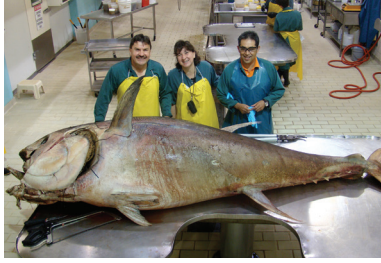


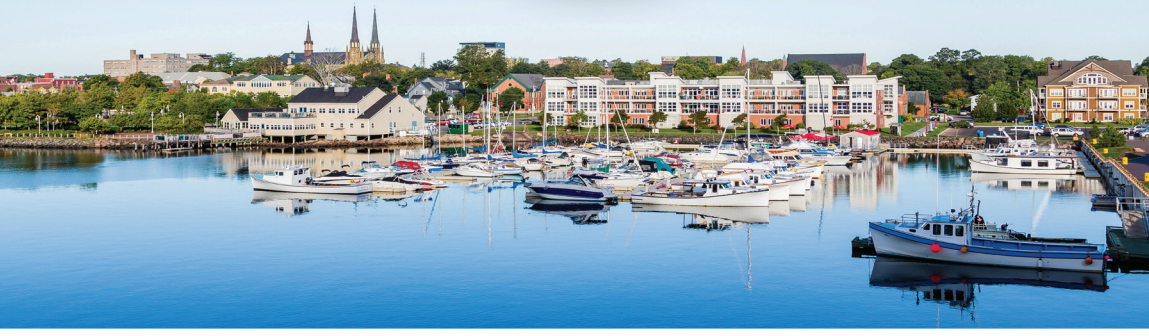


Celebrating 30 Years

ISAAH

CHARLOTTETOWN
PRINCE EDWARD ISLAND
2018

AFS Fish Health Section



8TH INTERNATIONAL SYMPOSIUM ON AQUATIC ANIMAL HEALTH

SEPTEMBER 2-6, 2018 · CHARLOTTETOWN, PEI, CANADA

HOSTED BY

American Fisheries Society Fish Health Section

University of Prince Edward Island

Prince Edward Island BioAlliance

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SYNAPSE CONNECT COLLABORATE CREATE

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UCDAVIS VETERINARY MEDICINE

USDA United States Department of Agriculture National Institute of Food and Agriculture

QASH (Quantitative Atlantic Salmon Health Assessment)

UF Environmental & Global Health College of Public Health & Health Professions UNIVERSITY OF FLORIDA

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Joan Turner-Adams, Business Development Officer, Innovation PEI

Peter Warris, Director of Projects and Industry Liaison, PEI Aquaculture Alliance

Thomas Ogilvie, Veterinarian, Marine Harvest Canada (formerly Northern Harvest)

Gerald Johnson, Director of Science, Halibut PEI Inc.

Randy Angus, Integrated Resource Management, Mi'Kmaq Confederacy of Prince Edward Island

NOTE: Final Scientific Program available at www.isaah2018.com

SUNDAY, SEPTEMBER 2ND – Registration & Pre-conference workshops

8:30AM – 12:30PM: **Registration Open for Pre-Conference Workshops**
Atlantic Veterinary College, University of Prince Edward Island

9:30AM – 5:00PM: **Pre-Conference Workshops** (Sponsored by the Atlantic Veterinary College)
Atlantic Veterinary College, University of Prince Edward Island

10:00AM – 11:30AM: **Fish Health Section Executive Meeting**
MacDonald Room, Delta Prince Edward Hotel Conference Centre

12:00PM – 6:30PM: **Registration / Partnering Help Desk Open**
Delta Prince Edward Hotel Conference Centre foyer

12:00PM – 6:30PM: **Poster Check-in & Set up**
Coles Room, Delta Prince Edward Hotel Conference Centre

5:00PM – 7:00PM: **ISAAH 2018 Student Reception** (Sponsored by the Fish Health Section)
Steeves McGee Hospitality Suite, Mezzanine Level
Delta Prince Edward Hotel

6:30PM – 8:30PM: **ISAAH 2018 Welcome Reception**
Harbourview Deck, Delta Prince Edward Hotel

MONDAY, SEPTEMBER 3RD – Prince Edward Island Day

7:30AM – 6:00PM: **Registration / Partnering Help Desk Open**
Delta Prince Edward Hotel Conference Centre foyer

7:30AM – 8:15PM: **Continental Breakfast**
Delta Prince Edward Hotel Conference Centre foyer

8:15AM – 8:30AM: **Welcome Remarks & Opening of ISAAH 2018**
Gray, Palmer, Pope Ballroom, Delta Prince Edward Hotel Conference Centre

8:30 – 9:15AM: **Keynote Presentation by Dr. Daniel R. Barreda**, Professor, Animal Immunity and Health, Department of Biological Sciences and Department of Agricultural, Food and Nutritional Science, University of Alberta, Canada

"Contributions of early cellular responses to immune protection in fish"

Gray, Palmer, Pope Ballroom, Delta Prince Edward Hotel Conference Centre

9:30AM – 5:00PM: **AAFV Sessions** (see page 23 for schedule)
Tilly Tupper Room

9:30AM – 12:15PM: **Industry Sessions** (Sponsored by Springboard / Synapse)
(see page 29 for presentation schedule)
MacDonald Room, Delta Prince Edward Hotel Conference Centre

9:30AM – 10:30AM: **Breakout Sessions**
Immunology I: Gray, Palmer, Pope Ballroom
Flavobacterium I: Archibald Campbell Breakout Room
Microbiome I: Langeve Cartier Breakout Room

9:30AM – 5:00PM: **Posters on Display**
Coles Ballroom, Delta Prince Edward Hotel Conference Centre

10:30AM – 10:45AM: **Refreshment Break**
Delta Prince Edward Hotel Conference Centre Foyer

10:45AM – 12:15PM: **Breakout Sessions**
Immunology II: Gray, Palmer, Pope Ballroom
Flavobacterium II: Archibald Campbell Breakout Room
Microbiome II: Langeve Cartier Breakout Room

12:15PM – 1:15PM: **Lunch**
Buffet served in the Delta Prince Edward Hotel Conference Centre Foyer

12:45PM – 3:05PM: **Partnering Meetings**
MacDonald Room, Delta Prince Edward Hotel Conference Centre

1:15PM – 3:00PM: **Breakout Sessions** (Tilapia Sessions sponsored by IDT/Ridgeway/Gallant Laboratories)
Vaccines I: Gray, Palmer, Pope Ballroom
Tilapia Health I: Archibald Campbell Breakout Room
Life Cycles: Langeve Cartier Breakout Room

3:00PM – 3:15PM: **Refreshment Break**
Delta Prince Edward Hotel Conference Centre Foyer

3:15PM – 5:00PM: **Breakout Sessions** (Tilapia Sessions sponsored by IDT/Ridgeway/Gallant Laboratories)
Vaccines II: Gray, Palmer, Pope Ballroom
Tilapia Health II: Archibald Campbell Room
Gill Health: Langeve Cartier Breakout Room

5:00PM – 6:00PM: **Official Poster Session-I**
Coles Ballroom, Delta Prince Edward Hotel Conference Centre (Cash Bar)

6:00PM: **Buses Depart for Off-site Cultural Event at Fort Amherst Historic Site**
(Buses will depart from the front of the Delta Prince Edward Hotel front doors at 6:00pm sharp)

6:30PM – 10:00PM: **Off-site Cultural Event & Feast**
Skmaqn–Port-la-Joye–Fort Amherst National Historic Site

TUESDAY, SEPTEMBER 4TH – Nova Scotia Day

7:30AM – 5:30PM: **Registration / Partnering Help Desk Open**
Delta Prince Edward Hotel Conference Centre foyer

7:45AM – 8:15AM: **Continental Breakfast**
Delta Prince Edward Hotel Conference Centre foyer

8:15AM – 8:30AM: **Announcements**
Gray, Palmer, Pope Ballroom, Delta Prince Edward Hotel Conference Centre

8:30 – 9:15AM: **Keynote Presentation by Dr. Henning Sørum**, Professor at the School of Veterinary Medicine, Norwegian University of Life Sciences, Oslo, Norway

"Bacterial skin ulcers in farmed fish; preventive effect of vaccines and probiotics"

Gray, Palmer, Pope Ballroom, Delta Prince Edward Hotel Conference Centre

9:30AM – 5:00PM: **WAVMA Sessions** (see page 24 for schedule)
Tilly Tupper Room, Delta Prince Edward Hotel Conference Centre

9:30AM – 12:15PM: **Ocean Supercluster Workshop Part I** (see page 27 for schedule)
MacDonald Room, Delta Prince Edward Hotel Conference Centre

9:30AM – 10:30AM: **Breakout Sessions**
Bacteriology I: Gray, Palmer, Pope Ballroom
Sea Lice I: Archibald Campbell Breakout Room
Zebrafish / Lab Animal: Langeve Cartier Breakout Room

9:30AM – 5:00PM: **Posters on Display**
Coles Ballroom, Delta Prince Edward Hotel Conference Centre

10:30AM – 10:45AM: **Refreshment Break**
Delta Prince Edward Hotel Conference Centre Foyer

10:45AM – 12:15PM: **Breakout Sessions**
Bacteriology II: Gray, Palmer, Pope Ballroom
Sea Lice II: Archibald Campbell Breakout Room
Invert/Shellfish I: Langeve Cartier Breakout Room

12:15PM – 1:15PM: **Lunch**
Buffet served in the Delta Prince Edward Hotel Conference Centre Foyer

12:45PM – 3:05PM: **Partnering Meetings**
MacDonald Room, Delta Prince Edward Hotel Conference Centre

1:15PM – 3:00PM: **Breakout Sessions** (Tilapia Sessions sponsored by IDT/Ridgeway/Gallant Laboratories)
Bacteriology III: Gray, Palmer, Pope Ballroom
Sea Lice III: Archibald Campbell Breakout Room
Cleaner Fish I: Archibald Campbell Breakout Room
Invert Shellfish I: Langeve Cartier Breakout Room

3:00PM – 3:15PM: **Refreshment Break**
Delta Prince Edward Hotel Conference Centre Foyer

3:15PM – 5:00PM: **Breakout Sessions** (Tilapia Sessions sponsored by IDT/Ridgeway/Gallant Laboratories)
Bacteriology IV: Gray, Palmer, Pope Ballroom
Cleaner Fish II: Archibald Campbell Breakout Room
Aquatic EPI I: Langeve Cartier Breakout Room

5:00PM – 6:00PM: **Official Poster Session-II**
Coles Ballroom, Delta Prince Edward Hotel Conference Centre (Cash Bar)

6:30 – 8:30PM: **Discover Charlottetown Reception** (Sponsored by Elanco Canada Limited)
Memorial Hall, Confederation Centre of the Arts

WEDNESDAY, SEPTEMBER 5 – Newfoundland Day

7:30AM – 6:30PM: **Registration/Partnering Help Desk Open**
Delta Prince Edward Hotel Conference Centre foyer

7:30AM – 8:15PM: **Continental Breakfast**
Delta Prince Edward Hotel Conference Centre foyer

8:15AM – 8:30AM: **Announcements**
Gray, Palmer, Pope Ballroom, Delta Prince Edward Hotel Conference Centre

8:30 – 9:15AM: **Keynote Presentation by Dr. Jerri Bartholomew**, Professor and Head of the OSU Department of Microbiology and Director of the John L. Fryer Aquatic Animal Health Laboratory

"Weapons of Micro-destruction: An interdisciplinary approach to understanding a parasitic cnidarian"

Gray, Palmer, Pope Ballroom, Delta Prince Edward Hotel Conference Centre

9:30AM – 12:15PM: **Ocean SuperclusterWorkshop Part II** (see page 27 for schedule)
MacDonald Room, Delta Prince Edward Hotel Conference Centre

9:30AM – 10:30AM: **Breakout Sessions**

Myxozoa I:	Gray, Palmer, Pope Ballroom
Ornamental I:	Archibald Campbell Breakout Room
Disease of Wild Fish I:	Tilly Tupper Breakout Room
Virology I:	Langeve Cartier Breakout Room

9:30AM – 5:00PM: **Posters on Display**
Coles Ballroom, Delta Prince Edward Hotel Conference Centre

10:30AM – 10:45AM: **Refreshment Break**
Delta Prince Edward Hotel Conference Centre Foyer

10:45AM – 12:15PM: **Breakout Sessions**

Myxozoa II:	Gray, Palmer, Pope Ballroom
Ornamental II:	Archibald Campbell Breakout Room
Disease of Wild Fish II:	Tilly Tupper Breakout Room
Virology II:	Langeve Cartier Breakout Room

12:15PM – 1:15PM: **Lunch**
Buffet served in the Delta Prince Edward Hotel Conference Centre Foyer

12:45PM – 3:05PM: **Partnering Meetings**
MacDonald Room, Delta Prince Edward Hotel Conference Centre

1:00PM – 1:45PM: **Keynote Presentation by Dr. Sabo-Attwood**, Associate Professor and Chair of the Department of Environmental and Global Health and a member of the Center for Environmental and Human Toxicology and Emerging Pathogens Institute at the University of Florida

"Nano-Evolution: Balancing safety and applications of nanotechnology in aquatic systems"

1:45PM – 3:00PM: **Breakout Sessions** (Sponsored by Skretting)

Myxozoa III:	Gray, Palmer, Pope Ballroom
Nutrition & Fish Health:	Archibald Campbell Breakout Room
Aquatic EPI II:	Tilly Tupper Breakout Room
Toxicology/Path:	Langeve Cartier Breakout Room

3:00PM – 3:15PM: **Refreshment Break**
Delta Prince Edward Hotel Conference Centre Foyer

3:15PM – 5:00PM: **Breakout Sessions**

Myxozoa IV:	Gray, Palmer, Pope Ballroom
Emergent Disease:	Archibald Campbell Breakout Room
Antibiotics/Pharma:	Tilly Tupper Breakout Room
Virology III:	Langeve Cartier Breakout Room

5:00 – 6:30PM: **Fish Health Section Business Meeting**
MacDonald Room, Delta Prince Edward Hotel Conference Centre

6:30PM – 11:00PM: **ISAAH 2018 Awards Banquet & Reception** (Sponsored by Marine Harvest Canada)
Keynote Presentation by Dr. Adel El-Mowafi, Aquaculture Technology Application Director, Cargill Aqua Nutrition, Norway

"Role of nutrition to mitigate diseases in aquaculture"

Gray, Palmer, Pope Ballroom, Delta Prince Edward Hotel Conference Centre

THURSDAY, SEPTEMBER 6TH – New Brunswick Day

7:30AM – 5:30PM: **Registration/Partnering Help Desk Open**
Delta Prince Edward Hotel Conference Centre foyer

7:30AM – 8:15PM: **Continental Breakfast**
Delta Prince Edward Hotel Conference Centre foyer

8:15AM – 8:30AM: **Announcements**
Gray, Palmer, Pope Ballroom, Delta Prince Edward Hotel Conference Centre

8:30 – 9:15AM: **Keynote Presentation by Alf-Helge Aarskog**, Chief Executive Officer (CEO) of Marine Harvest ASA

"Without good fish health – no blue revolution"

Gray, Palmer, Pope Ballroom, Delta Prince Edward Hotel Conference Centre

9:30AM – 5:00PM: **QASH Sessions** (see page 25 for schedule)
MacDonald Ballroom, Delta Prince Edward Hotel Conference Centre

9:30AM – 10:30AM: **Breakout Sessions**
Virology IV: Gray, Palmer, Pope Ballroom
Health Management: Archibald Campbell Breakout Room
Genomics I: Tilly Tupper Breakout Room
eDNA Metagenomics: Langeve Cartier Breakout Room

10:30AM – 10:45AM: **Refreshment Break**
Delta Prince Edward Hotel Conference Centre Foyer

10:45AM – 12:15PM: **Breakout Sessions**
Virology V: Gray, Palmer, Pope Ballroom
Health Management: Archibald Campbell Breakout Room
Genomics II: Tilly Tupper Breakout Room
Parasitology I: Langeve Cartier Breakout Room

12:15PM – 1:15PM: **Lunch**
Buffet served in the Delta Prince Edward Hotel Conference Centre Foyer

1:15PM – 3:00PM: **Breakout Sessions**
Virology VI: Gray, Palmer, Pope Ballroom
Health Management: Archibald Campbell Breakout Room
Confections: Tilly Tupper Breakout Room
Parasitology II: Langeve Cartier Breakout Room

3:00PM – 3:15PM: **Refreshment Break**
Delta Prince Edward Hotel Conference Centre Foyer

3:15PM – 5:00PM: **Breakout Sessions**
Virology/Emergent DIS: Gray, Palmer, Pope Ballroom
Miscellaneous: Tilly Tupper Breakout Room
Husbandry/Physio: Langeve Cartier Breakout Room

5:00PM – 6:00PM: **Closing Remarks & ISAAH 2018 Wrap-up**

FRIDAY, SEPTEMBER 7TH – Field Trip Day

8:00AM – 6:00PM: **Bus departs Delta Prince Edward Hotel for PEI Field Trip**

NOTE: Final Scientific Program available at www.isaah2018.com

8th International Symposium on Aquatic Animal Health

September 2-6, 2018 - Charlottetown, Prince Edward Island, Canada

2018 American Association of Fish Veterinarians Annual Conference

Monday Sept 3rd, 2018; Tilly/Tupper Room

09:30 AM	**Mitchell** - The Early Years of Salmon Farming Medicine in the US: Practice and Politics
10:00 AM	**Milligan** - Changing Practices and Environment Affecting Aquaculture: Overview of the Current Status and Challenges Facing A BC Atlantic Salmon Farm
10:15 AM	**Whitaker** - PRV from A BC Industry Vet
10:30 AM	**Refreshment Break**
10:45 AM	**Reichley** - Fish Health Challenges in Large-Scale Rainbow Trout Production
11:15 AM	**Morrison** - History and Future of Integrated Pest Management (IPM) In British Columbia
11:45 AM	**Hickey** - Western Washington Treaty Tribe's Pacific Salmon Enhancement Program
12:15 PM	**AAFV Business Lunch**
12:45 PM	One-to-One Partnering Program Meetings
1:15 PM	**Wyatt** - Restoration of Lake Sturgeon as a Bioindicator Species in Rochester, New York's Lower Genesee River EPA Area of Concern
1:45 PM	**Kebus** - Training Veterinarians for Fish Regulatory Medicine
2:15 PM	**Gaunt** - Fresh From the Field: Using Antimicrobials and Veterinary Feed Directive Drugs in Aquatic Medicine
2:45 PM	**Refreshment Break**
3:15 PM	**Whaley** - Offshore Aquaculture – A One Health Approach
3:45 PM	**Shelley** - Ornamental Aquaculture Medicine in the United States – Past, Present and Future
4:15 PM	**Sanders** - The Development and Sustainability of Private Aquatic Veterinary Practice; Anesthesia, Surgery and Pain Management in Pet Fish
4:45 PM	**Giffin** - Canada's Aquatic Animal Health Import Program: A Global Perspective
5:15 PM	Concluding Remarks

Fish Health Section – American Fisheries Society, 425 Barlow Place, Bethesda Maryland. USA 20814
Phone: (301) 897-8616 Fax: (301) 897-8096 Email: jsewell@fisheries.org

8th International Symposium on Aquatic Animal Health

September 2-6, 2018 - Charlottetown, Prince Edward Island, Canada

World Aquatic Veterinary Medical Association at ISAAH 2018

Tuesday Sept 4rd, 2018; Tilly/Tupper Room

09:30 AM	**Dhar** - The University of Arizona, Aquaculture Pathology Laboratory: A Worldwide Resource for Diagnostic Services and Collaborative Research to Shrimp Aquaculture Industry
10:00 AM	**Llano** - Parasitic Survey on Captive, Wild and Reintroduced Sirenians (Thrichechus inunguis and Trichechus manatus) in Brazil
10:30 AM	**Refreshment Break**
10:45 AM	**Scarfe** - Ensuring a Well-trained Aquatic Veterinary Workforce: Past, Present and Future Initiatives
11:15 AM	**Miller-Morgan** - Defining the Practice of Aquatic Veterinary Medicine – A Unique Approach for Establishing Day-1 Competencies
11:45 AM	Panel/Audience Discussion - Optimal Approaches for Ensuring a Well-Educated Aquatic Veterinary Workforce – Meeting the Needs of the Profession & Clients Served
12:15 PM	**Lunch**
1:15 PM	**Parker-Graham** - Pharmacokinetics of a Single Dose of Danofloxacin
1:45 PM	**Pulver** - Past, Present, and Future Perspectives on Fish Drug Development
2:15 PM	**Parker-Graham** - Effect of Anesthetic Time and Concentration on Blood Gasses, Acid-Base Status and Electrolytes in Koi (Cyprinus carpio) Anesthetized with Buffered Tricaine Methanosulfonate (MS-222)
2:45 PM	**Refreshment Break**
3:15 PM	**Parker-Graham** - Treatment of Severe Fishing Line Entanglement Injuries in a Free-Ranging Canada Goose, Branta canadensis
3:45 PM	**Hickey** - Size of Release? Time of release? What about health status at release?
4:15 PM	**Spark** - Sector Specific Biosecruity Plans: Development and Implementation
4:45 PM	**Nietlisbach** - Tilapia Health on Wisconsin's Aquaponics Farms
5:15 PM	Concluding Remarks

Fish Health Section – American Fisheries Society, 425 Barlow Place, Bethesda Maryland. USA 20814
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8th International Symposium on Aquatic Animal Health

September 2-6, 2018 - Charlottetown, Prince Edward Island, Canada

Quantitative Atlantic Salmon Health (QASH) assessment-workshop

Thursday Sept 6th, 2018; MacDonald Room

09:30 AM Welcome to QASH! – The QASH core team

Biomarkers session – Karin Pittman

09:35 AM **Powell** - A Healthy Fish Can Handle What Nature Throws At It: Allostasis in Fish Health

09:50 AM **Braceland** - Challenges in the Biomarker Pipeline

10:05 AM **Briceño** - A Risk Assessment Matrix for Smolt Welfare in Atlantic Salmon: Insights from Chile

10:20 AM **Auchterlonie** - Declining Marine Ingredient Inclusion Levels and a Hypothesized Link with Fish Health in Farmed Atlantic Salmon

Barriers and stressors session – Mark Powell

10:35 AM **Pittman** - Barrier Status in Skin, Gills and Guts: Mapping the Dynamics of the Innate Immune System throughout the Production Cycle with Statistically Robust Results

10:50 AM **Chikwati** - Gut Health Monitoring During the Seawater Phase of Farmed Atlantic Salmon in Different Production Regions of Norway - The GutMatters Project

11:05 AM **Mella** - Practical Applications of Quantitative Image-Based Assessment of Digital Pathology Slides in Chilean Salmon Industry

11:20 AM **Sveen** - Wound Healing and the Effect of Chronic Stress in Post-Smolt Atlantic Salmon (*Salmo salar*)

11:35 AM **WORKSHOP 1 - Biomarkers And Barriers – Criteria, Cutoff Levels, Long-Term Effects, And Remedial Actions? – Mark Powell and Karin Pittman**

12:00 PM **Lunch**

1:10 PM Welcome back!

Available tool boxes session

1:15 PM **Sandlund** - The Do's and Don'ts of Real Time RT-PCR As A Tool In Fish Diagnostics: Evaluating Important Parameters, Pitfalls and Results Bias

1:30 PM **Onken** - HealthPortal: Putting Production, Health and Environmental Data to Use For The Aquaculture Industry

1:45 PM **WORKSHOP 2 - Tool boxes available – ease of use, global applicability, time usage? Integrated pest management and threshold values to elicit a response? – Robin Shields**

2:15 PM Overview of Results, Comments, Volunteers for Core International QASH Proposal Work

2:45 PM The Knowledge Gaps And Missing Tools In The Toolbox; Core QASH Objectives For 2019

3:15 PM Concluding Remarks

8th ISAAH Program Overview

Sunday Sept - 2nd	Time	Room	Monday Sept - 3rd	Tuesday Sept - 4th	Wednesday Sept - 5th	Thursday Sept - 6th	Friday Sept - 7th
	7:30-8:15	Convention Foyer	Breakfast in Foyer Outside Main Ballroom				Buses for Field Trip
Buses To Workshops at AVC	8:15-8:30	Gray / Palmer / Pope	Announcements - Gray / Palmer / Pope Ballrooms				
	8:30-9:15		Keynote Speaker Dr. Daniel R. Barreda	Keynote Speaker Dr. Henning Sørum	Keynote Speaker Dr. Jerri Bartholomew	Keynote Speaker Alf-Helge Aarskog	
Pre-Conference Workshops at AVC	9:15-9:30	Transition to Simultaneous Sessions					
Publication Dos & Don'ts 9:00 am - 12:00 pm Diagnostic Testing 9:00 am - 5:00 pm	9:30-10:30	Gray / Palmer / Pope	IMMUNOLOGY-1	BACTERIOLOGY-1	MYXOZOA-1	VIROLOGY-4	
	9:30-10:30	Archibald / Campbell	FLAVOBACTERIUM-1	SEA LICE-1	ORNAMENTAL-1	HEALTH MANAGEMENT-1	
	9:30-10:30	Tilly / Tupper	AAFV	WAVMA	DISEASE of WILD FISH-1	GENOMICS-1	
	9:30-10:30	Langeve / Cartier	MICROBIOME-1	ZEBRA FISH / LAB ANIMAL	VIROLOGY-1	eDNA METAGENOMICS	
	9:30-10:30	MacDonald	INDUSTRY SESSIONS			QASH-1	
Fish Health Section Executive Meeting 10:00 am - 11:30 am MacDonald Ballroom	9:30-10:30	Coles	Posters on Display			OPEN	
	10:30-10:45	Foyer	Refreshment Break in Foyer				
Publication Dos & Don'ts 9:00 am - 12:00 pm Diagnostic Testing 9:00 am - 5:00 pm	10:45-12:15	Gray / Palmer / Pope	IMMUNOLOGY-2	BACTERIOLOGY-2	MYXOZOA-2	VIROLOGY-5	
	10:45-12:15	Archibald / Campbell	FLAVOBACTERIUM-2	SEA LICE-2	ORNAMENTAL-2	HEALTH MANAGEMENT-2	
	10:45-12:15	Tilly / Tupper	AAFV	WAVMA	DISEASES of WILD FISH-2	GENOMICS-2	
	10:45-12:15	Langeve / Cartier	MICROBIOME-2	INVERT / SHELLFISH-1	VIROLOGY-2	PARASITOLOGY-1	
	10:45-12:15	MacDonald	INDUSTRY SESSIONS			QASH-2	
	10:45-12:15	Coles	Posters on Display			OPEN	
POSTER SET UP (COLES BALLROOM) / Registration Open Delta Convention Center Foyer	12:15-1:15	Foyer	Lunch Outside Main Ballroom in Foyer				
Virtual Microscopy 1:00 pm - 5:00 pm Shellfish Necropsy 1:00 pm - 5:00 pm	1:15-1:45	Gray / Palmer / Pope	VACCINES-1	BACTERIOLOGY-3	Keynote Speaker Dr. Sabo-Attwood (1:00 Start)	VIROLOGY-6	Field Trip (All Day)
	1:45-3:00				MYXOZOA-3		
	1:15-1:45	Archibald / Campbell	TILAPIA HEALTH-1	SEA LICE-3		HEALTH MANAGEMENT-3	
	1:45-3:00			CLEANER FISH-1	NUTRITION & FISH HEALTH		
	1:15-3:00	Tilly / Tupper	AAFV	WAVMA		CO-INFECTIONS	
	1:45-3:00				AQUATIC EPI-2		
	1:15-3:00	Langeve / Cartier	LIFE CYCLES	INVERT / SHELLFISH-2		PARASITOLOGY-2	
	1:45-3:00				TOXICOLOGY / PATH		
	1:15-3:00	MacDonald	PARTNERING SESSIONS * (Partnering meetings will be held in from 12:45 – 3:05 Monday – Wednesday)			QASH-3	
	1:15-3:00	Coles	Posters on Display			OPEN	
	3:00-3:15	Foyer	Refreshment Break in Foyer				
	3:15-5:15	Gray / Palmer / Pope	VACCINES-2	BACTERIOLOGY-4	MYXOZOA-4	VIROLOGY / EMERGENT DIS	
	5:15-5:30					Closing Remarks	
	3:15-5:15	Archibald / Campbell	TILAPIA HEALTH-2	CLEANER FISH-2	EMERGENT DISEASE	OPEN	
	3:15-5:15	Tilly / Tupper	AAFV	WAVMA	ANTIBIOTICS / PHARMA	MISCELLANENOUS	
	3:15-5:15	Langeve / Cartier	GILL HEALTH	AQUATIC EPI-1	VIROLOGY-3	HUSBANDRY / PHYSIO	
	3:15-5:15	MacDonald	OPEN		OPEN	OPEN	
	5:30-6:15				FHS Business Meeting		
	3:15-5:15	Coles	Posters on Display		Poster Removal	OPEN	
Buses from Workshops to Convention Center	5:00-6:00		Official Poster Session	Official Poster Session			
Student Reception - Steeve/McGeen Room	6:00-6:30	Gray / Palmer / Pope	Buses to Off-Site Event		Banquet Reception	OPEN EVENING	
OPENING RECEPTION	6:30-8:30		Off-Site Event at Fort Amherst Historic Park	Discover Charlottetown Event at Confederation Center	ISAAH BANQUET Keynote Speaker Dr. Adel El-Mowafi		

American Fisheries Society Fish Health Section

University of Prince Edward Island

PRINCE EDWARD ISLAND BioAlliance

Keynote Presentation

Monday September 3rd

Contributions of Early Cellular Responses to Immune Protection in Fish

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The capacity of fish to defend against infection depends greatly on effective induction and control of cellular immune mechanisms. These are critical to short and long-term health, and since they are energetically costly, also relevant to fish performance. This presentation will summarize recent experiments from our lab looking to define the contributions of white blood cells to the induction and resolution of fish immune antimicrobial programs. Using combinatorial cellular and molecular approaches coupled with *in vivo* self-resolving immune challenge models (zymosan induced peritonitis and cutaneous live *Aeromonas* infection) we have defined key events in immune cell production, transport and function. These studies have also generated novel state-of-the-art tools for evaluation and promotion of fish immunity, setting the stage for valuable downstream applications in the aquaculture industry.

