Disease in Rainbow Trout (*Oncorhynchus mykiss*) in Puno, Peru

Yessica L. Ortega Asencios[1], Nieves N. Sandoval Chaupe[2]

[1]Pontifical Catholic University of Valparaiso, Valparaíso, Chile; [2]Department of Ictiopathology, College of Veterinary Medicine, San Marcos University, Lima, Perú

Peru is the fifth largest producer of rainbow trout in the world. In 2018 reported a production of 52,030.20 tons. However, health problems such as bacterial and viral diseases affect the production. Strawberry Disease is a non-lethal skin disease that has been reported in rainbow trout. It affects commercial size fish, producing skin lesions and even altering the quality of the fillet. It has been reported in various European countries, the United States and Chile. This report describes rainbow trout with average weight between 150 – 250 g. Bright red erythematous lesions, with loss of scales, located in different areas of the body without mortality record. Skin and muscle samples were taken for histopathology. Histopathology shows severe dermatitis with predominantly lymphocytic infiltration. It is concluded that the lesions are compatible with the Strawberry Disease. This is the first report that describes histopathological lesions compatible with Strawberry Disease in farmed rainbow trout in Peru.

Influence of Formalin Treatment Reduction on Survival and Egg Stress Response in *Oncorhynchus mykiss* During Incubation

Christine A. Parker-Graham[1], Ben Cross[2], Patricia Crandell[3], Nicole Hams[4], Ron Twibell[3], Dan Fielding[5], Nathan Eckert[6], Kristin Bates[7], Craig Thomas[8]

[1] Pacific Region Fish Health Program, US Fish and Wildlife Service, Lacey, WA 98503; [2] Western Washington Fish and Wildlife Conservation Office, US Fish and Wildlife Service, Lacey, WA 98503; [3] Abernathy Fish Technology Center, US Fish and Wildlife Service, Longview, WA 98632; [4] Columbia River Fish and Wildlife Conservation Officer, Vancouver, WA 98633; [5]Quinault National Fish Hatchery, US Fish and Wildlife Service, Humptulips, WA 98552; [6] Neosho National Fish Hatchery, US Fish and Wildlife Service, Neosho, MO 64850; [7] Makah National Fish Hatchery, US Fish and Wildlife Service, Neah Bay, WA 98357; [8] Alchesay National Fish Hatchery, US Fish and Wildlife Service, Whiteriver, AZ 85941

Formalin, an aqueous formaldehyde solution, is approved by the Food and Drug Administration (U.S.) for disease management on finfish hatcheries. Formalin is labeled for topical use on finfish eggs and is widely used during incubation to prevent oomycete (*Saprolegnia* spp.) colonization of eggs and egg loss. Formalin poses a significant public health risk to humans; it is a suspected carcinogen and exposure can cause irritation of the skin, eyes, and respiratory mucous membranes. Because of the health and environmental risks associated with formalin use and exposure, some U.S. states, like Wyoming and California, have dramatically reduced and discontinued formalin use in hatcheries in favor of less toxic chemicals like peracetic acid. For hatcheries with appropriate water sources, formalin reduction can reduce chemical costs, staff time, staff formalin exposure, and formalin effluent into surrounding waterways. This project evaluated the efficacy of formalin reduction in incubation practices at three different salmonid hatcheries in the U.S. raising *Oncorhynchus mykiss* by measuring percent fertilization, percent eye-up, percent hatch, percent of fry on feed, and cortisol levels in eggs and fry at different formalin exposure levels. Reducing the frequency and concentration of formalin treatment throughout the course of incubation did not have a significant impact of egg survival or egg stress at all three facilities in this study. Complete elimination of formalin, however, resulted in widespread egg loss to external saprolegniasis. For salmonid hatcheries with acceptable water sources, the reduction of formalin concentration or formalin treatment frequency yielded favorable results and reduced overall chemical usage and effluent during the incubation period.

A Stochastic Model to Investigate Atypical *Aeromonas hydrophila* Disease Dynamics in Catfish Aquaculture Ponds

Bradley M. Richardson[1], Michael E. Colvin[2], Matt J. Griffin[3,4], Charles C. Mischke[2,5], Terrence E. Greenway[2,5], David J. Wise[2,5]

[1]Warmwater Aquaculture Research Unit, USDA-ARS, Stoneville, MS; [2]Department of Wildlife, Fisheries, and Aquaculture, Mississippi State University, Mississippi State, MS; [3]Aquatic Research and Diagnostic Laboratory, Thad Cochran National Warmwater Aquaculture Center, Stoneville, MS; [4]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS; [5]Delta Research and Extension Center, Thad Cochran National Warmwater Aquaculture Center, Stoneville, MS

Catfish raised in earthen ponds are the largest commercial finfish aquaculture industry in the United States. The continuous push to maximize per-unit production risks pathogens proliferation. Dense stocking rates, water temperatures, and increased nutrient inputs increase the risk of disease outbreaks, despite management practices like intensive aeration and water quality monitoring designed to reduce stressors. An atypical strain of the pathogenic bacterium *Aeromonas hydrophila* (aAh) was first recorded on an Alabama farm in 2009 and spread across Mississippi by 2013. Difficulties recreating aAh outbreaks with tank studies limit the use of controlled experiments to increase understanding of disease dynamics. This study developed a stochastic susceptible-latent-infected-recovered model framework to investigate aAh disease dynamics within simulated catfish populations. The model used empirical data, expert elicitation, and information from related bacterial pathogens to evaluate the potential effects of pathogen reservoirs, disease characteristics, and management strategies on the disease and economic outcomes over the production season. The model successfully recreated acute and sub-acute disease outbreaks with mortality similar to outbreaks observed by the industry. Medicated feed was the most efficient and economical management strategy for outbreaks in model simulates despite increased feed cost. A pond monitoring strategy where fish are randomly sampled throughout the production season was effective in specific situations but was rarely profitable due to the low prevalence of the bacterium between outbreaks. This model serves as a framework for understanding commercially important diseases, particularly those lacking empirical data like aAh. Using expert opinion to bridge knowledge gaps allows this and similar modeling approaches to further understanding of disease dynamics in systems when data collection may be difficult or impossible and when reliably producing disease outbreaks in controlled experiments is challenging.

Evaluation of a New Biodegradable Technology Packaging for the Jumbo Squid *Dosidicus gigas*

Alejandro Sáez[1], Romina L. Abarca [2], Sebastián Escobar-Aguirre[1]

[1]Laboratorio Acuicultura, Departamento Ciencias Animales, Facultad de Agronomía e Ingeniería Forestal Pontificia Universidad Católica de Chile, Santiago, Chile; [2] Departamento de Ciencias Animales, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile

The jumbo squid *Dosidicus gigas* is the most abundant cephalopod species in the southeastern Pacific Ocean (Chile and Perú), representing the most significant cephalopod fishery globally. The giant squid's meat is a high protein resource that can be used to prepare preformed products based on surimi at the Asian market. Therefore, the quality of this product is necessary to maintain during long-distance transportation (i.e. quantitative and qualitative meat protein variations). This study aimed to develop biodegradable gelatin (280 bloom) and alginate-based films to find an active packaging candidate to stock the jumbo squid. To increase the food safety of the films, a nisin into polymer matrix was tested. An active packaging evaluation from cuttlefish pieces was performed at 4 °C, with a distribution given by the films and antimicrobial treatments. The consumer's perception was based on a colorimetric evaluation comparing the treatments with a frozen cuttlefish control sample. Our results showed that the alginate-based films showed a lower increase in the ΔE values than the gelatin-based films (α=0,05). RAM analysis showed that the nisin interacted with the films decreasing the colony count values on the samples. In this evaluation, the nisin-gelatin film showed no significant differences with the frozen cuttlefish control sample($p<0,05$), not so in the case of nisin-alginate and the polymers without nisin treatments. The dehydration rate showed no significant differences among treatments($p<0,05$). These results hint at a possible biodegradable film candidate for packaging cuttlefish. Further research on the barrier and dehydration properties of these films is needed.

Using Zebrafish to Disentangle the Impact of Environmental Exposure on Host-Microbiome Interactions

Thomas J. Sharpton

Department of Microbiology and Department of Statistics, Oregon State University, Corvallis, OR 97330

Extensive evidence demonstrates that the gut microbiome contributes to vertebrate physiology and that gut microbes can mediate how environmental factors, including nutrients, pollutants, and parasites, impact health. Consequently, the effort to manage the health of vertebrates benefits from understanding how different environmental factors interact with the microbiome to affect physiology. This effort, however, remains bottlenecked by the extensive scope and variation of environmental factors to which vertebrates are exposed. Researchers increasingly turn to the zebrafish model system to study these interactions, as it affords access to features that alleviate this bottleneck, including large sample sizes, automated exposure and phenotyping platforms, and short generation times. In this presentation, I summarize the utility and potential for the zebrafish model system to accelerate our understanding of how exposures interact with the microbiome to impact health. I also introduce key zebrafish research analytical and experimental tools we and others innovated to this end, including longitudinal sampling designs, high-throughput germ-free fish assays, and strategies for multi-omic data integration. Finally, I will discuss recent research we have conducted that exemplifies the utility and impact of this model system for microbiome research. This discussion will include our recent characterization of how exposure to polycyclic aromatic hydrocarbons affects zebrafish gut microbiome assembly to impact larval fish behavior, as well as our use of longitudinal multi-omic sampling to clarify mechanisms through which gut microbes may mediate helminthic infection outcomes in adult zebrafish.

Using Genomic Applications to Understand Wild Smallmouth Bass Immune Function

Cheyenne R. Smith[1,2], Christopher A. Ottinger[2], Heather A. Walsh[2], Vicki S. Blazer[2], and Patricia M. Mazik[3]

[1]West Virginia University, Davis College of Agriculture, Natural Resources and Design, Morgantown, WV, USA; [2]U.S. Geological Survey, Eastern Ecological Science Center at the Leetown Research Laboratory, Kearneysville, WV, USA; [3]U.S. Geological Survey, West Virginia Cooperative Fish and Wildlife Research Unit, West Virginia University, Davis College of Agriculture, Natural Resources and Design, Morgantown, WV, USA

A multidisciplinary approach has been implemented over the past two decades to investigate fish health issues in the Chesapeake Bay watershed. Investigations have focused on smallmouth bass *Micropterus dolomieu*, which is a popular sport fish and has been disproportionately affected due to their sensitivity to pollution and other stressors. As there are multiple stressors affecting wild fish populations, the approach integrates chemical exposure, climatic factors, and biological indicators at long-term monitoring sites with differing degrees of land-use and chemical inputs to assess potential adverse exposure affects and cumulative risk. Immune function is being assessed as one of the biological indicators to determine if immunosuppression relating to a complex mixture of stressors is playing a role. A suite of three *in vitro* immune function assays specific for smallmouth bass were developed and optimized to measure separate aspects of innate and adaptive immunity including bactericidal activity and respiratory burst of phagocytic cells and lymphocyte mitogenesis. These were integrated into comprehensive fish health assessments at a subset of long-term monitoring sites beginning in 2016 and performed twice a year – in spring before spawning and fall during recrudescence – using isolated anterior kidney leukocytes from 20 adult smallmouth bass at each site. The integration of immune function assays into full fish health assessments have allowed us to monitor and evaluate changes in immune status and relate to other aspects of health like parasite and pathogen prevalence in various tissues and concentrations of chemicals in plasma and other tissues. We are beginning to incorporate genomic applications to help understand mechanisms using Nanostring nCounter® custom CodeSets to look at transcript abundance of immune- and contaminant-related genes in RNAlater™-preserved anterior kidney and liver tissues archived from field samples. Immune function results alone have revealed yearly, seasonal, site and individual differences but we need to consider these results in the context of the whole organism to interpret the significance. We have observed correlations between immune function and microscopic indicators such as parasite prevalence and macrophage aggregates in liver and spleen tissues plus plasma concentrations of per- and polyfluoroalkyl substances but have not yet looked at correlations with gene expression. Correlations between immune function, various stressors, and transcript abundance of immune-related genes will be explored and discussed. The goal is to integrate lower level responses like gene expression into higher level responses like immune function to help us ultimately understand individual and population responses. We want to connect macromolecular and sub-organismal responses to impacts on whole animals.

Bivalves Transmissible Neoplasia (BTN) in *Macoma balthica* from the Baltic Sea

Katarzyna Smolarz [1], Anna Hallmann [2], Alicja Michnowska [1], Agnieszka Chomiczewska [2], Marcin Lipiński [1], Kaja Czajkowska [1]

[1]Department of Oceanography and Geography, University of Gdańsk, Gdynia, Poland; [2]Department of Pharmaceutical Biochemistry, Medical University of Gdańsk, Gdańsk, Poland

One of the symptoms of poor environmental health is the increase in number of pathologies in dominant species, including the occurrence of haemocyte (disseminated) neoplasia in marine bivalves, a leukemia-type disease. *Macoma balthica*, inhabiting the Baltic Sea and in particular the Gulf of Gdańsk, belongs to species in which the disease occurs at high frequency. Until know, several hypotheses explaining the aetiology of bivalve neoplasia were established so far with pollution and viral factor being widely recognised, but none fully explains the basis of the phenomenon. However, recent studies highlighted horizontal cancer cells spread among marine bivalves, suggesting the occurrence of contagious type of neoplasia. Within this presentation we will combine various facts related to the horizontal transfer of neoplastic cells with new data related to the biochemical and physiological characteristic of the disease. As neoplastic cells, after entering the host, must bypass its immune system, we studied if there are changes in selected immunological markers and corticosteroids levels (as the latter have the ability to reduce inflammatory processes) in neoplastic and healthy clams. Also, a set of markers highlighting the presence of antioxidative and physiological stress including the Total Antioxidant Capacity assay, acetylocholinesterase, and glutathione S-transferase activities. Levels of malondialdehyde and carbonyl content were assessed. Based on available flow cytometry protocols, DNA content (ploidy level), fagocytosis and calcium levels were studied in neoplastic and normal clams. Additionally, our goal was to seek pathways of contagious cancer transmission in marine environment. For that purpose, a co-habitation of healthy clams with neoplastic ones in controlled laboratory conditions was allowed for a period of six months. Also, mortality assessment of healthy and neoplastic hemocytes in artificial brackish water was conducted.
Activities of adenosine deaminase (ADA), alkaline phosphatase (ALP) were measured in haemolymph but no differences in ADA and ALP activities between healthy and neoplastic clams were found. Neoplastic clams were also characterized by significantly higher concentration of ROS, but no elevated response of the oxidative stress markers were found suggesting an efficient antioxidant response. High levels of corticosterone and lower amounts of dehydrocorticosterone, cortisol and cortisone in healthy clams and elevated cortisol level found in BTN individuals were found. Performed co-habitation experiment highlighted time-related increase in the frequency of neoplasia indicating the horizontal transmission of neoplastic cells between individuals kept in the same aquarium. A significant increase in the number of individuals with advanced stages of neoplasia was also observed most likely indicating the progression of neoplastic disease in some individuals during the experiment. The mortality of neoplastic hemocytes in seawater was lower than mortality of healthy ones. Both cell types were also able to survive in seawater for at least 4 hours after their release.

Genomics (Virtual)

Muñoz-Cerro	Front-Loading of Immune Genes Contributes to the Resistance of *Argopecten purpuratus* Scallop Larvae to *Vibrio bivalvicida* Infection
Sánchez	Use of Whole Genome Sequence Level Imputed Genotypes for Resistance to Salmon Rickettsiosis Syndrome (SRS) In Rainbow Trout (*Oncorhynchus Mykiss*).
Ulloa	RNA Sequencing Study Reveals the Genetic Variation (genes?SNPs) That Confer Dietary Tolerance and Favor Zebrafish (*Danio rerio*) Growth
Zhang	Genome-wide Analysis of Microsporidian ADP/ATP Carrier Proteins, with 2 Copies in *Ameson portunus* Infecting the Swimming Crab *Portunus trituberculatus*

Front-Loading of Immune Genes Contributes to the Resistance of *Argopecten purpuratus* Scallop Larvae to *Vibrio bivalvicida* Infection

Eduardo Jeria[1], Rodrigo Rojas[2] Rodolfo Farlora[3], Daniel Oyanedel[1], Katherine Muñoz-Cerro[1], Katherina Brokordt[4], Delphine Destoumieux-Garzón[5], Paulina Schmitt[1]

[1]Laboratorio de Genética e Inmunología Molecular, Instituto de Biología, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile; [2]Laboratorio de Patobiología Acuática, Departamento de Acuicultura, Universidad Católica del Norte, Coquimbo, Chile; [3]Laboratorio de Biotecnología Acuática y Genómica Reproductiva (LABYGER), Instituto de Biología, Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile; [4]Laboratorio de Fisiología y Genética Marina (FIGEMA), Departamento de Acuicultura, Universidad Católica del Norte, Coquimbo, Chile; [5]IHPE, Université de Montpellier, CNRS, Ifremer, Université de Perpignan Via Domitia, Montpellier, France.