Monday September 3rd – Gray / Palmer / Pope Ballroom
Immunology 1 & 2
Moderators – Susan Fogelson (Fishhead Labs) Bartolomeo Gorgoglione (Wright State University)

9:30 AM	Immunology 1	Adamek - Antiviral Actions Of 25 Hydroxycholesterol In Fish Cells
9:45 AM		Maekawa - Analysis Of Immune-Related Genes Expression Response To *Vibrio harveyi* Infection In Orange-Spotted Grouper (*Epinephelus coioides*)
10:00 AM		Kato - Immune Response Against Mycobacterium Gordonae In *Ginbuna carassius auratus langsdorfii*
10:15 AM		Li - Internalization Of Large Particles By Turbot (*Scophthalmus maximus*) Igm+ B Cells Mainly Depends On Macropinocytosis
10:30 AM		**Refreshments**
10:45 AM	Immunology 2	Braden - Should I Stay Or Should I Go? Testing The Divergent Immune Hypothesis In Steelhead Salmon *Oncorhynchus mykiss*
11:00 AM		**Kaneko - Uptake And Transportation of Bacterial Antigens In The Rainbow Trout Intestinal Epithelium**
11:15 AM		**Liu - Autophagy And Infectious Disease In Rainbow Trout**
11:30 AM		**Mori - Changes In Skin Mucus Protein Profile And Immune-Related Gene Expression In Skin Of Japanese Flounder *Paralichthys olivaceus* Fed A Diet Supplemented With High Concentrations Of Ascorbic Acid**
11:45 AM		Karsi - Live Attenuated *Edwardsiella ictaluri* Vaccines Stimulate Active Phagocytosis And Bacterial Killing Of Catfish B Cells

Antiviral Actions of 25 Hydroxycholesterol in Fish Cells

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Cholesterol is essential for building and maintaining cell membranes, is the main component of lipid rafts and by this modulates membrane fluidity. This makes cholesterol critical for several steps in the viral replication cycle, especially for enveloped viruses. Virus infections of mammalian cells, lead to the induction of the oxysterol 25 hydroxycholesterol (25HC), a soluble antiviral factor, which is produced from cholesterol by activation of cholesterol 25 hydrolase (CH25H). Immune responses based on CH25H were largely not studied in fish. Therefore, putative genes encoding for CH25H were identified and amplified in common carp and rainbow trout and an HPLC-MS method was established for measuring oxysterols in fish cells. Our results give substantial evidence that CH25H activation is an antiviral response against a very broad spectrum of viruses in both common carp and rainbow trout cells *in vitro*. Furthermore, the *ch25h* expression is also modulated *in vivo* in common carp during viral infections. HPLC-MS analyses showed that even fibroblastic cells are capable of producing 25HC and its metabolite 7a,25diHC. The 25HC had an antiviral activity by blocking the entry of *cyprinid herpesvirus 3* (CyHV-3) but not *spring viremia of carp virus* (SVCV) and common carp paramyxovirus (CCPV) in KFC cells and *viral haemorrhagic septicaemia virus* (VHSV) and *infectious pancreatic necrosis virus* (IPNV) in RTG-2 cells. The stimulation of RTG-2 cells with rainbow trout recombinant type I IFN provided further evidence that despite the fact that CH25H based antiviral response coincides with type I IFN responses; it is not type I IFN dependent. Interestingly, the vulnerability of CyHV-3 to 25HC is counteracted by a downregulation of the *ch25h* gene expression in carp fibroblasts during CyHV-3 infection.

Conference Session Designation: (Immunology General)
Presentation Format: (Oral)

Analysis of Immune-Related Genes Expression Response to *Vibrio harveyi* Infection in Orange-Spotted Grouper (*Epinephelus coioides*)

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Vibrio harveyi is a Gram-negative fish pathogenic bacterium. To investigate the expression of immune related genes responding to *Vibrio harveyi* infection, in this study, we performed transcriptome analysis of kidney and spleen in orange-spotted grouper (*Epinephelus coioides*) after *Vibrio harveyi* infection. A total of 79,128 unigenes were obtained after de novo assembly using the Illumina sequencing platform. The numbers of total differentially expressed genes (DEGs) in head kidney at 1 dpi, head kidney at 2 dpi, spleen at 1 dpi, and spleen at 2 dpi were 7918, 4260, 7887 and 8952, respectively. The differentially expressed genes were mainly annotated into signal transduction and immune system based on KEGG data base. The DEG were enriched in immune related pathway, such as Toll-like receptor signaling pathway, NOD-like receptor signaling pathway, NF-kappa B signaling pathway and Jak-STAT signaling pathway. In the spleen at 1 and 2 dpi, the expression levels of genes related to NF-kB signaling were up-regulated, including cytokines (IL-lb, RANKL), and NF-kB signaling mediators (Myd88, TAK1, IKKa, p50). These results indicated that NF-kB signaling was activated by *V. harveyi* infection. In the kidney at 2 dpi, while these immune-related genes were down-regulated. Therefore, it is suggested that the spleen is the primary organ for immune responses to *V. harveyi* infection rather than head kidney in orange-spotted grouper. These data will offer the functional analysis of immune-related genes in orange-spotted grouper against *Vibrio harveyi* infection.

Conference Session Designation: (Immunology General)
Presentation Format: (Oral)

Immune Response Against *Mycobacterium gordonae* in Ginbuna *Carassius auratus langsdorfii*

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Mycobacteriosis causes severe economic losses of fish production in aquaculture industry worldwide. Recently, we isolated Mycobacterium gordonae from diseased koi carp from some culture ponds in Niigata, Japan. In this study, we investigated the immune responses against M. gordonae in ginbuna crucian carp as a model fish species. An isogenic clone of ginbuna crucian carp (OB1 clone) were intraperitoneally injected with 2.0 × 107 CFU/fish of M. gordonae and cumulative mortality was recorded for 170 days. The trunk kidney was sampled from ginbuna injected with 1.9 × 108 CFU/fish at 1, 3, 7, 14, 21 and 28 days post-infection. Gene expression levels of CD4-1, CD4-2, CD8α, IFN-γ2 and T-bet in the trunk kidney were determined by quantitative RT-PCR. Furthermore, hematoxylin-eosin (HE), Ziehl-Neelsen (ZN), immunohistochemistry using anti-ginbuna IFN-γ and anti-ginbuna CD4-1 polyclonal antibody were performed using paraffin sections of the trunk kidney samples. Cumulative mortality was 50% in M. grodonae challenged fish at 170 days post-infection, while no mortality was observed in PBS-injected fish. CD4-1 and CD8α gene expression level was significantly up-regulated in M. gordonae-infected fish at 21 and 28 days post-infection, whereas the level of CD4-2 was not changed during the experiment. Gene expression levels of T-bet and IFN-γ2 was up-regulated during 7 to 28 days post-infection in the infected fish, suggesting the dominance of Th1 immunity. Granulomatous responses consisted of central macrophage accumulation and surrounding lymphocytes and ZN-positive bacterial cells were seen through the damaged area. Immunohistochemistry revealed that the marginal lymphocytes were positive for anti-ginbuna CD4-1 antibody, and the IFN-γ producing cells were also located surrounding the mycobacterial cells-laden phagocytes. These results clearly indicate that CD4-1 positive cells participate in granuloma formation in teleost fish. In addition, IFN-γ2 may play important roles in the granulomatous inflammation in teleost fish.

Conference Session Designation: (Immunology General)
Presentation Format: (Oral)

Internalization of Large Particles by Turbot (*Scophthalmus maximus*) Igm$^+$ B Cells Mainly Depends on Macropinocytosis

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Increasing evidence has demonstrated support for the endocytic capacities of teleost B cells. In the present study, the ability of turbot IgM$^+$ B cells to ingest microspheres of different sizes and the corresponding internalization pathways were investigated. The results showed that IgM$^+$ B cells exhibited relatively high endocytic capacities for 0.5μm and 1μm latex beads, and that different mechanisms were employed for IgM$^+$ and IgM$^-$ cells to uptake 0.5μm and 1m beads. For 0.5μm beads, IgM$^+$ B cells apparently employed macropinocytosis-dependent endocytic pathway, whereas IgM- cells utilized a different process involving both clathrin- and caveolae-mediated pathways. For the uptake of 1 μm beads, IgM$^+$ cells relied mainly on macropinocytosis and partially on caveolae-mediated pathway, while IgM- cells utilized the routes similar to that of internalizing 0.5μm beads. Consistently, the internalized microspheres were co-localized with high-molecular-mass dextran in IgM$^+$ phagocytic cells. In addition to latex beads, IgM$^+$ B cells could also ingest inactivated bacteria predominately through macropinocytosis and caveolae-mediated endocytosis. These results collectively indicated that macropinocytosis is principally responsible for particle uptake by turbot IgM$^+$ B cells.

Conference Session Designation: (Immunology General)
Presentation Format: (Oral)

Should I Stay or Should I go? Testing the Divergent Immune Hypothesis in Steelhead Salmon *Oncorhynchus mykiss*

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The process of smoltification in anadromous fishes is extremely energetically demanding, and so a common strategy is to temporarily divert energy from less important physiological systems in order to maximize survival during this stressful transition. Earlier work characterizing the transcriptomic response of rainbow trout, *Oncorhynchus mykiss*, suggested that the immune system of resident (fresh water, rainbow trout) was divergent from that of migratory (anadromous, steelhead salmon) *O. mykiss*. Resident fish showed divergent transcriptomic profiles in the gills compared to migratory fish, with several markers of dendritic cell (DCs) differentially expressed between the groups. However, the cell population responsible for this response in the gills was never characterized or identified. Thus, the aim for this present work was, 1.) to test the divergent immune hypothesis by characterizing more DC markers in an unknown population of *O. mykiss*, and, 2.) to localize and characterize the cells expressing these markers in the gills of this population. To achieve this, equal portions of the right gill arch of ten fish of unknown status (i.e., either resident or migratory) from the same population of the above study (Abernathy Creek, Columbia River system, Washington, US) were dissected from euthanized fish and placed in either RNAlater™ for downstream genetic analysis, or 10% buffered formalin for immunohistochemistry. Resulting transcript abundance of several markers of DCs (*cd83, cd80/86, mh□ii, clec4m,* and *cd209*) and smoltification (*s100a4, nka*) was used as input for principal component and clustering analysis. The data was clearly delineated into two distinct clusters composed of resident or transient fish, with expression of *s100a4* and *nka* positively correlated with expression of immature DC markers *cd209* and *clec4* while negatively correlated with expression of mature DC markers *cd83* and *cd80/86*. Furthermore, we identified $CD83^+$, $CD80/86^+$, and $MH□II^+$ cells in the gills of resident and migratory fish concordant with gene expression. Taken together, this present work supports the existence of a divergent immune strategy by *O. mykiss*, whereby the gills of migratory populations are characterized by immature DCs, while those of resident fresh-water populations are characterized by maturing DCs. The significance of these findings in respect to markers of smoltification and differential pathogen exposure will be discussed.

Conference session designation: (Immunology general)
Presentation format: (Oral)

Uptake and transportation of bacterial antigens in the rainbow trout intestinal epithelium

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In mammals, microfold (M) cells and goblet cells can take up bacterial antigen and transport it across the intestinal epithelium. In the teleost intestinal epithelium, M-like cells (lectin binding: UEA-1^+ WGA^-) and goblet cells (UEA-1^+ WGA^+) are considered to be antigen-sampling cells. However, the mechanisms underlying uptake and translocation of the bacterial antigen are still unclear. In this study, we investigated the antigen uptake and antigen transportation across the intestinal epithelium in rainbow trout after rectal administration of formalin-inactivated bacteria.

Rainbow trout were rectally administrated with five inactivated bacterial pathogens (*Aeromonas salmonicida* subsp. *salmonicida*, *Vibrio anguillarum* J-O-1, *V. anguillarum* J-O-2, *V. anguillarum* J-O-3 or *Streptococcus iniae*) at a dose of 2.2-5.0 × 10^8 CFU/ml, and the intestine was sampled at 30 min post-administration. Paraffin-embedded section of the intestine was subjected to immunofluorescent staining with rabbit anti-serum raised against each bacterium and subsequently with either anti-macrophage antibody, UEA-1 or WGA. Furthermore, rainbow trout were rectally administrated with five inactivated bacteria stained with syto61 and the intestine was sampled at 30 min post-administration. The epithelial cells were isolated from the intestine, stained with UEA-1 and analyzed by flow cytometry.

Immunofluorescent staining revealed that the bacterial antigens were taken up by goblet cells (UEA-1^+ WGA^+) in the intestinal epithelium. FS-SS plot of the intestinal epithelial cells in flow cytometry showed the increase of SS value in UEA-1^+ cells after the bacterin administration, suggesting that mucus granules increased in the goblet cells. However, antigen sampling by M-like cells (UEA-1^+ WGA^-) was not observed in this study. In addition, the inactivated bacterial antigens were also found in the lamina propria and were phagocytosed by macrophages. These results suggest that the goblet cells in the rainbow trout intestinal epithelium mainly take up bacterial antigen and transport it to macrophages in the lamina propria.

Conference Session Designation: (Immunology General)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Autophagy and Infectious Disease in Rainbow Trout

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Eukaryotic cells normally employ autophagy to degrade cellular components including proteins and organelles. This highly evolutionarily-conserved mechanism is present in eukaryotic cells from yeast to mammals and the most important autophagy-related genes have been identified in some teleosts. Some unfavorable conditions such as nutrient restriction can induce autophagy. Increasing evidence indicates that autophagy is involved with both the innate and adaptive immune responses and that it influences the success of infectious agents. Previous research in our laboratory revealed that feed restriction and deoxynivalenol (DON) reduced the mortality of rainbow trout to experimental *Flavobacterium psychrophilum* infection. The aim of our research is to elucidate the possible role that autophagy plays in rainbow trout resistance to *F. psychrophilum* and to viral hemorrhagic septicaemia virus (VHSV). The first objective was to confirm that autophagy can be regulated by nutritional restriction, selected chemicals and VHSV. Autophagy in rainbow trout gill epithelial cells (RTgill-W1) was induced by VHSV infection, and low serum concentration (2% FBS), but was blocked by chloroquine (CQ) and suppressed by DON. However neither the widely used autophagy inhibitor, 3-methyladenine, nor autophagy activator, rapamycin, acted consistently as expected. Similar *in vitro* results were also observed in a rainbow trout liver cell line, RTL-W1. The second objective was to apply ration restriction and chemical mediators, e.g. CQ and DON to examine their effect on survival in *in vivo* experimental infection trials with *F. psychrophilum*. The results from this research may help us to develop new directions and strategies for disease management in aquaculture.

Conference Session Designation: (Immunology General)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Changes in Skin Mucus Protein Profile and Immune-Related Gene Expression in Skin of Japanese Flounder *Paralichthys Olivaceus* Fed a Diet Supplemented with High Concentrations of Ascorbic Acid

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Ascorbic acid (AsA), commonly known as vitamin C, is an essential vitamin for normal growth and physiological function in fish. This dietary material is known to be beneficial for immune responses in fish, and increased disease resistance has been demonstrated in many fish species fed elevated levels of AsA. However, detailed knowledge concerning the beneficial effects of AsA supplementation using high concentrations of AsA with regard to the innate immune system of mucosal tissue remains to be determined. Therefore, the present study employed two-dimensional gel electrophoresis (2D-PAGE) to examine skin mucus of Japanese flounder *Paralichthys olivaceus* fed commercial diets supplemented with 2,000 mg AsA/kg diet (AsA2000) for 7 days. Compared to control fish fed a diet without AsA (AsA0), five factors were specifically detected from those fed AsA2000 and as identified by LC-MS/MS. We also performed quantitative reverse transcriptase PCR (qRT-PCR) of factors detected by 2D-PAGE and general immune-related genes from skin samples of AsA0 and AsA2000 fish. As a result, a factor with antibacterial activity was identified which was significantly up-regulated only following AsA supplementation. In conclusion, this research presents protein composition profiles of skin mucus and provides information demonstrating improvement of the mucosal immune system following high-concentration AsA supplementation in fish.

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Conference Session Designation: (Immunology General)
Presentation Format: (Oral)
Student Presentation: (Yes)

Live Attenuated *Edwardsiella ictaluri* Vaccines Stimulate Active Phagocytosis and Bacterial Killing of Catfish B Cells

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Edwardsiella ictaluri is a Gram-negative, facultative intracellular pathogen and the causative agent of enteric septicemia of catfish, a devastating disease of farmed channel catfish. B-1 and marginal zone B cells in mammals have phagocytic abilities and contribute to innate immune responses. The innate roles of B cells have been demonstrated in several teleost fish including zebrafish, rainbow trout, and channel catfish. Recently, our group developed two protective *E. ictaluri* live attenuated vaccines (LAVs). However, their interaction with catfish B cells has not been evaluated yet. In this study, we assessed the effects of *E. ictaluri* LAVs on B cells' survival, phagocytosis, and microbial killing by flow cytometry. Results indicated that *E. ictaluri* wild-type (*Ei*WT) opsonized with sera from vaccinated and non-vaccinated catfish were engulfed by catfish B cells. However, *Ei*WT opsonized with serum from catfish survived from *Ei*WT challenge significantly decreased the numbers of B cells compared to *Ei*WT opsonized with serum from vaccinated catfish. Furthermore, catfish B cells killed *Ei*WT opsonized with sera from vaccinated catfish more efficiently compared to *Ei*WT opsonized with normal serum. Finally, we demonstrated that *Ei*WT and two LAVs induced early and late apoptotic changes in catfish B cells. These results indicate that *E. ictaluri* LAVs were enhanced active phagocytosis and killing of internalized bacteria in catfish B cells.

Conference Session Designation: (Immunology General)
Presentation Format: (Oral)

Monday September 3rd – Gray / Palmer / Pope Ballroom
Immunology Vaccines 1
Moderator – Simon Menanteau-Ledouble (University of Veterinary Medicine – Vienna)

1:15 PM	Immunology Vaccines 1	Wallis - Autogenous Vaccines In Principle And Practice
1:30 PM		
1:45 PM		Aarattuthodiyil - Fish Vaccination – Factors To Consider
2:00 PM		**Delphino - Economic Evaluation Of Vaccination Against *Streptoccocus agalactiae* In Nile Tilapia Farms**
2:15 PM		Powell - Immersion Vaccination Research For Aquatic Animals Guided By Computer Assisted Laser Scanning Cytometry
2:30 PM		Braden - Vaccine-Induced Protection Against Infection With *Aeromonas salmonicida* Subsp *salmonicida* In Arctic Charr *Salvelinus alpinus* Involves Pre-Emptive Priming Of Humoral Immunity
2:45 PM		Bruce - Cross-Protective Ability Of A Live Attenuated Coldwater Disease Vaccine In Juvenile Rainbow Trout

Autogenous Vaccines in Principle and Practice

Timothy S. Wallis* and J. Gustavo-Ramirez Paredes

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Autogenous vaccines are farm-specific vaccines formulated with antigens derived from pathogens isolated from disease outbreaks on the farm. They can be used when fully licensed vaccines have proven to be ineffective or are unavailable. They should be used under the supervision of the responsible veterinary surgeon. The development of anti-microbial resistance is having a significant impact on antibiotic use in food producing animals. Autogenous vaccines can be a useful tool for the control of infectious diseases thereby reducing the need for undesirable antibiotic interventions. Autogenous vaccines are widely used in poultry, pig, cattle and aquaculture industries for disease control. We have developed and supplied immersion (dip & bath) and injectable mono- and multi-valent vaccines for the control of a wide range of pathogens (bacterial and viral) in farmed salmonids, sea-bass, tilapia and cleaner-fish i.e. ballan wrasse and lumpsuckers. New molecular typing methodology means that strain variation on farm can be readily understood relatively cheaply and bespoke vaccines can be formulated accordingly targeting the relevant variants on farm. Aspects relating to production and quality together with case studies will be presented. However autogenous vaccines are not an alternative to conventional vaccines if effective, good nutrition and good animal husbandry.

Conference Session Designation: (Vaccines)
Presentation Format: (Oral)

Fish Vaccination – Factors to Consider

Suja Aarattuthodiyil *, Todd Byars, Matt Griffin, Lester Khoo, Terrence Greenway, Ganesh Kumar, and David Wise

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Aquaculture industry is seriously impacted by several diseases. Although vaccination can minimize mortality due to some of these diseases, the effectiveness of a vaccine is dependent on the cross protection offered against multiple isolates. For example, in the case of Enteric Septicemia of Catfish (ESC), caused by the bacteria, *E. ictaluri*, traditionally, losses have been controlled by withholding feed from fish to reduce the oral route of infection combined with medicated feeds. Recently, a live attenuated ESC vaccine is delivered orally to catfish. Under laboratory conditions, the vaccine was shown effective against the parental wild-type strain and proved to be safe at 10 times the applied target dose. While live vaccines are very effective in providing long lasting immunity against disease, vaccine safety and efficacy could be compromised, if delivered to animals in suboptimal health or under stressful conditions. In a compromised animal, attenuated vaccine can cause infections leading to morbidity and mortality. Another key factor in field vaccinology is antigenic variation among pathogenic species, where immunization with a vaccine derived from one strain does not provide protection against genetic variants of the same species. Vaccinated and control fish were challenged with the wild type *E. ictaluri* isolate 30 days post-vaccination. Low oxygen stress did not induce any post-vaccination mortality in any of the vaccinated treatments. Similarly, all groups of vaccinated fish, regardless of stress treatment, were protected against virulent *E. ictaluri* infection. Data indicated that acute oxygen deprivation, before or after vaccination, does not alter vaccine safety and efficacy, however the effects of chronic long term stress have not been evaluated. Therefore, short acute stressors are unlikely to influence vaccine safety and efficacy and provides valuable insight in developing commercial vaccination protocols. Additional trials were conducted to determine if the attenuated isolate afforded protection against 23 archived field isolates collected over a time span of twenty years (1997-2016). Vaccination followed by bacterial challenge with archived isolates were conducted over a three year period. In all trials, vaccination was shown to protect catfish against all challenge isolates, regardless of host species, geographic region (state and farm location) or isolation year. While on farm vaccination greatly improved survival, yield and fish net-value, limited mortality was observed in vaccinated pond populations. Results indicated that mortality observed in farm vaccinated fish populations was not related to antigenic variations among isolates. The most likely cause of on-farm mortality was related to unequal distribution of vaccine laden feed to individual fish, an inherent problem with mass delivery of oral vaccines to large populations of animals. In order to differentiate between the 23 isolates, their clonal relation were determined. The PCR profile indicated relative homogeneity among the isolates dating back to 1997. This further confirmed the results which indicated no significant difference between the isolates. The clonal nature of *E. ictaluri* isolates demonstrated by our data negates the need to develop multivalent vaccines or construct new vaccines to account for antigenic variation occurring over time. Commercial vaccination trials are showing net economic benefit of $2000 to $3000/acre for channels and hybrid fingerling production phase.

Conference Session Designation: (Vaccines)
Presentation Format: (Oral)

Economic Evaluation of Vaccination Against *Streptoccocus agalactiae* in Nile Tilapia Farms

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Streptococcus agalactiae causes mortality and major economic losses in Nile tilapia (*Oreochromis niloticus*) farming worldwide. In Brazil, serotype strains Ia, Ib and III have been isolated in streptococcosis outbreaks, but serotype Ib is the most prevalent. Vaccination is considered an effective method to prevent economically-important diseases in aquaculture and has been associated with decreased usage of antibiotics and improvements in fish survival. We developed a simple and flexible partial-budget model to undertake an economic appraisal of vaccination against *Streptococcus agalactiae* in Nile tilapia farmed in net cages in large reservoirs. The model considers the benefits and costs that are likely to occur in one production cycle (time for fish to reach the marketable size), because of the proposed intervention. We analysed three epidemiological scenarios of cumulative mortality due to *S. agalactiae* (5%, 10% and 20%, per production cycle) in a non-vaccinated farm. For each scenario, we calculated the net return (benefits – costs) of vaccination, given a combination of values of "vaccine efficacy" and "gain in feed conversion ratio", in order to model uncertainty about the true value of such parameters. Results indicate that vaccination against *S. agalactiae* is likely be profitable in Nile tilapia farms, although in scenarios where cumulative mortality is lower than 10%, the profitability of vaccination would be more dependent on higher vaccine efficacy and/or better feed conversion ratio.

Conference Session Designation: (Latin American Fish Health)
Presentation Format: (Oral)
Student Presentation: (Yes)

Immersion Vaccination Research for Aquatic Animals Guided by Computer Assisted Laser Scanning Cytometry

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Immersion vaccination can be a rapid and cost-effective method to stimulate the immune system of fish against pathogens. Enhancement of antigen uptake during immersion vaccination may lead to increased vaccine efficacy and improved survival against disease. A variety of methods and materials have been reported to facilitate the uptake of foreign particulate antigens in fish, and fluorescent microspheres have been used in studies of particulate antigen uptake and processing by the host. Recent advances in solid phase laser scanning cytometry enabled development of a method to more accurately quantify adhesion and uptake of fluorescent microspheres by mucosal fish tissues. One-micron fluorescent microspheres were applied in combination with immersion vaccine formulations to quantify their adhesion to gill and skin tissues of rainbow trout under different vaccination conditions. We were particularly interested in the effect of adipose fin clipping on particle uptake by skin tissue and whether the wound site would be more susceptible to particle binding. A substantial increase in particle uptake occurred at the site of the fin clip wound compared to unclipped control fins. The use and concentration of hyperosmotic saline immersion treatments, MS-222 anesthesia, and Seppic Montanide™ adjuvants were also investigated. The results are relevant to decisions regarding the timing of immersion vaccination and potential methods used to improve their efficacy.

Conference Session Designation: (Immunology Vaccines)
Presentation Format: (Oral)

Vaccine-Induced Protection Against Infection with *Aeromonas salmonicida* Subsp *salmonicida* in Arctic Charr *Salvelinus alpinus* Involves Pre-Emptive Priming of Humoral Immunity

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With respect to salmonid aquaculture, one of the most important bacterial pathogens in terms of economic loss due to mortality and antibiotic usage is the causative agent of typical furunculosis, *Aeromonas salmonicida* spp. *salmonicida* (*Asal*). In Atlantic salmon, *Salmo salar*, the host response during infections with *Asal* is well documented; however, less is known about the host-pathogen interactions in the emerging aquaculture species, Arctic charr, *Salvelinus alpinus*. Furthermore, there is no data on the efficacy or response of this species during vaccination with commonly administered vaccines against furunculosis. To this end, we were interested in examining the immunological response of *S. alpinus* during infection with *Asal*, with or without administration of vaccines (Forte Micro®, Forte Micro® + Renogen®, Elanco Animal Health). Artic charr (vaccinated or unvaccinated) were i.p.-injected with a virulent strain of *Asal* (10^5 CFUs/0.1mL) and tissues were collected pre-infection/post-vaccination, 8, and 29 days post-infection. By 8 dpi, *Asal* bacterial load in sham fish, as assessed by *aopO* qPCR, was 4-fold higher than both vaccinates. Unvaccinated charr were extremely susceptible to *Asal* with 72% mortalities observed after 29 days. However, there was 72-82% protection in fish vaccinated with either the single or dual-vaccine, respectively. Protection in vaccinated fish was concordant with significantly higher *Asal*-specific serum IgM concentrations, and following RNA sequencing and transcriptome assembly, differential expression analysis revealed several patterns and pathways associated with the improved survival of vaccinated fish. Most striking was the dramatically higher basal expression of complement/coagulation factors, acute phase-proteins (APRs), and metal homeostasis proteins in pre-challenged, vaccinated fish. Interestingly, following *Asal* infection, this response was abrogated and instead, the transcriptome was characterized by a much weaker immune response compared to that of non-vaccinated fish. Furthermore, where pathways of actin assembly and Fc□R-mediated phagocytosis were significantly dysregulated in non-vaccinates, vaccinated fish showed either the opposite regulation (ForteMicro®), or no impact at all (ForteMicro®+Renogen®). The present data indicates that vaccine-induced protection against *Asal* relies on the priming of complement and other APRs, which influences cell-cell interactions, possibly in favour of B-cell survival and enhanced serum IgM production following challenge.

Conference session designation: (Immunology General)
Presentation format: (Oral)

Cross-protective Ability of a Live Attenuated Coldwater Disease Vaccine in Juvenile Rainbow Trout

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Bacterial coldwater disease (BCWD) caused by *F. psychrophilum* remains one of the most significant bacterial diseases of salmonids worldwide and impacts many government and commercial operations across North America and globally. A live attenuated immersion vaccine (B.17-ILM) has been developed at the University of Idaho and has been shown to confer significant protection to salmonids. To further characterize this novel vaccine, a series of challenge trials were carried out to determine the cross-protective efficacy of this live attenuated vaccine against 9 virulent *F. psychrophilum* isolates in comparison with a domestic, wild-type virulent strain. The 9 *F. psychrophilum* isolates of various sequence types (STs) were assessed for virulence using an injection-based challenge model prior to the initiation of a vaccination trial. To assess protection in juvenile rainbow trout, two separate 28-day challenge trials were conducted following immersion vaccinations with the B.17-ILM vaccine. All vaccinated fish developed an adaptive immune response (as measured by *F. psychrophilum*-specific antibodies) that increased out to the time of challenge (8 weeks post-immunization). All isolates demonstrated virulence at the time of initial testing, with cumulative percent mortality (CPM) rates ranging from 25.3% to 88.0%. Following vaccination and subsequent challenge, the immersion vaccine was shown to provide significant protection against all *F. psychrophilum* strains tested, with relative percent survival (RPS) values ranging from 51-72%. Similar RPS values were observed in fish challenged with ST10 group isolates. The ability of vaccine-specific antibodies to bind to similar antigenic proteins or LPS components for all *F. psychrophilum* strains was determined by Western blot analyses. It was shown that serum antibodies recognize a common 65 kDa antigen across all isolates, suggesting that this protective antigen is shared widely. Results suggest that this live attenuated vaccine will elicit a protective immune response to fish and provide a valuable tool for BCWD control even in aquaculture operations affected by diverse strains of *F. psychrophilum*. Additional studies to assess the cross-protective ability of this vaccine against other emerging Flavobacterial and non-Flavobacterial pathogens are underway.