Bivalve mollusks are in permanent contact with an abundant and diverse community of microorganisms from the aquatic environment. In turn, bivalves possess efficient innate defense mechanisms which protect them against potential pathogens. Still, mass mortality events have emerged in hatchery-reared bivalve larvae, which have been related to pathogenic *Vibrio* spp. Taking advantage of variations in the genetic component of host resistance, selective breeding is suggested as a feasible strategy to increase the resistance to diseases in bivalves, which in some cases is associated with the immune capacity of selected animals. Thus, we aimed to uncover host and pathogen determinants that contribute to different infection outcomes resulting from the interaction of pathogenic *Vibrio* with selected larvae from *Argopecten purpuratus* scallop. To achieve this, we produced 21 full-sibling families of mature larvae following a paternal half-sibling nested design, and we exposed each family to the pathogen *Vibrio bivalvicida* VPAP30. We analyzed the transcriptional response of larval families with contrasting resistance to the pathogen at an early stage of infection. We observed a significant variability in resistance among families, ranging from 18 to 85% of affected larvae at 24 h post infection. Then, we examined the gene expression dynamics in four larval families that showed the most contrasted resistance phenotypes, and in the pathogen simultaneously, by a dual RNA-seq approach. On the host side and before infection, the GO enrichment analysis of DEGs showed an overrepresentation of biological processes categories associated with immunity in the resistant families, such as regulation of macrophage activation, lymphocyte migration; while growth and metabolism were more represented in susceptible families. After 8 h of infection, positive regulation of response to cytokine production, response to iron ion, regulation and activation of immune cells among others were enriched in the resistant families, while lipid transport, regulation of wound healing, apoptosis, response to increased oxygen levels and receptor-mediated endocytosis were enriched in the susceptible families. This result strongly suggests that a front-loading of immune genes contributes to resistance to infection with pattern recognition related proteins, and antimicrobial effectors such as lysozyme and myeloperoxidase being common to the resistant phenotype, both before and after infection. On the pathogen side, DEG analysis showed a complete remodeling of the bacterial transcriptome after contact with the host. The Vibrio response was characterized by its adaptation to the host environment, independently of the host phenotype (resistant or susceptible). Several DEGs were associated with virulence in other bacterial pathogens, with no difference in terms of expression between resistant and susceptible hosts. Overall, we identified candidate genes for (i) host resistance/susceptibility markers for *A. purpuratus* scallop larvae and (ii) virulence factors for *V. bivalvicida*. Early expression of host immune genes appears as the main determinant of the disease outcome. Funded by Chilean government FONDECYT 1200129.

Use of Whole Genome Sequence Level Imputed Genotypes for Resistance to Salmon Rickettsiosis Syndrome (SRS) in Rainbow Trout (*Oncorhynchus Mykiss).*

Charles Sánchez-Roncancio[1,3], Baltasar F. Garcia[2,3], Jousepth Gallardo-Hidalgo[2,3], José M. Yáñez[2,3]

[1]Doctorado en Acuicultura, Programa Cooperativo Universidad de Chile, Universidad Católica del Norte, Pontificia Universidad Católica de Valparaíso, Chile; [2]Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, La Pintana, Santiago, Chile; [3]Center for Research and Innovation in Aquaculture (CRIA), Universidad de Chile, Santiago, Chile

Genome-wide association studies (GWAS) have become a practical approach to mapping quantitative trait loci (QTLs) and identifying genes associated with pathogen resistance. The investigation aimed to perform GWAS for resistance against salmonid rickettsial septicemia (SRS) under controlled challenges using imputed genotypes at the sequence level. 2,130 rainbow trout were challenged against *Piscirickettsia salmonis* using 57K SNP panels. One hundred two parents that were the whole genome sequenced (WGS) were imputed. GWAS was performed for the time-to-death (TD) and survival binary (SB) variables. 488,979 imputed WGS genotypes were found for the 2,130 individuals. Heritabilities of 0.27 (0.02) and 0.25 (0.02) for TD and BS. The GWAS revealed TD SNPs with genomic importance in (Omy02, Omy03, Omy25, Omy26, and Omy27, finding in the latter a peak with several SNPs) and for BS, a peak with several SNPs was found in the Omy26. These results highlight the polygenicity of this trait and the importance of the Omy 27 in TD, which should be further investigated.

RNA Sequencing Study Reveals the Genetic Variations (genes/SNPs) that Confer Dietary SBM Tolerance and Favor Zebrafish (*Danio rerio*) Growth

Pilar E. Ulloa[1]; Luis A. Valenzuela[2]; Gonzalo Rincón[3]; Felipe Gonzalez[1]; Natalia P. Lam[4,5]; Valentina R. Córdova-Alarcón[4,5]; Adrián J. Hernández[6]; Patricio Dantagnan[6]; Cristian M. Araneda[4,5]

[1]Núcleo de Investigaciones Aplicadas en Ciencias Veterinarias y Agronómicas, Facultad de Medicina Veterinaria y Agronomía, Universidad de Las Américas, Santiago Chile; [2]Omics Lab, Villavicencio 378, Oficina 32, Santiago, Chile; [3]Genus plc, Deforest, WI, 53532, USA; [4]Laboratorio de Genética y Biotecnología en Acuicultura, Departamento de Producción Animal, Facultad de Ciencias Agronómicas, Universidad de Chile. Santiago, Chile; [5]Food Quality Research Center, Departamento de Producción Animal, Facultad de Ciencias Agronómicas, Universidad de Chile. Santiago, Chile. [6]Departamento de Ciencias Agropecuarias y Acuícolas, Facultad de Recursos Naturales, Universidad Católica de Temuco, Chile.

Contrasting effects in growth fish and intestinal inflammation have been reported when fish fed high percentages of soybean meal (SBM) in the diet. This suggests that genetic variability confers tolerance to SMB in some individuals within the same species, favoring growth. The aim of this study was to identify differentially expressed genes of higher- and lower-growth zebrafish fed SBM-based diet supplemented with saponin (50SBM + 2SPN) and to identify SNPs associated to growth. We developed an approach where two replicates of 19 experimental families were fed fishmeal-(100FM, control diet) or 50SBM + 2SPN from juvenile to adult stages. The intestine sample collected were selected from families with higher (HG-50SBM + 2SPN, 170 ± 18 mg) or lower (LG- 50SBM + 2SPN, 76 ± 10 mg) weight gain on 50SBM+2SPN in relation to 100FM. Total RNA was extracted from individual intestine of five LG- 50SBM + 2SPN and five HG-50SBM + 2SPN. RNA-seq libraries were prepared individually and sequenced using the Illumina GAII Sequencer. Six hundred and sixty-five genes were differentially expressed (DEGs) between phenotypes (P-value < 0.01 and fold change values ≤-1.5 and ≥1.5). From these genes 43 SNPs were selected according to statistical filter (regression log10.P, p-adjusted<0.05 and slope regression) and genotyped in 340 fish samples. Marker-trait association results revealed 4 SNPs associated to growth in 3 key immunity-related genes in response to the 50SBM+2SPN diet ($p<0.05$). These genes correspond to aif1l, arid3c and cst14b.2. aif1l codes for the allograft inflammatory factor-1 protein, stimulating the synthesis of cytokines such as il-6, il-12, and TNF-α promoting cell migration. Arid3c encodes 2 isoforms, bright like and bright like Δ6, which modulate the binding of the B-cell antigen receptor. cst14b.2, is involved in neutrophil degranulation in the inflammatory response to infection in fish. Two SNPs belonging to aif1l y arid3c produce a positive (+19 mg) and negative (-26 mg) effect on fish growth, respectively. These SNPs can be used as markers to improve the early selection of tolerant fish to SBM diet or others plant-based diets, contributing to the aquaculture sustainability.

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Genome-wide Analysis of Microsporidian ADP/ATP Carrier Proteins, With 2 Copies In *Ameson portunus* Infecting the Swimming Crab *Portunus trituberculatus*

Zhaozhe Xin[1], Jiuyang Chen[1], Zhiaqiang Zhu[1], Xintong Zhang[1], Jinyong Zhang[1,2]

[1]The Laboratory of Aquatic Parasitology and Bioresources, School of Marine Science and Engineering, Qingdao Agricultural University, 266237 Qingdao, China; [2]Laboratory for Marine Biology and Biotechnology, Pilot National Laboratory for Marine Science and Technology (Qingdao), Qingdao, Shandong Province, China;

Microsporidia is a widely distributed obligate intracellular or intranuclear protist with wide host range of from invertebrates to vertebrates, including human being which cannot generally synthesize ATP on their own and rely on nucleotide transporters to obtain it from their hosts. ATP/ADP carrier proteins (AACs) are responsible for importing host-derived ATP to support the energy requirement for microsporidia. However, the knowledge of AACs of Microsporidia is still few, especially for aquatic microsporidia which account for more than half of the reported microsporidia. Here, we systematically identified and characterize AACs from all Microsporidia with available public genome data and *Ameson portunus* which is an obligate myocytes-infecting microsporidium and currently severely affects the Portunidae mariculture in China. The results showed that high AACs gene number variation occurred among all analyzed Microsporidia and two AACs were found from the genome of *A. portunus* which adjacently distributed in the same scaffold. Phylogenetic analysis showed that all microsporidian AACs formed 5 distinct clades and the 2 AACs of *A. portunus* phylogenetically grouped with those of *Cucumispora dikerogammari*. The AACs may be acquired by the common ancestor of *Rozella allomycis* and Microsporidia. Five motifs among microsporidian AACs were suggested to play conserved roles in the transport of host energy. The research results have important significances for revealing the mechanism of microsporidia-host interaction and developing the prevention and control technology of aquatic microsporidiosis.

Microbiome Applications in Fish Health (Virtual)

Divya	Diversity of Microbial Community Between Medicated and Non-Medicated Catfish Ponds in the Mississippi Delta
Koepper	The Shell Microbiome of American Lobster *Homerus americanus* in Atlantic Canada
Older	Nanopore Sequencing for Aquaculture Bacterial Microbiota Profiling
Venegas	Effect of Rearing Conditions on the Recruitment and Resilience of Atlantic Salmon's Microbiota (*Salmo salar*) Throughout its Different Developmental Stages
Yamamoto	The Intestinal Microbiota of Channel Catfish (*Ictalurus punctatus*) After Florfenicol Treatment Followed by Dietary Prebiotic or Probiotic Supplementation

Diversity of Microbial Community Between Medicated and Non-Medicated Catfish Ponds in the Mississippi Delta

Divya Rose[1,2], Caitlin Older[3], Brad Richardson[3], Cyndi Ware[1,2], Marsha Lewis[1,2], Geoff Waldbieser[3], Lester Khoo[1,2], Hasan Tekedar[4], Andy Perkins[5], Larry Hanson[4], T. Graham Rosser[4], David Wise[2,6] and Matt Griffin[1,2].

[1]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Stoneville, MS 38776; [2]Thad Cochran National Warmwater Aquaculture Center, Delta Research and Extension Center, Mississippi State University, Stoneville, MS 38776; [3]Warmwater Aquaculture Research Unit, Agriculture Research Service, U.S. Department of Agriculture, Stoneville, MS 38776; [4]Department of Comparative Biomedical Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762; [5]Department of Computer Science and Engineering, Mississippi State University, MS; [6]Mississippi Agriculture and Forestry Experiment Station, College of Forest Resources, Mississippi State University, MS 38776

Mississippi is the largest catfish producer in the United States, producing 58% of U.S farm-raised catfish; with active aquaculture ponds covering >36,000 acres. The majority of Mississippi catfish production is in "The Delta" region in the western part of the state. Bacterial disease accounts for most disease related losses. Feed restriction or antibiotic intervention are the most practiced management strategies to control bacterial outbreaks in catfish ponds. Reports from the Aquatic Research and Diagnostic Laboratory (ARDL) in Stoneville, MS, show an increase in antibiotic resistant (ABR) bacterial isolates over the past decade. A metagenomic assessment was performed on pond water samples from two adjacent catfish farms (<4 miles apart); each employing different strategies for antibiotic use. Both farms focus on channel catfish production and vaccinate their fingerling stocks using an orally delivered, live attenuated *E. ictaluri* vaccine. Farm A uses medicated feed in response to disease outbreaks, while Farm B employs feed restriction and does not rely on antibiotic intervention. Herein, the influence of on-farm antibiotic use on catfish pond microbial communities was assessed. High throughput 16S rRNA gene sequencing was performed on 56 pond water samples (33 from Farm A; 23 from Farm B) to assess bacterial community structure in ponds across both farms. Comparative analysis of relative abundance of multiple bacterial species revealed differences across the two farms. Visualization of beta diversity revealed differences in bacterial community structure between the two farms, while alpha diversity assessments demonstrated differences between medicated and non-medicated ponds. These analyses illustrate a possible difference in the bacterial community composition between farms that employ antibiotic interventions versus farms that rely on restricted feeding. These data lay the foundation for further research investigating the biological significance of the varied bacterial communities observed on these two farms.

The Shell Microbiome of American lobster *Homarus americanus* in Atlantic Canada

Svenja Koepper[1], K. Fraser Clark[2], Crawford W. Revie[3], Krishna K. Thakur[1]

[1]Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown C1A 4P3 PE, Canada; [2]Department of Animal Sciences and Aquaculture, Faculty of Agriculture, Dalhousie University, Truro, B2N 5E3 NS, Canada; [3]Department of Computer and Information Sciences, University of Strathclyde, Glasgow, G1 1XQ, UK

Due to current knowledge gaps regarding the shell microbial community – proposedly a key factor in the fast-spreading epizootic shell disease (ESD) – of American lobster (*H. americanus)* in Atlantic Canada, this study aims to describe and analyze the shell microbiome of healthy and diseased lobsters sampled from several locations in New Brunswick, Nova Scotia and Prince Edward Island. More than 300 lobster shell swab samples and associated data on biotic and abiotic factors have been collected from seven different lobster fishing areas. Long-read, next-generation 16S rDNA amplicon sequencing (PacBio) of cuticle samples followed by bioinformatic analyses will identify the shell associated bacteria to species level. Diversity indices will assess the microbial composition and diversity while network analyses explore bacterial interactions within the microbiome. Furthermore, multivariate analyses will detect any patterns in microbial species' abundances, composition or distribution based on biotic and abiotic factors. It is expected that spatial, temporal, and environmental variables as well as lobster characteristics such as sex, size, or molt stage to some extent influence microbial profiles on lobster cuticles. Bacterial taxa that have been associated with ESD are likely present in lobsters from Atlantic Canada as they are ubiquitous in the marine environment but may play a role in ESD proliferation. This study will help to assess and predict the risk of ESD outbreaks in Atlantic Canada and in turn will encourage the development of suitable fisheries management strategies in the future.

Nanopore Sequencing for Aquaculture Bacterial Microbiota Profiling

Caitlin E. Older[1], Fernando Y. Yamamoto[2], Matt J. Griffin[3], Cynthia Ware[3], Brian G. Bosworth[1], Geoffrey C. Waldbieser[1]

[1]Warmwater Aquaculture Research Unit, Agriculture Research Service, U.S. Department of Agriculture, Stoneville, Mississippi, USA; [2]Thad Cochran National Warmwater Aquaculture Center, Mississippi Agriculture and Forestry Experiment Station, Department of Wildlife, Fisheries, and Aquaculture, College of Forest Resources, Mississippi State University, Stoneville, MS, USA; [3]Thad Cochran National Warmwater Aquaculture Center, Aquatic Research and Diagnostic Laboratory, Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Stoneville, MS, USA

Bacterial community profiling in aquaculture can be useful for monitoring environmental conditions which may lead to disease outbreaks, understanding the impact of dietary treatments on fish health, and identifying microbes which may be useful as antibiotic alternatives. Microbiota characterization is most commonly performed using short-read platforms (i.e. Illumina) targeting informative fragments of the bacterial 16S rRNA gene. Comparably, long-read platforms (i.e. Oxford Nanopore Technology) enable sequencing of full-length 16S rRNA genes. While short-read platforms are highly accurate, long-read platforms sacrifice sequence quality in exchange for longer read lengths. Herein, a typical short-read approach (Illumina MiSeq; V4 region of 16S rRNA gene) was compared with two nanopore near full-length 16S rRNA protocols (ONT Custom and ONT 16S). Sequencing was performed on a mock community composed of fish-relevant bacteria, in addition to samples obtained from ten channel x blue hybrid catfish (digesta, gill, skin) and their environment (pond and tank). To assess the role of euthanasia on microbiota assessments, five fish were euthanized by overdose of MS-222, while the remaining fish were euthanized by cranial concussion and subsequent pithing. This enabled additional comparisons of these three sequencing protocols in the context of a realistic experimental design. Results from the ONT Custom protocol best recapitulated the theoretical composition of the mock community, followed by the Illumina MiSeq and ONT 16S protocols. At the phylum level, taxonomic composition was consistent within sample type across sequencing methods. However, at the lower levels there were biases for several taxa, particularly in pond samples. Euthanasia method also appeared to introduce bias, primarily on the cutaneous communities. The three sequencing methods consistently identified significant differences in beta diversity and taxonomic composition between euthanasia methods, with Illumina MiSeq revealing the greatest differences. Sample type also influenced results; while all three strategies performed comparably for digesta, skin and water (pond and tank), the ONT strategies underperformed for gill samples. Depending on application, results indicate near full-length 16S rRNA gene nanopore sequencing is a viable option for aquaculture microbiota studies.