Conference Session Designation: (Vaccines / Flavobacteria)
Presentation Format: (Oral)

Monday September 3rd – Gray / Palmer / Pope Ballroom
Immunology Vaccines 2
Moderator – Alex Primus (University of Minnesota)

3:15 PM	Immunology Vaccines 2	Karsi - Pathological And Immunological Assessment of Live Attenuated Vaccines Against Enteric Septicemia Of Channel Catfish
3:30 PM		**Kitiyodom - Mucoadhesive Nanoparticles As An Effective Delivery System For Fish Immersion Vaccination**
3:45 PM		**Sebastião - Evaluation Of Live Attenuated And Recombinant Subunit Vaccines Against Piscine Francisellosis**
4:00 PM		Sommerset - Comparative Analysis Of Performance In Vaccinated And Unvaccinated Atlantic Salmon Under Different O2 And Temperature Regimes
4:15 PM		Midtlyng - On The Way To New Batch Potency Tests For *Moritella viscosa* Vaccines: Antibody Response And Protective Immunity Correlate In A Dose–Response Manner
4:30 PM		Sandtro-Lunheim - Vaccination Against Yersiniosis In Atlantic Salmon - Experiences And Challenges
4:45 PM		Menanteau-Ledouble - Effect Of Immunostimulatory Feed Additives On The Response Of Rainbow Trout *Oncorhynchus mykiss* To A Commercial Vaccine Against *Yersinia ruckeri*
5:00 PM		Tingbo - Development Of Yersiniosis Vaccines For Atlantic Salmon

Pathological and Immunological Assessment of Live Attenuated Vaccines Against Enteric Septicemia of Channel Catfish

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Enteric septicemia of catfish (ESC) caused by *Edwardsiella ictaluri* is one of the most important bacterial diseases of farmed catfish in the United States. Use of live attenuated vaccines (LAVs) is an effective strategy for combating ESC mortalities in catfish farms. Our research group has developed two safe and efficacious live attenuated *E. ictaluri* vaccine strains (*Ei*Δ*evpB* and *Ei*Δ*gcvP*Δ*sdhC*Δ*frdA*) against ESC. In this study, we present mucosal uptake and pathology of LAVs in catfish fry. We also provide LAVs' effects on expression of innate and adaptive immune genes as well as pronephros lymphomyeloid cells in catfish fry. Results indicated that there were significant differences between the *E. ictaluri* wild-type (*Ei*WT) and vaccinated groups during vaccination and following *Ei*WT challenge of vaccinated catfish. Pathologically, LAVs were safer and showed no (Aquavac-ESC and *Ei*Δ*evpB*) or minor (*Ei*Δ*gcvP*Δ*sdhC*Δ*frdA*) pathological lesions during vaccination. However, *Ei*WT challenge of the vaccinated catfish fry indicated that *Ei*Δ*gcvP*Δ*sdhC*Δ*frdA* had less pathological lesions with fewer bacteria than Aquavac-ESC, *Ei*Δ*evpB*, and sham groups. Immunologically, in contrast to the vaccinated groups, a significant increase in expression of immune genes was observed in the *Ei*WT exposed control fry during vaccination, and the number of lymphomyeloid cells was reduced. Following *Ei*WT challenge of vaccinated catfish fry, a significant increase in expression of immune genes was observed, and the number of lymphomyeloid cells was increased. These findings support that *Ei*Δ*evpB* and *Ei*Δ*gcvP*Δ*sdhC*Δ*frdA* have improved vaccine properties compared to the commercial vaccine Aquavac-ESC.

Conference Session Designation: (Immunology Vaccines)
Presentation Format: (Oral)

Mucoadhesive Nanoparticles as an Effective Delivery System for Fish Immersion Vaccination

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It is well established that vaccination is the most effective approach for prevention of infectious diseases in fish. In fact, fish vaccines are mostly administered through major three routes of administration as bath or immersion, second through in-feed or oral and the third by injection. While immersion vaccination is more applicable, but this method suffers from low potency as the efficiency of uptake of antigens through the gills and skin are limited. In this study, we have successfully developed a mucoadhesive vaccine delivery system to circumvent this problem. We chose *Flavobacterium columnare*, the causative agent of columnaris disease, as a representative model antigen for a proof-of-concept study. The sonicated bacterial suspension was used to prepare nanovaccines through emulsification and homogenization techniques followed by coating with mucoadhesive polymer chitosan. The analysis of hydrodynamic diameter and zeta-potential also suggested the successful modification of nanovaccines by chitosan. The chitosan modified nanovaccines were positively charged and the overall diameter also increased. The prepared vaccines were nano-sized and spherical as confirmed by transmission electron microscopy (TEM). The *ex vivo* bioluminescence imaging showed excellent mucoadhesive property of nanovaccines coated with chitosan. Tilapia fishes were vaccinated with the prepared nanovaccines by brief immersion. The challenge test was then carried out 60 days post-vaccination and resulted in 90% mortalities in the control. Interestingly, the relative percent survival (RPS) of vaccinated fish was calculated at 89 for mucosal nanovaccine. In conclusion, we could use this system to deliver antigen preparation to the mucosal membrane of tilapia fishes and induce appropriate immune responses, resulting in a significant increase in survival compared to controls. Therefore, targeting mucoadhesive nanovaccines to the mucosal surface could be exploited as an effective method for immersion vaccination.

Conference Session Designation: (Immunology Vaccines)
Presentation Format: (Oral)
Student Presentation: (Yes)

Evaluation of Live Attenuated and Recombinant Subunit Vaccines Against Piscine Francisellosis

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Francisella noatunensis subsp. *orientalis* (*Fno*) is a bacterial pathogen of marine and fresh water fish worldwide. To date, there is no approved vaccine for this emergent disease. To better characterize immunodominant *Fno* antigens, proteomic analyses was performed in previous studies using serum collected from laboratory challenged Nile tilapia (*Oreochromis niloticus*). We hypothesized that some of these proteins could be used as recombinant subunit vaccines. In this study, the efficacy of recombinant subunit vaccine candidates was compared to two attenuated *Fno* strains previously proposed as plausible live vaccines and compared to non-vaccinated tilapia fingerlings against piscine francisellosis. Recombinant vaccine candidates included *Fno iglA, iglB, iglC,* and *vgrG* cloned into an *Escherichia coli* expression vector (pBAD (Life Technologies) inactivated with formalin or heat. Other treatments investigated were formalin and heat inactivated Top10 cells with empty vector, purified IglA (0.2 mg/ml), live attenuated Δ*iglC* and Δ*pdpA* strains, as well as mock-vaccinated (PBS) control. Approximately 10^7 CFU inactivated Top10 cells expressing the *Fno iglA, iglB, iglC,* and *vgrG,* or non-cloned pBAD vector mixed with 70% of Montadine adjuvant were used to immunize tilapia via intracoelomic injection. Similar amounts of live attenuated mutants were used for comparison. Each treatment consisted of 45 fish divided in triplicate tanks containing flow-through fresh water at 23±2°C. Approximately 690-degree days post-immunization, fish were challenged via immersion with 10^5 CFU/ml of wild-type *Fno* at 17±1°C. Mortality was monitored daily for 4 weeks. At the end of challenge, the mean percent mortality for each treatment were as follows: non-vaccinated (65%), *iglB*-F(58%), Top10-F (53%), *iglA*-P (51%), *iglB*-H (50%), Top10-H (47%), *iglA*-F (45%), *iglC*-F (44%), *vgrG*-H (38%), *iglA*-H (36%), *vgrG*-F (35%), Δ*pdpA1* (7%) and Δ*iglC* (4%), providing a relative percent survival for the vaccinated fish of 11%, 19%, 21%, 23%, 28%, 30%, 32%, 41%, 42%, 45%, 46%, 90% and 95%, respectively. Tilapia vaccinated with the recombinant subunit vaccines tested in this study and subsequently challenged with wild type *Fno* did not present significantly lower mortality when compared to non-vaccinated controls ($p>0.05$). However, significant differences were observed in fish vaccinated with the Δ*pdpA* and *Δig1C* attenuated strains when compared to non-vaccinated controls ($p<0.05$). This information demonstrates that live attenuated vaccines have higher efficacy at inducing a protective immune response in Nile tilapia fingerlings against francisellosis when compared to recombinant subunit vaccines developed so far.

Conference Session Designation: **(** Immunology Vaccines)
Presentation Format: (Oral)
Student Presentation: (Yes)

Comparative Analysis of Performance In Vaccinated and Unvaccinated Atlantic Salmon Under Different O2 and Temperature Regimes

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Growth-impairment is a well-known adverse effect of compulsory intraperitoneal immunisation with oil-based vaccines administered to commercially farmed Atlantic salmon (*Salmo salar L*). While the vaccine protection is well documented, there have been few studies focusing on the potential negative effects of vaccination and the environmental factors influencing the degree of these. As the temperature and the oxygen saturation of the water might fluctuate at and around the time of vaccination, a controlled study aimed to explore the effects of the variation of these parameters was carried out at a research facility resembling commercial farming conditions for the fish. Both vaccinated and sham-vaccinated salmon were exposed to combinations of different temperatures (12 and 17°C) and oxygen saturation (60 and 100% O2) in the post vaccination period. In addition, the different groups were exposed to a smoltification signal by switching from a 12h light – 12h darkness period to a 24h light regime one day after vaccination. Comparative measurements of body mass and length and Speilberg scoring of local adverse reactions in the abdominal cavity were carried out. Samples were secured from the gills, the brain and from the head kidney for qPCR to evaluate responses to the environmental parameters, and blood was sampled to assess the antibody response to the immunisation by an ELISA-assay. Growth-performance during the fresh water period and after transfer to sea for the various groups of fish exposed to various combinations of environmental factors will be presented, as well as the relative expression of candidate genes for environmental stressors, the smoltification process and the immune response.

Conference Session Designation: (Immunology Vaccines)
Presentation Format: **(** Oral)

On the Way to New Batch Potency Tests For *Moritella viscosa* Vaccines: Antibody Response and Protective Immunity Correlate in a Dose–Response Manner.

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The studies reported here were part of a project to lay the foundation for development of antibody-based tests that can replace experimental inoculation methods for routine batch potency testing of multivalent, adjuvanted salmon vaccines. Atlantic salmon pre-smolts were immunized with multivalent salmon vaccines that are commercially available in Norway or with experimental formulations, followed by splitting study groups for subsequent blood sampling or a waterborne challenge experiment that were carried out in parallel. Antibody activity against the *M. viscosa* antigen measured in an ELISA was clearly above the pre-vaccination level from 4 weeks of immunization. When being held at 15^{o}C, fish that had received experimental vaccine formulations with reduced content or completely lacking the *M. viscosa* antigen, formulations with reduced antigen content could be revealed by analyzing blood samples taken 6 and 9 weeks post vaccination. In the parallel waterborne challenge experiment, clinical protection induced by the same formulations was reduced correspondingly. The results suggest that antibody-based assay protocols for the *Moritella viscosa* antigen of multivalent salmon vaccines can replace current challenge tests for assuring batch quality, at the same time shortening the time to batch release by one month or more.

Conference Session Designation: (Immunology Vaccines)
Presentation Format: **(** Oral)

Vaccination Against Yersiniosis in Atlantic Salmon - Experiences and Challenges

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In recent years, there has been a drastic increase of yersinosis outbreaks in Norwegian salmon farming. The disease, caused by *Yersinia ruckeri*, has mainly been reported as a problem in juveniles and in smolts in the period after transfer to sea, but since 2015 an increase of outbreaks on larger fish (>1kg) at sea has been observed. In fact, more than 90 % of the reported outbreaks in 2017 were months or even years after transfer to sea. The outbreaks cause massive and acute mortality, up to 80% in some cages.

Today more than 100 million smolts are vaccinated annually against yersinosis in mid and northern Norway. The vaccine used, ALPHA DIP ERM Salar, is a water based inactivated vaccine authorized for bath or immersion use. Due to the emerging situation of outbreaks in the sea phase, the farming industry has rapidly implemented vaccination by intraperitoneal injection. The injection is done as co-injection with a multivalent basis vaccine. The presentation will show how the vaccinations are carried out, both manually and by machines.

Efficacy laboratory data from the various regimes of vaccination against yersinosis will also be presented along with results from field observations. Our studies have demonstrated good protection against *Yersinia ruckeri*. No yersinosis outbreaks have been registered in vaccinated fish and the injection of the water based vaccine did not show negative effect on the co-administrated multivalent vaccine.

Conference Session Designation: (Immunology Vaccines)
Presentation format: (Orai)

Effect of Immunostimulatory Feed Additives on the Response of Rainbow Trout *Oncorhynchus mykiss* to a Commercial Vaccine Against *Yersinia ruckeri*

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Immunostimulatory feed supplements are of great interest in fish farm management not only due to having a protective effect but also they can result in improved farming performances. However, most studies of these supplements have focussed on their effects on the innate immune system and relatively little consideration has been given to their potential effects on the specific immune system. Because one of the functions of the innate immune system is to present the antigens to initiate the specific response, it is plausible that an improvement in the innate response would also result in an improvement in the specific immune system. Consequently, the present study was designed to investigate two commercial feed supplements (Biotronic® Top 3 and Levabon® Aquagrow E) with a known protective effect against bacterial infections as well as a combination of both of these supplements. Their effects on the ability of rainbow trout (*Oncorhynchus mykiss*) to generate an antibody response was analysed using vaccination with a commercial vaccine against *Yersinia ruckeri* followed by sampling of the serum and ELISA. Afterwards, an infection trial was performed using *Y. ruckeri*. Finally, the effect of the supplements on the growth parameters of the fish was also investigated. While this effect on growth was not found statistically significant, the combination of both supplements was found to have a protective effect against infection, moreover, they were associated with slightly higher titters of specific anti-*Y ruckeri* antibodies and an improved response to the vaccine compared to the fish that had only received the control feed.

Conference Session Designation: (Immunology / Vaccine)
Presentation Format: **(** Oral)

Development of Yersiniosis Vaccines for Atlantic Salmon

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During recent years, the Norwegian aquaculture industry has experienced increasing numbers of yersinosis outbreaks in farmed Atlantic salmon after sea transfer and even into the second year in sea. Massive and acute mortalities have been registered in certain cages. The disease occurring at such a late stage in the production cycle causes huge economic losses in addition to the obvious animal welfare challenges. Not much reviewed literature describing salmon isolates of *Yersinia ruckeri* is available, despite the fact that salmon isolates are fairly different from trout isolates.

We will show that the salmon isolates previously serotyped to O1, more specifically belong to subgroup O1b, and no evidence of subgroup O1a in Atlantic salmon has to our knowledge thus far been found in Norway. Western blot of O-antigens from serotypes O1a, O1b and O2 showed different binding patterns when comparing polyclonal antibodies raised against O1a and O1b serotypes. Moreover, dose-response results for *Y. ruckeri* serotype O1b from water-based as well as oil-adjuvanted vaccine systems will be shown. Also, a cross protection study revealed that O1a immunization of salmon did not provide any protection against O1b challenge. The results demonstrate the importance of choosing the proper isolates for vaccination, as the different serotypes may provide limited cross-protection against others.

Conference Session Designation: (Immunology Vaccines)
Presentation format: (Oral)

Monday September 3rd – Archibald / Campbell
Flavobacterium 1 & 2
Moderator - Tom Loch (Michigan State University)

9:30 AM	Flavobacterium 1	Knupp - Novel MLST *F. psychrophilum* Genotypes Infecting North American Salmonids
9:45 AM		Beka - Detection of aquaculture pathogens *Flavobacterium columnare* and *F. psychrophilum* using 16S rRNA amplicon sequencing and high-resolution sequence variant typing
10:00 AM		LaFrentz - Identification Of Four Distinct Phylogenetic Groups In *Flavobacterium columnare* With Fish Host Associations
10:15 AM		**Cai - Unveiling The Genetic Diversity Behind The Species Complex *Flavobacterium columnare***
10:30 AM		**Refreshments**
10:45 AM	Flavobacterium 2	**Nakajima - Resistance against *Flavobacterium psychrophilum* in Ayu *Plecoglossus altivelis* Hatchery-Reared at Different Water Temperatures**
11:00 AM		**Sebastião - Characterization of *Chryseobacterium* spp. isolated from clinically affected fish in California**
11:15 AM		**Hu - Infectious disease caused by Elizabethkingia in farmed frogs, China**
11:30 AM		**Klakegg - Isolation, identification and characterization of a *Tenacibaculum dicentrarchi* like bacteria causing acute disease and mortality in Atlantic salmon in a Norwegian post smolt site.**
11:45 AM		Marsh - Natural antibiotic sensitivity and biofilm formation in Flavobacterium

Novel MLST *F. psychrophilum* Genotypes Infecting North American Salmonids

Christopher Knupp[1], Gregory Wiens[2], Mohamed Faisal[1,3], Douglas Call[4], Kenneth Cain[5], Pierre Nicolas[6], Danielle Van Vliet[7], Coja Yamashita[8], Jayde Ferguson[9], David Meuninck[10], Hui-Min Hsu[10], Bridget Baker[11], Ling Shen[12] and Thomas Loch[1,3]*

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Flavobacterium psychrophilum is the etiological agent of bacterial coldwater disease (BCWD) and is responsible for significant economic losses in salmonid aquaculture worldwide, particularly farm-raised rainbow trout (*Oncorhynchus mykiss*). Over the last decade, multiple researchers have investigated the genetic heterogeneity of this bacterium in Europe, Asia, South America, Oceania, and most recently, North America (NA), using multilocus sequence typing (MLST), which linked some genetic variation to geographic range, host specificity, and association with BCWD outbreaks. However, much remains unknown regarding the population structure of *F. psychrophilum* in the USA, a matter of concern for disease prevention and control. Therefore, MLST was used to genotype 314 North American *F. psychrophilum* isolates, which were recovered from 10 fish host species in 20 US states and 1 Canadian province over nearly four decades. Results revealed these isolates belonged to 66 sequence types (STs), 47 of which were novel. Furthermore, 7 novel NA CCs were discovered, which brings the total number of NA CCs to 12. Many of the identified CCs have only been detected in NA to date, whereas others have been recovered from NA and abroad. These CCs were diverse and varied in terms of host specificity, distribution, and association with BCWD outbreaks. The largest *F. psychrophilum* CC identified in this study was CC-ST10, whereby 10 novel genotypes were detected and primarily recovered from BCWD epizootics in *O. mykiss*. ST275 of CC-ST10 was recovered from wild/feral adult steelhead trout (*O. mykiss*) and the recovery of CC-ST10 from feral/wild fish in NA has not been reported previously. Furthermore, the progeny from these fish were found to harbor the same ST, thereby supporting that some *F. psychrophilum* strains are circumventing current egg disinfection techniques. Ongoing experiments are exploring how such diversity relates to *in vivo* virulence and *in vitro* antibiotic susceptibility. Study findings to date will be invaluable in devising improved and targeted prevention and control strategies to reduce BCWD-associated losses.

Conference Session Designation: (Flavobacteria)
Presentation Format: (Oral)

Detection of Aquaculture Pathogens *Flavobacterium columnare* and *F. psychrophilum* Using 16S Rrna Amplicon Sequencing and High-Resolution Sequence Variant Typing

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Flavobacterium columnare and *F. psychrophilum* cause high levels of mortality in the aquaculture industry and methods of detecting these pathogens in a facility are invaluable for the prevention of future outbreaks. Here, we use 16S rRNA amplicon deep sequencing and oligotyping to determine the presence of *F. columnare* and *F. psychrophilum*-specific sequences in a trout farm in the US. Water flowing into and out of the trout farm raceways was collected and filtered. Gill and intestinal samples were also collected from morbid and healthy fish in these raceways. DNA was extracted from water filters and fish tissue samples. The V4 region of the 16S rRNA gene was sequenced on an Illumina MiSeq. Single-nucleotide variant reads, SNVs, were identified and taxonomically assigned using the DADA2 analysis package with the Silva reference database. The identification was confirmed by aligning the SNVs to reference type strain sequences and those that matched 99.99-100% to pathogen reference sequences were quantified. Presence and absence results of pathogen-specific sequences in various sample types from the farm were further verified using a droplet digital PCR (ddPCR) analysis, which targeted a different genetic marker. This approach allowed us to discriminate between and determine the relative abundance of *F. columnare* and *F. psychrophilum* sequences in samples collected at this farm over two years. *F. psychrophilum* and *F. columnare* were detected in water entering the raceways, in gill samples from sick fish (up to 1,764 sequences per 10,000 reads), and in swabs from baffles placed within the raceways. This method not only allows us to determine the microbial community composition of various samples but also shows great potential for identifying *Flavobacterium* pathogens at aquaculture facilities. Amplicon sequencing allows us track the source of these infectious agents and importantly, to survey raceways before an outbreak occurs which can be useful for deciding on an intervention.

Conference Session Designation: (Flavobacteria / Diagnostics)
Presentation Format: (Oral or Poster)

Identification of Four Distinct Phylogenetic Groups in *Flavobacterium columnare* with Fish Host Associations

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Columnaris disease, caused by the Gram-negative bacterium *Flavobacterium columnare*, is one of the most prevalent fish diseases worldwide. An exceptionally high level of genetic diversity among isolates of *F. columnare* has long been recognized, whereby six established genomovars have been described to date. However, little has been done to quantify or characterize this diversity further in a systematic fashion. The objective of this research was to perform phylogenetic analyses of 16S rRNA and housekeeping gene sequences to decipher the genetic diversity of *F. columnare*. Fifty isolates and/or genomes of *F. columnare*, originating from diverse years, geographic locations, fish hosts, and representative of the six genomovars were analyzed in this study. A multilocus phylogenetic analysis (MLPA) of the 16S rRNA and six housekeeping genes supported four distinct *F. columnare* genetic groups. There were associations between genomovar and genetic group, but these relationships were imperfect indicating that genomovar assignment does not accurately reflect *F. columnare* genetic diversity. To expand the dataset, an additional ninety 16S rRNA gene sequences were retrieved from GenBank and a phylogenetic analysis of this larger dataset also supported the establishment of four genetic groups. Examination of isolate historical data indicated biological relevance to the identified genetic diversity, with some genetic groups isolated preferentially from specific fish species or families. It is proposed that *F. columnare* isolates be assigned to the four genetic groups defined in this study rather than genomovar in order to facilitate a standard nomenclature across the scientific community. An increased understanding of which genetic groups are most prevalent in different regions and/or aquaculture industries may allow for the development of improved targeted control and treatment measures for columnaris disease.

Conference Session Designation: (Flavobacterium)
Presentation Format: (Oral)

Unveiling the Genetic Diversity Behind the Species Complex *Flavobacterium columnare*

Wenlong Cai* and Cova R. Arias

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Flavobacterium columnare is the causative agent of columnaris disease, which causes significant losses in cultured freshwater finfish species across the world. The instraspecies genetic diversity found in *F. columnare* was first revealed by DNA-DNA hybridization that divided the species into 3 genetic groups or genomovars. Later studies further subtyped the species into 5 genetic groups based on 16S rDNA polymorphisms. Virulence studies in channel catfish showed that genovar II strains were more virulent than genomovar I strains, suggesting the presence of more than one patovar within the species. On the contrary, all *F. columnare* strains are biochemically similar. The objective of this study was to elucidate if *F. columnare* was a species complex that harbors more than one cryptic species or if the observed genetic diversity was within the definition of bacterial species. Based on polyphasic data previously obtained by our group, we selected three strains representing 3 different lineages within the species (ATCC 23463 (type strain), ARS-1, and BGFS-27) for whole genome sequencing using PacBio RS long-read sequencing platform. *De novo* genome assembly of filtered reads was performed using PacBio PBcR HGAP 2.3 pipeline with default settings, which yielded 5 (ATCC 23463), 7 (ARS-1), and 16 (BGFS-27) contigs with 214x, 182x, and 184x coverage, respectively. Average nucleotide identity (ANI) were 85.55%, 85.69, and 91.3% for groups ARS1 & ATCC 23463, ARS1 & B27, and B27 & ATCC 23463, respectively. All ANI values were lower than the recommended cut-off point of 95% for species delineation. ANI results validated previous MLST and MALDI-TOFF phylogenetic analyses. Comparative genomic analysis (CGA) identified 1,876 genes in the core genome (shared by all 8 strains), which accounted for 34.2% of the total pangenome (gene repertoire= 5,491 genes). Strains within lineage 1 (represented by ATCC 23463) contained 61 unique genes while lineage 3 (represented by ARS-1) harbored up to 459 unique genes. Lineage 2 (represented by BGFS-27 and highly virulent for catfish) contained 52 unique genes including several genes encoding for putative virulence factors (O-antigen polymerase, glycosyltransferase, streptococcal hemagglutinin protein, type II toxin-antitoxin system toxin, and subtilisin-like serine protease). Based on our data, three species of *Flavobacterium* can cause columnaris disease in fish: *F. columnare* and two nomen nudum species that warrant full taxonomic description. Our results have direct implications in control and prevention of columnaris disease in farms.

Conference Session Designation: (Bacteriology / Flavobacteria)
Presentation Format: (Oral)
Student Presentation: (Yes)

Resistance against *Flavobacterium psychrophilum* in Ayu *Plecoglossus altivelis* Hatchery-Reared at Different Water Temperatures

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Ayu *Plecoglossus altivelis* is one of the most important fish species in Japanese inland water fishery; however, several pathogenic bacteria cause mass mortalities of the cultured and wild fish. Although rearing at high water temperatures (17°C–20°C) promotes growth of ayu seedlings, thymus development is remarkably inhibited under the high water temperature condition. In this study, we aimed to investigate disease resistance of ayu reared at different water temperatures against *Flavobacterium psychrophilum*, the pathogen of bacterial cold-water disease. Ayu (mean body weight = 0.85 g) were reared at 12°C or 18°C for 2 months, followed by acclimatization at 15°C for 1 month. Cubic volume of the thymus was measured by computed tomography scanning. Fish were intraperitoneally injected with *F. psychorphilum* (1.6 × 107 CFU/fish and 6.6 × 106 CFU/fish), and the cumulative survival rate was statistically analyzed with Log-rank test. Furthermore, the trunk kidney was sampled from the infected fish at 0, 1 and 2 days after the infection, and gene expression analysis of IL-1β and in the trunk kidney was performed. The thymus volume of 18°C group was significantly lower than that of 12°C group ($p < 0.01$), when the rearing at 12°C or 18°C was over. Although all experimental fish died in both 12°C and 18°C groups, the survival times of 12°C group were significantly longer than 18°C group in high-dose and low-dose challenge ($p < 0.05$). Gene expression level of IL-1β was significantly up-regulated at 1 day post-infection compared with uninfected fish in 12°C group, whereas significant difference was not observed in the gene expression level in 18°C group. These results suggest that rearing at the high-water temperature negatively influences on resistance of ayu to *F. psychrophilum.* Further, fish reared at the high-water temperature could not induce normal inflammatory responses to the infection.

Conference Session Designation: (Immunology / Flavobacteria)
Presentation Format: (Oral)
Student Presentation: (Yes)

Characterization of *Chryseobacterium* spp. Isolated From Clinically Affected Fish in California

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Some members of the family *Flavobacteriaceae* are recognized as emergent fish pathogens, including *Chryseobacterium* spp. In this study, seven bacterial strains recovered from 2015-2018 from spleen of diseased rainbow trout, *Oncorhynchus mykiss* (*n*=1), green sturgeon, *Acipenser medirostris* (*n*=1), white sturgeon, *Acipenser transmontanus* (n=2), blue cichlid (n=1) and fall chinook salmon, *Oncorhynchus tshawytscha* (n=2) were characterized by phenotypic and molecular taxonomic methods. After 24-48 hrs incubation at 20°C, colonies on tryptone-yeast extract-salts agar media were yellow, mucoid, circular in shape with entire margins. The isolates were Gram negative, rod-shaped, catalase and oxidase positive. Amplification and partial sequence analysis of 900 bp of the 16S rRNA gene allocated the microorganisms to the genus *Chryseobacterium*, with isolates presenting 98.1%, 99.6%, 97.5%, 97.2% 98.9% and 99.7% homology to *C. viscerum*, *C. aquaticum*, *C. sediminis*, *C. culicis*, *C. ureilyticum*, and *C. indologenes*, respectively. In order to investigate the pathogenicity of the recovered isolates, five isolates (e.g, *C. viscerum*-like, *C. aquaticum*-like, *C. sediminis*-like, *C. culicis*-like, and *C. indologenes*-like) were selected to challenge rainbow trout, brown trout *Salmo trutta* and white sturgeon under laboratory conditions. Fish were acclimated and challenged in flow-through freshwater at 18°C. Approximately $5x10^7$ CFU/fish of each *Chryseobacterium* strain were intramuscularly injected in the epaxial musculature of anesthetized animals (n=10 per treatment). No mortality occurred in fish challenged with *C. aquaticum*-like, *C. sediminis*-like, and *C. indologenes*-like isolates. White sturgeon exposed to the *C. viscerum*-like strain, and brown trout exposed to *C. culicis*-like strain experienced 10% mortality (1/10). However, the bacterium was not reisolated from the posterior kidney of these fish. Thirty days post-challenge, survivors were euthanized and multiple tissues were collected and fixed for histopathological analysis. Although results suggest that the recovered *Chryseobacterium* sp. may be opportunistic microorganisms, further research is warranted to better understand the role of these bacteria in fish diseases.

Conference session designation: **(** Flavobacteriaceae)
Presentation format: (Oral)
Student presentation: (Yes)

Infectious Disease Caused by *Elizabethkingia* in Farmed Frogs, China

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Frog farming, as a large proportion in aquaculture, has been practiced in many countries. The black-spotted frog *Pelophylax nigromaculatus*, endemic to East Asia, is one of the most widely farmed frogs in south-central China in the last five years. Since 2016, epidemic meningitis-like disease outbreaks in cultured black-spotted frogs occurred in separate farms.

To figure out the pathogenesis of this disease, a total of 213 abnormal black-spotted frogs were collected from seven separate farms in Hunan, China, during May to October 2016. After euthanasia, a routine necropsy and histopathology were performed. Bacteria isolation, microscopic parasites examination, PCR test for fungus and viruses were conducted for etiology detection. Histopathologic examination demonstrated chronic severe meningitis with denatured incrassated meninges. Bacterial infections (190/213) were confirmed in the etiological examination, and 90% of the isolates were identified as *Elizabethkingia miricola* according to 16S rRNA gene and gyrB gene. The pathogenicity of *E. miricola* was been verified by experimental challenges. *Elizabethkingia* was reported to be occasionally associated with human clinical infections with high mortality. Whole-genome sequencing revealed that this amphibian *E.miricola* is closely related to human clinical isolate, indicating that *E. miricola* can be epizootic and may pose a threat to humans. PFGE is ongoing to try to study more about the epidemiology of this pathogen.

We described infectious disease in amphibians caused by *Elizabethkingia* genus. As we known, *Elizabethkingia* has been reported to infect Chinese sturgeons (*Acipenser sinensis*) (Wei, 2018) and African catfish (*Clarias gariepinus*) (Laith, 2016). These studies indicate that *Elizabethkingia* is an emerging pathogen in aquatic animal, and the pathogenic mechanism needs to be further studied.

Conference Session Designation: (Flavobacteria)
Presentation Format: (Oral)
Student Presentation: (Yes)

Isolation, Identification and Characterization of a *Tenacibaculum dcentrarchi* Like Bacteria Causing Acute Disease and Mortality in Atlantic Salmon in a Norwegian Post Smolt Site.

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Particularly due to the challenges of sea lice, *Lepeophtheirus salmonis*, post smolt sites for aquaculture production of Atlantic salmon (*Salmo salar*) on land is desirable and the number of sites has grown significantly. At the post smolt sites the salmons can grow larger without exposure of sea lice and then make the exposure time for sea lice at sea shorter. However, it has been found that wounds and increased mortality is becoming a challenge at several post smolt sites.

This study reports an investigation of a case of disease outbreak at a Norwegian post smolt site, with 600 000 smolts divided in eight 750m^3 tanks, with the primary objective of isolation and characterization of the causative agent. A few days after transfer from freshwater to seawater at the post smolt site, the mortality at the site increased and soon the mortality was increased in all tanks. The salmon that died and found moribund had severe lesions, often 2-6 cm wide, particularly behind the pectoral fins. The lesions penetrate the skin as well as deep into the musculature. In some of the moribund salmon, the lesions were penetrating into the abdominal cavity and exposed internal organs as gut and liver which occasionally penetrate out of the wounds. We also saw fin rot, particularly on the pectoral fins. Culturing specimen taken from lesions on marine agar showed huge growth of one dominant bacteria colony. Microscopy showed rod shaped *Tenacibaculum* like bacteria. 16S rRNA showed that the dominant bacteria was a *Tenacibaculum dicentrarchi* like bacteria. Genetic characterization employing Multi Locus Sequence Analyze (MLSA) using seven housekeeping genes: atpA, dnaK, glyA, gyrB, infB, rlmN and tgt, was conducted. The MLSA analysis indicated that the isolates obtained in the outbreak belong to *T. dicentrarchi* where all were highly phylogenetically related to the *T. dicentrarchi* type strain (USC39/09^T) from Spain. *Tenacibaculum dicentrarchi* is known to be associated with tail roots, frayed fins and wounds in severe outbreaks in Atlantic salmon in Chile. In Norway as far as we know this is the first described outbreak of *Tenacibaculum dicentrarchi* in Atlantic salmon.

Conference Session Designation: (Bacteriology / Mycology)
Presentation Format: (Oral)
Student Presentation: (Yes)

Natural Antibiotic Sensitivity and Biofilm Formation in *Flavobacterium*

Terence L. Marsh*[1], Marceline Stevens[1], Thomas Loch[2], Donna Ye[1] Mariane Mota Cavalcante[1], John Bauman[3], Roshan Angoshtari[1], Masanori Fujimoto[4] and Kim Scribner[5]

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Our interest in *Flavobacterium* stems from investigations into the microbiome of sturgeon eggs and biofilm formation by environmental bacteria. Our interrogation of the sturgeon egg microbiome revealed that among the diverse phylogenetics of freshwater *Flavobacterium/Chryseobacterium*, a well-defined subset contributed to the egg microbiome. Moreover, treating hatchery eggs with formalin or peroxide shifted the community from 10-15% to as high as 82% *Flavobacterium*. Screening several hundred of isolates from the egg's microbiome identified six with antimicrobial activity, of which four had significant activity against fish pathogens *Aeromonas* spp., *Yersinia ruckeri* and *Flavobacterium* spp., as measured with a soft agar overlay test. We then tested the robustness of biofilm formed by these fish pathogens to challenge by our most aggressive antimicrobial-producing isolate, a *Pseudomonas* sp., and found that biofilm of one *Aeromonas* sp. was reduced but biofilm was elevated when *Flavobacterium* sp., *Yersinia ruckeri* and *F. columnare* were co-incubated with an antimicrobial-producing *Pseudomonas*.

In a separate line of investigation into environmental signals that trigger biofilm formation, we identified exogenous protein as a factor. Elevated protein concentrations in media were found to stimulate biofilm formation by *Serratia* spp., *Aeromonas* spp., and *Flavobacterium columnare*. A more detailed analysis of *Flavobacterium/Chryseobacterium* isolates from fish has identified 3 phenotypes in response to elevated protein. Under our laboratory conditions, some strains produce little biofilm, regardless of changes in nutritional conditions. A second phenotype produced reasonably robust biofilm under our standard conditions, but produced diminishing amounts of biofilm as the concentration of exogenous protein increased. Finally, *F. columnare* and *C. nakagawai* produce robust biofilms at an exogenous protein concentration of 1%. These increases in biofilm productivity as measured by crystal violet staining were accompanied by an increase in cells within the biofilm and an increase in protein in the biofilm matrix. *F. columnare* biofilm formed under elevated exogenous protein was robust and visualized with fluorescent microscopy, revealed a highly proteinaceous biofilm, indicating recruitment of exogenous protein from the media into the biofilm matrix.

Conference Session Designation: (Flavobacterium)
Presentation Format: (Oral)

Monday September 3rd – Archibald / Campbell
Tilapia Health 1
Moderators – Win Surachetpong (Kasetsart University) Paola Barato (Copavet – Colombia)

1:15 PM	Tilapia Health 1	Ramirez-Paredes - The Potential Of Autogenous Vaccines For Controlling Infectious Diseases In Tilapia Aquaculture
1:30 PM		**Delphino - Seasonal Dynamics Of Bacterial Pathogens Of Nile Tilapia Farmed In A Brazilian Reservoir**
1:45 PM		Barato - Outbreaks Of *Edwardsiella anguillarum*-Associated Edwardsiellosis In Farmed Tilapia (*Oreochromis* Sp.) In Colombia
2:00 PM		Lafrentz - Resistance Of Nile Tilapia *Oreochromis niloticus* To *Streptococcus Iniae* And *S. agalactiae* Is Heritable But Not Correlated
2:15 PM		**Pulpipat - Phenotyping, Genotyping, And Pathogenicity Of *Francisella noatunensis* Subsp. *orientalis* Isolated From Cultured Tilapia Oreochromis Sp. In Taiwan**
2:30 PM		Surachetpong - Current Situiation Of Tilapia Lake Virus: What Arte The Impact To Tilapia Aquaculture?
2:45 PM		Gómez-Sánchez - Tilapia Lake Virus (Tilv) Emergency Disease In Wild Tilapia In Peru
3:00 PM		**Refreshments**

The Potential of Autogenous Vaccines for Controlling Infectious Diseases in Tilapia Aquaculture

J. Gustavo Ramirez-Paredes[1]* and Timothy S. Wallis[1]

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The farming of tilapia species (*Oreochromis niloticus*, *O. aureus*, and *O. mossambiccus*) is one of the most important sectors in aquaculture worldwide. This activity has spread throughout dozens of developing countries in Africa, Asia and Latin America where it boosters local economy and constitutes an affordable source of animal protein for human consumption. Losses due to infectious disease are one of the major challenges that this industry currently faces. Outbreaks of disease are predominantly caused by co-infections where two or more pathogens trigger mortalities after husbandry handling procedures. The most common tilapia pathogens are: *Streptococcus* spp., *Flavobacterium columnare*, *Edwardsiella tarda*, *Aeromonas* sp., *Plesiomonas shigeloides*, *Francisella noatunensis orientalis* and Tilapia Lake Virus. Despite the relevance that these agents possess there are no licensed vaccines commercially available to prevent or treat these coinfections. Autogenous vaccines (AV) are farm-specific inactivated formulations that have the potential to be rapidly developed and deployed when novel pathogens emerge and off-the-shelf fully licensed vaccines do not exist. In this context the use of AV arises as the most efficacious solution to control mortalities caused by these pathogens in the field. Ridgeway Biologicals Ltd (RBL) is the UK leading supplier of AV's for veterinary use and holds a range of mono and multivalent aquaculture vaccines. These include bacterins for tilapia successfully used in West Africa and the first viral auto vaccine for farmed fish i.e. a Nodavirus AV for European sea bass widely used in Greece. In an attempt to gain a better understanding on the predominant pathogens affecting tilapia farmed in Latin America, RBL has conducted a series of bacteriological and viral surveys in farms suffering mortalities in Colombia, Honduras, Peru and Mexico. In the present study a summary of these outcomes will be presented and compared to our results from Africa where regular disease surveillance has been established since 2016 by RBL. Moreover the significance of maintaining these surveillance programmes and their impact on the success that auto-vaccines have will be discussed and exemplified with a study case from Ghana.

Seasonal Dynamics of Bacterial Pathogens of Nile Tilapia Farmed in a Brazilian Reservoir

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Fish aquaculture is rapidly growing in Brazil. Nile tilapia is the most cultivated species, mainly through an intensive production system carried out in floating cages installed in large reservoirs. However, fish pathogens pose a major challenge to production chain sustainability, and tilapia farmers often have limited knowledge of prevailing health problems and rarely implement biosecurity practices to prevent introduction of economically important infectious agents. This study aimed to identify the key disease risks of tilapia farming in a tropical reservoir and characterize the dynamics of the prevalent pathogens, as a basis for development of effective control measures for tilapia health and surveillance programs. From August 2015 to October 2016, a longitudinal study was carried out at the Três Marias reservoir, in the municipality of Morada Nova de Minas in the southeast of Brazil. Daily and monthly data were collected from six out of 32 existing fish farms, including fish samples, mortality counts, and measurements of temperature and water quality parameters. The main bacteria detected were *Streptococcus agalactiae*, infecting mostly adult tilapia throughout the period, with higher frequency as the average temperature increased, and *Francisella noatunensis* subsp. *orientalis* (Fno), infecting mainly younger tilapia, only during the cooler months. Coinfections with multiple pathogens were detected in 33 fish. The detection of Fno in one farm in two consecutive winters, after months of unfavorable water temperature conditions and without evidence of sustained introduction of infected stock, strengthens the case for investigating if this pathogen can survive and remain infective causing new outbreaks. Furthermore, variation in mortality was likely associated with the dynamics of the studied pathogens.

Conference Session Designation: (Tilapia Disease)
Presentation Format: (Oral)
Student Presentation: (Yes)

Outbreaks of *Edwardsiella anguillarum*-Associated Edwardsiellosis in Farmed Tilapia (*Oreochromis* sp.) in Colombia

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Edwardsiellosis in fish can be caused by members of the genus Edwardsiella, including *Edwardsdiella ictaluri*, *E. tarda*, *E. piscicida* and *E. anguillarum.* While *E. ictaluri* and *E. tarda*-associated edwardsielliosis have been reported in tilapia (*Oreochromis* sp.), these reports occurred prior to the recent reorganization of *E. tarda* and recognition of *E. piscicida* and *E. anguillarum* as valid taxa. Herein, we describe two outbreaks of *E. anguillarum* associated edwardsiellosis in farmed tilapia in Colombia. The first outbreak was reported by the Departmente of Meta in January of 2017, a mortality event in tilapia (*Oreocrhomis* sp.) raised in a biofloc system approached 40%. Similarly, in November 2017 in the Department of Huila, mortality in pond-reared red (*O. mossambicus* X *O. niloticus*) and Nile tilapia (*O. niloticus*) alevins approached 30%. For each outbreak, 15 live tilapia alevins (~10g) were submitted for diagnostic workup. Fish were euthanized upon submission and immediately subjected to post-mortem examination. Brain, eyes, gills, heart, liver, spleen, stomach, intestine, kidney and skin were processed for histopathological analysis. Also, spleen, liver, brain and eyes from five fish were pooled aseptically, homogenized and aerobically cultured on blood agar for microbiological analysis. Recovered isolates consistent with *Edwardsiella* spp. were archived for later molecular analysis. In both cases, histopathology demonstrated systemic granulomatous infection (granulomatous splenitis, hepatitis, nephritis, encephalitis and choroiditis) compatible with previous reports of edwardsiellosis. Three isolates were recovered from the Biofloc case: two were identified molecularly as *E. anguillarum*, which was confirmed molecularly by PCR and *gyrB* sequence analysis; while one isolate was confirmed as *E. tarda*. One *E. anguillarum* isolate was recovered from the Huila case with the same molecular analysis. One of the confirmed *E. anguillarum* isolates was used to fullfill Koch's postulates by intragastric (IG; 10^7 CFU/fish), intracoelomic (IC; 10^7 CFU/fish) and immersion (IMM; 10^8 cfu/ml) challenges in nile tilapia fingerlings. Each exposure group consisted of 10 tilapia alevins (approximately 8g). All tilapia exposed by IC died during the first 24 h post-challenge. Similarly, all fish exposed by IG died within 72 h. Comparibly, four tilapia exposed by IMM infection died within 72 h. The six remaining survivors were euthanized 7 days post challenge. Only one control fish (IC challenge) died. Tissues from fresh dead IG and IMM infected fish, as well as the survivors from the IMM challenge and controls were evaluated by histopathology. Systemic granulomatous infection was observed in all exposed fish, with no relevant lesions present in any controls fulfilling Koch's postulates. This is the first description of *E. anguillarum* associated edwardsiellosis in tilapia.

Conference Session Designation: (Tilapia Diseases)
Presentation Format: (Oral)

Resistance of Nile Tilapia *Oreochromis Niloticus* to *Streptococcus Iniae* and *S. Agalactiae* is Heritable But Not Correlated

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Tilapia (*Oreochromis* sp.) are an important source of protein with an economic value approaching US $8 billion yearly. Streptococcal disease, caused by *Streptococcus iniae* and *S. agalactiae* are emerging or re-emerging diseases that negatively affecting tilapia aquaculture worldwide. Because of the difficulty controlling these pathogens in tilapia production, selective breeding for resistance *S. iniae* and *S. agalactiae* is a potential tool to limit the impact of streptococcal disease. The objectives were: 1) to verify additive genetic variation in resistance of Nile tilapia (*Oreochromis niloticus*) to *S. iniae*; 2) to determine if realized genetic gain in resistance and/or susceptibility to *S. iniae* is possible following positive assortative mating based on estimated breeding values (EBV); and 3) to determine if resistance to *S. iniae* and *S. agalactiae* Ib is genetically correlated. A total of 144 full and paternal half-sib families were challenged intraperitoneally with *S. iniae* using PIT tagged fish in a common tank. For *S. agalactiae* challenge, 130 full and paternal half-sib families were intramuscularly injected. Cumulative mortality was 46% for *S. iniae* and 68% for *S. agalactiae*. There was a high additive genetic component found for survival in fish injected with *S. iniae* (estimated heritability 0.52 ± 0.12) validating our previous results. The estimated heritability for *S. agalactiae* was 0.38 ± 0.11 based on the univariate linear animal model. Positive assortative mating further demonstrated resistance to *S. iniae* was heritable with mean survival of 88% (range 60 – 100%) for families produced on high EBV (*S. iniae* resistant parents) and mean survival of only 10% (range 0 -42%) for families produced using low EBV (*S. iniae* susceptible parents). No genetic correlation was noted amongst resistance to *S. iniae* and *S. agalactiae* Ib. Selective breeding of tilapia to improve survival to *Streptococcus* sp. will require knowledge of the pathogen(s) prevalent in the region so that custom genetic material may be formulated for individual farms.

Conference Session Designation: (Tilapia Disease)
Presentation Format: (Oral)

Phenotyping, Genotyping, and Pathogenicity of *Francisella Noatunensis* subsp. *Orientalis* Isolated from Cultured Tilapia (*Oreochromis* Sp.) in Taiwan

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Francisella noatunensis subsp. *orientalis* (*Fno*) has been reported as a causative agent of systemic granulomatous disease in tilapia and ornamental cichlids in Taiwan for the past 26 years. However, the phenotypic and genotypic diversities, and also the pathogenicity of Taiwanese *Fno* strains are still poorly understood. In this study, phenotypic and genetic characteristics, as well as pathogenicity of *Fno* isolates obtained from diseased fish in different geographical locations in Taiwan were examined. Bacterial colonies were isolated from Tilapia (n=17) and Green Texus cichlid (*Herichthys cyanoguttatus*) (n=1) on cysteine heart agar supplement with 1% bovine hemoglobin, and identified as *Fno* using polymerase chain reaction with species-specific primers. An assessment of enzymatic profile of *Fno* isolates was carried out under the API ZYM system. Genotypic determination of *Fno* isolates was performed by phylogenetic analysis based on 16S rRNA and housekeeping genes, together with pulsed-field gel electrophoresis (PFGE) using *Xho*I and *Bam*HI restriction enzymes. The phylogenetic tree showed that 16S rRNA and housekeeping genes of Taiwanese isolates possessed a very high nucleotide similarity (99-100%) to that of other *Fno* references from GenBank database. All of the *Fno* isolates in this study revealed identical enzymatic and PFGE profiles which discriminated from *F. philomiragia* isolated from marine fish. The clinical isolates from diseased tilapia were further confirmed for their pathogenicity and virulence by inoculation in cultured tilapia. Systemic granulomatous lesions were presented in spleens and head kidneys, concomitant with high mortalities similar to that observed in a natural outbreak. Based on the cumulative mortalities found at day 21 after challenged with *Fno*, the observed median lethal dose (LD_{50}) for the intraperitoneal challenge in tilapia and red tilapia were 9.06×10^3 CFU/fish and 2.08×10^2 CFU/fish, respectively. Taken together, our data provided a basis for characterization, epidemiology of Taiwanese *Fno* isolates, and future vaccine development.

Conference Session Designation: (Bacteriology or Tilapia Diseases)
Presentation Format: (Oral)
Student Presentation: (Yes)

Current Situation of Tilapia Lake Virus: What are the Impact to Tilapia Aquaculture?

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Tilapia is the second most important aquaculture fish species worldwide with the annual production of 5.6 million tonnes. Although tilapia has been recognized as the disease resilient finfish species, mass mortality of an unidentified etiology has been recently observed in major tilapia producing countries. Since 2011, the investigation of mass mortality in wild and farmed tilapia in Israel led to the identification of a novel RNA virus called Tilapia Lake Virus **(**TiLV). Subsequently, the virus has been detected in tilapia cichlid in Ecuador, Colombia, Egypt, Thailand, Taiwan, Malaysia, and India. In Thailand, the disease has been called **"**Tilapia One-Month Mortality Syndrome**" (**TOMMS**)** as the mortality usually occur during a month period after juvenile tilapia have been transferred from hatchery to the grow-out ponds or cages. The clinical signs of diseased fish included skin redness and erosion, exophthalmos, abdominal distension, scale protrusion, multiple skin hemorrhages, pale and liver contraction. Generally, multiple infections of external parasites and opportunistic bacteria has been found in TiLV-infected fish**.** The predisposing factors such as inappropriate handling, transportation, poor water quality, and fluctuation of water temperature associate with TiLV outbreaks. Moreover, high stocking density, frequent used of pond, size of fish and genetic background of fish are important risk factors for TiLV epidemic. Currently, the detection of TiLV relies on the molecular methods including RT-PCR, nested RT-PCR, and RT-qPCR together with the clinical signs, histopathology and virus isolation in the susceptible cell culture. To reduce the impact of this emerging viral disease, rapid diagnostic assay, strict biosecurity, and vaccine development could support disease containment and limit virus spreading within the endemic region or to a new geographical area**.** Furthermore, fundamental research on the pathogenesis, route of disease transmission, susceptible host species and vectors await further investigation**.** Such knowledges are important to gain better understanding of the TiLV biology that could lead to better control strategies.

Conference Session Designation: (Tilapia Disease)
Presentation format: (Oral)

Tilapia lake virus (TiLV) emergency disease in wild Tilapia in Peru

(WITHDRAWN)

Muriel Gómez-Sánchez[1*] and Katherine Zapata[1]

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Tilapia lake virus disease (TiLVD) is an emerging disease reported in tilapias farmed and wild, since the first report in Israel (Eyngor et al., 2014). Subsequently the virus has been found in South America, Africa and Asia (Bacharach et al., 2016; Fathi et al., 2017; Surachetpong et al., 2017). The world Organization for Animal and Health (OIE) published a technical card in February 2018 to describe clinical signs of TiLV including ocular alterations, skin erosions, congestion of the spleen and hemorrhages in the leptomeninges. In this study, we described the reports of TiLV outbreak in wild tilapias in Peru. Since November 2017, local fisherman's notified massive mortality of all life stages wild tilapias at four different water sources (reservoirs and lakes) in Piura and San Martín regions. The affected fish had clinical signs with skin erosions and redness, ocular injury and unusual behavior. The PCR diagnosis confirmed TiLV positive in all of the four cases. This is the first report of multiples outbreaks of TiLV in wild tilapias in South America. The emergence of the virus could be related to several factors including illegally movement of fingerlings from positives countries, the spreading of virus in water and potential vectors such as wild fish, birds, crustaceans, others. Meanwhile, ongoing actions are focusing on reduce the virus spread and surveillance tilapias farms and others water sources.

Conference Session Designation: (Tilapia Disease)
Presentation Format: (Oral)

Monday September 3rd – Archibald / Campbell
Tilapia Health 2
Moderators – Win Surachetpong (Kasetsart University) **Paola Barato** (Copavet – Colombia)

3:15 PM	**Tilapia Health 2**	Sanguinetti - Identification of Tilapia Lake Virus In Fish Farms of The Rainforest Region of Peru
3:30 PM		Barato - Epidemiologic Assessment and Dna Sequencing of Tilv From Colombian Tilapia Farms Using Motif Fingerprints
3:45 PM		**Liamnimitr - Mucus as a Source of Horizontal Transmission and Non-Lethal Sampling For Tilapia Lake Virus Detection**
4:00 PM		Soto - Tilapia Lake Virus Susceptibility to Iodine and Chlorine Disinfectants

Identification of Tilapia Lake Virus in Fish Farms of the Rainforest Region of Peru

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Tilapia (*Oreochromis sp.*) are an increasingly important protein source worldwide due to their omnivorous diet, high-stress tolerance and disease resistance. In recent years, the emergence of Tilapia lake virus (TiLV) disease, caused by a novel Orthomyxo-like virus that affects *Oreochromis sp.*, has been reported in Israel, Egypt, Thailand, India, Colombia and Ecuador producing high mortalities and important economic losses for tilapia aquaculture. By the end of 2017 several outbreaks of high mortality have been observed in wild and farmed tilapias in the north of Peru. Our first report confirmed the presence of TILV in Piura region. Two months later, high mortalities were reported in the Peruvian northeast, in the rainforest area of San Martin region. For this study samples from two affected tilapia farms in San Martin region were taken, consisting of 210 fish from different productive stages. Samples of liver, brain and intraocular fluid were collected from juvenile and adult tilapia and processed in pools of all organs every 3 fish. In the case of younger productive stages (fry), whole fish were processed in pool of 100 individuals approximately. In order to diagnose the etiological agent, molecular and histopathological assays were performed. Pools of tissue samples were processed for ARN extraction and analyzed through RT-PCR nested technique to amplify segment 3 of TiLV genome. PCR products were sequenced and compared with Israeli isolate of TiLV (KU751816.1) to determine identity between all strains. Results showed, for the second time, the presence of TiLV in Peruvian tilapia, evidencing high nucleotide identity (96.9-97.1%) to Israeli strain at the sequence analysis and the presence of typical syncytial giant cells in liver observed in histopathology. This results evidence the distribution capability of TiLV through South America as an important emerging disease for tilapia aquaculture, and its rapid dissemination inside Peruvian territory. Tilapia culture is a raising economic activity in Peru and its production is principally distributed in the north and northeast regions of the country. Our results must offer important information in order to stablish and improve control and prevention of dissemination strategies in Peru.

Conference Session Designation: (Emergent Disease or Tilapia Disease)
Presentation Format: (Oral)

Epidemiologic Assessment and DNA Sequencing of TiLV from Colombian Tilapia Farms using Motif Fingerprints

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The Tilapia Lake Virus (TiLV) is a novel orthomyxo-like virus which causes high mortalities and economical losses among tilapia (***Oreochromis* sp.)** producers worldwide. While TiLV was reported in May 2015 from a Colombian tilapia fish farm, little is known of the virus distribution within the country. To determine the specific frequency of TiLV disease, several fish farmers and Corpavet, began to collect tilapia samples with lesions associated TiLV infection. From June 2016 to March 2018 we evaluated 463 cases from 23 (72%) of 32 Departments of Colombia. Necropsy evaluations at several developmental stages were performed using 5 to 10 tilapias per case (50 cases of larvae, 310 of alevins, 18 of pre-growth-out, 28 of growth-out fish and 57 of adults for reproduction). Four hundred thirty-three (433) cases had mortality history and 30 did not report clinical signs. Spleen, liver, brain and eyes from five fish of each batch were pooled aseptically, homogenized and frozen in PBS or RNA later at -40ºC to latter RT-PCR for TiLV described by Eyngor et al., (2014). Brain, eyes, gills, heart, liver, spleen, stomach, intestine, kidney and skin were also processed for histopathological analysis. Molecular assays showed that 109 cases (23%) yield positive amplification of the segment 3 of TiLV genome. *On site* metagenomic sequencing of three positive samples was performed using the MinION portable DNA sequencing device. This approach yielded more than > 3 million reads that were scanned against a library of 8.5 billion motif fingerprints covering all know organisms. This approach mapped thousands of reads to different segments of the TiLV genome. Six of 109 cases had not mortality or clinical signs, they came from regular health monitoring. Ninety-eight (98) cases positive to TiLV for RT-PCR were alevins, 3 were larvae, 3 adults for reproduction, 3 were pre-growth-out fish and 2 in growth-out phase. Alevins with mortality history and RT-PCR positive (92 cases) had histopathological lesions compatible with TiLV infection (syncytial hepatitis, encephalitis and/or keratitis). This is the first epidemiological assessment and on site DNA sequencing-based confirmation of the presence of TiLV in several regions of Colombia. This information will be very useful to propose a plan to minimize the impact of TilV in the country, improve the epidemiological biosurveillance and optimize the sensitivity of molecular diagnostics.

Conference Session Designation: Virology / Emergent Disease
Presentation Format: Oral presentation

Mucus as a Source of Horizontal Transmission and Non-Lethal Sampling for Tilapia Lake Virus Detection

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A recent emerging orthomyxo-like virus called Tilapia lake virus (TiLV) has been discovered in wild and farm-raised tilapia in Israel in 2014. Later, multiple outbreaks of TiLV were reported in three continents and eight countries including Ecuador, Colombia, Egypt, Thailand, Malaysia and India. The cumulative mortality rate of TiLV in affected farm are as high as 80-90% depend on the management practices, breed of fish and other environmental factors. Although studies of TiLV receive more attention, little is known about the mode of transmission and how the virus spread in fish population. In this study, we demonstrated that cohabitation of TiLV-infected tilapia and susceptible tilapia led to high mortality of 55.71% within 14 days. Interestingly, TiLV genomic RNA could be detected in liver and mucus of cohabitation challenge fish. The material prepared from TiLV-infected mucus caused CPE formation in E-11 cells within 5-7 days, suggesting that live viruses are present in the infected fish mucus. Our results also revealed that TiLV RNA persists in fish mucus up to 12 days post infection, allowing the possibility of virus to spread in fish population. For the diagnostic purpose, mucus could provide a non-lethal sampling procedure for TiLV detection in valuable broodstocks or large fish. Horizontal transmission via direct contact of carrier or TiLV-infected fish is important route of virus transmission. Taken together, our data provide important insight that could be applied for the control and implementation of biosecurity program for TiLV control.

Conference Session Designation: (Tilapia Disease)
Presentation Format: (Oral)
Student Presentation: (Yes)

Tilapia Lake Virus Susceptibility to Iodine and Chlorine Disinfectants

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Tilapia Lake Virus (TiLV) is an emergent orthomyxo-like virus affecting the tilapia (*Oreochromis* sp.) aquaculture industry worldwide. Mortality in affected farms typically reach 80-90% in affected systems and there is no current vaccine or therapy against the diseases. The aim of this study was to identify the biocide efficacy of two commonly used disinfectants in aquaculture, namely, house hold bleach (free-chlorine) and Buffered Povidone-Iodine (PVP Iodine) complex against TiLV. Cultured TiLV was grown on endothelial *Oreochromis mossambicus* bulbus arteriosus cell line (TmB) and a stock stored at -80C. Chlorox (The Clorox Company, Oakland, California, USA) and Ovadine (Syndel, Ferndale, Washington, USA) ranging from 10-100 mg/L (ppm) were diluted the day of the assay in autoclaved water collected from an aquaculture facility in California, USA and added to TiLV for 0.5, 1, or 24h. At each timepoint, sodium thiosulphate was added to inactivate the available iodine and dilutions in media containing 10% FBS were used to inactivate the free chlorine. All aliquots were then titrated on TmB cells to determine the $TCID_{50}$/ml. Virucidal reductions in titre of >4 $\log_{10}$ $TCID_{50}$/ml after 0.5 and 1h were only obtained at concentrations of ≥20ppm free chlorine and ≥50ppm available iodine. When contact time with disinfectant increased to 24h, virucidal reductions in titre of >4 $\log_{10}$ $TCID_{50}$/ml were obtained at concentrations of ≥10ppm free chlorine and ≥25ppm available iodine. In conclusion, chlorine and iodine were found effective for the disinfection of fish farming equipment at the manufacturer's recommended dose for 30 min duration. This information should be taken into consideration when developing biosecurity protocols in tilapia aquaculture.