Effect of Rearing Conditions on the Recruitment and Resilience of Atlantic salmon's Microbiota (*Salmo salar*) Throughout its Different Developmental Stages

Lucas Venegas[1,2], Camille Lavoie[3], José M. Yañez[1], Nicolas Derome[2]

[1]Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, 8820000, Chile; [2]Institut de Biologie intégrative et des Systèmes, Université Laval, G1V 0A6, Québec, Canada; [3]WSP, H3H 1P9, Montréal, Canada.

Wild Atlantic salmon populations in North America, essentially Canada, have faced a continuous declining process over the last 35 years. Current government programs to restore salmon stocks in rivers have not achieved the expected results, in terms of survival rates and fitness, of re-introduced individuals with respect to the wild born relatives. In this context, molecular ecology approaches like metabarcoding studies have highlighted the relevance of including the microbiota composition indicators of artificially raised fish when compared with wild ones, especially in the early steps of microbiota ontogeny, on supportive fish breeding programs. The aim of this study was to recapitulate the ontogeny of the egg-to-smolt microbiota for wild and farmed Atlantic salmon (*Salmo salar*) from the same genetic population to identify the characteristic bacteria present throughout the development of Atlantic salmon individuals. Using the V4 region of the 16S rRNA gene, the microbial composition and structure analysis (evaluated with the core microbiota, alpha and beta diversity, and network interactions) were performed and compared between individuals from four developmental stages (egg, fry, parr, and smolt) and two different conditions (stocked and wild) of Atlantic salmon. In addition, stocked eggs were incubated in the Laboratoire Régional en Sciences Aquatiques (LARSA) or the Societé Saumon Rivière Romaine (SSRR) to evaluate the long-term effects of the early life-associated environment on the later stages of microbiota recruitment. Main results showed a microbial convergence between eggs incubated in LARSA and SSRR, suggesting a non-negligible effect of vertical transmission of parental microbiota. Interestingly, more than twice as many significant correlations were found in the SSRR fries compared to the LARSA ones (390 vs 160), like previous results found in wild parr network compared to the stocked individuals, denoting the higher resilience capacity of the microbiota in eggs incubated at SSRR condition. Also, a persistent taxon was found in the egg, fry, and parr stages, in both wild and captive conditions, belonging to the *Beijerinckiaceae* family, a free-living aerobic nitrogen-fixing acid tolerant bacterium. Finally, the microbiota composition of smolt individuals was totally different to the previous developmental stages, indicating that much of the differences in bacterial community dynamics during *Salmo salar* development is more related to the developmental stage than the rearing conditions on the early bacterial colonization in salmon life. A major understanding over the interaction between microbiota components in the early ontogeny with the physiological and metabolic processes of salmonids is necessary to understand the role of bacterial communities in the declining of salmonids for improving current supportive fish programs.

The Intestinal Microbiota of Channel Catfish (*Ictalurus punctatus*) After Florfenicol Treatment Followed by Dietary Prebiotic or Probiotic Supplementation

Fernando Y. Yamamoto[1,2], Caitlin E. Older[3], Matt J. Griffin[1,4], Bradley M. Richardson[3], Jonathan G. Reifers[1], Penelope Lucas[1], Delbert M. Gatlin III[5], David J. Wise[1,2]

[1]Thad Cochran National Warmwater Aquaculture Center, Mississippi Agriculture and Forestry Experiment Station, Mississippi State University; [2]Department of Wildlife, Fisheries, and Aquaculture, College of Forest Resources, Mississippi State University, Stoneville, MS, USA; [3]Warmwater Aquaculture Research Unit, Agriculture Research Service, U.S. Department of Agriculture, Stoneville, Mississippi, USA; [4]Thad Cochran National Warmwater Aquaculture Center, Aquatic Research and Diagnostic Laboratory, Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Stoneville, MS, USA; [5]Department of Ecology and Conservation Biology, Texas A&M University, College Station, TX, USA

The intestinal microbiota of healthy individuals can be heavily disrupted by antibiotic therapy. And the industry has been reporting increasing cases of enteric septicemia shortly after florfenicol administration. The present study evaluated the intestinal microbiota of channel catfish after a 10-day florfenicol dietary treatment, followed by the supplementation of a commercial probiotic (Bactocell®) or a commercial prebiotic (GroBiotic-A®). The feeding trial included a negative control, which consisted of a group fed the basal diet and no antibiotic, and a positive control consisted of a group fed the basal diet after the antibiotic treatment. Six hundred naïve channel catfish juveniles (~20.7 g) were equally distributed in 20 aquaria (110-L). The dietary treatments were randomly distributed in a completely randomized block design (n=5). After the florfenicol administration, fish were fed the experimental diets for 12 days at a fixed percentage of the body weight, and digesta samples were collected 20 h postprandial from three fish from each tank on days 0, 4, 8, and 12. The remaining fish were challenged with a virulent strain of *Edwardsiella ictaluri* through immersion at a concentration of 1.7×10^7 CFU per mL, and survival was monitored for 32 days. DNA was extracted from the digesta samples and were subjected to Illumina sequencing (Illumina MiSeq) of the V4 region of the 16S rRNA gene. Alpha and beta diversity metrics were significantly impacted after the florfenicol therapy based on samples collected at day 0. Interestingly, based on the diversity metrics and the relative abundance of the bacteria taxa, the intestinal microbial population seemed to recover as early as 4 days after the florfenicol administration. No differences were detected for the alpha and beta diversity metrics on days 4, 8, and 12. A higher relative abundance of *Mycobacterium* spp. was observed for the fish treated with florfenicol on day 0, and *Pediococcus* spp. was significantly higher for fish fed the probiotic treatment at day 8 and 12. No differences were observed in survival after the bacterial challenge; nevertheless, it is noteworthy mentioning that a higher survival trend was observed for fish fed the probiotic treatment compared to the positive control (P=0.053). In conclusion, the intestinal microbiota of healthy channel catfish is disrupted by the florfenicol treatment but seems to restore to homeostasis after 4 days posttreatment, and the supplementation of probiotics may improve catfish bacterial resistance after the antibiotic therapy.

Immunology/Vaccinology

Aceituno	A Novel VHSV Subunit Vaccine: A New Prophylactic Tool with Oral Delivery Potential
Balami	Effect of *Lactobacillus plantarum* on growth performance, immune responses, and disease resistance of striped catfish (*Pangasianodon hypophthalmus*)
Gomaa	Developing a Dual Live Attenuated Vaccine to Prevent Motile Aeromonas Septicemia and Enteric Septicemia of Catfish
Nguyen	Glucan Immunostimulation Against Columnaris and Streptococcosis in a White Sturgeon (*Acipenser transmontanus*) Model
Tattiyapong	Development an Indirect ELISA to Measure Anti-Tilapia Lake Virus Antibodies in Tilapia Serum
Wolter	Characterization of the Innate and Acquired Immune Response Associated with Use of a Heterologous Vaccine with Immunomodulatory Activity in Atlantic Salmon

A Novel VHSV Subunit Vaccine: A New Prophylactic Tool with Oral Delivery Potential

Patricia E. Aceituno[1], Mauricio E. Rojas[1], Marlid Garcia-Ordoñez[1], María E. Salvador[2], María del Mar Ortega-Villaizan[2], Luis Perez[2], Nerea Roher[1,3,4]

[1]Institute of Biotechnology and Biomedicine (IBB), Universitat Autònoma de Barcelona, 08193 Bellaterra (Cerdanyola del Vallès), Spain; [2]Instituto de Investigación, Desarrollo e Innovación en Biotecnología Sanitaria de Elche (IDiBE), Universidad Miguel Hernández (UMH), Elche, Spain; [3]Departament de Biologia Cel·lular, Fisiologia Animal i Immuologia, Universitat Autònoma de Barcelona, 08193 Bellaterra (Cerdanyola del Vallès), Spain; [4]CIBER-BNN

Despite the impact of viral diseases in the aquaculture sector, there is still a lack of effective commercial vaccines against relevant viral diseases. Currently, our group is developing a new vaccine for oral administration based on modular nanostructured recombinant proteins named Nanopellets (NPs). This biotechnological platform for vaccine production has already been tested with different viral recombinant antigens with promising results for SVCV and VNNV (Rojas-Peña et al., 2022; Thwaite *et al,* 2018; 2020). These biomaterials are biologically active, non-toxic, inexpensive and stable at gastrointestinal pH, thus not needing further encapsulation. To increase the antiviral response, we develop a novel oral vaccine made of VHS viral antigen plus an extra functional protein domain coding for recombinant interferon-gamma (IFNg). Here we present the structural and functional characterization of VHSV-INFg^{NP} tested *in vitro* in intestinal cells (RTGut-GC) and primary macrophages (RT-HKM). Further, *in vivo* VHSV^{NP} and VHSV-INFg^{NP} intubation in *rainbow trout* provided relevant information about the administration route, showing that the NPs were correctly absorbed by the gut epithelia and were biodistributed to key immune tissues.

Effect of *Lactobacillus plantarum* on Growth Performance, Immune Responses, and Disease Resistance of Striped Catfish (*Pangasianodon hypophthalmus*)

Bui T. B. Hang[1], Sujita Balami[1], and Nguyen T. Phuong[1]

[1]College of Aquaculture and Fisheries, Can Tho University, Can Tho city, Viet Nam.

A feeding trial was carried out to investigate the effect of a potential probiotic bacterial strain, *Lactobacillus plantarum* (LP) supplemented in diets on survival and immune response of larval striped catfish (*Pangasianodon hypophthalmus*). The feeding trial was 8 weeks long in which three treatments were supplemented with 10^6, 10^7, and 10^8 CFU LP/g of feed and one control group without probiotic supplementation. The growth parameters were evaluated at the end of the feeding trial. In terms of immunity, fish blood was sampled for hematological parameters and lysozyme activity. After the feeding trial, the fish were challenged with *Edwardsiella ictaluri* for evaluating the bacterial resistance. Fish supplemented with *L. plantarum* diets showed significantly higher growth compared to those supplemented with the control diets. The weight gain, daily weight gain, and specific growth rate of treatment supplemented with 10^7 CFU LP/g were the highest. However, survival rates were not significantly different between the treatments. Results showed that hematological parameters (total white blood cells, monocytes, lymphocytes, neutrophils, and thrombocytes were significantly higher ($p < 0.05$) in the probiotic supplemented groups compared to those of the control group whereas total red blood cells were not significantly different between the treatments. Total lysozyme activity was significantly higher in the probiotic supplemented groups compared to those of the control group ($p < 0.05$) indicating higher immunity in the probiotic supplemented group. Accumulated mortalities after bacterial challenge were 0, 33.33, 20, 23.33, and 23.33 % for the negative control, positive control, 10^6, 10^7, and 10^8 CFU LP/g respectively. The results suggest that *L. plantarum* has a great potential for improving the survival of the striped catfish larvae as well as modulating the immune response. Hence, further research should be conducted to find the effect of *Lactobacillus plantarum* in different stages of striped catfish.

Developing a Dual Live Attenuated Vaccine to Prevent Motile Aeromonas Septicemia and Enteric Septicemia of Catfish.

Basant M. Gomaa[1], Hasan C. Tekedar[1], Hossam A. Abdelhamed[1], Mark L. Lawrence[1]

[1]Department of Comparative Biomedical Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, Mississippi, USA

Sales from catfish aquaculture totaled $386 million in 2020, and Mississippi produced 54% of total farm-gate sales. *Aeromonas hydrophila* is a pathogen that affects farm-raised catfish causing motile aeromonas septicemia (MAS). Since 2009, outbreaks caused by a genetic clonal group of *A. hydrophila* (virulent *A. hydrophila* or vAh) caused significant losses of market-size catfish in Alabama and Mississippi. There are limitations in the current therapeutic and preventative strategies against vAh. Our preliminary data revealed that recombinant vAh surface proteins (Fim, FimMrfG, ATPase, Tdr, and OmpA1) are effective in protecting catfish against MAS. Furthermore, live attenuated *Edwardsiella ictaluri* vaccine strain ESC-NDKL1 is an efficacious vaccine for enteric septicemia of catfish (ESC), and it is an effective vector for expressing vAh antigens. Our hypothesis is that expression of vAh surface proteins in a live attenuated *E. ictaluri* vaccine will provide significant protection against both MAS caused by vAh and ESC. Three pMEG-375 suicide plasmids were constructed and used for conjugation and integration of gene combinations encoding vAh antigens into gene deletion sites in the ESC-NDKL1 chromosome. A total of 32 stable recombinant ESC-NDKL1 strains expressing one, two, or three vAh surface antigens were successfully constructed. Specific pathogen free catfish fingerlings were used for vaccination trials with five replicate tanks per treatment. Fish were vaccinated by immersion, and 21 days after vaccination, fish were experimentally infected with $1X10^5$ CFU vAh strain ML09-119 by IP injection. Recombinant ESC-NDKL1 strains expressing two vAh antigens showed significant protection against MAS and improved protection compared to recombinant ESC-NDKL1 strains expressing one vAh antigen and non-recombinant ESC-NDKL1. Additionally, recombinant ESC-NDKL1 strains expressing three vAh antigens showed further improvement in protection against MAS. The efficacy of candidate vaccines will be further evaluated using oral and immersion vAh challenge methods. We expect to identify a promising vaccine candidate that will yield an important tool to control two diseases that impact the catfish industry.

β-Glucan Immunostimulation Against Columnaris and Streptococcosis in a White Sturgeon (*Acipenser transmontanus*) Model

Diem Thu Nguyen[1,2], David Marancik[2], Esteban Soto[1]

[1]Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, California, USA; [2]Department of Pathobiology, St. George's University School of Veterinary Medicine, True Blue, Grenada

Flavobacterium columnare and *Streptococcus iniae* represent the two most important bacterial pathogens of cultured sturgeon. However, at present there are no commercially available vaccines to prevent against infections and treatment options are limited. β-glucans have been shown to be potent immunostimulants that can provide fish protection against infectious disease. In this study, the effects of dietary β-glucan supplementation on disease susceptibility were examined by exposing 0.3% β-glucan-fed white sturgeon (*Acipenser transmontanus*) to the pathogens *Flavobacterium columnare* and *Streptococcus iniae* in laboratory-controlled challenges. Morbidity and mortality were monitored for 15 (*F. columnare*) or 30 (*S. iniae*) days post-challenge (dpc). Additionally, transcript levels for pro-inflammatory cytokines, regulatory cytokines and acute phase proteins (APP) were investigated in the spleen and gills at different time points post-challenge. No evidence of protection was observed in β-glucan-fed fish and challenged with the bacteria. Moreover, significantly greater mortalities were observed in β-glucan-fed fish challenged with *F. columnare* ($p<0.05$), likely associated with acute inflammatory response as haptoglobin and serotransferrin transcripts in the gills were significant higher in fish within this group at 1 dpc. Similarly, serotransferrin transcript in the gills was significant higher in β-glucan- fed fish challenged with *S. iniae* when compared to β-glucan- fed non-exposed control at 1 dpc. Transcript levels for all tested cytokines and APP in the spleen were similar amongst treatment groups in both *F. columnare* and *S. iniae* experiments. The results from this study suggest that β-glucan supplementation at the concentration and rate investigated provides no-benefit to white sturgeon against *F. columnare* or *S. iniae*.

Development an indirect ELISA to measure Anti-Tilapia lake virus Antibodies in Tilapia Serum

Puntanat Tattiyapong[1,2] and Win Surachetpong[1,2*]

[1]Interdisciplinary Program in Genetic Engineering and Bioinformatics, Graduate school, Kasetsart University, Bangkok, Thailand; [2]Department of Veterinary Microbiology and Immunology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand

Tilapia lake virus (TiLV) or *Tilapia tilapinevirus* is a novel RNA virus that causes Tilapia Lake Virus Disease (TiLVD) in tilapia and its hybrid species. Currently, no commercial vaccine or prophylactic treatment is yet available to prevent TiLVD. Hence, understanding tilapia immunity and the response against TiLV is crucial for drug and vaccine development. Here, we developed an indirect ELISA by using whole TiLV virus as antigen for plate coating, commercially mouse anti-tilapia IgM antibody, and goat anti-mouse IgG antibody conjugated with horseradish peroxidase enzyme to determine the reaction. The assay was applied to evaluate specific anti-TiLV IgM antibodies in serum of unexposed tilapia (n=41) and TiLV-exposed tilapia (n=44) collected from both farmed and experimental challenge fish, with significant OD value to separate unexposed and TiLV-exposed fish ($p<0.05$). The sensitivity and specificity of the assay were 61.36% and 80.49% respectively. Application of a new indirect ELISA to monitor the antibody response in tilapia challenged by intraperitoneal injection showed a rapid antibody response within 7 days post infection (dpi). The antibody response peaks at 15 dpi and gradually declined until 42 dpi. Interestingly, TiLV specific antibody persisted in some fish until 110 dpi. In conclusion, the new ELISA assay could distinguish serum of fish previously expose to TiLV and unexposed fish serum. Indeed, the assay could be applied as a tool for future vaccine development and determines the status of TiLV in tilapia farms. The sensitivity and specificity of the assay were optimized using fish serum exposed to TiLV or collected from naive fish. Our results revealed that serum collected from TiLV exposed fish showed specific detection of anti-TiLV IgM antibodies were observed in TiLV-exposed tilapia serum. No reaction was observed in unexposed tilapia serum.

Characterization of the Innate and Acquired Immune Response Associated with the use of a Heterologous Vaccine with Immunomodulatory Activity in Atlantic Salmon

Juan Carlos Rodríguez-Lecompte[1], Marilyn Wolter[2], Juan Pablo López[2]

[1]University of Prince Edward Island, Canada; [2]Elanco Animal Health, Puerto Varas, Chile

Renogen® is a heterologous live vaccine composed of live *Arthrobacter davidanieli* as microbial stimulation for the immune system. *A. devidanieli* shares antigenic determinants with both *Renibacterium salmoninarum* and *Piscirickettsia salmonis* (Salmon Rickettsial Septicemia; SRS); in Chile, Renogen is indicated for preventation of disease caused by both agents. The objective of the present study was to characterize the immune response of Atlantic salmon after one of four vaccination strategies incorporating Renogen or saline control. 508 fish were injected intraperitoneally at 15 g and 45 g bodyweight with Renogen, saline and/or a multivalent oil-adjuvanted vaccine. Fish were terminally sampled at one of seven timepoints up to 900 degree days (dd) post initial injection (300 dd post second injection). Anterior kidney was sampled and stored in RNAlater until analysis by real-time PCR (rt-PCR) for quantification of mRNA gene expression for Interleukin (IL)-1 beta, IL-12, IL-2, CD4, CD8, IL-10, and Interferon (IFN)-gamma. Expression changes were evaluated against the endogenous expression gene (housekeeping gene) (ELF1 + Bactin). Gene expression data will be presented across study time points. Results indicate pro-inflammatory and anti pro-inflammatory effects associated with the use of Renogen, suggesting Renogen helps the activation of macrophages and activates cellular immunity, increasing the spectrum of the overall immune response, both cellular and humoral. Results suggest a modulating or activating effect of immune response upon revaccination with heterologous antigens; presumably because they share similar antigens.

Parasitology (Virtual)

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Field Performance of Lufenuron (IMVIXA™) In Chilean Atlantic Salmon from 2016-2021

Julio Boettiger[1], Patricio. Peña[1], Janet Allen[2], Juan Pablo Lopez[1] and Alicia Macdonald[3]

[1]Elanco Animal Health, Puerto Varas, Chile; [2]Elanco US Inc., Greenfield, IN, USA; [3]Elanco Canada Limited, Mississauga, Ontario, Canada

IMVIXA™ (lufenuron) has been marketed in Chile since 2016 for the prevention and control of *Caligus rogercresseyi* on salmonids. The product is for oral administration in fresh water prior to sea transfer, with Chilean clinical studies and industrial use confirming a long duration of protection. Since commercialization, Elanco's technical support program has monitored field use of the product from different sites, seasons and treatment occasions through a comprehensive sampling program conducted across freshwater and seawater. The time from sea transfer until the first follow up treatment with a different lousicide (t_{FUT}) is additionally provided by the fish farm. These post-marketing data (to 31 Dec 2020) were filtered to exclude incomplete datasets, populations assessed as potential off-label dosing or incorrect administration and populations with concomitant treatment prior to loss of effect of IMVIXA, resulting in a dataset representing 69 farming cycles and over 67 million treated fish. Data were analyzed with summary statistics and for visualization of trends over time. Under commercial field conditions without negative control populations, t_{FUT} provides one indication of product performance however interpretation of t_{FUT} requires due consideration to the significant effect of uncontrolled environmental factors e.g. louse pressure. Results indicate a median time to follow up treatment of 2321 dd [Interquartile range 2116 - 2725 dd] with no apparent trend for decreasing product performance (t_{FUT}) over time, although limitations in sample size of the included dataset impacted the potential for statistical analysis. Best practice recommendations to maximize IMVIXA performance will additionally be discussed.

Epidemiology of Myxosporean Infections in Economically Important and Dietary Freshwater Fishes in The Sudano-Guinean Zone of Cameroon

Fonkwa Georges[1,3,4], Kouam K. Marc[2], Tomedi Eyango Minette[1], Tchuinkam Timoléon[3] and Tchoumboue[4]

[1]Laboratory of Aquaculture and Demography of Aquatic Resources, Institute of Fisheries and Aquatic Sciences, Department of Aquaculture, University of Douala, P.O. Box 7236 Douala-Cameroon; [2]Physiology and Animal Health Research Unit, Department of Animal Production, Faculty of Agronomy and Agricultural Science, University of Dschang, P.O. Box 118, Dschang-Cameroon; [3]Vector Borne Diseases Laboratory of the Applied Biology and Ecology Research Unit, Department of Animal Biology, Faculty of Science, University of Dschang P.O. Box 67, Dschang-Cameroon; [4]Applied Hydrobiology and Ichthyology Research Unit, Department of Animal Production, Faculty of Agronomy and Agricultural Science, University of Dschang, P.O. Box 222, Dschang-Cameroon

The epidemiology of Myxosporean infections was carried out from May 2016 to May 2017. Thus, 857 Teleosts (350 *Oreochromis niloticus*, 305 *Barbus callipterus*, 118 *Hemichromis fasciatus* and 84 *Clarias gariepinus*) from the Mayo –Banyo Division in the Sudano-guinean zone of Cameroon were sampled and the prevalence of myxosporean infections was determined after examination of fishes. A total of 21 myxosporean species belonging to four genera (*Myxobolus*, *Myxidium*, *Henneguya* and *Thelohanellus*) were recorded. The genus *Myxobolus* exhibited the highest prevalence (37.11%) and *Henneguya* (0.35%) the lowest. All the four fish species were infected with the genus *Myxobolus* with the highest ($P < 0.001$) prevalence in *O. niloticus* (45.43%) and *B. callipterus* (45.90%). A total of 325 fishes were infected (37.92%). *B. callipterus* (48.20%) followed by *O. niloticus* (45.43%) were the most infected ($P < 0.001$) compared to *C. gariepinus* (9.52%) and *H. fasciatus* (9.32%). The prevalence of parasite species was not correlated ($P > 0.05$) with the condition factor and fish size except that of *Myxobolus tilapiae* which was positively correlated with *O. niloticus* size ($r = + 0.17$; $P < 0.01$). Males were significantly ($P < 0.001$) more infected than females in *H. fasciatus* only. The overall prevalence was insignificantly ($P > 0.05$) higher in the dry season than in the rainy season. Out of 12 parasitized organs, *O. niloticus* and *C. gariepinus* exhibited the highest (10 organs) and lowest (3 organs) number of infected organs respectively. This study provided some baseline data useful in myxosporean infections prevention and control in fishes from Adamawa-Cameroon.

Myxozoan Diversity Infecting the Freshwater Fishes in the Sea of Galilee, Israel

Aditya Gupta[1,2], Michal Haddas-Sasson[1] & Dorothee Huchon[1,2]

[1]School of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv-6997801, Israel; [2]Steinhardt Natural History Museum, Tel Aviv University, Tel Aviv-6997801, Israel

Myxozoans are parasitic cnidarians that can cause severe damage to fish, resulting in economic losses to aquaculture and fisheries. In Israel, only a few taxonomic studies have investigated freshwater myxozoans. Specifically, five and six myxozoan species have been described from catfish (*Clarias lazera*), and three cultured tilapias (*Oreochromis aureus* x *Oreochromis niloticus* hybrids, *Oreochromis niloticus* and *Sarotherodon galilaeus*), respectively. The 18S rRNA sequence is only known for three of these 11 species. We here investigated the diversity of myxozoans present in the Sea of Galilee (Lake Kinneret), the largest freshwater body in Israel, using both molecular and morphological approaches. For the past two years, a total of 338 fishes from 11 species were examined for the presence of myxozoan parasites. The prevalence of infection was 21.59% (73/338). Parasites were found in one tilapia species (*Oreochromis aureus*), and three barb species (*Capoeta damascina, Carasobarbus canis,* and *Luciobarbus longiceps*), which are native Levantine species. Parasites were also found in two introduced species: the common carp (*Cyprinus carpio*) and the thinlip mullet (*Chelon ramada*). In total, 11 myxozoan species belonging to two genera i.e., *Myxobolus* (9 species) and *Myxidium* (2 species) were identified. Ten species show novel 18S rRNA sequences. Phylogenetic analyses indicated that the sequences of these species belong to five different lineages. Surprisingly, all 11 species are new records in Israel. Interestingly, our results indicate that the parasites infecting the thinlip mullet belong to a lineage of myxozoans infecting mugilids. This suggests that these parasites are alien species that were introduced with their fish hosts to the Sea of Galilee. To conclude, our study indicates that myxozoan infections are prevalent in the Sea of Galilee and involve various myxozoan species and hosts. Furthermore, the stocking of alien fish co-introduced myxozoan species, the long-term effect of such introductions remains to be determined.

Development of Alternative, Ecologically Safe, Effective, and Well-tolerated Control Strategies Against *Ichthyophthirius multifiliis*

Verena Jung-Schroers[1], Dennis Kallert[2], Felix Teitge[1], Marcus Zielasko[3], Christina Loy[2], Gregor Schmidt[3], Helmut Wedekind[3], Dieter Steinhagen[1]

[1]Fish Disease Research Unit, Institute for Parasitology, University of Veterinary Medicine, Hannover, Germany, [2]Kallert & Loy GbR, Adelsdorf, Germany and [3]Bavarian State Research Center for Agriculture, Institute for Fisheries, Starnberg, Germany

Infections of fish with *Ichthyophthirius multifiliis* can lead to severe losses. Currently, no effective drugs for the treatment of this parasite in edible fish are authorized in the EU. Considering animal welfare, this is not justifiable; and additionally, a disease outbreak can lead to considerable economic losses and often endangers the existence of traditional farms. The development of a non-therapeutic-based control strategy can therefore make an important contribution to promote sustainable aquaculture and preserve traditional pond management. Three alternative control strategies for the reduction of *Ichthyophthirius multifiliis* in fish keeping facilities were tested. The number of infectious parasite stages in the water and their distribution should be significantly reduced by nanofiltration and by blocking the transmission of the parasite by methods which inactivate the parasite stages in the water, prevent the host recognition or trap parasite stages. Additionally, new vaccine strategies against the parasite were developed. The results show that the number of parasites can be reduced in small water amounts by nanofiltration. Nevertheless, for larger ponds this method is not suitable. We identified solubilized and matrix-bound natural stimulants that trigger theront host finding behavior with high efficacy. Utilizing these activating compounds gave positive results for an effective transmission breach in both experimental laboratory trials and semi-field challenge setups with juvenile trout. Vaccination of fishes with preparations from *I. multifiliis* resulted in a sufficient immunization. In conclusion, it is possible to reduce infection intensities with *Ichthyophthirius multifiliis* in fish without a therapeutic treatment. In small units, for example in hatcheries, nanofiltration can reduce the number of theronts. In larger tanks matrix-bound stimulants can prevent theront transmission. Vaccination strategies are promising as well and must be evaluated further.

Morphological, Molecular and Histopathological Characterization of *Dermocystidium* sp. in Redspot Darters *Etheostoma artesiae* in Mississippi, United States

Jonah A. Nguyen[1], Justin M. Stilwell[2], Ethan T. Woodyard[2], Celene M. Slifka[1], Divya Rose[1], Thomas G. Rosser[1]

[1]Department of Comparative Biomedical Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762; [2]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762.

The cosmopolitan genus *Dermocystidium* contains organisms on the taxonomic boundaries of both animals and fungi. These mesomycetozoeans infect fish where some species have been documented to cause mortality. The biodiversity, life cycle and pathogenicity of many *Dermocystidium* spp. remains poorly understood. A total of 41 *Etheostoma artesiae* were collected from a small branch of Catalpa Creek in Oktibbeha County, Mississippi, US over multiple sampling events between April 10 and May 8, 2022. Of these, 4 harbored large ovoid cysts of a *Dermocystidium* sp. beneath the skin. Spherical spores containing a prominent refractile body were morphologically examined using fresh and formalin fixed spores released from ruptured cysts. The small subunit ribosomal DNA, 18S, was amplified and sequenced. Comparisons with other mesomycetozoeans supported the morphological identification with the highest sequence identity being with *Dermocystidium anguillae*, a species which has not been reported in the United States to the best of our knowledge. While 18S is the standard marker for molecular characterization of *Dermocystidium*, additional molecular markers will be attempted to be sequenced in order to potentially bring more clarity to the taxonomy and identification of the *Dermocystidium* sp. in redspot darters from this study. Microscopically, round to oblong, hypnospore-filled cysts formed space occupying masses within the epidermis, dermal and hypodermal stroma, skeletal muscle, and retroperitoneal and peritoneal cavities. While intact cysts elicited minimal to no inflammation, ruptured cysts elicited mild to moderate, localized granulomatous inflammation and necrosis. Spores were round with a brightly eosinophilic refractile body and thin, clear cytoplasm. This account represents a new geographic locality and host record for a *Dermocystidium* sp. Further study of this obscure group of aquatic parasites is critical to understand its impact on fish. The morphological and molecular data found herein along with histopathological observations aim to bring clarity to the pathological effect of this pathogen and identify additional molecular markers useful in for phylogenetic assessment of this genus.

The Effect of Temperature, Host, and Parasite Traits on Parasite-Induced Mortality in Fisheries: A Meta-Analysis

Megan M. Tomamichel[1,2], Kaitlyn I. Lowe[3], Richard J. Hall[1,2,4], Craig W. Osenberg[1], James E. Byers[1,2]

[1]Odum School of Ecology, University of Georgia, Athens, Georgia, 30602, USA; [2]Center for the Ecology of Infectious Diseases, University of Georgia, Athens, Georgia, 30602, USA; [3]Franklin College of Arts and Sciences, University of Georgia, Athens, Georgia, 30602, USA; [4]Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, Georgia, 30602, USA

Since climate warming could drastically impact host survival and parasitism, a growing body of research has addressed how increasing temperatures will affect fisheries host-parasite relationships both theoretically and empirically. However, to date we lack a computational analysis of how warming will affect parasite induced mortality across diverse host and parasite taxa, and across host ecological traits. We conducted a meta-analysis of 43 laboratory studies that manipulated temperature to quantify mortality of both infected and uninfected hosts in controlled experiments. We then estimate the slope of the relationship between temperature and the ratio of infected to uninfected host mortality risk, and statistically evaluate which host and parasite traits alter the sign and magnitude of this slope value. The papers included in our analysis represent 38 unique species from 28 families. Species from *Salmonidae* and *Ostreidae* are most prevalent, comprising a combined total of 34% of our studies. Studies are most often performed on juvenile hosts (45%) and use viral (32%) or bacterial (53%) microparasites. The temperature range represented in our meta-analysis is from 4° to 34° C. We found that infected hosts experienced significantly higher mortality than their uninfected counterparts. Preliminary results indicate a small, positive effect of temperature, with rising temperatures increasing the risk of infected host mortality. Host family strongly correlates with mortality risk, with species from the *Arcidae, Cyprinidae, Oplegnathidae,* and *Soleidae* families having a steeper increase in risk of parasite-induced mortality with temperature, while species from *Paralichthyidae* and *Sinipercidae* did not experience an increase in parasite-induced mortality risk with increasing temperature. We also find that viruses were less likely to cause increased mortality risk in their hosts with rising temperatures compared to bacteria, myxozoas or protists. Our results suggest that while rising temperatures are likely to increase the risk of parasite induced mortality in most harvested species, the magnitude and direction of this relationship varies substantially with both host and parasite traits.