Conference Session Designation: (Tilapia Diseases)
Presentation Format: **(** Oral)

Monday September 3rd – Langeve / Cartier
Microbiome 1 & 2
Moderator - Matt Griffin (Mississippi State University)

9:30 AM	Microbiome 1	Marsh - Microbiome of The Sturgeon Egg
9:45 AM		Arias - The Channel Catfish (*Ictalurus Punctatus*) Microbiome
10:00 AM		**Ahasan - Fecal Microbiota of Wild Captured And Stranded Green Turtles on The Great Barrier Reef**
10:15 AM		**Pathirana - The Role Of Pacific Oyster *Crassostrea Gigas* Microbiome In The Pathogensis of Ostreid Herpesvirus-1 Infection**
10:30 AM		**Refreshments**
10:45 AM	Microbiome 2	Amthor - Efficacy Of A Bath Probiotic Application Before Seawater Exposure In Atlantic Salmon
11:00 AM		Fauske - Compatability of Application of A Probiotic Treatment In Anesthetic Bath Using Benzocaine or Tricaine Methanesulfonate (Tms)
11:15 AM		**Parker-Graham - Effect of Oxytetracycline Treatment on The Gastrointestinal Microbiome of Critically-Endangered White Abalone *Haliotis sorenseni* Treated For Withering Syndrome**
11:30 AM		Open
11:45 AM		Open

The Microbiome of the Sturgeon Egg

Terence L. Marsh[1]*, John Bauman[2], Roshan Angoshtari[1], Donna Ye[1], Masanori Fujimoto[3]
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Sturgeons are an imperiled group of fishes and conservation efforts have focused on all life stages but in particular during early development where losses are high. To better understand egg mortality caused by microbes, our work has investigated the assembly of the egg's microbiome. The egg, which is essentially sterile when extruded into river water during spawning, becomes coated within 15 minutes with a diverse collection of bacteria dominated by *Pseudomonas*, *Aeromonas*, *Geobacillus* and *Bacillariophyta.* At 135 minutes the community shifted modestly and was dominated by *Pseudomonas*, *Aeromonas*, *Geobacillus*, *Comamondacea* and *Burkholderia*. After 24 hours exposure, the community shifted significantly and was dominated by *Comamonadaceae*, *Rheinheimera*, *Undibacterium*, *Bacillariophyta*, *Rhodobacteraceae* and *Methylophilus*. Differences were also detected between different mating pairs of sturgeon, indicating that variation in the egg surfaces can select for different bacterial populations. Community analysis with 18S revealed ciliates, algae and *Saprolegnia* at low concentrations while analysis targeting the ITS region identified 30 genera in 20 sampled eggs. *Aureobasidium*, *Cryptococcus*, *Neobulgaria*, *Pythium* and two unidentified groups dominated.

Disinfection of eggs with formalin or peroxide to quench microbial pathogenesis is common practice in hatcheries. These treatments reduce the bacterial load but also alter the community. Both peroxide and formalin treated eggs had increases in *Flavobacterium* species and losses to *Sphaerotilus* and *Rheinheimera* populations. Community shifts are also detected when eggs are supplemented with putative probiotics or sugars that compete with glycan binding motifs on the surface of the egg during fertilization. The addition of an *Acidovorax* sp. isolated from the egg reduced mortality by 25% and the addition of glucose and galactose shifted the communities in statistically significant ways, diminishing the attachment of select aquatic populations. In addition, we have isolated and characterized over 400 bacterial strains from stream-captured sturgeon eggs. The isolates were dominated by *Pseudomonas* and *Aeromonas*, of which 65% were positive for extracellular cellulose and 52.3% positive for ß-hemolysin. Several isolates have antimicrobial and antifungal activities and have been considered for use as a probiotic.

These investigations point to possible interventions during the assembly of egg- associated microbial communities that will reduce mortality and the use of harmful chemicals in the hatchery. Controlling the community assembly early in the process appears critical to altering the phylogenetic structure of the community and improving mortality rates and larval robustness.

Conference Session Designation: (Microbiomes)
Presentation Format: (Oral)

The Channel Catfish (*Ictalurus punctatus*) Microbiome

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Nowadays it is well accepted that the community of microbes occupying the gastrointestinal (GI) tract of vertebrates (gut microbiome) plays a critical role in host development, physiology, and health. One of the main reasons for studying the gut microbiome in fishes is the idea that those communities can be modified to improve host health. A prerequisite to this approach is the characterization of the gut microbiome of the species of interest. Many factors contribute to the composition of the gut microbiome in vertebrates including host genetics, environment, and nutrition among others. Discovering a core microbiome, i.e. members of the microbial community present in all individuals of a species, has been a primary goal for many researchers interested in understanding gut microbial communities. Our group has been studying the gut microbiome of channel catfish (*Ictalurus punctatus*) for several years in order not only to identify its core microbiome but also to characterize how the gut microbial community assemblies during ontogenesis. Channel catfish (*Ictalurus punctatus*) is the top farmed raised fish in United States with a production of more than 750 million pounds per year. The demand for farmed catfish is strong and growing worldwide. However, the US catfish industry faces losses during the entire commercial cycle due primarily to infectious diseases. In many cases, bacterial diseases affecting channel catfish are common opportunistic pathogens that fail to cause disease in healthy hosts. In order to elucidate if fish rearing under intensive conditions displayed an altered gut microbiome, we performed a series of studies that assessed changes in the gut microbiome of fish exposed to medicated feed, vaccination, and mechanical injury (some of those experiments were carried out in the animal model zebrafish *Danio rerio*). We utilized High throughput Illumina MiSeq of the 16S rRNA V4 region to characterize the gut microbiome.

Our results showed that the gut microbiome of hatchery-reared channel catfish was dominated by Firmicutes (~50% of the community) followed by Fuseobacteria (~25%). This differs from what we have reported previously in wild catfish in which >90% of their gut community was comprised of Fuseobacteria. As expected, the administration of medicated feed containing the antibiotic florfenicol elicited a drastic shift in the gut microbiome even after fish resumed normal feeding. The gut microbiome of treated fish experienced a significant decrease in diversity and an overall increase in Proteobacteria, which are typical traits of a dysbiotic gut. Most importantly, dysbiotic fish were more susceptible to infection by an opportunistic pathogen (*Aeromonas hydrophila*) than control groups. By contrast, vaccination practices did not have a significant effect on the gut microbiome (or on the skin and gill microbiomes). Finally, and this was an unexpected result, mechanical injury (adipose fin clipping) did produce a significant change in the gut community. The 'gut-brain axis' has been the subject of many studies in humans and mice and our results suggest that a similar pathway exists in channel catfish by which the gut microbiome can sense and react to external stimuli or assaults not directly infringed upon the gut microbiome.

Conference Session Designation: (Microbiome)
Presentation Format: (Oral)
Student Presentation: (Yes)

Fecal Microbiota of Wild Captured and Stranded Green Turtles on the Great Barrier Reef

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Green turtle (*Chelonia mydas*) is an endangered marine herbivore that breaks down food particles, primarily sea grasses, through microbial fermentation. However, the microbial community and its role in health and disease are still largely unexplored. In this study, we investigated and compared the fecal bacterial communities of wild-captured green turtles to stranded turtles by PCR amplification of a hypervariable region (V1-V3) of the bacterial 16S rRNA gene**.** A total of 12 samples were sequenced using next generation high-throughput sequencing technology on an Illumina MiSeq platform. At a phylum level, Firmicutes predominated among wild-captured green turtles, followed by Bacteroidetes and Proteobacteria. In contrast, Proteobacteria (Gammaproteobacteria) was the most significantly dominant phylum among all stranded turtles, followed by Bacteroidetes and Firmicutes. In addition, Fusobacteria was also significantly abundant in stranded turtles. No significant differences were found between the wild-captured turtles from two different locations. At a family level, 25 of the 53 families were identified in both the wild-captured and stranded green turtles, while 14 families were found only in stranded turtles. At the OTU level, 256 (48.7%) of the total OTUs (>1% abundance) were shared between the wild-captured groups of turtles, while absent in stranded turtles. The predominance of *Bacteroides* in all groups indicates the importance of this bacteria in turtle gut health. In terms of microbial diversity and richness, wild-captured green turtles showed the highest microbial diversity and richness compared to stranded turtles. The marked differences in the bacterial communities between wild-captured and stranded turtles suggest the possible dysbiosis in stranded turtles in addition to potential causal agents.

Conference Session Designation: (Microbiomes)
Presentation format: (Oral)
Student presentation: (Yes)

The Role of Pacific Oyster (*Crassostrea Gigas*) Microbiome in the Pathogenesis of *Ostreid Herpesvirus-1* Infection

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Mass mortality disease outbreaks have caused severe economic loss in the global Pacific oyster industry. *Ostreid herpesvirus-1* (OsHV-1) has emerged as an important cause of disease outbreaks, yet the outcome of this infection is impacted by many factors. A polymicrobial pathogenesis may be associated with mass mortality outbreaks associated with OsHV-1 including the influence of the commensal microbiota of oysters. As filter-feeders in variable estuarine environments, oysters host a microbiota that is influenced by the environment. Changes in the microbiome provide an indirect pathway by which the environment can alter susceptibility to disease and might explain some of the variability in mortality caused by OsHV-1. This study aimed to: (1) compare the microbiome of Pacific oysters from a common hatchery but grown in geographically distinct estuaries; and (2) evaluate changes in the microbiome with particular reference to *Vibrio* spp., during progression of an experimental OsHV-1 infection. Pacific oysters sourced from a single hatchery were grown by commercial farming methods in three geographically distinct estuaries. The oysters (10-16 months of age) were acclimated to the laboratory and challenged with a measured dose of OsHV-1. Samples were collected: A) before OsHV-1 challenge; B) soon after OsHV-1 challenge; C) from moribund oysters; D) from survivors 7 days after OsHV-1 challenge and; E) from unchallenged control oysters at the end of the experiment. Total bacterial, OsHV-1 and *Vibrio* genomic DNA were quantified from each sample, using real-time PCR assays. The bacterial community composition in oysters was identified by sequencing the bacterial 16S rRNA gene and the relative abundance, diversity and evenness of bacterial families were calculated. Non-metric multidimensional scaling was used to visualize the dissimilarity in bacterial community structures between samples, and was analysed using one-way permutational multivariate analysis of variance (PERMANOVA). The initial diversity and evenness of bacterial families was different for oysters grown in the three estuaries. Mortality after OsHV-1 challenge was also variable between the batches. A difference in relative abundance of bacterial families and an increase in diversity, after the viral challenge ($p<0.05$) occurred for oysters from two estuaries. In contrast, the same bacterial community structure was maintained by oysters challenged with a negative control and those that survived the viral challenge, for two sites of origin. Both OsHV-1 and *Vibrio* DNA concentration was higher in dead oysters compared to live oysters. A strong correlation was observed between the OsHV-1 DNA load and *Vibrio* in OsHV-1 infected oysters. In conclusion, the bacterial community composition in oysters was different for each geographic site at which they were grown. The bacterial community changed with the outcome of OsHV-1 challenge. A potential opportunistic role of *Vibrio* spp. in the disease associated with OsHV-1 was demonstrated. This study provided insights on the potential for different estuarine environments to shape the Pacific oyster microbiome and how different commensal bacterial populations are associated with different outcome of OsHV-1 infection.

Conference session designation: (Microbiomes)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Efficacy of a Bath Probiotic Application before Seawater Exposure in Atlantic Salmon

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This laboratory study was undertaken to evaluate and verify significant results from large scale field trials with probiotic bath treatment on weight gain, survival and wound development in Atlantic salmon with exposure to natural water borne challenge *Moritella viscosa* and *Aliivibrio wodanis*. Nine (9) weeks before seawater entry, duplicate tanks of PIT tagged fish (weight range 40-60 g, n=200 per group) were bathed in three dilutions of selected probiotic strains of *Aliivibrio* species or no bacteria as a control in brackish rearing water containing 25 ppt salt. The treatment was applied for 30 minutes as a static bath and was ended by a return to normal water flow.

During the acute phase of the outbreak with *M. viscosa* a significant reduction in the mortality of fish in the 1/100 treatment group was observed. Mortality observed 9-45 days after seawater introduction resulted 40% and 32% loss in the control groups, and 23.4% and 23.4 % in the treated groups. The relative percent survival was of 35% in the reduction of impact of acute infection. In contrast, there was no significant reduction in overall survival in the groups treated with 1/600 or 1/1000 dilution of the treatment seeding. To evaluate the possibility of an immunological effect, we analyzed blood plasma samples from treated and control fish. The result was that the anti-*M. viscosa* titers were inversely proportional to dose, i.e., the 1/100 dilution group had the lowest titer and the controls, followed by the 1/1000 and 1/600 group had the highest titer. This indicates that benefit afforded by the probiotic treatment was not related to a humoral antibody response to the microbial introduction. As a measure of overall effectiveness and tertiary growth factors, the incremental weight increase over the whole study period was analyzed against the start weight. The mean weight of fish in the treatment groups was not statistically significant from the control groups. However, due to the higher survival rate at the effective treatment concentration, the 1/100 treated group had a total biomass of 16.2% over that of the control group and an absolute weight gain difference from the start to end of study of 14.2%, over 229 days (7.6 months).

The results of the probiotic bacteria application clearly indicate that a good effect is afforded by seeding in terms of pathogen displacement and general overall health to combat a naturally occurring epidemic outbreak with ulcerative bacterial infection. The infection progressed after the acute phase period with more chronic losses including the involvement of *A. wodanis*, but a clear benefit to avoiding to a higher degree the infection in treated fish was evident. Selected strains of *Aliivibrio sp.* were protective at the effective concentration of 1 x10 ^{7} cfu-mL and supported significantly better growth in fish challenged with ulcerative disease.

Conference Session Designation: (Microbiome)
Presentation Format: (Oral)

Compatibility of Application of a Probiotic Treatment in Anesthetic Bath Using Benzocaine or Tricaine Methanesulfonate (TMS)

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This laboratory study was undertaken to determine the safety and efficacy of application of live probiotic bacteria in combination with anesthesia commonly used for sedation prior to vaccination. The parameters assessed were survival by cultivation of the probiotic bacteria in the presence of the anesthetics with prolonged exposure (3,5 h), blood uptake of the bacteria in treated fish within 5 minutes of exposure, survival and safety of the treatment for 21 days after treatment, and time to stage II anaesthesia with and without the concurrent application of probiotic bacteria. The probiotic, Stembiont™ is a bath treatment of active cultures of specified strains of bacteria for microbial enhancement of the microbiome and has been shown to reduce the disease, and increase growth and survivability after exposure to sea water in treated smolt (Amthor *et al*., this session).

For the survivability determination *in vitro*, fresh cultures of the probiotic were exposed with Benzocaine (0,89 ml/L) or TMS (80 mg/L) in 1 % (10 ppt) salinized tap water and samples plated on blood agar plates at 30 min intervals. An initial decrease in cfu to time 60 minutes of 0.5 $x10^1$ in both the anesthetic groups and the controls was observed. Following 60 min, all groups maintained stabile end-point viability >1 x 10^8 cfu/mL to time 3.5 h.

Groups of fish (n=20) were introduced to anesthetic bath containing the same concentrations of anesthetic as above. Individual fish were observed to determine behavior and the time to loss of ability to right Stage II anesthesia). The mean time to reach anesthesia varied from t=36 sec to t=41 seconds and was not significantly different between the treatments.

Blood uptake by positive cultivability was sampled from 5 fish per group 5 min of introduction to the baths containing probiotic with or without anesthetic. Bacterial counts of 2000-10,000 cfu/mL blood were recorded and no biologically significant differences between anesthetic and without anesthetic were observed. In addition, no adverse behavioral characteristics or mortality were observed over 21 days in any of the treated groups.

The results of the combined application of specified probiotic strains and either Benzocaine or TMS did neither effect the effective concentration of anesthesia or probiotic and can be considered compatible for co-administration. Blood uptake of probiotic bacteria is rapid from bath application and easily applied at the time of anesthesia before vaccination where it is useful to augment the microbiome in the face of handling stress and vaccination.

Conference Session Designation: (Microbiome)
Presentation Format: (Oral)

Effect of Oxytetracycline Treatment on the Gastrointestinal Microbiome of Critically-Endangered White Abalone (*Haliotis sorenseni*) Treated for Withering Syndrome

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The white abalone (*Haliotis sorenseni*) is a critically-endangered marine gastropod native to the northeastern Pacific and is at risk of extinction in the wild due to overfishing. Despite closure of the white abalone fishery in 1996 there has been no significant recruitment in remaining wild populations. Captive-rearing programs in California have been successful in culturing white abalone with the intent to re-establish wild populations throughout the species' native range. Withering syndrome (WS) is a fatal disease caused by colonization of the gastrointestinal tract by an intracellular prokaryotic Rickettsiales-like parasite (WS-RLP), identified as *Candidatus xenohaliotis californiensis.* In other abalone species, induction of disease following infection with WS-RLP is significantly accelerated with increased water temperatures, making the disease of special interest with regards to climate change and ocean warming. Studies have shown that white abalone have the highest susceptibility and the lowest intrinsic resistance to WS of all Pacific abalone species. WS-RLP has been identified in wild white abalone populations and poses a considerable threat to captive culturing operations. Oxytetracycline (OTC) is effective in eliminating WS-RLP infections from the gastrointestinal tract of affected abalone. OTC concentrates in the digestive gland of exposed abalone and provides protection against reinfection with WS-RLP for up to six months after the completion treatment. OTC baths (500 mg/L) are used to treat and protect captive populations from WS. Clinically, OTC treatment is well-tolerated by abalone and there is no significant difference in growth rates between treated and untreated abalone. While the genetic composition of the normal white abalone gastrointestinal biome has not been fully characterized, as kelp-eaters they are also suspected to rely on a balanced microbiome for optimal feed utilization; dysbiosis could result in reduced fitness of white abalone in the wild. Because many white abalone that are destined for release into the wild undergo OTC treatment during their captive-culture phase it is important to investigate the long-term impacts that this antimicrobial may have on the fitness of these animals. This study is a metagenomic comparison between the gastrointestinal microbiomes of OTC-treated and untreated control captive-cultured white abalone to evaluate the impact of OTC treatment on the gut microbiome. Gastrointestinal tracts from five treated individuals and five untreated controls were sampled at each time point: time 0, at the end of the 21-day OTC treatment, and 200-days post-treatment to coincide with the withdrawal period of the OTC. Gastrointestinal tracts were analyzed via 16S metagenomics and compared to evaluate for any statistical differences between bacterial populations between the groups. This study achieves two goals: to characterize the gastrointestinal microbiome of normal white abalone and to evaluate the potential for long-term impact of OTC treatment for WS-RLP on individuals that are part of a captive-breeding program with the intent to release into the wild.

Conference session designation: (Invertebrate and Shellfish Disease)
Presentation format: (Oral)
Student presentation: (Yes)

Monday September 3rd – Langeve / Cartier
Parasitology Life Cycle
Moderator – Isaure de Buron (College of Charleston)

1:15 PM	Parasitology: Life Cycle	Cole - Life Cycle of *Truttaedacnitist truttae* (Nematoda: Cucullanidae) in Rainbow Trout (*Oncorhynchus mykiss*) in the Colorado River Drainage in Grand Canyon, Az
1:30 PM		De Buron - First Evidence of Polychaete Intermediate Hosts for Marine Turtle Blood Flukes (Trematoda: Spirorchiidae)
1:45 PM		Geist - Host-Parasite Interaction Between Salmonids and Larvae of the Freshwater Pearl Mussel
2:00 PM		Alberson - Experimental Elucidation of the Life Cycle of *Drepanoscephalus auritus* (Digenea: Echinostomatidae) in the Double-Crested Cormorant (*Phalacrocorax auritus*), the Marsh Rams-Horn Snail (*Planorbella trivolvis*), and the Channel Catfish (*Ictalurus punctatus*)
2:15 PM		Nowak - Understanding Parasitic Life Cycles Contributes to an Improved Health Management of Ranched and Farmed Bluefin Tuna
2:30 PM		Rosser - Investigations into the Life Cycles of Trematodes in Catfish Aquaculture Systems in Mississippi, USA
2:45 PM		**Woodyard - Morphological and Molecular Data Linking the Life Stages of *Sebekia mississippiensis* (Pentastomida: Sebekidae) from the American Alligator *Alligator mississippiensis* and the Spotted Gar *Lepisosteus oculatus*, with Notes on Pathology in the Intermediate and Definitive Hosts**

Life Cycle of *Truttaedacnitis truttae* (Nematoda: Cucullanidae) in Rainbow Trout (*Oncorhynchus mykiss*) in the Colorado River Drainage in Grand Canyon Arizona.

Rebecca A. Cole[1]* and Anindo Choudhury[2]

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Truttaedacnitis truttae is a cucullanid nematode of primarily salmonine fishes. Brown trout (*Salmo trutta*) in Europe reportedly become parasitized by ingesting lampreys (*Lampetri planeri)* carrying infective larvae. However, our field and laboratory observations suggested that North American *T. truttae* has an alternative life cycle. High abundance and potential impact of *T. truttae* in rainbow trout, *Oncorhynchus mykiss*, in the Colorado River drainage in Grand Canyon prompted a study on the transmission dynamics of this nematode. Eggs of *T. truttae*, collected from live gravid females, were incubated in the laboratory, and snails (*Physa gyrina* and *Lymnaea* sp.) were exposed to *T. truttae* larvae three to four weeks later. Active larvae of *T. truttae* were observed penetrating the intestinal wall of exposed snails, and worm larvae were found in the visceral tissues one week after exposure. Larvae in snails showed little growth and development two weeks later and corresponded to L3 larvae. Infected snails were fed to hatchery-reared juvenile rainbow trout in which developing stages were subsequently found in the mucosal lining and lumen of trout intestines. Adult male and female (gravid) worms were found in the ceca of trout examined five to six months after consuming infected snails. Larvae found in pepsin/trypsin digests and mucosal scrapings from wild, naturally infected trout corroborated laboratory findings. Screening of *Physa* sp. and gammarids collected from the Colorado River, Grand Canyon for natural infections with *T. truttae* using ITS-1 primers gave positive results. *Trutteadacnitis truttae* is the second species, after *T. clitellarius* of lake sturgeon, capable of using a snail as a first intermediate/paratenic host, and is similar to several other cucullanids in having a histotropic phase of development in the definitive fish host.

Conference Session Designation: (General Parasitology)
Presentation Format: (Oral)

First Evidence of Polychaete Intermediate Hosts for Marine Turtle Blood Flukes (Trematoda: Spirorchiidae)

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Spirorchiids that infect the vascular system of turtles are not well studied. Few life cycles of these blood flukes have been elucidated and all intermediate hosts reported are mollusks, regardless of whether the definitive host is a freshwater or a marine turtle. During a recent survey of blood fluke larvae in polychaetes on the coast of South Carolina USA, sporocysts and spirorchiid-like cercariae were found to infect the terebellid *Amphitrite ornata* and polycirrid *Enoplobranchus sanguineus*. Cercariae were furcate and had a ventral acetabulum, but no ocelli were observed. Partial sequencing of D1-D2 domains of the large ribosomal subunit (LSR), ITS2, and *cox1* genes allowed the identification of two *Neospirorchis* species reported from green turtle, *Chelonia mydas* in Florida USA. *Neospirorchis* sp. (Neogen 13) and *Neospororchis* sp. (Neogen 14) infected individuals of *A. amphitrite* and *E. sanguineus,* respectively. Phylogenetic analysis showed that infection of annelids by blood flukes evolved separately in aporocotylids and spirorchiids. This finding demonstrates that specificity of spirorchiid for their intermediate hosts is broader than it was thus far assumed and that survey of annelids in turtle habitats is necessary to further our understanding of the life history of these parasites.

Conference Session Designation: (Parsitology General or WAVMA)
Presentation Format: (Oral)

Host-Parasite Interaction Between Salmonids and Larvae of The Freshwater Pearl Mussel

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Endangered freshwater pearl mussels (*Margaritifera margaritifera*) have a complex life cycle which involves a larval glochidium stage at which the mussel larvae need to attach to the gills of a suitable salmonid host fish that develops immunity after first infestation. In this study, the suitability of different salmonid species and strains as hosts for the freshwater pearl mussel was investigated using standardized infestation procedures. Also, the temperature-dependency of glochidia metamorphosis on the gills, the transfer of nutrients from the host to the mussel larvae based on stable isotope analyses, and the impacts of different infestation intensities on the performance of brown trout (*Salmo trutta*), were analyzed. In addition, genetic co-evolutionary patterns between host fish and mussels were investigated. The results suggest that freshwater pearl mussel larvae can clearly be considered parasites of their salmonid hosts as evident from the observed mortality and reduced swimming performance of hosts at high infestation densities, as well as the unidirectional nutrient transfer from the host to the growing glochidium. On the other hand, salmonids may also benefit from the presence of mussels due to their filtration activity and effects on water clarity. Timing of metamorphosis and transformation success was strongly temperature-dependent, with pronounced differences among host strains within the same species. The genetic population structure from pearl mussel and their main host fish, brown trout, suggest co-evolutionary colonization patterns revealing stronger differentiation among mussel populations and more pronounced genetic drift effects in the parasite compared to the more mobile host fish. The better understanding of this unique host-parasite relationship in which the maximum age of the mussel exceeds the maximum age of the host by a factor of 30 is useful for the conservation of both host fishes and mussel.

Conference Session Designation: (Parasitology General)
Presentation Format: **(** Oral)

Experimental Eucidation of the Life Cycle of *Drepanoscephalus auritus* (Digenea: Echinostomatidae) in the Double-crested Cormorant (*Phalacrocorax auritus*), the Marsh Rams-horn Snail (*Planorbella trivolvis*), and the Channel Catfish (*Ictalurus punctatus*)

Neely R. Alberson[1], Thomas G. Rosser [1], D. Tommy King[2], Lester H. Khoo[3], David J. Wise[3], Ethan T. Woodyard[1], Fred L. Cunningham[2] and Matt J. Griffin[3]*

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Drepanocephalus auritus is a trematode parasite of the double-crested cormorant *Phalacrocorax auritus*, and in North America, the marsh rams-horn snail *Planorbella trivolvis*, and ghost rams-horn snail *Biomphalaria havanensis*, both commonly found to inhabit catfish aquaculture ponds in east Mississippi. Previous infectivity challenges demonstrated *D. auritus* is infective to channel catfish *Ictalurus punctatus*, although infection begins to resolve quickly. A 2-year study was undertaken to experimentally elucidate the life cycle of *D. auritus.* In both studies, *P. trivolvis* were collected from a commercial catfish operation in east Mississippi, of which several were releasing *D. auritus* cercariae. Juvenile channel catfish were exposed individually to ~150 cercariae/fish. Double-crested cormorants (DCCO) were live captured, housed individually, and given praziquantel to clear gastrointestinal helminth infections. During the first study, 3 experimental DCCO were fed *D. auritus* infected fish. Fecal samples were collected daily and observed for trematode ova. At 18 days post-exposure (dpe) birds were sacrificed, and gravid, adult trematodes morphologically and molecularly consistent with *D. auritus* were recovered from experimental DCCO. During the second study, 2 experimental DCCO were fed infected fish. Daily fecal sampling continued as in the previous year's study, and trematode ova was observed in experimental birds beginning 8 dpe. Once ova were observed, birds were allowed to defecate into clean water tanks containing naïve *P. trivolvis*. Birds were then removed and snails transferred to a laboratory aquarium for holding. Eggs from experimental DCCO feces were recovered by sedimentation and placed into a second aquarium housing 15 naïve *P. trivolvis*. In addition, eggs were placed individually in 24 well cell-culture plates and checked daily for miracidia, which were observed, on average, after 16 days at 25C. All birds were sacrificed on study day 60 and adult trematodes were again recovered from experimental birds. Recovered adults were morphologically and molecularly confirmed as *D. auritus*. One snail from the DCCO tanks shed *D. auritus* cercariae 97 dpe, while another snail, directly exposed to trematode eggs, began shedding *D. auritus* 89 dpe. This work is the first experimental confirmation of the *D. auritus* life cycle and sheds new light on the complex biology of this parasite. Moreover, this work resolves unknown developmental timelines and provides critical baseline information for the development of management practices to minimize the effects of digenetic trematodes on US catfish aquaculture.

Conference Session Designation: (Parasitology –Life Cycle)
Presentation Format: (Oral)

Understanding Parasite Life Cycles Contributes to an Improved Health Management of Farmed and Ranched Bluefin Tunas

Barbara F. Nowak

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Bluefin tunas, including Atlantic Bluefin Tuna (ABT), *Thunnus thynnus*, Pacific Bluefin Tuna (PBT), *Thunnus orientalis* and Southern Bluefin Tuna (SBT), *Thunnus maccoyii* are commercially important species. Bluefin tunas are either ranched (wild fish fattened in sea pens – ABT, PBT and SBT) and farmed (life cycle closed, commercial hatchery production – PBT) in a number of countries. Parasites, in particular blood flukes *Cardicola orientalis*, *Cardicola opisthorchis* and *Cardicola forsteri* are the main health problems for ranching and farming of bluefin tunas. Husbandry is the key in bluefin tuna health management. Our knowledge of blood fluke life cycles, in particular the intermediate hosts and their habitats has contributed to the minimisation of the risk of infection with blood flukes. This is also true for other parasitic infections of SBT. For example, adult *Caligus chiastos* but few chalimi were observed on SBT. Proper feed management reduces interactions between SBT and wild Degen's leatherjacket *Thamnaconus degeni* which carry chalimi and adults *Caligus chiastos* and as a result decreases infections of SBT with sealice and minimises the risk of eye damage. Moving of SBT further off shore resulted in the reduction of parasitic loads. Another parasitic disease, swimmer syndrome is caused by scuticociliate *Miamensis avidus* but this infection is now rare. Improved husbandry and management of SBT health based on our understanding of the biology of parasites, including their life cycles, have increased sustainability of SBT production

Conference Session Designation: (Parasitology General or Tuna Diseases)
Presentation Format: (Oral)

Investigations into the Life Cycles of Trematodes in Catfish Aquaculture Systems in Mississippi, USA

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In the southeastern United States, farm-raised catfish is the most extensively cultured freshwater food fish. These earthen ponds are open to the external environment and provide an ideal system for the propagation of parasite life cycles. The American white pelican *Pelecanus erythrorhynchos*, double-crested cormorant *Phalacrocorax auritus*, great egret *Ardea alba*, and great blue heron *Ardea herodias* are the primary piscivorous bird hosts plaguing the industry through depredation and while doing so also introduce trematode parasites into the ponds. These complex trematode life cycles involve avian definitive hosts, aquatic snail first intermediate hosts and fish second intermediate hosts. *Bolbophorus damnificus* is the most damaging trematode and is responsible for production deficits due to mortality and parasite induced inappetence. However, in more recent years the true diversity of the trematode species occurring in these aquaculture systems has grown considerably. Surveys of snail first intermediate hosts, fish, and piscivorous birds utilizing these ponds have uncovered previously unknown life cycles. Using classical morphological, experimental, and molecular techniques we have elucidated complete or partial life cycles of >5 trematode species in catfish ponds and have evaluated impacts on the fish hosts through experimental infection studies. Two *Austrodiplostomum* sp. are known to infect catfish and other forage fish species found in catfish ponds and are found in the eyes and brain. One of these species (*A. ostrowskiae*) utilizes the double-crested cormorant as a definitive host and *Biomphalaria havanensis* as a first intermediate host. In addition to diplostomids, catfish ponds also play a role in the propagation of at least two *Clinostomum* spp. The "yellow grubs" *Clinostomum marginatum* and *Clinostomum album* are parasites of the great egret and infect catfish, silversides, and minnows in catfish ponds. The great egrets are also hosts to a multiple species of diplostomid and strigeid trematodes, many with unknown life cycles. In addition to trematodes with fish second intermediate hosts we have identified species infectious to native amphibians and have begun exploring their impacts on the host. Discussions on methods of elucidation of life cycles will be discussed.