Report on the Occurrence of a New Putative *Henneguya* Species, a Gill Parasite of the *Astyanax lacustris*, Based on Morphological and Molecular Evidence

Diego H. M. D. Vieira, Rodrigo B. Narciso, Reinaldo J. Silva

Division of Parasitology, Institute of Biosciences, São Paulo State University – UNESP, Botucatu, São Paulo, Brazil.

Myxozoans are mandatory parasitic metazoans with potential to cause economic impacts on some species. There is still much to be explored on the life cycle, the great diversity of species and evolution, and the parasite-host interaction in this group. In this study, *Henneguya* sp. parasitizing the gills of *Astyanax lacustris*, a characiform fish, was reported based on the myxospore morphology, histology, and ssrDNA. Fifty specimens of *A. lacustris* were collected from June 2020 to December 2021 in the Pardo River, Brazil, using nets for the collection of hosts. All internal organs of the fish were analyzed. Some plasmodia found were collected and freshly examined between slide and coverslip, photographed, and measured, while other plasmodia were separated for further processing for histological and molecular analysis. Phylogenetic analysis was used to compare the new *Henneguya* species with genetically similar species. Round and whitish plasmodia, measuring about 0.01 mm, were observed in the secondary filaments of the gill lamellae. The prevalence was 20%. Mature myxospores were oval and elongated in frontal view and biconvex in sutural view measuring: 10.3 ± 0.7 (9.1 – 11.1) µm in body length, 9.7 ± 1.3 (8.5 – 11.6) µm in tail length, 19.5 ± 1.4 (17.8 – 21.4) µm in total spore body length, 4.3 ± 0.2 (4.0 – 4.7) µm in the width of the spore body, 3.7 ± 0.6 (3.1 – 4.2) µm in thickness. Two equally sized, pyriform polar capsules at the anterior pole of the myxospore, occupying 1/3 of the body, measuring 4.1 ± 0.6 (3.2 – 4.8) µm in length and 1.5 ± 0.2 (1.2 – 1.6) µm wide with 5-6 turns of polar tubules. The histological analysis showed that the plasmodia are interlamellar and small deformations in the gill lamellae caused by the infection. Two partial sequences of the ssrDNA gene from *Henneguya* sp. of 1594-pb and 1536-bp were obtained. When aligned, the partial sequences were identical. The research carried out in Blastn with partial sequences of species deposited in GenBank, showed that none of the available sequences is identical to the partial sequences obtained in this study. The species that most resembled *Henneguya* sp. was *Henneguya lacustris* with 93.5% similarity. Phylogenetic analysis showed that *Henneguya* sp. groups in a clade formed by species that parasitize Characiformes from Brazil. Our analysis was consistent with previous studies suggesting that orders and families of the hosts are strongly correlated with phylogenetic signals in the Myxobolidae. These data supported the diagnosis of the parasites as distinct and novel species. Using molecular and morphological characterization, this species was identified as a putative new species of the genus *Henneguya*. The present study contributes to the knowledge of myxozoan biodiversity in Brazil.
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The Use of Histopathology and Laser Capture Microdissection (LCM) for Myxozoan Identification from Multiple Fishes in the Eastern United States

Heather L. Walsh[1] and Vicki S. Blazer[1]

[1]U.S. Geological Survey Eastern Ecological Science Center, Leetown Research Laboratory, 11649 Leetown Rd., Kearneysville, West Virginia 25430

To date, over 2,100 species of myxozoan parasites have been described from fishes worldwide; however, many more remain undiscovered and/or undescribed. In recent fish health assessments conducted by the U.S. Geological Survey in parts of the eastern United States, multiple myxozoans were identified with histopathology from warm, freshwater fishes. Following identification, laser capture microdissection (LCM) was used to cut out myxozoans from fish hosts and 18S rDNA gene sequences were subsequently analyzed with Sanger sequencing for species identification. Multiple myxozoans were identified from three different host species with differences in tissue tropism. In banded sunfish *Enneacanthus obesus* sampled from the New Jersey Pine Barrens for a study on wastewater exposure, myxozoan plasmodia were observed in the muscle connective tissue below fins and in the operculum, in and around the heart and eye, and in the gills. In fantail darter *Etheostoma flabellare* sampled from multiple tributaries of the Chesapeake Bay in Virginia and West Virginia for a study on agricultural best management practices, myxozoan plasmodia were observed in the muscle connective tissue below fins and the operculum and in the brain cavity. Lastly, in white sucker *Catostomus commersonii* (collected concurrently with fantail darters), myxozoan plasmodia were observed in the muscle connective tissue, cartilage near the brain, gills, and glomeruli of the posterior kidney. The effects these myxozoa may impose on host health remains unclear; however, other myxozoan species that infect cartilage in/or around the brain can have detrimental implications. These preliminary findings will help to expand the current knowledge of known myxozoa and provide additional information on host specificity and land use associations.

Hepatospora eriocheir is a Usual Microsporidium of *Eriocheir sinensis*, but not Associated with the Recent Nationwide Epidemic of Hepatopancreatic Necrosis Disease of Farmed *E. sinensis* in China

Xintong Zhang[1], Meiqi Weng[2,3], Qianqian Zhang[2], Aihua Li[2,3], Jinyong Zhang[1,2,4]

[1]The Laboratory of Aquatic Parasitology and Bioresources, School of Marine Science and Engineering, Qingdao Agricultural University, 266237 Qingdao, China; [2]Key Laboratory of Aquaculture Diseases Control, Ministry of Agriculture and State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China; [3]The University of Chinese Academy of Sciences, Beijing 10049, China; [4]Laboratory for Marine Biology and Biotechnology, Pilot National Laboratory for Marine Science and Technology (Qingdao), Qingdao, Shandong Province, China;

Hepatopancreatic necrosis disease or syndrome (HPND or HPNS) is a recent emerging disease of the cultured Chinese mitten crab, *Eriocheir sinensis* in China. The underlying factors to induce HPND, however, remains controversial. Although *H. eriocheir* was suggested to be the causative agent of HPND, several subsequent researches suspected this viewpoint. It is well-known that the pathogen load is positively related with the disease occurrence. Here, we developed a new quantitative PCR method with 98.3% amplification efficiency to monitor the *H. eriocheir* infection for the low amplification efficiency (79.1%) of the previously reported QPCR by Ding et al. (2017). Then, we applied this newly developed method to perform an in-depth molecular epidemiological investigation of the *H. eriocheir* infection in Chinese mitten crab of all geographical populations in China and of all developmental stages in the epizootic region of HPND in Jiangsu province to infer the correlation of HPND and the *H. eriocheir* infection. Our results clearly indicated: 1) the infection of *H. eriocheir* in Chinese mitten crab of cultured and wild populations was widely distributed; 2) the infection of *H. eriocheir* firstly occurred in juvenile stages and no infection is found in eggs and larval stages of Chinese mitten crab; 3) the infection of *H. eriocheir* was not directly associated with HPND for high percentage of individuals with HPND was negative for the infection of *H. eriocheir*, on the contrary the high *H. eriocheir* load was found in individuals without HPND. Furthermore, heathy crabs which were fed or infected with *H. eriocheir*-infected tissues showed no symptoms of HPND. Based on the literature review and present results, we support the viewpoint that HPND is not an etiological disease, although other possible pathogenic microorganisms should be further excluded.

An Abbreviated the Recent 20 Year History of Myxosporean and Myxosporidiosis Research

Jinyong Zhang[1,2], Xinhua Liu[3]

[1]The Laboratory of Aquatic Parasitology and Bioresources, School of Marine Science and Engineering, Qingdao Agricultural University, 266237 Qingdao, China; [2]Laboratory for Marine Biology and Biotechnology, Pilot National Laboratory for Marine Science and Technology (Qingdao), Qingdao, Shandong Province, China; [3]Laboratory of Hydrobiology, Hunan Agricultural University, Changsha 410128

Since the publication the monograph of Fauna Sinica, Myxozoa: Myxosporea by Chen & Ma (1998), some progress has been achieved in this field by researches of newly generation in China. Firstly, the integrative taxonomic approach has been widely employed to the identification of novel species, validation of cryptic species and delimitation of morphologically similar species. About 100 species new to science have been reported with robust taxonomic criteria during the past above 20 years. The geographical distribution of myxosporean was dramatically extended, especially from freshwater habitat to marine, however, most of concerns were given on cultured fish species and few were putted on wild fish, amphibian, bird and mammals. Additionally, malacosporean has not yet been discovered in China. The phylogenetic relationships of some myxosporean taxa is increasingly reasonable, e.g. Sphaerosporidae, Chloromyxidae and Myxobolidae with more data available. The study on life cycle of myxosporeans in China has achieved much. About 20 novel actinospores have been identified and some of them were identified to be the developmental stage in invertebrates host. Especially, the vertical transmission has been proven for *Myxobolus honghuensis* which possibly represent a novel radiation way for myxosporean. Further, genome, transcriptome and proteome of more myxobolids in China have been sequenced and annotated which greatly promote the molecular mechanisms underlying the adaptive evolution, transmission and host-myxosporean interaction. Also, a comprehensive prophylaxis and control approach of combing SPF fingerling production, chemical agents' application and culture management for fish myxosporidiosis has been suggested based on the knowledge of life cycle, pathobiology and ecological roles of pathogenic myxosporean. Although the above significant progress achieved, we should acknowledge that big gap on knowledge of myxosporean biology is still there. My three suggestion are: 1) deep usage of the obtained omic data to uncover the function of virulence, invasion and interaction-related genes; 2) wide sampling of species locating the key nodes of phylogenetic trees to conduct wide phylogenomic analysis to draw clear and reasonable evolutionary trajectory of myxosporea; 3)the development of in vitro culture system for myxosporean. Hope sincerely that much novel and exciting achievement on myxosporean biology in China can be obtained with the communication and aid of international counterparts.

Genetic Diversity of the Fish-infecting Microsporidian Parasite *Pseudokabatana alburnus* (Microsporidia) Provides New Insights into the Tissue Tropism

Meiqi Weng[1,2,3], Xintong Zhang[1], Qianqian Zhang[2], Aihua Li[2,3], Jinyong Zhang[1,2,4]

[1]The Laboratory of Aquatic Parasitology and Bioresources, School of Marine Science and Engineering, Qingdao Agricultural University, 266237 Qingdao, China; [2]Key Laboratory of Aquaculture Diseases Control, Ministry of Agriculture and State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China; [3]The University of Chinese Academy of Sciences, Beijing 10049, China; [4]Laboratory for Marine Biology and Biotechnology, Pilot National Laboratory for Marine Science and Technology (Qingdao), Qingdao, Shandong Province, China;

Pseudokabatana alburnus is a xenoma-forming fish microsporidium, firstly described from the liver of the *Culter alburnus* from Poyang Lake in China. In the present study, the infection sites of *P. alburnus* extends to the ovary, with first record from various Xenocypriid fish (Cyprinidformes), including *Squaliobarbus curriculus*, *Hemiculter leucisclus*, *Cultrichthys erythropterus*, *Pseudolaubuca engraulis* and *Toxabramis swinhonis*. High genetic diversity was observed among *P. alburnus* isolates of different hosts and geographical locations by comparing their ITS and Rpb1 sequences. The Rpb1 variation loci mainly occurred in the 1400-1800 bp region. The presence of a wide variety of Rpb1 sequence variation within an isolate from an individual fish host suggested that the genome of *P. alburnus* possibly cover multiple Rpb1 copies. High sequence variation in ITS sequence suggest that ITS can be a suitable molecular marker for conducting the population genetic analysis of *P. alburnus*. Our data confirm the broad geographical distribution and host range of *P. alburnus*. And, the liver should be removed from the taxonomic criterion of the genus *Pseudokabatana* and the ovary may be the common infection site for *P. alburnus*.

Mass Mortality of Pond-cultured *Scylla serrata* (Decapoda: Portunidae) Associated with *Ameson portunus* (Microsporidia) in China

Xintong Zhang[1], Meiqi Weng[2,3], Zhaozhe Xin[1], Jinyong Zhang[1,2,4]

[1]The Laboratory of Aquatic Parasitology and Bioresources, School of Marine Science and Engineering, Qingdao Agricultural University, 266237 Qingdao, China; [2]Key Laboratory of Aquaculture Diseases Control, Ministry of Agriculture and State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China; [3]The University of Chinese Academy of Sciences, Beijing 10049, China; [4]Laboratory for Marine Biology and Biotechnology, Pilot National Laboratory for Marine Science and Technology (Qingdao), Qingdao, Shandong Province, China

Ameson portunus is a recently reported muscle-infecting microsporidium responsible the notorious toothpaste disease of the farmed *Portunus trituberculatus* in China. Here, we firstly identified that *A. portunus* is also the aetiological agent of a newly occurred disease of the pond-farmed giant mud crab, *Scylla serrata*, designated as slurry crab syndrome by farmers, in South China based on morphological, ultrastructural and molecular data. The infection prevalence and mortality rate can be up to 100% and 60%, respectively. The gross appearance of infected *S. serrata* include yellow gills, atrophied hepatopancreas, fuzzy internal organs, opaque articular membrane of swimming legs and distinctly white turbid abdominal and leg muscle. Histopathological analysis clearly showed the most muscle fibers were replaced by the variable developmental stages, predominately mature spores. The infection of *A. portunus* significantly reduced the condition factor of *S. serrata*. Three polymorphic sites were found among the rRNA gene of *P. trituberculatus* isolate and *S. serrata* isolate of *A. portunus*. This is the first report of *A. portunus* in *S. serrata* where the infection represents some different clinical symptoms from those in *P. trituberculatus*.

Virology (Virtual)

Adamek	Long Persistence of Carp Edema Virus Contributes to an Increased Potential for the Virus to Spread – A Case Study on Fish Population Affected by Koi Sleepy Disease
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Long Persistence of Carp Edema Virus Contributes to an Increased Potential for the Virus to Spread – A Case Study on a Fish Population Affected by Koi Sleepy Disease

Mikolaj Adamek[1], Felix Teitge Anne-Carina Miebach[1], Dieter Steinhagen[1]

[1]Fish Disease Research Unit, Institute for Parasitology, University Of Veterinary Medicine Hannover, Hannover, Germany

Poxviruses are known for persistence in the environment and their ability to reinfect their hosts at a suitable time point. Carp edema virus (CEV) is a very successful fish poxvirus and often causes very severe disease with high mortality in carp populations. Its worldwide distribution is driven by international trade with koi and common carp. In Germany, the virus was repeatedly found in fish shipments in high abundance with completely asymptomatic fish. Furthermore, some fish farmers and ornamental fish keepers experienced repeated outbreaks of the disease without introduction of new fish. This raised the question if the virus could persist for longer time in a population of fish after an outbreak. An ornamental fish population consisting of 23 koi was followed up after an outbreak of koi sleepy disease (KSD). Individual fish were recognized based on their colour pattern and their virus load was measured by qPCR in gill swabs collected during routine health check-ups. The fish population initially experienced mild outbreak of KSD in May/June with the fish showing lethargic behaviour characteristic for this disease. All 23 fish were confirmed to be positive for CEV with a virus load from 5 to 1600 virus copies. Immediately after diagnosis, a 12-day long salt bath (0.5%) was administered, which led the clinical signs to subside and only one fish died. However, after this treatment the fish were free from clinical signs, but 14 from 22 fish remained positive for CEV with a virus load from 3 to 160 copies. During a routine autumn health check in October 3 from 11 fish remained CEV positive with, a virus load from 12 to 251 copies, without clinical signs. A further check-up performed one year after the outbreak showed that all fish were negative for CEV. Thanks to the ability to follow a small population of fish, it was shown that CEV seems to be persisting in fish for at least 5 months with some individuals being long haulers of the virus infection. That long persistence of the virus could explain the successful spread of the virus and should be an imperative for checking fish before purchase as the virus seems to be difficult to get cleared from a susceptible population.

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Resistant to Everything? A Comprehensive Approach to the Development of Common Carp Crosses Resistant to the Diseases Caused by Infections with CyHV-3, CEV and SVCV.