Conference Session Designation: **(** Parasitology General)
Presentation Format: **(** Oral)

Morphological and molecular data linking the life stages of *Sebekia mississippiensis* (Pentastomida: Sebekidae) from the American alligator *Alligator mississippiensis* and the spotted gar *Lepisosteus oculatus*, with notes on pathology in the intermediate and definitive hosts

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Pentastomes are an enigmatic group of parasitic crustaceans found mostly as internal parasites in reptilian and fish hosts. In the southeastern United States, *Sebekia mississippiensis* parasitizes the lungs of the American alligator *Alligator mississippiensis* as an adult. While many freshwater fish have been reported as intermediate hosts, few of these accounts have provided morphological descriptions of the nymphs and to date no molecular data have been generated for *S. mississippiensis*. During Mississippi's 2016 and 2017 alligator hunting seasons, *S.mississippiensis* were recovered from American alligators collected from a commercial alligator processor in Mississippi. Concurrently, nymphs were collected from spotted gar *Lepisosteus oculatus* from Louisiana. Recovered adult and nymphal pentastomes were morphologically identified as *S. mississippiensis* and molecular data of ribosomal (18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA) and mitochondrial (cytochrome c oxidase subunit 1) DNA were generated. Using these molecular data, the nymphal stages from fish hosts and adults from alligators were confirmed to be conspecific. In the future, these data will allow further investigations into the molecular systematics of *S. mississippiensis* and its placement within the Pentastomida as well as more robust life cycle studies of the species. Histopathological analysis of lungs from alligators and alligator gar tissues was performed and the impacts on the hosts will be discussed. The presented data will help resolve many ambiguities in the literature regarding this species and its life cycle.

Conference Session Designation: **(** Parasitology General)
Presentation Format: (Oral)
Student Presentation: **(** Yes)

Monday September 3rd – Langeve / Cartier
Gill Health
Moderator - Mark Powell (Institute of Marine Research - Norway)

3:15 PM	Gill Health	Papanna - Gill Diseases In Meditarranean mariculture: Factors That Exacerbate Gill Pathologies Under Fish Farming Conditions
3:30 PM		**English - Possible Multi-Amoeba Aetiology of Amoebic Gill Disease (AGD) Of Farmed Atlantic Salmon**
3:45 PM		Powell - Does Exposure To Cnideria Increase The Susceptibility Or Severity Of Amoebic Gill Disease In Atlantic Salmon?
4:00 PM		De Jourdan - Assessing The Avoidance And Preference Behavior Of Atlantic Salmon *Salmo salar* To Varying Oxygen Saturation Waters
4:15 PM		Iqbal - Parasitic Infection Of Red Tiger Oscar *Astronotus ocellatus* Imported Into Pakistan
4:30 PM		Adamek - When Hypnos Meets Thanatos - Physiological Impact Of A Carp Edema Virus Infection Of Common Carp Gills During Koi Sleepy Disease
4:45 PM		**Bright - Histopathological Changes In The Gills And Skin of *Clarias gariepinus* Challenged With *Ichthyophthirius multifilis* And Treated With Aqueous Leaves Extract of *Moringa oleifera***
5:00 PM		Powell - Functional Feed Ingredients Against Amoebic Gill Disease: Efficacy And Effects on Gill Inflammatory Responses

Gill Diseases in Meditarranean Mariculture : Factors That Exacerbate Gill Pathologies Under Fish Farming Conditions

Kantham K. Papanna

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Gills are the most delicate structures of fish that are in their simplest of structure, and in constant exposure to the environment they live-in. As a consequence of this they are easily pliable to the influences of the external environmental factors and internal pathophysiological alterations. Gill Diseases caused by pathogenic and environmental agents are on the increase in the Mediterranean Marine fish farming in recent years. This presentation will highlight on the important gill diseases in Mediterranean marine aquaculture and the present control methods and limitations in executing suitable pathogen specific treatments. The gill diseases were minimal in the beginning of Med aquaculture, however over the years various gill pathogens with specific pathological effects have appeared in the cultivated fish. Over the years the gill disease causing agents have also accumulated in the culture environments. With present environmental changes associated with global warming and constantly changing feeds due to the limitations on the availability of fish meal and increases reliance unsustainable plant protein quality in the present diets, fish are increasingly more susceptible to gill diseases and exhibit poor immune competence to cope with the stresses.

Conference Session Designation: (Gill Health)
Presentation Format: (Oral)

Possible Multi-Amoeba Aetiology of Amoebic Gill Disease (AGD) of Farmed Atlantic Salmon

Chloe J. English[1,2*], Paula C. Lima[2], James W. Wynne[3], Andrew C. Barnes[1], Mathew T. Cook[4]

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Amoebic gill disease (AGD) is a parasite-mediated gill condition affecting many teleost fish globally, and it is the biggest health issue impacting farmed Atlantic salmon in Tasmania's expanding aquaculture industry. To date, *Neoparamoeba perurans* is considered the only aetiological agent of AGD, based on laboratory trials that confirmed its pathogenicity, and its frequent presence on the gills of infected farmed Atlantic salmon. However, the development of gill disease in salmonid aquaculture is complex and multifactorial, and is not always tightly associated with the presence of *N. perurans*. Moreover, multiple other amoeba species colonise the gills and their role in AGD is unknown. Previous reports of these accompanying amoebae on AGD-affected salmon based their taxonomic assessments on gross morphology alone, and are therefore likely inaccurate. The aim of this study was to more accurately document the diversity of amoebae colonising the gills of AGD-affected Atlantic salmon using a combination of morphological and sequence-based taxonomic methods. Amoebae isolated from AGD-affected salmon gills were characterised morphologically via light microscopy and transmission electron microscopy, and by phylogenetic analyses based on the 18S rRNA gene and COI gene. Apart from *N. perurans*, 11 other amoebozoans were found on the gills, and were classified within the genera *Neoparamoeba*, *Paramoeba*, *Vexillifera*, *Pseudoparamoeba*, *Vannella* and *Nolandella*. This comprehensive documentation of amoeba species highlights there is a far greater diversity of amoebae colonising AGD-affected gills then what is currently considered. Ongoing research which is investigating whether these accompanying amoebae are involved in AGD development, or can act as the primary agent in the absence of *N. perurans* will also be discussed.

Conference Session: (Gill Health)
Presentation Format: (Oral)
Student Presentation: (Yes)

Does Exposure to Cnideria Increase the Susceptibility or Severity of Amoebic Gill Disease in Atlantic Salmon ?

Mark D. Powell[1,2*], Nina Bloecher[2], Sigurd Hytterød[4], Mona Gjessing[4], Haakon Hansen[4], Anne-Gerd Gjevre[4]

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Amoebic gill disease (AGD) is a significant disease affecting Atlantic salmon, *Salmo salar* aquaculture in Europe, North America, and Australia and is often a component of what is now referred to as "complex gill disease" – a multifactorial response to multiple potentially gill pathology causing agents. Of these potential agents, cniderians (hydroids and scyphozoa) are known to cause gill pathology alone where contact with the nematocysts has the potential to cause irritation and pathological damage to salmon gills. This study examined the potential implications of pre- exposure of Atlantic salmon (*Salmo salar* L). smolts to sublethal levels of hydrozoa (*Ectapleura larynx*) or scyphozoan jellyfish (*Cyanea capillata*) 24h prior to challenge with *Neoparamoeba perurans*, the causative agent of AGD. This study used laboratory challenge trials to: (1) characterise the gill pathology resulting from the exposure of salmon *to E. larynx* or *C. capillata*, and (2) investigate if such exposure can predispose the fish to secondary infection – using *N. perurans* causing amoebic gill disease (AGD). Gill health (AGD gill scores, non-specific gill scores, lamellar thrombi, epithelial hyperplasia) was monitored over 5 weeks and compared to an untreated control group. In both cases, higher average numbers of gill lamellar thrombi occurred in fish up to 7 days after exposure to hydroids or jellyfish. However, gill pathologies caused by hydroids did not affect the infection rates of *N. perurans* or the disease progression of AGD based upon gross gill score or histopathology. On the other hand, pre-exposure to jellyfish appeared to retard the development of AGD over the initial 3 weeks post-challenge based upon gross gill score. Thereafter, gross gills scores between *N. perurans* only and *C. capillata* and *N. perurans* combined were equivocal. Similarly, the prevalence of *N. perurans* positive fish (based upon qPCR analysis of *N. perurans* mRNA) indicated that there were fewer amoebae on the gills of fish in the combined challenge, compared with those of the *N. perurans* only group. This study indicated that cnidarian pre-exposure prior to infection *with N. perurans* did not enhance the rate of infection, not severity of disease and potentially may have lessened the impact of infection. The reasons for this are not clearly understood but may reflect the nature of the inflammatory responses occurring in the gills in response to cnidarian envenomation with nematotoxins.

Conference Session Designation: (Gill Health)
Presentation Format: **(** Oral)

Assessing the Avoidance and Preference Behaviour of Atlantic Salmon *Salmo Salar* to Varying Oxygen Saturation Waters

Ben de Jourdan[1], Anne McCarthy[1] and Michael Beattie[2]

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Temperature has a significant role in governing the metabolic rate in poikilotherms like fish, with increases in temperature corresponding to increases in metabolic rate and subsequently oxygen demand. Ironically, increases in temperature also lowers the solubility of oxygen, thereby reducing the supply of dissolved oxygen available for respiration. These two intrinsically linked water quality parameters have a major impact on the ecology of aquatic species, and it is not uncommon for them to be negatively affected in the presence of hydroelectric dams. This may, in turn, negatively impact migration and result in lower returns of culturally and economically important fish species.

This study was designed to provide behaviour data of Atlantic salmon (*Salmo salar*) as it relates to avoidance or preference in a two-current flume choice system with waters of varying oxygen saturation. Study water was made up on demand using a proprietary gas infusion system to infuse either oxygen or nitrogen to raise or lower the measured dissolved oxygen concentrations, respectively, while maintaining water total gas pressure. A total of 27 trials were conducted with groups of 10 fish (total = 270 fish) tested in a two-current choice flume. Each current (left and right) had varying concentrations of dissolved oxygen (ranging from 68 to 125% saturation). The trials were conducted at three different temperatures (8, 10, and 12^{o}C) and involved nine combinations of flumes, for a total of 81 individual runs. Each run was 10 minutes in duration and was recorded by an overhead GoPro Hero3+ camera. Video analysis of each run was performed using ImageJ and ToxTrac software. From the video analysis, gross and net avoidance were calculated for each group of fish relative to oxygen saturation, and swimming performance metrics (e.g., swimming speed, acceleration, and exploration rate) were determined in each treatment flume.

While the data did not support a preference or avoidance related to freshwater oxygen saturation within the tested concentrations, we noted significant increases in swimming speed and acceleration with increasing concentrations of dissolved oxygen. These behaviours, and the benefits of higher oxygen saturated freshwater, may be more important to fish ladder performance compared with fish preference. Greater swimming capacity displayed within high oxygen saturation may enhance fish abilities to cross ladders and move past dams during their incoming annual migration. Discussion of these laboratory results will be augmented with data from ongoing field trials involving the deployment of this innovative technology in a fish ladder at a hydroelectric dam.

Conference Session Designation: **(** Climate Change & Aquatic Animal Health)
Presentation Format: (Oral)

Parasitic Infection of Red Tiger Oscar, *Astronotus ocellatus* Imported into Pakistan

Zafar Iqbal and Asna Fatima

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The aim of present study was to observe parasitic infection in red tiger Oscar, *Astronotus ocellatus*, an ornamental fish imported into Pakistan. Total 30 specimens of red tiger oscar, were obtained from a pet shop in Lahore and were examined clinically and histopathologically. The mean total length and mean body weight of the fish were 7.82 ± 2.99cm and 8.59 ± 6.07g respectively. Clinically infected fish showed eroded dorsal and caudal fins. One fish did not have left eye ball and two fish had curved vertebral column. One fish had white spot on the body. Skin, fins and gills were observed under microscope by wet mount preparation. Gills were also examined histologically for detailed observations.

A total of 7,503 parasites were recorded in red tiger oscar fish. The parasites observed were protozoans; *Ichthyophthirius multifiliis* (13.3%, MI=257) and *Piscinoodinium pillulare* (10.0% MI=480.6); monogeneans, *Dactylogyrus* sp. (96.66%, MI=139.3), *Gyrodactylus* sp. (10%; MI=196.6); digeneans; *Postodiplostomum* sp.(6.66%; 151.5) and encysted metacercaria of trematode (6.66%; 49.5). Gill infection by *Dactylogyrus* sp. was the most prevalent compared to infection by other parasites. Histological results of infected gills showed; hyperplasia, fusion of secondary lamellae, swollen nodules on tips of gill filaments as a result of attachment of *Dactylogyrus* sp. and *Ichthyobodo* sp. on the gills. Gill infection may be categorized as low, mild and severe. Large fish showed low infection as compared to small fish. The present observations raise issues with regard to veterinary inspection of the imported fish. Bio-security measures including strict quarantine must be adopted to avoid transmission of parasites into local fish fauna of Pakistan.

Conference Session Designation: (Gill Health or Ornamental Fish Diseases)
Presentation Format: (Oral)

When Hypnos Meets Thanatos - Physiological Impact of a Carp Edema Virus Infection of Common Carp Gills During Koi Sleepy Disease

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Koi sleepy disease, caused by an infection with carp edema virus is a unique virus model which predominantly affects carp gills. Initially infected fish are lethargic, start laying at the bottom of the tank. With the progress of the disease, the activity of the fish decreases until nearly complete stillness, followed by death. We hypothesized that these clinical signs are related to gill dysfunction. Therefore, the pathophysiological impact of the infection was measured, including an analysis of the respiration, ammonia excretion and the hydro-mineral balance. Furthermore the blood plasma metabolome of KSD affected fish was studied. The experimental setup included two strains of carp (AS and koi) with different susceptibilities to KSD. All carp were cohabitated with koi infected with a CEV variant from genogroup IIa. During four infection experiments performed at 18 °C 100% of the koi developed severe KSD, which led to a complete immobilisation of the animals at the bottom of the tank between days 6 and 12 post infection (p.i.) with a peak at days 6 p.i.. In blood collected at days 6 and 9 p.i., the oxygen content was slightly reduced, sodium and calcium concentration extremely decreased (e.g. Na^+ dropped from 130 mmol l^{-1} in control to 82 mmol l^{-1} in infected group), and ammonia levels severely increased from 212 µmol l^{-1} in controls to 658 µmol l^{-1} in infected fish. Analyses of over 2,500 metabolites showed changes in the pyrimidine and urea cycle as well as the beta-alanine and amino acid metabolism in blood plasma at day 6 p.i.. These changes occurred only in clinically affected koi while clinically healthy AS strain carp remained unaffected. This correlated with a much higher virus load and the onset of histopathological changes in the gills of koi. Furthermore, a 0.6% NaCl supplementation to keeping water was able to prevent the fish form developing clinical sighs of KSD including the sleepy behaviour, the elevated ammonia level and the loss of ions measured in blood. The bath however, did not stop the virus infection and virus load did not differ between fish kept on the NaCl and not supplemented groups. Taken together the results suggest that the sleepiness of the fish is not related a lack of oxygen but to a disruption of the waste removal from the amino acid metabolism which leads to intoxication with ammonia. While the death is most likely caused by the severe osmotic imbalance.

Conference Session Designation: (Gill Health)
Presentation Format: (Oral)

Histopathological Changes in the Gills and Skin of *Clarias gariepinus* Challenged with *Ichthyophthirius multifilis* and Treated in Dip Bath Treatment with Aqueous Leaves Extract of *Moringa oleifera*

Ikele, C Bright[*1], Mgbenka, B Obialo[1] and Ikele, C Faith[2]

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Ichthyophthirius multifilis (Ich) is a ciliate protozoan pathogenic parasite in the wild and freshwater system that parasitizes gills and skin of freshwater fish, *Clarias gariepinus*. The use of chemotherapeutants has promoted residual chemical drugs on the body of the fish, therefore the need for the use of environmental friendly herbal therapy has a great potential as a suitable replacement for chemotherapeutants and a good pharmacopeia in aquaculture. A study was conducted to investigate the effects of aqueous leaves extract of *Moringa oleifera* in the histology of the skin and gill of *Clarias gariepinus* challenged with *Ichthyophthirius multifilis*. Six concentrations of aqueous leaves extract of *Moringa oleifera* were exposed to ich-infested fish for 1h to limit the impact of the adult parasite (trophont) in juveniles of *Clarias gariepinus*. The cumulative incidence of the Ich infestation was significantly lowered in the skin and gill of the treated fish compared to the negative control ($p<0.05$). The major histopathological alterations revealed abnormal and some significant morphology characteristics in the skin; fatty degeneration, abscess formation, degeneration of the muscle fibers while presence of matured adult parasite embedded on the gill lamellae, degenerated secondary lamellae, edema and epitheliocystis were observed in the gill. An irreversible lesion was observed in the skin and gill of the negative control. Meanwhile, the overall lesion scores analyzed using kruskal wallis revealed asymptotically significant changes; oedema ($p=0.041$), severe destruction of the secondary lamellae ($p=0.025$), fatty degeneration ($p=0.041$) and inflammatory infiltrates ($p=0.02$) to be the most observed damage among the groups before the treatment began. Ich parasitic infestations in the organs of *C. gariepinus* are very dangerous due to observed lesions that are putative routes for secondary infection and subsequent manifestation of diseases. These results indicate that the use of aqueous leaves extract of *Moringa oleifera* reduced the adult parasite in the gills and skin of Ich-infested fish, although with clear tissue damage but cannot be ruled out as an environmental friendly herbal therapy for controlling ichthyophthiriasis.

Conference Session Designation: (Aquatic Animal Health Management)
Presentation format: (Oral)
Student Presentation: (Yes)

Functional Feed Ingredients Against Amoebic Gill Disease: Efficacy and Effects on Gill Inflammatory Responses

Mark D. Powell[1,2*], Sindre Rosenlund[3], Ole Høstmark[3] Christopher Hawes[4] and Simon Wadsworth[4]

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Amoebic Gill Disease (AGD) is rapidly becoming a significant health issue in Norwegian fish farming. The causative agent, *Neoparamoeba perurans* is often seen as a single agent disease, AGD is also observed as a component of a complex of potential gill pathogens resulting in a multifactorial gill disease. The primary treatment of the disease has only two commercially available options; freshwater and hydrogen peroxide. Investigation of potential functional feed candidates to be used to slow disease progression and form part of an integrated pathogen management program has been undertaken. Firstly, an assay for investigating the efficacy of a feed candidate was developed for screening potential candidate compounds supplied by Cargill innovation. After the screening process, three candidates was picked for in-vivo direct challenge pilot trials and incorporated into test diets. A strong correlation between results of in-vitro screening, and in-vivo challenge trials, based on gill scores and RT-PCR for *Neoparamoeba perurans*. Pilot testing of two of the potential candidate compounds showed significant reductions in gross gill pathology and histological lesions. For one compound, gill gene expression was examined and showed significant changes supporting the development of gill inflammation and suppression of epithelial cell hyperplasia consistent with the histological findings. These results suggest that functional dietary feed ingredients against *Neoparamoeba perurans* can also modulation of gill inflammatory processes.

Conference Session Designation: (Gill Health)
Presentation Format: **(** Oral)

Monday September 3rd – Tilly / Tupper
American Association of Fish Veterinarians (AAFV)
Moderator – Thomas Waltzek (University of Florida)

9:30 AM	AAFV	Mitchell - The early years of salmon farming medicine in the US: Practice and Politics
9:45 AM		
10:00 AM		Milligan - Changing practices and environment affecting aquaculture: overview of the current status and challenges facing a BC Atlantic salmon farm
10:15 AM		Whittaker - The Risk to Farmed Atlantic Salmon of Wild Piscine *Orthoreovirus* (PRV) in British Columbia
10:30 AM		**Refreshments**
10:45 AM		Reichley - Fish health challenges in large-scale rainbow trout production
11:00 AM		
11:15 AM		Morrison - History and future of Integrated Pest Management (IPM) in British Columbia
11:30 AM		
11:45 AM		Hickey - Western Washington Treaty Tribe's Pacific Salmon Enhancement Programs
12:00 PM		
12:15 PM		**AAFV Business Lunch**
12:30 PM		

The Early Years of Salmon Farming Medicine in the US: Practice and Politics

Hugh Mitchell

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Salmon farming veterinary medicine began in Maine and Washington State in the mid- to late '80's. Initially in Maine, the major farms were venture capital funded, with uneven stockmanship acumen together with a host of colorful characters. Fish health was very "pathogenocentric" and policies and regulations did not often encompass a fully-integrated approach to fish health. The message that practitioners seemed to have to continually promulgate was one of: "Presence of the pathogen does not mean the presence of disease". The fish health learning curve was steep, and many of the current issues (e.g.: sea lice dermatitis and Infectious salmon anemia) hadn't yet entered the picture. One by one, existing disease issues of the early days were figured out and solved. How to cost-effectively injection vaccinate large numbers of hatchery fish was a key component in managing several diseases. Furunculosis and cold water vibriosis were two diseases mitigated with successfully integrated strategies. The dearth of FDA-approved pharmaceuticals for farmed fish was alleviated by ready and quick access to Emergency INAD solutions from the FDA. Anti-salmon farming activism was present but extremely low key and interaction of farmed and wild salmon diseases was rarely voiced as a concern. The lecture will conclude with a flash-forward to the future, a quick review of current top disease issues, current regulatory policies, politics and anti-farming activism efforts, especially with respect to the focus on disease issues. With the advancement in genomic technology, a reinterpretation of the message from the early days might be: "Presence of a pathogen's DNA doesn't necessarily mean infection or disease".

Conference Session Designation: (AAFV)
Presentation Format: (Oral)

(Withdrawn)

Potential Impacts of Environmental Change on Atlantic Salmon in British Columbia.

Barry Milligan

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Two potentially significant production concerns in Atlantic salmon include “winter ulcers” and maturation. Depending on severity both types of downgrades can have significant impacts on health, growth and sales price resulting in more than 20 percent loss in stock value. In the last seven years there has been a significant increase in the prevalence of both winter ulcers and maturation in stocks farmed by Cermaq Canada in open saltwater net pens. During this period the level of downgrades due both to winter ulcers and maturation more than doubled. Maturation levels have been successfully reduced through photo manipulation. Winter ulcer levels appear to have been successfully reduced through importation of intra-peritoneal vaccines for Moritella viscosa. The broader potential impact of environmental variability (oxygen, temperature, salinity) on fish health, production, and husbandry will be discussed.

Conference Session Designation: (American Association of Fish Veterinarians)
Presentation Format: (Oral

The Risk to Farmed Atlantic Salmon of Wild Piscine *Orthoreovirus* (PRV) in British Columbia

Patrick Whittaker*, Matthew Patterson, Tim Hewison

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Grieg Seafood BC Ltd, has tested all groups of smolts for piscine orthoreovirus (PRV) before going to the marine environment since Aug 2015 (n=88 pools by PCR). All samples have tested negative for PRV, whether taken from fish produced within our hatcheries, or externally purchased smolts (5 hatchery sources). Smolts were then tested from each region within three months of entry into the marine environment. This is followed up by regional testing until samples become positive. Grieg Seafood BC farms with a single year class stock in some geographically unique regions, most not bordering other salmon farming companies. The source of infection is theorized to be wild fish, potentially salmonids. Despite evidence of eventual PRV infection, no clinical disease attributable to PRV has been seen in any of the populations of fish.

Conference Session Designation: (AAFV)
Presentation Format: (Oral)

Fish Health Challenges in Large-Scale Rainbow Trout Production

Stephen R. Reichley*, Stacy G. King and Andy Morton

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Rainbow trout farming has a long history in the United States. In September of 1966, Ted Eastman and a group of investors started Clear Springs Trout Company. In 1991, the company name was changed to Clear Springs Foods, Inc. In August of 2000, ownership changed from a closely held private company to a privately-held employee-owned company. Today, Clear Springs Foods is the leading producer of premium rainbow trout, processing over 20 million pounds a year and has over 300 employee-owners. Clear Springs is 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. This talk with provide an overview of the fish health challenges due to infectious diseases (viral, bacterial, and parasitic) facing the largest producer of freshwater rainbow trout in the United States. Prevention and control strategies for these infectious diseases will also be discussed.

Conference Session Designation: (AAFV)
Presentation Format: (Oral)

History and future of Integrated Pest Management (IPM) in British Columbia

Diane B Morrison*

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Salmon farming in the coastal waters of British Columbia (BC) Canada has been in operation for over 30 years. In the 1980's the industry moved from rearing Pacific salmon (*Oncorhynchus tshawytscha, Oncorhynchus kisutch*) to the species of choice for farming, Atlantic salmon (*Salmo salar*). Around the same time there was an increase in environmental activisms against salmon farming, which has resulted in extreme polarization of opinions on the value and risk of salmon farming in BC.

Sea lice (*Lepeophtheirus salmonis*) will be used as an example to illustrate how opponents of salmon farming have driven research dollars and regulatory focus, how the industry has responded and how a lack of political support has hindered pest management on the farms. The required components of IPM will be discussed with examples of how the BC industry is able to apply them. Data from sea lice monitoring, treatment efficacy, bioassay and genetic testing will be used to highlight the need for full implementation of IPM components. In 2014 the first hydrogen peroxide (Interox® Paramove 50®) permit was granted for one production area. Data from areas where hydrogen peroxide has been incorporated into an IPM plan will be presented and discussed.

Conference Session Designation: (American Association of Fish Veterinarians)
Presentation Format: (Oral)

Western Washington Treaty Tribes' Pacific Salmon Enhancement Programs

Nora O. Hickey

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The 1974 *United States v. Washington* case, also known as the Boldt decision, reaffirmed the right of treaty tribes in western Washington to co-manage their natural resources, including Pacific salmon, alongside state and federal agencies. Today, over forty years later, the western Washington treaty tribes are leading the effort to preserve Pacific salmon populations in the Pacific Northwest. Tribes maintain sustainable fisheries, restore destroyed and degraded habitat, keep captive brood populations for threatened and endangered stocks, and run hatchery programs to enhance wild runs. These activities require fish health services, and ultimately resulted in the creation of the Northwest Indian Fisheries Commission (NWIFC) and its Tribal Fish Health Program.

The NWIFC is a tribal natural resources management support service organization for 20 treaty Indian tribes in western Washington. One service provided to tribes by the NWIFC is the Tribal Fish Health Program (TFHP). The TFHP consists of a fish health program manager, a program veterinarian, two field fish pathologists, and two microbiologists, as well as a fish health laboratory with cell culture and molecular diagnostic capabilities. The TFHP provides a number of different fish health services to member tribes, including broodstock spawning surveillance, routine fish health checks, disease investigation, and educational workshops.

Tribes are becoming increasingly involved in managing their natural resources, and with this increased involvement comes new opportunities for veterinarians to provide fish health services, as well as opportunities for collaboration for veterinarians providing fish health services for local, state, and federal agencies.

Conference Session Designation: (American Association of Fish Veterinarians)
Presentation Format: (Oral)

Monday September 3rd – Tilly / Tupper
American Association of Fish Veterinarians (AAFV)
Moderator – Thomas Waltzek (University of Florida)

1:15 PM	AAFV	Wyatt - Restoration of Lake Sturgeon as a Bioindicator Species in Rochester, New York's lower Genesee River EPA Area of Concern
1:30 PM		
1:45 PM		Kebus - Regulatory Fish Health Documents for Live Fish Movement
2:00 PM		
2:15 PM		Gaunt - Fresh from the Field: Using Antimicrobials and Veterinary Feed Directive Drugs in Aquatic Medicine
2:30 PM		
2:45 PM		**Refreshments**
3:00 PM		
3:15 PM		Whaley - Offshore Aquaculture – A One Health Approach
3:30 PM		
3:45 PM		Shelley - Ornamental Aquaculture Medicine in the United States – Past, Present and Future
4:00 PM		
4:15 PM		Sanders - The Development and Sustainability of Private Aquatic Veterinary Practice; Anesthesia, Surgery and Pain Management in Common Pet Fish
4:30 PM		
4:45 PM		Giffin - Canada's Aquatic Animal Health Import Program: A Global Perspective
5:00 PM		

Restoration of Lake Sturgeon as a Bioindicator Species in Rochester, New York's Lower Genesee River EPA Area of Concern

Jeffrey D. Wyatt[1,2]* and Dawn E. Dittman[3]

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The lake sturgeon, a contemporary of the dinosaur, came swimmingly close to extirpation in Lake Ontario due to overfishing and spawning habitat degradation over the past two centuries of industrialization and exploitation. Rochester's industrial waste discharged historically into the Genesee River significantly damaged the aquatic environment of the river and Rochester embayment warranting designation in 1987 by the U.S.-Canada Great Lakes Water Quality Agreement as one of forty-three Great Lakes Binational Areas of Concern. After fifteen years of industrial waste reduction, river & watershed remediation and habitat assessments, U.S. Federal and New York State governmental agencies partnering with academic and conservation minded community organizations launched an experiment to test the health of the Genesee River by reintroducing the lake sturgeon. In 2003 and 2004, we released 1,900 hatchery-reared sturgeon to determine if the lower Genesee River would once again support this legendary, native fish. Morphometric data collected from our mark and recapture studies over the following ten years demonstrated that the sturgeon were growing at impressive rates from four inches to four feet matching healthy populations in unpolluted river systems of the Great Lakes. An EPA Great Lakes Restoration Initiative (GLRI) grant funded research where we compared blood levels of persistent chemical contaminants (PCBs, dioxins/furans, mirex, mercury, cadmium, silver and nickel) in our repatriated, ten-year old sturgeon with age-matched controls from a Lake Ontario reference tributary, the Oswegatchie River, not located in an EPA Area of Concern. Our results indicate that the blood levels of persistent chemical contaminants responsible for fish consumption advisories are attributed to lake-wide effect and not from the city's historic, industrial discharging into the Rochester Embayment Area of Concern. With such positive bioindicator data, we began a program annually releasing 1,000 hatchery-reared sturgeon into the lower Genesee River totaling 4,000 more repatriates so far. Our sturgeon restoration success and bioindicator data renew community hope and pride in our healing watershed and aquatic ecosystem.

Conference Session Designation: (American Association of Fish Veterinarians)
Presentation Format: (Oral)

Regulatory Fish Health Documents for Live Fish Movement

Myron J. Kebus

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In Wisconsin, a valid "Fish Health Certificate" must be provided prior to import of live fish to Wisconsin, or introduction into waters of the state. Wisconsin has trained and listed private veterinary practitioners who are available to issue these certificates to private aquaculture, state-run aquaculture, and tribal fish hatchery producers. Wisconsin's fish health program is administered by the Wisconsin Department of Agriculture, Trade & Consumer Protection (WDATCP), Division of Animal Health.

The WDATCP has developed a veterinary-training short-course, "Aquaculture Veterinary Medicine for Practitioners' with the UW-Madison School of Veterinary Medicine, and national fish health experts, It is an intensive program designed to provide practical training in field techniques for sample collection and field diagnostics. This course has attracted over 300 veterinarians from throughout the world.

http://vetmedce.vetmed.wisc.edu/FishCertificate/

A second online Fish Health Certificate Program for Fish Producers is has been developed by the Wisconsin Department of Agriculture, Trade & Consumer Protection (WDATCP), University of Wisconsin-Stevens Point, University of Wisconsin–Madison, and the Iowa State University. The course is constructed as a series of modules for fish producer leading to certification. The modules include discussions to help fish producers understand fish health testing and fish health certificates and prepare them for fish health work that may be conducted at their facility on their fish. The six modules can be taken anytime and anywhere using narrated PowerPoint presentations and supplemental reading materials delivered using new educational technology software. This online course has attracted over 500 producers worldwide.

This presentation will also discuss Certificates of Veterinary Inspection (CVI), the predominant form of health certificate for animals in the United States. These certificates are issued by accredited veterinarians for terrestrial and avian livestock to meet the requirements for animal movement throughout the country. In recent years the need for a CVI for aquatic animal movement has been discussed. Along with other aquatic animal health groups, the American Veterinary Medical Association has worked to assist in the development of a CVI for aquatic animals. This presentation will present some of the efforts in this area to-date.

Conference Session Designation: (American Association of Fish Veterinarians)
Presentation Format: (Oral)

Fresh from the Field: Using Antimicrobials and Veterinary Feed Directive Drugs in Aquatic Medicine

Patricia S. Gaunt

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Use of antibiotics in food animals has been debated for decades, and in response to continued concern over antimicrobial resistance in humans, FDA issued several guidelines which revised animal antimicrobial usage. In 2012, FDA finalized the "Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals" (Guidance #209) which represented FDA's thinking on antimicrobial drugs that are medically important in human medicine and also used in food-producing animals. The two main principles covered by this guidance were to (1) limit medically important antimicrobial drugs to uses in animals that were necessary for assuring animal health, and (2) include veterinary oversight on medically important antimicrobial drugs used in animals.

Another FDA guideline was Guidance #213 "Recommendations for Drug Sponsors for Voluntarily Aligning Product Use Conditions with GFI #209" finalized in 2013, to provide pharmaceutical sponsors with recommendations to voluntarily modify use of their medically important antimicrobial drug products to support the two principles in Guidance #209. These guidances changed the marketing status of antibiotics in aquatic medicine from over the counter to either a prescription (if dispensed in water) or VFD (if dispensed in feed) effective January 2017 and to withdraw production uses. Based on these guidances, FDA revised the First VFD Rule and formulated the New or Second VFD Rule.

This presentation will explore implementation of the Second VFD Rule in aquatic medicine. Although many presentations have discussed major provisions of this rule, there are still many unanswered questions from veterinarians and producers. MSU CVM has received many inquiries on the use of antimicrobials in aquatic animals which will be discussed in this presentation.

Conference Session Designation: (American Association of Fish Veterinarians)
Presentation Format: (Oral)

Offshore Aquaculture – A One Health Approach

Janet Whaley

NOAA National Marine Fisheries Service, Offices of International Affairs, Seafood Inspection and Aquaculture 1315 East West Highway, Silver Spring, MD 20910 Janet.Whaley@noaa.gov

The United States imports between 85 to 90% of its seafood from both wild-caught and aquaculture sources. This has resulted in a trade deficit of about $14 billion in 2016. The new U.S. Administration has set a goal to increase seafood exports in order to close this gap. According to the 2016 United Nations Food and Agriculture Organization report of the State of the World Fisheries and Aquaculture, the U.S. ranks 17th in total aquaculture production despite having the one of the world's largest coastlines. Boosting aquaculture in the U.S. may meet the expanding global demand for seafood and to decrease our trade deficit. Seafood safety starts with the aquaculture farmer or fisherman and continues with the Federal government, seafood importers and exporters, retailers, restaurants, and consumers. NOAA Fisheries is one federal agency working with industry to close the gap by expanding opportunities for marine aquaculture in the open ocean. With this expansion, the opportunities for veterinarians will grow in the areas of aquatic animal health, seafood safety and conservation medicine – a One Health approach. Just like the terrestrial setting, farmer education, biosecurity, disease surveillance and reporting, and judicious use of veterinary pharmaceuticals are vital components in aquaculture all of which are key areas for veterinary involvement and leadership. When developing site- specific biosecurity plans, the veterinarian can also assess the risk of disease transfer to and from wild populations thus addressing risks to important fisheries. Another area that is ripe for veterinary involvement is public health and seafood safety where the veterinarian has the skill set to apply the hazards assessment and critical control points (HACCP) approach to mitigate risks. Finally, the veterinarian can assist with selection of sites considering relevant hazards and risks to production animals, seafood safety and wild populations.

NOAA Fisheries is currently collaborating with USDA APHIS Veterinary Services and FDA to develop guidance for ocean aquaculture famers (finfish and shellfish) to assist them with management of aquatic animal health and seafood safety issues. The guidance supports development of site-specific aquatic animal health plans that address training and communication, biosecurity measures, disease detection and mitigation, biomonitoring and surveillance, disease reporting, disease investigation, depopulation, and follow-up activities (cleaning and disinfection). NOAA Fisheries is also working with the AVMA to further develop policy on licensing veterinarians for practice in federal waters (i.e., outside state water jurisdiction). The overall approach presented here in managing aquatic animal health in offshore aquaculture endeavors is likely to serve as a template for other U.S. offshore regions.

Conference Session Designation: (American Association of Fish Veterinarians)
Presentation Format: (Oral)

Ornamental Aquaculture Medicine in The United States – Past, Present and Future

John P Shelley[1]*, Roy P E Yanong[2], Ruth Francis-Floyd[3] and Gregory A Lewbart[4]

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Ornamental fish aquaculture is a multimillion dollar industry in the United States concentrated in Florida. With roots in the 1930s, Florida's weather, water, and transport hubs fostered major expansion by the 1970s. As with other burgeoning livestock industries, health and disease management became increasingly important. In the 1970s John B. Gratzek of the University of Georgia pioneered aquarium fish medicine, becoming the go-to veterinarian for Florida fish farmers. In 1987, Ruth Francis-Floyd began working with Florida producers on health issues via the University of Florida's Institute of Food and Agricultural Sciences (UF/IFAS) and College of Veterinary Medicine (CVM). In 1988, extension agent Craig Watson joined Francis-Floyd at UF/IFAS to work on health and production issues. The two initiated a Fish Health Management Workshop for the ornamental fish producers in the late 1980s that is still taught to this day. In 1988, Greg Lewbart, who received training from Gratzek, began working for aquarium fish wholesaler O'Beirne Tropical in Philadelphia, PA as the first veterinarian employed full-time in the ornamental fish industry. Lewbart helped establish an import station in Naples, FL and developed contacts with the Florida fish producers. In 1992, Lewbart facilitated Roy Yanong's employment at 5D Tropical Inc. as the first full-time veterinarian employed by an ornamental fish commercial production facility. In 1995, Segrest Farms, one of the largest wholesalers in the U.S. hired veterinarian Denise Petty, who also worked with partner farms. John Slaughter worked with the industry through the University of Florida CVM from 1995-96. In 1996, under the leadership of Watson, the University of Florida opened the Tropical Aquaculture Laboratory (TAL) in Ruskin, FL; Yanong began work there as an extension veterinarian later that year. In 1997, TAL began its aquaculture veterinary student externs program. In 2003, based at TAL, Kathleen Hartman joined USDA APHIS Veterinary Services (VS) as its first field aquaculture Veterinary Medical Officer (VMO). In 2015, after Hartman's promotion to National Aquaculture Program Leader, Kat Starzel joined as an import/export VMO. Concurrently, with TAL and others, USDA APHIS VS began more targeted biosecurity programs with Florida producers. Various diseases of concern with widespread financial implications have cropped up over the years, but through veterinary involvement the ornamental aquaculture industry has been able to identify and address these issues. UF/IFAS continues to support the industry through diagnostics, research and training support; state and federal agency engagement; aquaculture training for current and future veterinarians; and by other venues including online publications, social media and webinars.

Conference Session Designation: (American Association of Fish Veterinarians)
Presentation Format: (Oral)

The Development and Sustainability of Private Aquatic Veterinary Practice

Jessie M Sanders, DVM, CertAqV

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There a significantly fewer aquatic private practitioners in comparison to small and large animal veterinarians. The divide may be due to a lack of standardized education, perceived opportunities or simply not knowing how to get involved in aquatic practice. With the substantial number of pet fish, aquaculture, research and aquarium facilities, many opportunities are available for veterinarians to be involved in aquatic medicine. The journey of Aquatic Veterinary Services can offer potential aquatic clinicians an inside look at the triumphs and hurdles of private aquatic veterinary practice. Looking forward, private aquatic veterinary practice needs to involve more practicing veterinarians in order to serve the fish requiring veterinary care and oversight.

Conference Session Designation: (American Association of Fish Veterinarians)
Presentation Format: (Oral)

Anesthesia, Surgery and Pain Management in Common Pet Fish

Jessie M Sanders

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Treatment options available to private and public aquatic veterinary practitioners expand beyond traditional medical therapy. Surgical treatment of fish has clear benefits for all fish, including common pet fish, including koi, goldfish and betta fish. With surgical treatment comes important considerations for anesthesia and pain management. Depending on the situation, anesthetic protocols can vary and practitioners need to be familiar with what is available and how to measure anesthetic depth in fish. Pain management should always be considered in a complete surgical protocol. Special considerations may be taken with patients that remain in their home tank or pond post-surgery versus remaining in a controlled hospital environment.

Conference Session Designation: (American Association of Fish Veterinarians)
Presentation Format: (Oral)

Canada's Aquatic Animal Health Import Program: A Global Perspective

Bernita Giffin

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Canada's import controls are designed to meet international aquatic animal health standards to protect Canadian aquatic resources (both wild and farmed aquatic animals) from the introduction of reportable, immediately notifiable and emerging diseases. Prevention of disease introduction allows Canadian exporters to maintain competitive international market access.

Canada's Aquatic Animal Health Import Program controls imports of live and dead, cultured and wild aquatic animals that are susceptible to diseases of concern to Canada and is consistent with the World Organization for Animal Health (OIE) guidelines. Import controls for susceptible species are based on the end use and the levels of controls are commensurate with the risk of introducing disease into natural populations. The Canadian Food Inspection Agency negotiates export certification requirements with exporting countries and can be specific to the animal health situation, regulatory framework, and aquatic animal health programs present in the exporting country.

Various regulatory issues and international trade issues which must be overcome to facilitate trade have included differing scope of regulations and aquatic animal health requirements, inclusion of wild animals, competent authority oversight of exports and specific test requirements. Other considerations for Canada's Aquatic Animal Health Import program have included OIE reference lab certification requirements and OIE changes in approach for determining susceptible species and diagnostic testing guidelines.

Conference Session Designation: (Aquatic Animal Health Programs)
Presentation Format: (Oral)

Keynote Presentation

Tuesday September 4th

Bacterial Skin Ulcer in Farmed Fish; Preventive Effect of Vaccines and Probiotics

Henning Sørum*, Aud Kari Fauske, Gaute Skogtun

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Ulcers and fin rot has been recognized as an animal welfare problem and as a cause of both acute and chronic mortality in various farmed fish species like carp, eel, flounder, goldfish, and salmonids. In farmed Atlantic salmon *Moritella viscosa* was included in the intraperitoneal vaccines from the last half of the mid-90's to control winter ulcer disease in farmed Atlantic salmon. Vaccines with *M. viscosa* antigens reduced the acute outbreaks of winter ulcer in the cold periods of the year. However, there has still been chronic mortality caused by ulcer disease ever since and at least half of the mortality in the marine phase of the salmon production is connected to ulcer and fin rot disease.

Challenge and vaccine trials have documented that *A. wodanis* can cause ulcer disease and septicemic mortality. It is known that *A. wodanis* can produce a bacteriocin that reduces the growth and metabolism of itself and a number of other bacteria in its vicinity including *M. viscosa* at physiological salt levels. The bacteriocin production is not activated at higher salt concentrations in the marine water. The bacteriocin production by *A. wodanis* explains the chronic character of many winter ulcer outbreaks compared to the peracute mortality observed when *M. viscosa* is the single challenge organism in bath or injection challenge. During the last ten years four other novel ulcer pathogens have been discovered in addition to *M. viscosa* that are potentially controlled by the bacteriocin produced in *A. wodanis* during the infection.

Probiotic approaches have demonstrated some protective effects against ulcer development in farmed Atlantic salmon. Experimental vaccine trials also demonstrate a potential to improve the vaccine protection against ulcer disease.

Tuesday September 4th – Gray / Palmer / Pope Ballroom
Bacteriology / Mycology 1 & 2
Moderators – **Cova Arias** (Auburn University) **John Hawke** (Louisiana State University)

9:30 AM	**Bacteriology 1**	**Coleman - Susceptibility of the Emerging Pathogenic Mold *Veronaea botryosa* to Natamycin**
9:45 AM		**Powell - Culture and Identification of *Exophiala* spp. Isolated From Aquaculture Reared Lumpfish (*Cyclopterus lumpus*) in Newfoundland & Labrador, Canada**
10:00 AM		Sarowar - *Saprolegnia* Diversity Among Farmed Salmonids in Nova Scotia, Canada and Their Response to NaCl and Clotrimazole
10:15 AM		Rhodes - Emergence of Mucormycosis Among Marine Mammals in Pacific Northwest
10:30 AM		**Refreshments**
10:45 AM	**Bacteriology 2**	Kalindamar - T6SS Effector Protein *EvpP* Is Essential for *Edwardsiella ictaluri* Virulence in Catfish
11:00 AM		**Griggs - *Edwardsiella ictaluri* Type Three Secretion System Effector *EseK* Interacts With the Invariant Chain of the Channel Catfish MHC Class II Complex**
11:15 AM		Katharios - *Edwardsiella anguillarum* Infecting Farmed Sharpsnout Seabream *Diplodus puntazzo* in Greece; Genomic Characterization and Virulence
11:30 AM		Sandlund - Bacterial Ulcer Infections in Land Based Production of Large Post Smolts of Atlantic Salmon – A Case Study
11:45 AM		Garland - Refinement of *Moritella viscosa* Challenge Model End Points

Susceptibility of the Emerging Pathogenic Mold *Veronaea botryosa* to Natamycin

Denver J Coleman[1*], Beatriz Martinez-Lopez[2] and Esteban Soto[2]

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Sturgeon aquaculture is particularly important since wild populations are significantly affected due to overfishing, habitat destruction and pollution. In the Pacific North-west, White Sturgeon (*Acipenser transmontanus*) culture is a multi-million-dollar industry. The production of globally recognized, high-quality caviar makes this product one of the few aquaculture-generated exports for the United States. *Veronaea* is a small genus of saprobic fungi found in soil and on plant material, belonging to the family *Herpotrichiellaceae*, order *Chaetothyriales*. *Veronaea botryosa* is the etiologic agent of fluid belly; one of the most important emergent diseases in sturgeon aquaculture and has also been associated with disease in other aquatic animals as well as humans. Despite its impact on the caviar industry, there are no commercially available therapeutants against systemic *V. botryosa* infections and little is known regarding its disease pathogenesis. Additionally, there is currently very little published data regarding antifungal susceptibility *in vivo* or *in vitro*, and at present there are no known efficacious chemotherapeutants or vaccines available. Natamycin, also known as pimaricin, is a macrolide polyene antifungal agent produced by the bacterium *Streptomyces natalensis*. It targets ergosterol in the cell wall of fungi and has been used for food preservation and treatment of fungal infections in over 150 countries. As a food additive, levels beneath 40mg/kg in the finished product are considered safe for human consumption. In the present study, we established microbroth kinetic protocols, based on turbidity measurements, to analyze the growth characteristics of *V. botryosa* in seven nutrient media using modified published protocols.

Optimal *in vitro* fungal growth was observed in Potato or Sabouraud Dextrose base for *V. botryosa* incubated at 25°C. The generated protocol was then used to test the susceptibility of nine different *V. botryosa* isolates to natamycin. SBD and RPMI containing 1, 2, 4, 8, 16, 32□g/ml natamycin were inoculated with purified *V. botryosa* spores and growth curves were generated using a 96-well plate reader over a 120-hour period at 25°C. A cubic smoothing spline model compared the generated areas under the curve (AUC) for the different treatments in the different broth media. All concentrations of natamycin significantly lowered the AUC when compared to the respective positive controls ($p<0.05$). However, at least 4 and 16□g/ml of natamycin were needed to decrease the AUC by 70% for fungal growth in SBD and RPMI media respectively. These novel protocols can be used to investigate susceptibility of pathogenic fungus to antimicrobials and disinfectants as well as support future therapeutic protocols against emerging fungal diseases like fluid belly.

Conference Session Designation: **(** Antibiotic Use / Pharmacology)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Culture and Identification of *Exophiala* spp. Isolated from Aquaculture Reared Lumpfish (*Cyclopterus lumpus*) in Newfoundland & Labrador, Canada

Ashley L. Powell[1,2*]. Matthew E. Saab[1,2], Jan Giles[1,2], David Overy[3], Nicole O'Brien[4] and David Groman[1]

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In winter 2018 a population of lumpfish (*Cyclopterus lumpus*) from a land based research facility in Newfoundland and Labrador presented with clinical signs described as dark skin lesions. The clinical signs progressed to systemic disease resulting in darkened and necrotic gills as well as dark internal organs such as the heart. Swabs and tissues from kidney, heart, eye and skin were submitted to Aquatic Diagnostic Services at the Atlantic Veterinary College for culture and histology. Swabs were plated on blood agar with 2% NaCl (BAS), and incubated at 15°C and 22°C.

Culture results did not yield any significant bacterial pathogens, but growth of a black fungus was observed within 7 days at 22°C from kidney (2 fish), heart (1 fish) and eye (1 fish). Histological examination of body wall lesions exhibited multiple regions of necrosis and mixed leucocyte infiltrate and fungal hyphae colonization (4 fish), and multifocal to coalescing regions within ventricular spongiosum of endocardial hyperplasia and a mixed leucocytic infiltrate with affected cardiac myofibers colonized by segmented, melanized fungal hyphae in the heart tissue of two fish.

Morphological examination of the fungal colony and conidia confirmed the identification as *Exophiala* spp., known to cause systemic fungal infections in fish. DNA from the fungal isolate was extracted using a commercial kit, the ITS region was amplified, and the amplicon was sent for Sanger sequencing. Analysis of the sequenced ITS region revealed the isolate had only 3 bp differences compared with the type strain of *Exophiala psychrophila* with 99% identity, and is the closet species match of all *Exophiala* species. Further work with the isolate is in progress. The challenges in identifying aquatic fungal pathogens in a diagnostic laboratory will be discussed.

Conference Session Designation: (Cleaner Fish Diseases)
Presentation Format: **(** Oral)
Student Presentation (Yes)

Saprolegnia Diversity Among Farmed Salmonids in Nova Scotia, Canada and Their Response to NACL and Clotrimazole

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Saprolegniosis, caused by the oomycete genus *Saprolegnia*, causes considerable economic losses in the salmonid aquaculture industry. The pathogen diversity was determined by sequencing the Internal Transcribed Spacer (ITS) region of the rRNA gene among five salmonids (*Salmo salar, S. trutta; O. mykiss; Salvelinus alpinus, S. fontinalis*). The phylogenetic analysis of the sequences using the maximum likelihood method identified seven species: *S. parasitica* (n=82 samples, including four strains, S-1, S-2, S-3 and S-4), *S. ferax* (n=8 samples, including 2 strains, F-1 and F-2), *S. diclina* (n=5), *S. aenigmatica* (n=1), *S. torulosa* (n=4), *Saprolegnia* sp. (n=4) and *Pythiopsis cymosa* (n=2). Cyst diameter was similar among all isolates (7 to 9µm), but the presence/absence and length of specialized hair attachment structures on the cysts differed greatly, possibly a measure of pathogenicity between species. *In vitro* exposure to 3% NaCl for 24 hours killed zoospores/cysts from all isolates, but fully grown mycelia were resistant and resumed growth post-exposure in all species except two strains of *S. parasitica* (S-1 and S-3). The high salinity tolerance of mycelia may significantly limit the efficacy of NaCl treatments on infected fish in Nova Scotia farms. By contrast, Clotrimazole exposure *in vitro* (20°C) of two of the most abundant *S. parasitica* strains (S-1 and S-2) at 4 µg/ml killed >93% of cysts within 24h, and mycelial growth was greatly inhibited by 8 µg/ml. Clotrimazole targets the ergosterol biosynthesis pathway in *S. parasitica* and is a promising potential therapeutant.

Conference Session Designation: (Bacteriology / Mycology)
Presentation Format: (Oral)

Emergence of Mucormycosis Among Marine Mammals in Pacific Northwest

Linda D. Rhodes[1]*, Jessica L. Huggins[2], Dyanna M. Lambourn[3], Stephen A. Raverty[4], M. Bradley Hanson[1], Stephanie A. Norman[5], Joseph K. Gaydos[6], Jennifer K. Olson[7], Martin Haulena[8], and Michael M. Garner[9]

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Mucormycosis is a fungal infection described in humans as an indication of compromised immunity or debilitated health, and it has a poor prognosis (fatality rate 46-96%). Although mucormycosis is infrequently reported among marine mammals, there has been an increasing trend of cases in the northwestern coast of the United States and southwestern British Columbia, Canada. The first case in the region was reported in 2012 in a dead stranded harbor porpoise (*Phocoena phocoena*), and since then, has been the confirmed cause of death in twelve additional harbor porpoises, one Southern Resident killer whale (*Orcinus orca*), and one harbor seal (*Phoca vitulina*). In two other harbor seals, mucormycosis was detected but was not the cause of mortality. Histologically, granulomatous inflammation and necrosis with intralesional fungal hyphae has been detected in brain, lung, spleen, pancreas, kidney, lymph nodes, thyroid, and skin. DNA sequencing of the internal transcribed regions of ribosomal DNA from infected tissues have so far identified *Rhizomucor pusillus* and *Lichtheimia corymbifera* as etiologic agents. These and other species of fungi that cause mucormycosis in humans are common in the terrestrial environment, but their occurrence in marine waters is unknown. Applying a molecular diagnostic procedure for mucormycetes in clinical specimens, we survey water samples from nearshore coastal areas of Washington State (USA) and British Columbia (Canada) as well as inland waters of Puget Sound (USA) occupied by these marine mammal species to assess their exposure risk. This information, in conjunction with additional contextual data such as individual health status and concentrations of contaminants in tissues, can contribute to understanding the epidemiology of mucormycosis in these aquatic mammals.

Conference Session Designation: (Emergent Disease or Aquatic Mammals – WAVMA)
Presentation Format: (Oral)

T6SS Effector Protein Evpp is Essential for *Edwardsiella Ictaluri* Virulence in Catfish

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Edwardsiella ictaluri is a facultative intracellular fish pathogen that can survive inside macrophages, and its survival mechanisms are not well known. The type six secretion system (T6SS) is a special nanomachine that is used for active transport of effector proteins from bacteria to the host environment and inter-bacterial competitions. EvpP is one of the T6SS related secreted effector proteins. However, its role in *E. ictaluri* virulence is not known yet. In this study, we mutated and characterized the *E. ictaluri evpP* mutant (*Ei*Δ*evpP*). Results indicated that *Ei*Δ*evpP* resisted complement killing, but its attachment and invasion in catfish epithelial cells were significantly less than that of *E. ictaluri* wild-type (*Ei*WT). Uptake of mutant and wild-type strains as well as their survival inside peritoneal macrophages were similar. *Ei*Δ*evpP* and *Ei*WT were tolerant of both sodium nitroprusside and hydrogen peroxide stresses. The apoptosis assay indicated that survival rate of catfish head kidney macrophages was significantly higher in *Ei*Δ*evpP* group compared to *Ei*WT group at 24 h post-exposure. However, at the same time point, there were no significant differences in the early and late apoptotic changes. Remarkably, the necrosis rate was significantly less in the *Ei*Δ*evpP* group compared to the *Ei*WT group at 24 h post-exposure. *Ei*Δ*evpP* was less virulent in catfish compared to *Ei*WT, and vaccination of catfish with *Ei*Δ*evpP* protected them against *Ei*WT infection. Our results demonstrated that *evpP* plays a vital role in attachment and invasion of catfish epithelial cells, survival in head kidney macrophages, and virulence in catfish.

Conference Session Designation: (Bacteriology / Mycology)
Presentation Format: (Oral)

Edwardsiella ictaluri Type Three Secretion System Effector EseK Interacts with the Invariant Chain of the Channel Catfish MHC Class II Complex

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Edwardsiella ictaluri is a Gram-negative bacterium that causes enteric septicemia of channel catfish (*Ictalurus punctatus*). Replication of *E. ictaluri* in catfish head-kidney-derived-macrophages (HKDM) is dependent on an *E. ictaluri* encoded Type III Secretion System (T3SS) that translocates effector proteins from the bacteria in the *Edwardsiella* containing vacuole (ECV) through the bacterial cell wall and the vacuolar membrane directly to the host cytoplasm, where they bind host-cell target proteins in order to modify host physiology to favor infection. Of the nine *E. ictaluri* T3SS effectors identified to date, five contain a translocation domain, a leucine rich repeat (LRR) domain, and an E3 ubiquitin ligase domain. LRR's are solenoid-shaped protein binding domains whose shape determines the target protein. The *E. ictaluri* effectors differ in the number of LRR's that they contain, meaning that they have different shapes and target different host cell proteins with different functional effects on the host cell. The overall LRR proteins, however, are very similar to each other at the amino acid level with 71.4 to 79.5 percent identity. The high level of identity precludes the use of recombinantly expressed proteins to produce specific antibody for each protein because of cross-reactivity. In order to identify individual mutants, an epitope fusion approach was developed in which the FLAG tag was fused to the carboxyl terminus of EseK, and antibody to the FLAG tag was used in a co-immunoprecipitation assay to identify the host target protein for EseK. Previous work showed that EseK is translocated by the T3SS in HKDM and suggested that EseK binds to the invariant chain of the major histocompatibility complex class II (MHC class II), also known as CD74. Binding of EseK with CD74 would allow binding of MHC class II with endogenous peptides and potentially prevent binding of exogenous peptides. The goal of this study was to create a tagged EseK that could be specifically detected with antibody to confirm the interaction between EseK and CD74.The EseK::FLAG fusion strain was constructed and the interaction of CD74 and EseK was confirmed by co-immunoprecipitation using FLAG antibody-coated magnetic beads. Syber Ruby Red staining detected two distinct bands in a polyacrylamide-gel of the co-immunoprecipitated sample. Mass spectrometry analysis confirmed the two proteins to be EseK and CD74. The EseK::FLAG band was also confirmed for the presence of FLAG by immunoblot using anti-FLAG antibody. This assay confirmed the interaction of EseK and CD74. CD74 plays an important role in initiation of the adaptive immune response by binding the major groove of MHC class II to prevent binding of endogenous peptides during MHC class II assembly and transport to the endosome. Binding of EseK to CD74 could suppress presentation of foreign peptides on the surface of the antigen presenting cell and subsequently suppress antibody production. Work is ongoing to evaluate this hypothesis.