Mikolaj Adamek[1], Marek Matras[2], Krzysztof Rakus[3], Verena Jung-Schroers[1], Veronika Piackova[4], D David Gela[4], Martin Kocour[4], Jerzy Adamek[5], Rafal Kaminski[6], Michal Reichert[2], Dieter Steinhagen[1]

[1]Fish Disease Research Unit, Institute for Parasitology, University Of Veterinary Medicine Hannover, Hannover, Germany; [2]Laboratory of Fish Diseases, National Veterinary Research Institute, Pulawy, Poland; [3]Department of Evolutionary Immunology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Krakow, Poland; [4]South Bohemian Research Centre of Aquaculture and Biodiversity of Hydrocenoses, Faculty of Fisheries and Protection of Waters, University of South Bohemia in Ceske Budejovice, Vodnany, Czech Republic; [5]The Stanislaw Sakowicz Inland Fisheries Institute in Olsztyn, Experimental Farm in Zator, Zator Poland; [6]The Stanislaw Sakowicz Inland Fisheries Institute in Olsztyn, Experimental Farm in Żabieniec, Piaseczno, Poland

Common carp (*Cyprinus carpio*) aquaculture is affected by several viral diseases caused by cyprinid herpesvirus 3 (CyHV-3), carp edema virus (CEV) and spring viremia of carp virus (SVCV) infections. Especially the koi herpesvirus disease (KHVD) caused by CyHV-3 and koi sleepy disease (KSD) caused by CEV can lead to extremely high losses of common carp at all age stages. However, common carp strains with a different genetic background present diverse susceptibility to some pathogens and this fact could be utilised for developing a strain with a broad resistance to virus infections. Here we present results from a first comprehensive evaluation of viral disease resistance of common carp strains and initial steps of the development of crosses with increased resistance to multiple viral diseases. Common carp strains Amur wild carp (AS), Ropsha scale carp (Rop), Prerov scale carp (PS) and koi carp (Koi) were tested for resistance to CyHV-3, CEV and SVCV using bath or cohabitation infection models. Two carp strains (Rop and AS) presented the highest resistance after challenge experiments were selected to crossing with Zator strain (Zat), which has a very good productive parameters. The three new Rop×Zat, AS×Zat and Rop×Zat×AS crosses were tested for resistance to CyHV-3, SVCV, and CEV. Mortality and viral load in gills were measured. Furthermore the survival of F2 generation specimens was measured during rearing in experimental ponds. The new crosses showed a higher survival during infections with CEV and CyHV-3, and showed no mortality during SVCV infection. The higher resistance was reflected by significantly lower virus loads in gills. The crosses showed higher survival in experimental ponds in the first year of production, indicating that the challenges caused by viral diseases in common carp can be addressed by selective breeding.

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Proteomics Give New Insight into Pathology and Immunosuppression Observed During Carp Edema Virus-Induced Gill Disease

Mikolaj Adamek[1], Mariola Dietrich[2], Krzysztof Rakus[3], Maria Zawisza[3], Felix Teitge[1], Natalia Kodzik[2], Verena Jung-Schroers[1], Veronika Piackova[4], David Gela[4], Martin Kocour[4], Magdalena Chadzinska[4], Alberto Falco[5], Andrzej Ciereszko[5], Dieter Steinhagen[1]

[1]Fish Disease Research Unit, Institute for Parasitology, University of Veterinary Medicine Hannover, Hannover, Germany; [2]Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Olsztyn, Poland; [3]Department of Evolutionary Immunology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Krakow, Poland; South Bohemian Research Centre of Aquaculture and Biodiversity of Hydrocenoses, Faculty of Fisheries and Protection of Waters, University of South Bohemia in Ceske Budejovice, Vodnany, Czech Republic; [5]Institute of Research, Development, and Innovation in Healthcare Biotechnology in Elche (IDiBE), Miguel Hernández University (UMH), 03202 Elche, Spain,

Koi sleepy disease (KSD), caused by the gill infecting poxvirus carp edema virus (CEV), is a unique model to study a branchial disease in fish. Gill disfunction caused by CEV resulted in impaired respiration, osmoregulation and nitrogenous waste excretion. Affected common carp (*Cyprinus carpio*) individuals are lethargic, lying at the bottom of the tank and with progressing disease, the activity of the fish decreases until almost complete stillness, followed by death. In the present study, we performed 2D-DIGE based proteomics of gills, followed by Ingenuity Pathway Analysis (IPA) based bioinformatics to evaluate biological pathways affected by the disease. The proteome results were supplemented by measuring mRNA expression of the genes encoding all changed proteins. CEV-infected fish developed classical clinical signs of infection related to gill dysfunction, resulting in ion dysregulation: decrease of sodium level under 90 mmol L^{-1} and an accumulation of ammonia to over 600 µmol L^{-1}. The proteomic analysis indicated that the abundance of 86 proteins was significantly changed in gills during the onset of a severe KSD. The IPA analysis indicated changes in following regulatory pathways: post-translational modifications, cellular assembly and organization and protein synthesis, which were most probably are related to the pathology of the proliferative gill disease. The pathological changes were reinforced by an activation of regulatory networks responsible for the response to cellular compromise, infectious diseases, inflammatory response and connective tissues disorders. The results linked also KSD with the alteration of metabolic pathways like small molecule biochemistry, nucleic acid metabolism and carbohydrate metabolism. Furthermore, CEV-infection affected: regulatory networks for drug metabolism and glutathione depletion as well as activation of the xenobiotic metabolism CAR signaling pathway. The changes in these networks indicate a response to ammonia intoxication and point to the effort of gill cells to handle and eliminate this toxic metabolic by-product. Moreover, observed in infected animals, down-regulation of the antimicrobial peptide NK-lysin-like suggests a lower activity of NK cells. Together with an activation of transforming growth factor beta 1 (TGFB1) this points to infection-induced immunosupression. In turn, down-regulation of calpain and caspase indicated a pro-apoptotic effect of the infection. Increased inflammation was mediated by a down-regulation of anti-inflammatory proteins like gelsolin (GSN), annexin A1 (ANXA1) and scinderin (SCIN). Increased concentrations of several heat shock proteins and activation of the upstream heat shock factor protein 2 (HSP2) could indicate elevated stress in infected gills. All these changes are accompanied by the increased expression of viral genes responsible for immunosupression like multiple paralogues of B22R and the viral HSP70. Taken together, the results allowed to establish a closer link beween pathology and immunosuppression occuring during the gill disease caused by carp edema virus. This research was supported by Deutsche Forschungsgemeinschaft (DFG project number 426513195) and by the National Science Centre of Poland (NCN project number UMO-2018/31/F/NZ6/02311).

NK-Lysin Inhibits the Replication of Several Fish Viruses From Different Families With Low pH-Mediated Entry

Mikolaj Adamek[1], Patricia Pereiro[2], Beatriz Novoa[2], José Antonio Encinar[3], Win Surachetpong[4], Alberto Falco[3]

[1]Fish Disease Research Unit, Institute for Parasitology, University of Veterinary Medicine Hannover, Hannover, Germany; [2]Institute of Marine Research (IIM-CSIC), Vigo, Spain; [3]Institute of Research, Development, and Innovation in Healthcare Biotechnology in Elche at the Miguel Hernández University (IDiBE-UMH), Elche, Spain; [4]Department of Veterinary Microbiology and Immunology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand

Antimicrobial peptides (AMPs) show extraordinary diversity in sequence and structure and, hence, in mode of action. Among them, NK-lysins are attracting special attention because of their broad-spectrum antimicrobial, but also antitumor, activity. NK-lysin orthologs have been identified in all vertebrate groups. The tertiary structure of these peptides shows helical regions stabilized by disulfide bridges. It also exhibits a face with abundant lysine residues, responsible for its interaction with anionic lipid membranes. In our previous reports, the peptide corresponding to such region in the turbot (*Scophthalmus maximus*) ortholog (Nkl_{71-100}) showed membrane-disrupting activity against the parasite *Philasterides dicentrarchi*, as well as inhibition of the spring viremia of carp virus (SVCV) infection *in vitro,* via the hampering of the pH-dependent fusion step of its entry phase. In this sense, biophysical data have shown that the Nkl_{71-100} peptide interacts preferentially with anionic phosphatidylserine lipid bilayers at acidic pH. The present study aims to investigate whether such effect is extensible to other fish viruses with the same entry strategy. For this task, the susceptibility of a diverse battery of fish viruses to Nkl_{71-100} has been determined *in vitro*. In general, the results obtained corroborate the proposed hypothesis to explain the mode of action of the peptide. In addition, SVCV and tilapia lake virus (TiLV) particularly showed high susceptibility to this treatment, while the effect on cyprinid herpesvirus 3 (CyHV-3) was only present in higher concentration and common carp paramyxovirus (CCPV) entry was not affected. In conclusion, apart from the selective antiviral activity of NK-lysin, the fusion step of the viral entry phase is postulated as promising target for the treatment of some viruses with high economic impact on aquaculture.

What is the role of virulence in the evolution of endemic pathogens?

Rachel B. Breyta[1,2], Douglas G. McKenney[1], Gael Kurath[1]

[1]USGS Western Fisheries Research Center, Seattle, WA 98115; [2]School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA98195

The fish rhabdovirus infectious hematopoietic necrosis virus (IHNV) is endemic in the native freshwater range of its host species, Pacific salmonid fishes. One feature of IHNV evolution in the past is that the adaptive process of moving into a new host has been associated with increased virulence in the new host. However, within a single host species discrete genetic variants have also demonstrated increased virulence over evolutionary time. Decades of genetic typing surveillance of IHNV in Columbia River basin of North America in *Oncorhynchus mykiss* (*O. mykiss*, steelhead and rainbow trout) has revealed four different genetic variants that emerged between 1980-2003. All four emerged within a small section of the Columbia River basin that has the highest concentration of steelhead trout culture facilities. The first three variants each grew in prevalence until it was the dominant type during a discrete time period, and always in the same region. Each variant was then subsequently displaced by another variant that was able to become dominant in its turn. The fourth variant did not follow this pattern, it emerged but did not displace the previous variant nor did it become dominant. Instead it translocated to a different, upstream region where it did become dominant. In controlled laboratory studies measurable viral phenotypes like infectivity or competitive fitness did not correlate with observed displacement patterns, but virulence did. For all three viruses that did displace the previous variant, the later emergent virus was more virulent (caused higher mortality). Additional studies showed that the pattern of increasing virulence with later evolutionary emergence was not limited to individual virus isolates but was a reproducible trait of a viral variant. These results and evolutionary inference of the viral genomes will be presented, reminding us of the value for understanding human viral evolution that can be gained from careful surveillance of aquatic animal viruses.

A New Sturgeon Herpesvirus Associated with Epithelial Skin Lesions in Juvenile Lake Sturgeon *Acipenser fulvescens* From Manitoba, Canada

Sharon C. Clouthier[1], K. Marek Tomczyk[2], Tamara L. Schroeder[1], Cheryl N. Klassen[3] and Eric D. Anderson[1]

[1]Fisheries & Oceans Canada, Freshwater Institute, 501 University Crescent, Winnipeg, MB R3T 2N6, Canada; [2]Manitoba, Agriculture & Resource Development Veterinary Diagnostic Services, 545 University Crescent, Winnipeg, MB R3T 5S6, Canada; [3]Manitoba Hydro, 360 Portage Ave, Winnipeg, MB R3C 0G8

A new virus, sturgeon herpesvirus 3 (AciHV3), was detected in juvenile Lake Sturgeon *Acipenser fulvescens* propagated by a conservation stocking program for this endangered species in Manitoba, Canada. Mortality was not observed but epithelial skin lesions that resembled blisters were detected on the ventral surface of pectoral fins and the abdomen among hatchery-reared progeny of wild Lake Sturgeon. Lesions, initially found between the months of August and October, reached prevalence levels of 0.2 to 35% and then eventually regressed. Cellular changes were characterized by epidermal hyperplasia or focal thickening of the epithelial layer due to cells growing on top of each other. Although tissue samples from sturgeon displaying lesions produced limited cytopathic effect on established white sturgeon cell lines, notable cytoplasmic vacuoles were observed in a primary Lake Sturgeon gonad cell line inoculated with the same samples. A 144 kbp contiguous dataset of AciHV3 DNA sequence from Lake Sturgeon tissue contained twelve core genes conserved across members of the *Alloherpesviridae* family. Bayesian inference of phylogeny reconstructed with the major capsid protein sequence revealed that AciHV3 formed a new evolutionary lineage within the family. Investigation into the ecology of the virus using a new qPCR test provided evidence that the virus is endemic in wild Lake Sturgeon (n=1,167) in the Hudson Bay drainage basin. The 100% prevalence and titer of $10^{7.5}$ equivalent plasmid copies mg^{-1} tissue suggest that the virus has established a persistent, latent type of infection that involves integration of AciHV3 DNA into the Lake Sturgeon chromosome.

Susceptibility of Native Amphibians from the U.S. Pacific Northwest to Spring Viremia of Carp Virus

Eveline Emmenegger[1], Emma Bueren[1,2], Tamara Schroeder[3], Sharon Clouthier[3], Sanders, GE[4], and Conway, CM[1]

[1]USGS, Western Fisheries Research Center, Seattle, Washington, USA; [2]Department of Biological Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA; [3]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Manitoba, Canada; [4]Department of Comparative Medicine, University of Washington, Seattle, WA, USA

Spring viremia of carp virus (SVCV) is a rhabdovirus that infects primarily cyprinid finfish with common carp *Cyprinus carpio carpio* considered to be the most susceptible species. The disease is notifiable to the World Organization for Animal Health which lists fish (and possibly shrimp) as SVCV susceptible host species. In 2015, distressed ornamental Chinese firebelly newts *Cynops orientalis* imported into the U.S. were suspected to be infected with chytrid fungus *Batrachochytrium salamandrivorans*. The newts tested negative for the fungal agent, but SVCV was detected and appeared to be responsible for the observed morbidity. This discovery represented the first isolation of a rhabdovirus in an amphibian species. To better understand the host range of this virus, susceptibility testing of amphibians native to the Pacific Northwest was initiated. Pacific tree frog *Pseudacris regilla* tadpoles and larval long-toed salamander *Ambystoma macrodactylum* were exposed to the virus by either intra-peritoneal injection, immersion, or co-habitation with SVCV-infected fish. Cumulative mortality in virus-exposed amphibians ranged from 0 – 100% and many animals that died exhibited clinical signs of disease. SVCV was detected by plaque assay and RT-qPCR assay in both amphibian species regardless of the virus exposure/transmission method. Convalescent amphibians contained measurable levels of viable virus at targeted sampling time-points including 28 days following exposure. These results suggest that SVCV can be transmitted and cause disease culminating in mortality in amphibian species. As such, amphibians may serve as virus carriers in an ecosystem and pose a risk for sympatric fish and amphibian populations vulnerable to SVCV.

Factors Influencing the Pathogenesis of White Spot Syndrome Virus (WSSV) in Louisiana Red Swamp Crayfish *Procambarus clarkia*

John P. Hawke[1], Meredith Paisant[2], Justice Lopez Merrifield[2], Ramesh Subramanian[3], Stephen Midway[4], and Christopher Green[2]

[1]Louisiana State University School of Veterinary Medicine, Department of Pathobiological Sciences, Baton Rouge, Louisiana 70803; [2]Louisiana State University Agricultural Center, Aquaculture Research Station, Baton Rouge, Louisiana 70820; [3]Louisiana State University School of Veterinary Medicine, Division of Biotechnology & Molecular Medicine and Department of Pathobiological Sciences, Baton Rouge, Louisiana 70803; [4]Louisiana State University, Department of Oceanography and Coastal Sciences, Baton Rouge, LA 70803

Crayfish farming is the most economically valuable sector of the Louisiana Aquaculture Industry and relies on the productive yearly harvest of red swamp crayfish, *Procambarus clarkii*. The presence of the highly pathogenic white spot syndrome virus (WSSV) since 2007 in Louisiana threatens the success of the industry due to sporadic outbreaks that cause significant mortality in aquaculture ponds. In the current study, we utilized an intramuscular (IM) infection model to deliver specific concentrations of WSSV particles per gram body weight and record cumulative mortality over an eight-day period. The influence of temperature, crayfish size, and total ammonia nitrogen (TAN) on time to lethargy, time from lethargy to mortality, time to mortality, and cumulative mortality of crayfish from IM inoculation of WSSV particles was examined. Larger crayfish were found to have a delayed mortality compared to small and medium crayfish, and large crayfish were found to have a longer period between lethargy and mortality than small crayfish. Increased time to mortality was observed for crayfish held at 20°C when compared to 26°C and 32°C groups. Two-week exposures of TAN of concentrations up to 1.5 mg/L were not observed to impact cumulative mortality or timing of lethargy, morbidity, and mortality.

Characterization of a Novel Acipenserid Herpesvirus (Family *Alloherpesviridae*) Recently Recovered from Great Lakes Lake Sturgeon (*Acipenser fulvescens*)

Amber E. Johnston[1,2], Megan Shavalier[1,2], Kim Scribner[2], Esteban Soto[3], Matt Griffin[4], Geoffrey Waldbieser[5], Andrew Winters[6], Susan Yun[3], Zeinab Yazdi[3], Thomas Loch[1,2,7]

[1]Aquatic Animal Health Lab, Aquatic Animal Disease Ecology Program, Michigan State University, 1129 Farm Lane Rm 340G, East Lansing MI, 48824; [2]Department of Fisheries and Wildlife, College of Agriculture and Natural Resources, Michigan State University; [3]Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California-Davis; [4]College of Veterinary Medicine, Mississippi State University; [5]United States Department of Agriculture - Agricultural Research Service, Warmwater Aquaculture Research Unit; [6]Wayne State University School of Medicine; [7]Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University

The lake sturgeon (*Acipenser fulvescens*, LST) is the only native sturgeon species in the Great Lakes (GL), where it is the largest and longest living fish therein. However, multiple factors have contributed to substantial declines in abundance and geographic distributions of GL-LST populations. Recently, we detected an Acipenserid Herpesvirus (AcHV; Family *Alloherpesviridae*) from GL-LST collected from the St. Clair and Black Rivers (Lake Huron and Erie watersheds), and, for the first time, isolated the virus from the latter. Though originally recovered from wild adult LST with skin lesions, the capacity of this virus to cause disease/mortality in juvenile LST is unknown, leaving managers questioning its potential impacts on GL-LST conservation efforts. To address this unknown and guided by pilot experiments, juvenile LST (10 weeks post-hatch) were either exposed or mock-exposed to the GL-AcHV via immersion and subsequently monitored for disease signs and mortality for 112 days under controlled laboratory conditions. Less than two weeks post-infection (PI), gross signs of disease, including lethargy, perioral hemorrhaging, and ulceration of the caudal fin, developed in AcHV-exposed LST. Thereafter, mortality began 19 days PI and continued until 76 days PI, reaching a mean cumulative percent mortality of 33.3% (vs. 0% in mock-exposed fish). Histopathological and virological analyses of exposed and mock-exposed fish are ongoing, though the virus has been re-isolated from exposed and diseased LST. In addition to virulence experiments, genomic analyses of the newly-isolated AcHV using Nanopore technology supports our previously conducted phylogenetic analyses, in that this GL-AcHV is distinct from all previously described AcHVs and likely comprises a novel virus taxon. Now alerted to the potential negative health effects of this newly recognized sturgeon-pathogenic virus, ongoing studies are seeking to arm fisheries managers with practical tools to reduce transmission risk among GL-LST and support ongoing conservation efforts of this iconic fish species.

Within-host Replication Kinetics of Specialist and Generalist IHN Virus Strains in Three Salmonid Host Species

Gael Kurath[1], David J. P'aez[2], Douglas McKenney[1], Maureen K. Purcell[1], Kerry A. Naish[2]

[1]USGS Western Fisheries Research Center, Seattle, WA 98115; [2]School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA98195

The rhabdovirus infectious hematopoietic necrosis virus (IHNV) occurs naturally in several Pacific salmonid fish species in North America. Based on field prevalence and virulence, IHNV viruses in phylogenetic subgroups UP, MD, and L are specialists on sockeye salmon (*Oncorhynchus nerka*), steelhead (*O. mykiss*), and Chinook salmon (*O. tshawytscha*), respectively. The UC subgroup evolved naturally from a UP ancestor and has a generalist host specificity, infecting all three host species without causing severe disease. Here we examine the mechanistic basis of varying host specificity by quantifying within-host replicative fitness from the onset of infection to the beginning of virus clearance for IHNV in specialist, generalist, and non-specialist interactions. We test three IHNV strains from each of four viral subgroups, in each of three fish host species. Viral kinetics over 14 days post-infection in each host revealed that viruses in specialist interactions had higher peak and mean viral loads that non-specialist viruses in the same host, while generalist UC viruses typically had intermediate values. However, in sockeye salmon UC viruses were nearly equivalent with ancestral UP viruses, indicating low or no-cost generalism. Viral kinetics clearly indicated that viral fitness involves both the ability to maximize early virus replication and to avoid clearance at later times, with different mechanisms of specialization evident in different host-virus combinations. Overall, our results support major elements of specialist-generalist theory, quantify evidence of a specialist-generalist continuum in a vertebrate pathogen, and define within-host replicative fitness tradeoffs resulting from the natural evolution of specialist and generalist virus lineages in multi-host ecosystems.

Novel Adomavirus Identified in Proliferative Skin Lesions in a Sand Tiger Shark *Carcharias taurus*

Ashley L. Powell, Alvin C. Camus, John H. Leary, Terry Fei Fan Ng

Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602

A captive sand tiger shark *Carcharias taurus* presented with progressive, localized, raised, skin lesions along the lateral trunk and peduncle in July 2021 that persisted over six months. Histopathologic evaluation of biopsy samples revealed proliferation of epithelial elements within dermal denticles producing malformed tooth-like structures, including dentin production, resembling odontogenic neoplasms in other vertebrates. Deep sequencing of the affected tissue revealed a novel DNA virus related to the adomaviruses. "Adomavirus" is a proposed family of viruses, that has been discovered in fish over the past decade. Structural and replicative genes of this proposed viral family share a complex evolutionary history with small DNA tumor viruses including the papillomaviruses, polyomaviruses, and adenoviruses. The first adomavirus was reported from Japanese eels *Anguilla japonica* with endothelial cell necrosis in 2011, followed by marbled eels *Anguilla marmorata* experiencing an outbreak of hemorrhagic gill disease in 2016. Since then, adomaviruses have been described from proliferative skin lesions in a giant guitarfish *Rhynchobatus djiddensis* and smallmouth bass *Micropterus dolomieu*. Most recently, a novel adomavirus has produced necrohemorrhagic gill disease in American eels *Anguilla rostrata* cultured in China. A growing list of additional adomavirus sequences and genomes have been identified within the whole genome sequence data of multiple fish species. While final genome assembly and annotation was incomplete at the time of abstract submission, protein BLAST analysis of Illumina MiSeq sequence data revealed viral sequences with greatest similarity (<40%) to that of guitarfish adomavirus (GAdoV) and lesser similarities to other piscine adomaviruses. In contrast to the papillomatous skin lesions induced by (GAdoV), nuclear inclusion bodies were not present and viral particles were not observed with transmission electron microscopy (TEM). Lesions in the index animal regressed moderately but persisted for approximately 1 year, and four additional sand tiger sharks in the same enclosure developed similar skin proliferations. Additional sampling of these sharks and an RNAscope in situ hybridization assay is currently in development. To our knowledge, this is the second report of an adomavirus characterized from an elasmobranch and the first report of an adomavirus characterized from a sand tiger shark. Adomavirus infections in elasmobranchs warrant further attention and investigation in confined aquatic systems.

Phylogenomic Characterization of Ranavirus Isolated from Wild Smallmouth Bass (*Micropterus dolomieu*)

Hannah Quail[1,2], Pedro H. O. Viadanna [1,2], Jordan A. Vann [1,2], Scott Hansen [3], Nicole Nietlisbach [3], Danielle Godard [3], Thomas B. Waltzek [1,2,#], Kuttichantran Subramaniam [1,2,]

[1]Department of Infectious Diseases and Immunology, College of Veterinary Medicine, University of Florida, Gainesville, Florida, USA; [2]Emerging Pathogens Institute, University of Florida, Gainesville, Florida, USA; [3]Bureau of Fisheries Management, Wisconsin Department of Natural Resources, Madison, Wisconsin, USA; [#]Current affiliation: Animal and Plant Health Inspection Services, U.S. Department of Agriculture, Gainesville, Florida, USA

Largemouth bass virus (LMBV; species *Santee-Cooper ranavirus*, SCRV; genus *Ranavirus*, family *Iridoviridae*) is associated with disease of North American black bass species (*Micropterus spp.*). The first LMBV mortality event occurred in 1995 at the Santee Cooper Reservoir, South Carolina, where at least 1000 largemouth bass (*M. salmoides*) died. Since then, LMBV outbreaks have been recorded throughout the Midwestern and Southern United States and Asia. Clinical signs of LMBV disease include ulcerated skin lesions and over-inflation of the swim bladder, altering the equilibrium of the infected host. Since 2005, LMBV has been reported from an increasing number of smallmouth bass (SMB; *M. dolomieu*) in Pennsylvania, Michigan, and Wisconsin. In September 2021, 14 wild SMB with ulcerated skin lesions were collected from the waters surrounding Door County, Wisconsin, and submitted for diagnostic evaluation. All samples tested positive for LMBV by conventional PCR. A homogenized skin sample was inoculated into *Epithelioma papulosum cyprini* cells, and cytopathic effects characterized by enlarged and refractile cells detaching from the monolayer were observed 24 hours post-inoculation at 25°C. The infected cell culture media was then clarified by centrifugation prior to DNA extraction, DNA library generation, and sequencing using an Illumina MiSeq sequencer. The *de novo* assembly of paired-end reads using SPAdes v3.15.3 resulted in a 99,354 bp LMBV genome. Maximum Likelihood (ML) phylogenetic analysis based on the 21 core iridovirus genes supported the LMBV isolated from SMB (21117) as a member of the species SCRV. A separate ML phylogenetic tree, based on the complete major capsid protein gene (MCP) alignment, grouped LMBV isolate 21117 with other LMBV isolates reported from the United States and China, as well as doctorfish virus (DFV) and guppy virus 6 (GV6). In addition, pairwise nucleotide comparison of the MCP gene showed that LMBV isolate 21117 is identical to LMBV reported from the United States and nearly identical to DFV and GV6 (99.2%), and LMBV from China (99.1%). Thus, the LMBV isolate generated in this study represents a different strain within the species SCRV and is closely related to previously isolated strains from the United States and Asia.

Establishment of a Citizen Science Virus Biosurveillance Network: Read the Room

Clayton Raines[1,2], Pat Mazik[2], Cynthia Fox-Holt[3], Blayk Michaels[4], and Luke Iwanowicz[1]

[1]United States Geological Survey, Eastern Ecological Science Center, Kearneysville, WV 25430, [2]West Virginia University, West Virginia Cooperative Fish and Wildlife Research Unit, Morgantown, WV 26506, [3]Texas Parks and Wildlife, Dallas/Fort Worth Inland Fisheries, Fort Worth, TX 76114, 4Bass Pro Shops Operation Center, Springfield, MO 65898

Blotchy bass syndrome (BBS) is characterized by the manifestation of variable, discrete areas of hyperpigmentation (melanosis) on the external surface of black basses. This is a condition that has received increased attention from anglers and resource managers during the past decade, and it is a frequent topic of discussion and reporting on angling websites and blogging platforms. Advances in discovery and diagnostic capabilities using "next generation sequencing", coupled with *de novo* assembly approaches, have augmented surveillance efforts, and subsequently lead to the discovery that this condition is associated with multiple viruses of the family *Adomaviridae*. A coordinated biosurveillance network was deemed necessary to understand the geographical extent, seasonality, and biological threat of this viral disease to black basses. Establishing image-based crowdsourced biosurveillance increases augments geographical and temporal sampling coverage beyond realistic management agency resources, such that observations can be vetted. A proposed three-year plan to establish a nationwide biosurveillance network was initiated as follows : i) Creation of a blotchy bass syndrome task force that includes federal (USFWS and U.S. Forest Service) and state fisheries managers across states where black basses are managed; ii) Development of minimally invasive (non-lethal) sampling methods and focal prevalence studies in selected states; and iii) Crowdsourced detection of blotchy bass occurrence and prevalence using citizen science approaches. Citizen science approaches consisted of solicitation of images of presumptive cases of BBS via social media. Grassroots campaigns targeting special interest groups such as NANFA (North American Native Fishes Association) garnered modest outputs of generally <50 images per post. Ultimately, leveraging messaging through larger social media channels hosted by state agencies (e.g., Texas Parks and Wildlife) or national retail chains (Bass Pro Shops) was observed to be considerably more effective. This poster will outline the advances made and pitfalls encountered navigating this nationwide effort.

Infectious Salmon Anemia Outbreak in Farmed Atlantic salmon (*Salmo salar* L.) in Iceland, First Detection of an ISAV HPRdel Variant

Sigurdardottir HS[1], Casás Casal S.[1], Bjornsdottir TS[1], Kristmundsson A[1]

[1]Keldur Institute for Experimental Pathology, University of Iceland, Reykjavik, Iceland

Infectious salmon anemia (ISA) is a serious viral disease of Atlantic salmon (*Salmo salar* L.) caused by the ISA virus (ISAV) and is notifiable to the World Organization for Animal Health (OIE). Virulent strains ISAV-HPRdel have deletions in a highly polymorphic region (HPR) of the hemagglutinin-esterase (HE) gene on segment 6, whereas avirulent strains ISAV-HPR0 have none. Routine targeted samplings for Real-time RT-PCR analyses have been performed since 2009. Icelandic Atlantic salmon broodfish farms are formally declared free of ISA by the fish health authority of the European Union. (https://www.mast.is/static/files/aaetlanir/aquaculture-surveillance-programme-fish-diseases-2020.pdf) ISAV screening with RT-qPCR started at Keldur in 2011. The few samples that have been ISAV positive from broodfish are HPR0 genotype. A research project carried out at Keldur from 2015-2018 screened both wild and cultured salmon juveniles, cultured salmon in sea pens and wild salmon from rivers (a total of about 800 fish) for ISAV, which was not detected. In November 2021, an increased mortality was experienced in farmed Atlantic salmon in sea pens in Reyðarfjörður, East Iceland. The fish showed macroscopic clinical signs suggestive of ISAV. Tissue samples and organs were sent to Keldur for diagnosis, using ISAV RT-PCR and histopathological analysis. The histopathology of the diseased fish was consistent with previous descriptions of ISAV-del infections in Atlantic salmon, i.e., characterized by extensive hemorrhage and congestion in most organs, associated with varying degree, often significant, pathological changes. Erythrophagocytosis was commonly observed, due to extensive immune reaction. RT-qPCR results for ISAV were positive with Ct. values ranging from 14-27. The samples were also run in a ISAVseg6 RT-PCR and further analysed by capillary electrophoresis. Sequencing of the ISAVseg6 PCR amplicons showed that it was an ISAV HPRdel variant. This is the first time that an HPRdel variant of ISAV has been detected in Iceland. The complete HE-gene was sequenced and when aligned with published sequences it showed greatest similarity to HPR0 and HPRdel sequences from northern Norway and HPR0 sequences from The Faroe Islands.

Development of a Concentration Method for Recovery of Viruses from Marine Water

Simran B. Singh[1], Tiong G. Aw[2], Joan B. Rose[1]

[1]Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI 48823;
[2]Department of Environmental Health Sciences, School of Public Health and Tropical Medicine, Tulane University, New Orleans, LA 70112.

The prevalence of viruses affecting marine mammals is an under investigated subject. The Office of Naval Research in conjunction with the U.S. Department of Defense run a marine mammal program involving bottlenose dolphins (*Tursiops truncates*) and California sea lions (*Zalophus californianus*). Sustaining healthy marine mammals is of the utmost importance and the health of these animals is closely related to the environments they are exposed to. Viral diseases of marine mammals have been much more difficult to study, and this has led to a limited knowledge on emerging known and unknown viruses. Therefore, the purpose of this study was to develop an automated and simple concentration method using the Innovaprep Concentrating Pipette Select™ (CP) for the recovery of marine mammalian viruses. Bacteriophages MS2, P22, Phi6, and PhiX-174 were seeded in artificial seawater and then tested for recovery post CP concentration. These bacteriophages vary in characteristics and were selected to be surrogates for animal viral families. Recovery for all bacteriophages was tested using a plaque assay, while additional analyses were performed for MS2 and Phi6 with Droplet Digital PCR (ddPCR). The average plaque assay recovery for each bacteriophage are as follows: P22 46.08% ± 12.86 (n=8), Phi6 30.55% ± 20.52 (n=9), PhiX-174 7.26% ± 6.61 (n=11) and MS2 3.15% ± 4.28 (n=8). The average ddPCR recovery is as follows: Phi6 16.93% ± 19.68 (n=11) and MS2 2.19% ± 1.12 (n=10). Thus far, P22 has the highest recovery percentage followed by Phi6, then PhiX-174 and MS2. This may be due to the structure of each virus's compatibility with the CP ultrafilter. In the future, animal viruses Human Coronavirus OC43, Adenovirus, and Morbillivirus CDV will be tested in artificial seawater as well as for naturally occurring viruses from the San Diego Bay and Shedd Aquarium.

Interlaboratory Reproducibility of a TaqMan RT-qPCR Assay for Detection of Tilapia Lake Virus

Kuttichantran Subramaniam[1,2,], Dorothea V. Megarani[1,2], Jordan A. Vann[1,2], Christopher Tong[3], Janet V. Warg[4], Thomas B. Waltzek[1,2,5]

[1]Department of Infectious Diseases and Immunology, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610, USA; [2]Emerging Pathogen Institute, University of Florida, Gainesville, FL 32610, USA; [3]Center for Veterinary Biologics, VS, APHIS, USDA, Ames, IA 50010; [4]Diagnostic Virology Laboratory, National Veterinary Services Laboratories, VS, APHIS, USDA, Ames, IA 50010, USA; [5]Current affiliation: Live Animal Imports & Exports, VS, APHIS, USDA, Gainesville, FL 32606, USA

Tilapia is the second most important aquaculture species globally and a primary source of protein in many developing countries. Although tilapia are known for rapid growth and general hardiness, they are susceptible to common finfish pathogens such as viruses, bacteria, fungi, water molds, and parasites when reared intensively. Tilapia lake virus (TiLV) is the causative agent of an emerging viral disease associated with high morbidity and mortality in cultured tilapia worldwide. Since the first outbreak in 2012 in Ecuador, TiLV has spread globally, causing variable mortality in all ages of tilapia species in Asia, Africa, and the Americas. Although diagnostic assays to detect TiLV (or exposure to TiLV) have been developed, these assays have not been fully validated. The University of Florida Wildlife and Aquatic Disease Veterinary Laboratory has developed and partially validated (analytic and diagnostic performance) a TaqMan RT-qPCR assay to detect TiLV. In the current study, the reproducibility of the TiLV TaqMan RT-qPCR assay was evaluated through a collaborative effort involving six laboratories. All participating laboratories received a standard operating protocol for the RT-qPCR assay and a blinded test panel consisting of 31 positive and 19 negative RNA samples. Seventeen positive RNA samples were extracted from striped snakehead (SSN-1; E11 clone) cell culture supernatant (n=14) and fish tissues (n=3) infected with the same TiLV isolate. Seven positive RNA samples were generated from a single *in vitro* transcription event by preparing 7 aliquots of 10^6 copies *in vitro* standards from a single 10^7 copies tube; each was then diluted separately down to 10^4 copies. Furthermore, each of these 7 samples was duplicated within the panel (two vials aliquoted from the same tube) for a total of 14 samples. Nineteen negative RNA samples were extracted from fish tissues unexposed to TiLV. Performance measures, including variation between- and within-laboratory, were evaluated for cell culture supernatant and tissue RNA extracts. For the RNA samples generated by *in vitro* transcription, variations between-laboratory and within-laboratory were evaluated, including the variation within-vial and between-vial for the latter. All laboratories reliably detected both positive and negative samples, except for one laboratory reporting a negative sample as a suspect. For cell culture supernatant and tissue RNA extracts, the estimated standard deviation (SD) of mean Ct values between-laboratory was nearly double (0.39) that of the within-laboratory (0.20). However, the magnitude of this variation is relatively small, with both SDs being less than a full cycle threshold. The estimated SD of mean Ct values between-laboratory was 0.70 for *in vitro* transcript samples. For within-laboratory, the estimated SD of mean Ct values between-vial was nearly double (1.24) that of the within-vial (0.65); note that the former is an artifact of the sample preparation rather than the testing process. Thus, standard deviations reflecting the testing process all fell below a single cycle threshold for each sample type. This interlaboratory validation trial provided data to support the reproducibility (stage 3) of the post-extraction component of the TiLV TaqMan RT-qPCR assay as outlined by the World Organisation for Animal Health (OIE) for diagnostic assay validation.

Virulence and Immunogenicity of Novel Blue Catfish Alloherpesvirus in Channel, Blue and Blue × Channel Hybrid Catfish

Arun Venugopalan[1,2], Matt J. Griffin[2,3], David J. Wise[2,4], Danielle' White[1], Lorelei Ford[1], Adrián López-Porras[2,3], Alvin C. Camus[5], and Larry A. Hanson[1]

[1]Department of Comparative Biomedical Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762; [2]Thad Cochran National Warmwater Aquaculture Center, Delta Research and Extension Center, Mississippi State University, Stoneville, MS 38776; [5]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Stoneville, MS 38776; [3]Department of Wildlife, Fisheries and Aquaculture, College of Forest Resources, Mississippi State University, Mississippi State, MS 39762; [4]Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602

Blue catfish alloherpesvirus (BCAHV) is a novel virus isolated from the blue catfish (Ictalurus furcatus). To date, the ultrastructure, virulence and immunogenicity of BCAHV have not been reported. Given the importance of blue catfish in producing channel ♀ (I. punctatus) × ♂ blue (I. furcatus) catfish hybrids and the increasing demand for hybrid catfish in the US catfish industry, the susceptibility of blue, channel and hybrid catfish to BCAHV was assessed. Further, the cross-protective potential of BCAHV against Ictalurid herpesvirus 1 (IcHV1) was investigated in channel and hybrid catfish that survive BCAHV exposure. Neutralization assays revealed BCAHV is refractive (neutralization index [NI] = 0) to anti-IcHV1 monoclonal antibody Mab 95, compared to IcHV1 (NI = 1.8). Exposure of blue catfish fingerling to 1.3×10^5 $TCID_{50}$ /L BCAHV produced cumulative mortality of 51.67 ± 0.70% and pathologic changes similar to disease induced by IcHV1. Blue catfish fingerlings exposed to BCAHV revealed necrotic focus in trunk kidney hematopoietic tissue, congested and oedematous spleen and hepatocellular necrosis. Comparably, no mortality was observed in channel or hybrid catfish, which were asymptomatic throughout the challenge. Twenty-eight days post-challenge, surviving channel and hybrid catfish were exposed to 9.4×10^4 $TCID_{50}$ /L IcHV1 (LC_{50} dose), resulting in 100% relative percent survival compared to naïve cohorts. These data provide baseline information for BCAHV and lay the groundwork for future studies. Data also identify BCAHV as a potential vaccine candidate against IcHV1. Based on host range and immunogenicity evaluations, in addition to genome sequence data from previous studies, BCAHV should be given consideration as a new species of Ictalurivirus.

Spatiotemporal Survey of Two Discrete Genotypes of Latent Channel Catfish Virus (IcHV1) Using Quantitative Real-Time Assays

Arun Venugopalan[1,2] Matt J. Griffin[2,3], David J. Wise[2,4], Andrew D. Perkins[5], Cynthia Ware[2,3], Charles M. Walker[2,4], Bradley M. Richardson[6], Geoffrey C. Waldbieser[6], Lorelei Ford[1], Kuttichantran Subramaniam[7], Thomas B. Waltzek[7], Esteban Soto[9], and Larry A. Hanson[1]

[1]Department of Comparative Biomedical Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS; [2]Thad Cochran National Warmwater Aquaculture Center, Delta Research and Extension Center, Mississippi State University, Stoneville, MS; [3]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Stoneville, MS; [4]Department of Wildlife, Fisheries and Aquaculture, College of Forest Resources, Mississippi State University, Mississippi State, MS; [5]Department of Computer Science and Engineering, Bagley College of Engineering, Mississippi State University, Mississippi State, MS; [6]USDA, Warmwater Aquaculture Research Unit, Stoneville, Mississippi State, MS; [7]Department of Infectious Diseases and Immunology, College of Veterinary Medicine, University of Florida, Gainesville, Florida, USA; [8]Department of Epidemiology and Medicine, Davis-School of Veterinary Medicine, University of California, Davis, California, CA;

Channel catfish virus (IcHV1) and the recently described blue catfish alloherpes virus (BCAHV) are alloherpesviruses associated with US catfish aquaculture. Channel catfish virus disease (CCVD) can be catastrophic, with mortality approaching 100% in severe outbreaks. Previous work reported the prevalence of latent IcHV1 in swim-up channel catfish fry to be ~10%. Restriction length polymorphism analysis (RFLP) identified two discrete genetic variants of IcHV1 among clinical isolates, although the prevalence of these newly recognized IcHV1 variants is unknown. In the current study, the genomes (~135 kb) of representative IcHV1 variants (RFLP group 1A; RFLP group 1B; BCAHV) were sequenced. Initially, two separate probe based quantitative PCR assays were designed and validated following MIQE guidelines. While the qPCR targeting RFLP group 1A was specific to its target, due to a high level of conservation across the three viral groups, the RFLP group 1B primers co-amplified BCAHV. To account for this cross-reactivity, a subsequent High Resolution Melt Curve assay was developed to differentiate between RFLP group 1B and BCAHV, offering for the first time, a rapid molecular diagnostic test to confirm identify of BCAHV. Catfish fry were collected in 10-12 day intervals from six commercial hatcheries in the Mississippi Delta during the spawning season. At each sampling, sixty swim-up fry were collected from discrete hatching troughs, and individual fry were screened for the latent virus. Survey results indicate IcHV1/BCAHV prevalence ranged from 0 – 96% in the channel and hybrid catfish fry, depending on farm and time of sampling. Importantly, there were no disease/mortality events reported from cooperating laboratories, indicating CCV/BCAHV can be present in the absence of disease. These assays offer a useful research tool for investigating the epidemiology of IcHV1/BCAHV in catfish aquaculture and rapid molecular confirmatory tests to identify IcHV1a, IcHV1b and BCAHV.

Complete Genome Sequencing of Infectious Spleen and Kidney Necrosis Virus from Farmed Tilapia in Brazil

Pedro H. O. Viadanna[1*], Ulisses P. Pereira[2], Arthur R. da Costa[2], Breno Manzini[3], José D. Neto[3], Kuttichantran Subramaniam[1], Thomas B. Waltzek[1;4]

[1]Department of Infectious Diseases and Immunology, College of Veterinary Medicine, University of Florida, Gainesville, FL 32611 USA; [2]Laboratory of Fish Bacteriology (LABBEP), Department of Preventive Veterinary Medicine, State University of Londrina, Londrina, PR, Brazil ; [3]PREVET-Sanidade Aquícola, Av. Olga Morello de Stéfani, 260, CEP 14887-364 Jaboticabal, SP, Brazil
[4]Current affiliation: Live Animal Imports & Exports, VS, APHIS, USDA, Gainesville, FL 32606, USA

Tilapia aquaculture provides an important source of protein for communities around the globe, including Brazil. As the number of aquaculture facilities rearing tilapia intensively has increased, infectious diseases have emerged as a significant impediment to production. Although several bacterial and parasitic diseases have been reported in farmed tilapia in Brazil,viral infections have been limited to the iridovirus (genus *Megalocytivirus*), infectious spleen and kidney necrosis virus (ISKNV). ISKNV can cause severe systemic disease resulting in high morbidity and mortality outbreaks. This virus has been detected in numerous marine and freshwater fish species produced for food and ornamental purposes. In October 2019, three farms in São Paulo and one in Mato Grosso do Sul, Brazil, experienced mass mortalities among fingerlings reared in net cages or ponds. Moribund fish displayed discoloration of the body/fins, and internal abnormalities included splenomegaly, ascites, and redness of the intestine. Histopathological examination and conventional PCR confirmed the tilapia were infected with ISKNV. Subsequently, next-generation sequencing and phylogenomic analysis revealed the tilapia were infected with the ISKNV genotype (clade 1). A genetic comparison of the partial major capsid protein nucleotide sequences of the tilapia strain with ISKNV strains from previous reports confirms the virus is circulating in both ornamental and food fish industries in Brazil. Repeated detection of ISKNV in ornamental and food fish species warrants further surveillance efforts to determine the prevalence and potential impact of ISKNV strains on Brazilian aquaculture.

Insight Towards Salmonid Alpha Virus Infection and Tropism in Atlantic Salmon Pseudobranch

Haitham Tartor[1], Lisa-Victoria Bernhardt[1], Saima Nasrin Mohammad[1], Sonal Patel[1], Ole Bendik Dale[1], Atle Lillehaug[1] and Simon C Weli[1]

[1]Norwegian Veterinary Institute, 1433 ÅS, Norway.

Salmonid alphavirus (SAV) is an etiological agent of pancreas disease (PD) which is associated with farmed Atlantic salmon and causes significant losses in salmon aquaculture. Necrosis and loss of exocrine pancreatic tissue, as well as degeneration and inflammation of the heart and skeletal muscle characterize SAV infection. However, SAV3 particles have also been detected in the pseudobranch of experimental infected salmon; and despite several years of research work on SAV tropism, the role of pseudobranch in the pathogenesis of PD in SAV-infected Atlantic salmon has not been fully illustrated. In severe cases of PD, fish appear lethargic and unresponsive to visual challenge that may suggest blindness. Also, swollen or papillate pseudobranch has been observed in PD cases in Atlantic salmon that may underline association with the virus. Here, we studied the infection kinetics of SAV3 in the pseudobranch and heart of Atlantic salmon and demonstrate the distribution of SAV3 in pseudobranch tissue using an experimental infection model. In addition, the immune response of pseudobranch against SAV3 infection was studied by quantifying the expression of sixteen immune-related genes in fish exposed to low or high dose of SAV3. Cohabitant challenge trial with high and low dose SAV subtype 3 (SAV3) was performed for six weeks using post-smolt Atlantic salmon (weighing 110g). At 16 different time point, pseudobranch tissue was collected from fish in control, low and high dosage tanks and analyzed by histology and *in situ hybridization*. SAV3 detection and immune genes expression were analyzed by reverse transcription quantitative PCR (RT-qPCR). Pseudobranch tissues collected from fish exposed to SAV3 showed gross pathology and histopathology, associated with PD. *In situ hybridization* staining with RNAscope kit on pseudobranch tissues from SAV3 challenged fish were positive for SAV3 using a probe targeting the SAV-nsp1 gene. Heart and pseudobranch tissues from SAV challenged fish were positive for SAV3 at days 12-29 post-challenge as judged by RT-qPCR. The expression analysis of immune genes including viperin, MX, MHC-I, sIgM and sIgT-B, were significantly upregulated in pseudobranch tissues collected from SAV-challenged fish as compared with the control group at days 16-29 post challenge. The detection of SAV3 in pseudobranch tissues of Atlantic salmon and the change in the expression of different immune genes in the tissues after fish exposure to the SAV3 suggests the pseudobranch tissues may have a role in the pathogenesis of SAV3 in Atlantic salmon.

The First Report of Tilapia Parvovirus in Thailand and Co-infection With *Tilapia Tilapinevirus* in Red Hybrid Tilapia

Jidapa Yamkasem[1,2] Puntanat Tattiyapong[2,3] Bartolomeo Gorgoglione[4] and Win Surachetpong[1,2,3*]

[1]Graduate Program in Animal Health and Biomedical Science, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand; [2]Department of Veterinary Microbiology and Immunology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand; [3]Interdisciplinary Program in Genetic Engineering and Bioinformatics, Graduate school, Kasetsart University, Bangkok, Thailand; [4]Fish Pathobiology & Immunology Laboratory, Department of Pathobiology and Diagnostic Investigation, CVM & Department of Fisheries and Wildlife, CANR - Michigan State University, East Lansing, MI, USA

Tilapia parvovirus (TiPV) is an emerging virus in tilapia and a novel *Parvovirus* identified in fish. TiPV was only reported in Nile tilapia (*Oreochromis niloticus*) associate with high morality in China in 2015. In this study, we detected and characterized TiPV for the first time in Thailand in farmed red hybrid tilapia (*Oreochromis* spp.). During the study of the unusual mortality of ten tilapia farms from April 2019 to December 2020, multiple pathogens including viruses, bacteria and parasites were detected in moribund fish. Interestingly, out of ten fish farms investigated, we found the coinfection of *Tilapia tilapinevirus* and a novel parvovirus TiPV in a farmed juvenile red hybrid tilapia. The affected fish exhibited clinical signs of infection, including abnormal swimming, scale protrusion, skin and muscle haemorrhaging, exophthalmia, and generalized anaemia reaching a cumulative mortality of 36%. Histological changes included extensive infiltration of lymphocytes, with increased melanomacrophage centres in the anterior kidney and spleen, erythrocyte depletion in the spleen and hepatic syncytial cells. Both TiLV and TiPV were detected in most tissues of red hybrid tilapia including liver, gills, heart, intestine, muscle, eye, spleen, kidney, brain, and mucus, suggesting that both viruses cause systemic infection and that a panel of organs could be applied for routine diagnostics. Analysis of the near-complete TiPV genome isolated from Thailand revealed that the isolate was closely related to the formerly virus reported from China (97.01 to 100% nt and 97.18 to 100% aa sequence identity). Our study suggested that diagnostic investigations during tilapia disease outbreaks should include the screening for TiPV. Further studies are needed to elucidate TiPV genomic variance, pathobiology, including focusing on the outcomes of TiLV-TiPV co-infection patterns. Further work is necessary to enable risk assessment for the worldwide spreading of TiPV, and to design adequate control measures against these emerging viruses in tilapia.

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