Conference Session Designation: (Bacteriology/ Mycology)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Edwardsiella Anguillarum Infecting Farmed Sharpsnout Seabream (*Diplodus Puntazzo*) in Greece; Genomic Characterization and Virulence

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Edwardsiellosis is a serious disease affecting a wide range of cultured fish species both in marine and freshwater environment. It is caused by *Edwardsiella tarda*, *E. ictaluri*, *E. piscicida* and *E. anguillarum*. We have recently described the first incidence of Edwardsiellosis in the Mediterranean Sea, in cultured sharpsnout seabream, *Diplodus puntazzo*. We have analyzed the strain EA011113 isolated from Greece, using whole genome sequencing focusing on its phylogenetic position, presence of prophages and virulence. Following multilocus sequence typing and whole genome comparisons with other bacteria of the *Edwardsiella* genus we showed that the strain belongs to the newly described species *E. anguillarum* and is closely related to the *E. piscicida*-like strain isolated from diseased grouper in the Red Sea, with which it shares an Average Nucleotide Identity of 99.95%. Furthermore, the isolate contained an intact prophage that could be induced spontaneously and seems to be widespread in several other bacteria. The prophage belonged to the Myoviridae family and its genome, which was individually sequenced, was 40844 bp in size with 76 putative ORFs, two of which were *in silico* predicted to encode pathogenic proteins. The virulence of the isolate was studied *in vivo*, using adult zebrafish and by *in silico* analysis of virulence-related genes. The LD50 of the EA011113 was 8.9 x 10^3 cfu/fish at 48h post injection and 5.3 x 10^2 at 72h. Using comparative genomic analysis, the genome of EA011113 was shown to contain 3 distinct T6SS and 2 T3SS clusters which are major virulence factors of the species. In total, the genome of EA011113 contained 94 putative virulence-related genes. The strain is non-motile contrary to the type strain and this is possible due to a large deletion in the flagellar biosynthetic protein flhB. This is the first confirmed report of *Edwardsiella anguillarum* in the Mediterranean Sea affecting farmed fish. It is a highly virulent strain that may constitute a significant threat for the aquaculture industry of the region.

Conference Session Designation: **(** Bacteriology / Mycology or Genomic Applications)
Presentation Format: (Oral)

Bacterial Ulcer Infections in Land Based Production of Large Post Smolts of Atlantic Salmon - A Case Study

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Production of Atlantic salmon (*Salmo salar*) in Norway amounts to about 1.4 milllion tons yearly. New and improved production methods are constantly being developed to meet demands of further increased production rates and efficiency. Currently, further growth is limited by disease during the growth period at sea, caused mainly by the parasitic salmon lice *Lepeophtheirus salmonis* and viral pancreas disease (PD). One approach to deal with these problems is to shorten the production time at sea by keeping the fish longer in facilities on land after smoltification. But the development of ulcers has challenged this approach. The bacterium *Moritella viscosa* has long been known to cause winter ulcers, but lately also *Tenacibaculum* spp. has frequently been isolated from sick and diseased fish suffering from ulcers. Mortality rates due to ulcers are variable, but ulcers are problematic as they may lead to declassification of the fish, reduced growth, and thereby economical losses. More importantly, however, ulcers represent a serious fish welfare problem that must be addressed to ensure sustainable production.
This present work is a result of a close cooperation with a commercial fish farm. The goals have been to identify bacteria causing ulcers in the facilities and to enhance the production protocol to mitigate its development. Both bacteriological and histological samples as well as samples from water and biofilm have been taken in addition to registration of ulcers. Results from this case study will be presented.

Conference Session Designation: (Cleaner Fish Diseases)
Presentation Format: **(** Oral)

Refinement of *Moritella Viscosa* Challenge Model End Points

Amy N. Garland *, Bryan E. Linton and Thomas E. Wright

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In Norwegian salmonid aquaculture, *M. viscosa* infection and the resulting winter ulcer disease is a major animal welfare concern. The most prominent clinical sign of an infection with *M. viscosa*, is the deep sores which can penetrate into underlying musculature of fish. These sores, or ulcers, open the animal to secondary infections, interfere with osmotic regulation, and can result in death.

A clinical laboratory study was conducted to produce a number of salt-water challenge models using *M. viscosa* isolates, that could reliably produce at least 60% cumulative mortality, and clinical signs in the form of sores of winter ulcer disease in Atlantic salmon. Sores were categorized as follows: S0= no sores, S1= superficial sores, which do not penetrate the skin to underlying muscle, and S2= deep sore penetrating the underlying muscle. One model objective was to assess mortality kinetics of Atlantic salmon following a bath challenge with *M. viscosa* at two concentrations. An additional objective of this model was to assess if removal of fish with observable S2 sores would negatively affect or skew mortality data.

Challenge concentrations were ran in duplicate; one replicate allowed fish to reach a moribund state or die, and one replicate removed and humanely euthanized fish with obvious S2 ulcers. Any fish with S2 ulcers that were euthanized, were counted as mortalities. Once challenged, fish were monitored daily for mortalities, moribund fish, or development of S2 ulcers (in relevant tanks) for 15 (High Concentration) to 22 (Low Concentration) days. No difference was noted in cumulative mortality for fish challenged with the Low Concentration (78.8%), and a difference of 6.2% (93.8 – 100.0%) was noted between replicates for fish challenged with the High Concentration. All replicates produced acceptable mortality levels according to pass criteria required by the study objectives. All fish counted as mortalities produced clinical signs of an infection with *M. viscosa*, and all fish tested for specificity to *M. viscosa* via agglutiantion with *M. viscosa antibodies*, tested positive. Additionally, removing S2 ulcerated fish from one replicate of fish challenged with Low Concentration, reduced that tanks challenge duration by two days compared to the replicate tank where ulcerated fish were not removed immediately.

This study demonstrated that removing fish with S2 ulcers from a challenge tank does not negatively affect the challenge kinetics and, it is acceptable, recommended, and humane to remove fish challenged with *M. viscosa* once S2 ulcers develop.

Conference Session Designation: (Bacteriology / Mycology)
Presentation Format: (Oral)
Student Presentation: (Yes)

Tuesday September 4th – Gray / Palmer / Pope
Bacteriology / Mycology 3
Moderator – Ben LaFrentz (USDA – Agricultural Research Service)

1:15 PM	Bacteriology 3	Gulla - Detection and Epizootiology of *Yersinia ruckeri*
1:30 PM		Kumar - Proteomic Changes of Rainbow Trout Intestinal Mucosa in Response to *Yersinia ruckeri*
1:45 PM		Menanteau-Ledouble - Host Genes Involved in Intracellular Invasion by the Enterobacterium *Yersinia ruckeri* in Fish Cell Cultures
2:00 PM		Welch - Flagellar Regulation Is Required for Virulence in *Yersinia ruckeri*
2:15 PM		Katharios - *Aeromonas veronii* bv *sobria*: An Emerging Threat for European Seabass Aquaculture. Virulence and Vaccination Trials
2:30 PM		**Richardson - Evaluating Atypical *Aeromonas hydrophila* (aAh) in Catfish Aquaculture of the Mississippi Delta Region**
2:45 PM		Xiao - A new Isolate of *Aeromonas salmonicida* Caused Furunculosis in Atlantic Salmon (*Salmo salar*) From Recycling Aquaculture System in China

Detection and Epizootiology of *Yersinia ruckeri*

Snorre Gulla[1*], Andreas Riborg[1,2], and Duncan J. Colquhoun[1]

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Yersinia ruckeri is a significant pathogen of farmed salmonid fish worldwide. Following its first identification in Norwegian farmed salmon in 1985, yersiniosis rapidly became a serious problem with nearly sixty farms affected in 1987. By the mid-90's, however, the number of annual outbreaks were drastically reduced, largely through improved production conditions and increased focus on biosecurity. The situation remained stable until the mid-2000's when the number of outbreaks yet again began to increase, both in freshwater and marine sites.

While the bacterium can easily be cultured and serotyped using standard techniques, or detected by PCR, diagnostic investigations have not always correlated well with the clinical yersiniosis situation, possibly indicating strain differences in virulence. To improve our understanding of the epizootiology of yersiniosis we have focused our research on molecular typing and improved PCR detection of virulent *Y. ruckeri* infections. We have developed both a high-resolution Multi-Locus Variable number of tandem repeat Analysis (MLVA) scheme and a series of specific qPCR assays for *Y. ruckeri* detection at the species, serotype and clonal complex levels.

Our research has revealed that nearly all yersiniosis outbreaks in Norwegian aquaculture over the last twenty years or so have been caused a single clonal complex of serotype O1, apparently exclusive to Norway. Similarly, distinct clones appear to dominate in other salmon-producing countries, while another serotype O1 clonal complex dominates the disease situation in international rainbow trout farming. While most historic and present yersiniosis outbreaks worldwide have been associated with serotype O1 strains, we find an increasing body of evidence for the widespread existence of putatively low-virulent or avirulent environmental strains belonging to serotype O1.

Conference Session Designation: (Bacteriology / Mycology)
Presentation Format: (Oral)

Proteomic Changes of Rainbow Trout Intestinal Mucosa in Response to *Yersinia Ruckeri*

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Yersinia ruckeri is the causative agent of enteric redmouth disease in salmonids. The signs of the disease include exophthalmia, subcutaneous hemorrhages, splenomegaly and inflammation of the lower intestine. The intestinal epithelium is a primary site of enteric pathogens infection. The intestine is a multifunctional organ that plays a crucial role in nutrient uptake, host–pathogen interactions and immune system. Little is known about intestinal proteomic changes in rainbow trout in response to *Y. ruckeri*. In this study, we examined proteome changes in the intestine of rainbow trout after exposure to *Y. ruckeri*. Fish were challenged by immersion of *Y. ruckeri* strains and sampled at different time points. Each lower intestine was opened and washed three times with phosphate-buffered saline. Intestinal mucosa was scraped and immediately frozen in liquid nitrogen. Intestinal mucosa samples were resuspended in denaturing lysis buffer and disrupted by sonication. The lysates were centrifuged and supernatants were collected. Protein digestion was performed using a standard in-solution protocol. Resulting peptides were analyzed by nano LC-MS/MS directly coupled to a high resolution quadrupole time of flight mass spectrometer (TripleTOF 5600). Quantification of proteins was performed using SWATH MS2 data independent technology. Statistical analysis was performed in R programming language to identify differential expression of intestinal mucosa proteins. Sophisticated statistical evaluation revealed 62 up-regulated and 75 down-regulated proteins in intestinal mucosa of rainbow trout during *Y. ruckeri* infection. These proteins mostly are related to exopeptidase and endopeptidase activities, defense response, ion binding and metabolic process. The findings of this study provide new insight to understanding defence mechanisms and host-pathogen interactions of intestine during *Y. ruckeri* infection. These advanced proteomic data expand our knowledge on effects of *Y. ruckeri* on intestine of rainbow trout.

Conference Session Designation: (Bacteriology)
Presentation Format: (Oral)

Host Genes Involved in Intracellular Invasion by the Enterobacterium *Yersinia Ruckeri* in Fish Cell Cultures

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Yersinia ruckeri is an important fish pathogen. Like other members of the genus *Yersinia*, it is a facultative intracellular bacterium. While little is known about the mechanisms of intracellular invasion in *Y. ruckeri*, several mechanisms of invasion have been described in other bacterial pathogens, including within the genus *Yersinia*. Interestingly, the bacterium triggers its own uptake by the host cells and therefore relies on the apparatus of the host cells, for example, its cytoskeleton.

Consequently, the role of several host genes in the invasion process of *Y. ruckeri* was investigated. 17 genes that are known to play a role in the invasion of other facultative intracellular bacterial pathogens were silenced in Chinook Salmon Embryo using small inhibitory RNA. The cells were then exposed to *Y. ruckeri* and their susceptibility to infection was assessed using a gentamycin assay.

Inactivation of all 17 genes resulted in a decreased number of bacteria recovered at the end of the assay. However, in only 13 of these 17 genes were the differences statistically significant (Sumo 2, CDC 42, arhgap18 and, β-cadherin Actin were not statistically significant). The fact that multiple genes appear required for the invasion of *Y. ruckeri* is consistent with our previous findings that this pathogen makes use of multiple mechanisms of entry into the host.
The present results will contribute to our understanding of the virulence mechanisms in *Y. ruckeri* and in particular of the bacterium's interactions with the host cells.

Conference Session Designation: (Bacteriology / Mycology)
Presentation Format: **(** Oral)

Flagellar Regulation is Required for Virulence in *Yersinia ruckeri*

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The gram-negative Enterobacterium *Yersinia ruckeri* is the etiologic agent of enteric redmouth disease (ERM), a septicemia affecting primarily farmed rainbow trout (*Oncorhynchus mykiss*, Walbaum). Expression of the flagellin locus (*fliC)* is repressed during the course of infection and subsequently up-regulated upon host mortality in a motile strain of *Y. ruckeri*. We have recently used a selective method to identify a spontaneous *Y. ruckeri* mutant strain (TW32) that displays elevated and constitutive expression of the flagellar motility phenotype. Strain TW32 is non-virulent and exposure of rainbow trout to this strain induces a specific anti- *Y. ruckeri* IgM antibody response and non-specific anti-*Flavobacterium pschrophillum* immunity of unknown duration. Virulence in TW32 is restored when the flagellar secretion system is inactivated through mutation of the *filR* gene in the TW32 background. This demonstrates that the attenuating mutation in TW32 exerts its effect through the flagellar secretion system and is thus dependent on either a component of the system (flagellin) or a secreted factor. Genome sequencing of the TW32 strain and marker-exchange experiments revealed that a single mutation in the promoter region of the flagellar master regulator *FlhDC* is responsible for this phenotype. These results suggest that the inappropriate expression of flagellar secretion during infection triggers host recognition and thus immune stimulation resulting in attenuation of virulence. The repression of flagellin expression during infection likely occurs in order to evade host recognition and is critical for *Y. ruckeri* virulence. We also hypothesize that these unique properties of TW32 could make this strain an ideal live-attenuated vaccine.

Conference Session Designation: (Bacteriology / Mycology)
Presentation Format: **(** Oral)

Aeromonas Veronii bv _Sobria_: An Emerging Threat for European Seabass Aquaculture. Virulence and Vaccination Trials

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European seabass, *Dicentrarchus labrax* is one of the most important species for the Mediterranean Aquaculture. The past years, morbidity and mortality of cage-cultured seabass due to infections by *Aeromonas veronii* bv *sobria* have been reported from Greece and Turkey which are the main producers of the species. More than 50 strains of the pathogen were isolated from various locations in Greece and Turkey and were partially characterized. The genomes of nine strains were fully sequenced. These strains were representative of the geographic origin of isolation, but also of the phenotypes of the bacteria since isolates have differences in motility and pigment production. Virulence of the sequenced strains was examined in adult zebrafish where LD50 values ranged between 4.3×10^5 – 1.3×10^6 cfu/fish at 24h following intraperitoneal (i.p.) administration. Differences in the phenotypes and virulence of the strains were studied through comparative genomic analysis. Two of the sequenced strains were tested in adult seabass, in which virulence was significantly higher. Following a 2.5h immersion challenge in 10^5 cfu/mL, seabass suffered mortality of 100% within 7 and 10 days post challenge for the two strains, respectively. The two strains were used as the basis of a bivalent autogenous vaccine using Montanide ISA 763 A VG as an adjuvant. Following i.p. vaccination and subsequent immersion challenge with *Aeromonas veronii* bv *sobria* 30 days post vaccination, the autogenous vaccine resulted in high protection with RPS_{60} equal to 100%. Vaccination could be the method of choice for the management of disease, however the variance in the phenotypic and genomic traits of the bacterial strains indicates that a cautious choice of the appropriate antigen is required to achieve a global protection.

Conference Session Designation: **(** Bacteriology / Mycology or Genomic Applications)
Presentation Format: (Oral)

Evaluating Atypical *Aeromonas hydrophila* (aAh) in Catfish Aquaculture of the Mississippi Delta Region

Bradley M. Richardson[1,4*], Michael E. Colvin[1,5], David J. Wise[1,2,6], Matt J. Griffin[2,3,7], Charles C. Mischke[1,2,8] and Terrence E. Greenway[1,2,9]

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Atypical *A. hydrophila* (aAh) has been plaguing channel catfish *Ictalurus punctatus* aquaculture farms in the southeastern US since the late 2000s. Multiple serotypes of aAh effect various parts of Alabama and Mississippi, and clinical symptoms vary with serotype. Our study aimed to investigate the status of aAh in catfish aquaculture ponds of Mississippi. Number of aAh outbreaks varies between years, and anecdotal evidence suggests some temperature-dependence. Water samples and culture swabs were collected from disease and non-diseased ponds. Once notified of a potential aAh outbreak from a farm manager, samples were collected from the infected pond, as well as one adjacent pond and one non-adjacent pond. Samples were subjected to quantitative polymerase-chain reaction (qPCR) to determine aAh pathogen load within each sample. Pond outbreaks appeared random, and in most cases, only one pond showed signs of an active outbreak, at any given time. Using occupancy models, our results showed as much as 60 % or more of the population of a pond may be infected with aAh with no visual signs of disease outbreak. The results of this study suggest aAh outbreaks in catfish aquaculture ponds are not isolated incidences, but that multiple ponds may be infected, making outbreak prediction more difficult. Ongoing studies are focusing on outbreak predictors, such as environmental drivers, and possible vaccines. At this point, only one antibiotic is available for treating aAh outbreaks. This lack of treatment options increases the risk of antibiotic resistance in the pathogen and could exasperate the issue even further.

Conference Session Designation: **(** Emergent Disease)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

A New Isolate of *Aeromonas salmonicida* Caused Furunculosis in Atlantic salmon (*Salmo salar*) from Recycling Aquaculture System in China

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Atlantic salmon (*Salmo salar*) is the most successful cultured fish species in world aquaculture. Considering of its rich nutrition and good taste as an excellent ingredient, a Chinese company imported fertilized eggs of Atlantic salmon from Norway and started hatching and culturing the fish in 2010. That was the first attempt to culture Atlantic salmon in land based recirculating aquaculture systems both in freshwater and seawater period. Years later, disease became a predominant restriction for the industry. We isolated many bacterial isolates from diseased Atlantic salmon samples and they were all identified as *Aeromonas salmonicida* by molecular methods. Physiological and biochemical characteristics results showed that the isolates were *Aeromonas salmonicida* subsp. *masoucida*. In vitro cultured bacteria induced furuculosis like symptoms in Atlantic salmon. And the bacteria could be re-isolated from these infected fish. The clinical isolate performed strong virulence to Atlantic salmon when been intramuscularly injected into experimental fish. The LD50 was $9.67\times10^{2.18}$ CFU/fish. These findings showed that an *A. salmonicida* subsp. *masoucida* was the causative agent of the furuculosis like disease as each of Koch's postulates were fulfilled. An inactivated vaccine was prepared and it provided protection with relative protection percentages of 80% against C4 and 40% against NCIMB1102 respectively. This was the first report of *A. salmonicida* subsp. *masoucida* causing Atlantic salmon with furuculosis in recirculating aquaculture system in China. And the vaccine developed in this study protected Atlantic salmon against furuculosis.

Conference Session Designation: (Immunology Vaccines)
Presentation Format: (Oral)

Tuesday September 4th – Gray / Palmer / Pope
Bacteriology / Mycology 4
Moderator – Stephen Reichley (Clear Springs Foods)

3:15 PM	Bacteriology 4	**Pomaranski - *Erysipelothrix* spp. Virulence to Tiger Barbs (*Puntigrus tetrazona*) Is Associated With Surface Protective Antigen (*Spa*) Genotype**
3:30 PM		**Elliott - Review of Epizootic Ulcerative Syndrome in Louisiana**
3:45 PM		Soto - Investigating the Role of the Type VI Secretion System (T6SS) in the Emergent Fish Pathogen *Francisella noatunensis* Subsp. *Orientalis*
4:00 PM		**Kalatzis - Going Viral Against Bacteria: Implications for Phage Therapy in Aquaculture**
4:15 PM		Laurin - Bayesian Latent Class Analysis of the Accuracy of RT-qPCR and Elisa Testing for *Renibacterium salmoninarum* Bacterial Kidney Disease in Atlantic Salmon *Salmo salar* Broodstock in British Columbia, Canada
4:30 pm		Saab - Rapid Identification of *Piscirickettsia salmonis* Using MALDI-TOF Mass Spectrometry
4:45 PM		Barato - Attenuation of an Unencapsulated *Streptococcus agalactiae* Mutant in Tilapia (*Oreochromis* sp.) Model of Infection
5:00 PM		**Heckman - Characterization of *Streptococcus iniae* Isolates From Diseased Wild and Farmed Fish Across the North American Continent**

Erysipelothrix spp. Virulence to Tiger Barbs (_Puntigrus tetrazona_) is Associated with Surface Protective Antigen (_spa_) Genotype

Eric K. Pomaranski[1*], Matt J. Griffin[2], Alvin C. Camus[3], Abigail R. Armwood[3], Johnny Shelley[4], Geoffrey C. Waldbieser[5], Esteban Soto[1]

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An emergent, systemic disease causing low to moderate mortality in ornamental and aquarium fish is associated with an *Erysipelothrix* sp. positive for the *spaC* gene. The aim of this study was to investigate the genetic relationships of *Erysipelothrix* spp. with different *spaABC* genotypes and determine the virulence of representative members of each genotype in laboratory controlled challenges. Whole genome sequencing was performed on 5 of the *spaC Erysipelothrix* sp. isolates associated with disease outbreaks in ornamental fish. In addition, *spaC Erysipelothrix* sp. were compared to *spaA, spaB* and *spaC* positive *Erysipelothrix* spp. isolated from terrestrial and marine mammals, avian species, and fish mucosa using multi-locus sequencing typing (MLST). Comparative genomics identified the fish pathogenic *spaC* isolates are genetically distinct from *E. rhusiopathiae*, with 87% average nucleotide identity (ANI) and 32% digital DNA-DNA hybridization (dDDH) estimations. Comparably, *spaC* isolates from fish are conspecific (99% ANI; 91% dDDH) with the uncharacterized *spaC* positive *Erysipelothrix* sp. strain 2 isolated from swine and represent a previously unrecognized taxon. Phylogenies inferred from MLST sequences confirmed this trend, but indicated slight genetic differences between the *spaC* isolates from fish and the *Erysipelothrix* sp. strain 2 isolate. The relationship between *spaABC* genotype and virulence was assessed in tiger barbs (*Puntigrus tetrazona*) via bath immersion using nine different *Erysipelothrix* spp., representing three isolates from each *spaABC* type. Tiger barbs (n=20 fish per tank) were held in flow-through fresh water at 26 ± 1 ^{o}C and exposed to 10^{7} CFU/ml for 1 h. Fish were observed daily for morbidity and mortality for 30d post-challenge. Cumulative mean percent survival was 37% for *spaA*, 100% for *spaB*, and 13% for the *spaC* isolates, suggesting differences in virulence among the different *spa* genotypes in fish. Based on these genetic findings, in addition to observed differences in virulence, it is put forward the fish pathogenic *spaC* isolates represent a novel species within the genus *Erysipelothrix*, for which the name *Erysipelothrix piscicida* sp. nov. is proposed.

Conference Session Designation: **(** Bacteriology / Mycology)
Presentation Format: (Oral)
Student Presentation: (Yes)

Review of Epizootic Ulcerative Syndrome in Louisiana

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Epizootic Ulcerative Syndrome (EUS) is an important fungal disease of freshwater and brackish water fish, affecting more than 100 species worldwide. The disease is also known by other names including red spot disease (RSD), mycotic granulomatoses (MG), ulcerative mycosis (UM), and epizootic granulomatous aphanomycosis (EGA). It was first reported in farmed ayu (*Plecoglossus altivelis*) in Japan in 1971 and is particularly problematic in Southeast Asia. The causative agent is a fungal-like oomycete known as *Aphanomyces invadans.* Invalid synonyms found in the literature include *A. piscicida*, *A. invaderis*, and ERA (EUS-related Aphanomyces). Presumptive diagnosis can be made based on gross lesions including open dermal ulcers and the observation of aseptate hyphae in squash preparations of muscle underlying ulcerated skin lesions. Definitive diagnosis requires histological demonstration of granulomatous inflammation around invasive fungal hyphae and/or isolation of *Aphanomyces invadans* from underlying muscle. In recreational ponds in Louisiana, the infection results in an ulcerative mycosis of the skin and muscle and high rates of morbidity and mortality in channel catfish, brown bullhead, bluegill and largemouth bass. Occasional cases have occurred in red drum, black drum and sheepshead in estuaries in southwest Louisiana following heavy rainfall events. Recreational ponds in Louisiana that have experienced outbreaks are watershed ponds (no well water supply) located on sandy loamy soils that are poorly buffered, with waters that have consistently low pH (6.0-7.0), low hardness (0-17 ppm) and low alkalinity (0-17 ppm). In 2017, Booker Fowler Fish Hatchery in Forrest Hill, LA experienced mortality in populations of largemouth bass broodfish cultured in lined ponds. These fish were affected by a dual infection of *Aphanomyces invadans* and *Edwardsiella piscicida*, a gram negative bacterial agent that affects various species of fish. Fungal granulomas were seen histologically within the skeletal muscle and eye. Approximately 50% of the existing broodstock population was lost. The outbreak was successfully treated via hypochlorite disinfection of ponds and formalin baths for remaining fish prior to restocking. So far, no recurrence has been seen in 2018. EUS is an endemic and economically significant disease affecting recreational fishing ponds in southeastern Louisiana.

Conference Session Designation: (Bacteriology / Mycology)
Presentation Format: (Oral)
Student Presentation: (Yes)

Investigating the Role of the Type VI Secretion System (T6SS) in the Emergent Fish Pathogen *Francisella noatunensis* subsp. *orientalis*

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Francisella noatunensis subsp. *orientalis* (*Fno*) is an emergent fish pathogen and the etiologic agent of piscine francisellosis. Besides persisting in the environment in both biofilm and planktonic forms, *Fno* is known to infect and replicate inside tilapia macrophages and endothelial-derived cells. However, the mechanism used by this emergent bacterium for intracellular survival is unknown. Additionally, the basis of virulence for *Fno* is still poorly understood. Several potential virulence determinants have been identified in *Fno*, including homologues of the recently described *F. tularensis* Type VI Secretion System (T6SS). In order to gain a better understanding of the role the T6SS might play in the pathogenesis of piscine francisellosis, we performed transcriptional analysis of *Fno* T6SS gene-homologues under temperature, acidic, and oxidative stress conditions. Few transcriptional differences were observed at different temperatures, growth stages and pHs; however, a trend towards higher expression of *Fno* T6SS-homologue genes at 25°C and under oxidative stress was detected when compared to those quantified at 30°C and under no H_2O_2 ($p<0.05$). Results from this study suggest that several of the *F. tularensis* T6SS-homologues may play an important role in the virulence of *Fno*, particularly when the bacterium is exposed to low temperatures and oxidative stress.

Conference session designation: (Bacteriology/Mycology)
Presentation format: (Oral)

Going Viral Against Bacteria: Implications for Phage Therapy in Aquaculture

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Bacterial infections are a serious problem in aquaculture since they can result in massive mortalities of farmed fish and invertebrates. Administration of antibiotics is the most commonly applied method to control pathogenic bacteria but their excessive use has given rise to concerns about development and spreading of antibiotic-resistant strains in the environment. The idea of using bacteriophages (or phages), which are viruses that infect bacteria, as therapeutic agents against bacterial diseases is known as phage therapy. Phage therapy constitutes a promising alternative not only as a treatment method but also as a preventive weapon against bacterial outbreaks in aquaculture, since bacteriophages are able to biologically control the population of their host and subsequently to lower the risk of a potential disease outbreak. Development of resistance against bacteriophages can be an issue; however, this process is often accompanied by a significant fitness cost for the host bacteria.

Our primary focus has been on pathogens belonging to the genera of *Flavobacterium* (https://www.bonusportal.org/projects/blue_baltic_2017-2020/flavophage) and *Vibrio* (http://www.proaqua.dk/ & https://en-fishphage.weebly.com/) because of their high significance on Baltic and Mediterranean aquaculture. Several scientific and technological challenges still need further investigation before reliable, reproducible treatments with commercial potential are available for the aquaculture industry; however, according to the obtained results there is a strong potential of phage-based alternatives for treatment of bacterial diseases in aquaculture. Our progress in the use of phages in aquaculture will be presented with emphasis on applying lytic phages at the hatchery level, either as disinfectants of live feeds or as a treatment for fish larvae. Moreover, we will introduce our research on the diverse mechanisms that drive the development of resistance, both genetic and phenotypic, against bacteriophages as well as the fitness costs at which they come.

Conference Session Designation: (Aquatic Animal Health Management)
Presentation Format: (Oral)
Student Presentation: (Yes)

Bayesian Latent Class Analysis of the Accuracy of Rtqpcr And ELISA Testing for *Renibacterium Salmoninarum* (Bacterial Kidney Disease) in Atlantic Salmon (*Salmo Salar*) Broodstock in British Columbia, Canada

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Infection with *Renibacterium salmoninarum* causes Bacterial Kidney Disease (BKD) in both freshwater and saltwater lifestages of salmonids and can lead to severe financial losses for the aquaculture industry. Prevention of vertical transmission of the bacterium from infected broodstock to the eggs is a key management strategy for this disease. Both quantitative real-time PCR and ELISA methods have been used to identify BKD-infected fish, but these tests are imperfect, and previous studies comparing these methods often used different methods or target analytes and did not target Atlantic salmon broodstock in particular. Therefore, our study focused on analyzing diagnostic sensitivity (DSe) and diagnostic specificity (DSp) for both reverse transcriptase (RT) qPCR (RNA target) and ELISA (p57 antigen target) in Atlantic salmon broodstock from BC, Canada, and to assess the repeatability of ELISA. As there is no perfect (100% DSe and 100% DSp) reference standard assay for detecting *R. salmoninarum*, we used Bayesian latent class analyses to compare the diagnostic accuracy of these two tests. In our study, 4385 broodstock Atlantic salmon (no clinical signs or gross lesions) were sampled for ELISA screening of kidney tissue (anterior, middle, and posterior sections). Two groups of the ELISA positive samples (n=132) and two groups of a random sample of the ELISA negatives (n=137) were retested with RTqPCR and repeat ELISA testing. Based on the results of our study (Bayesian analyses and repeatability of ELISA), we recommend that ELISA testing of broodstock provides the best DSe and thereby less chance for false negative results. Using both RTqPCR and ELISA improves DSe if a positive result on either equates to a positive result for the sample. However, this is costlier and may depend on the value of the broodstock and progeny. Using these testing schemes in combination with management practices could decrease the likelihood of vertical transmission of *R. salmoninarum* from subclinically-infected broodstock.

Conference Session Designation: **(** Aquatic Animal Health Management)
Presentation Format: (Oral Presentation)
Student Presentation: **(** No)

Rapid Identification of *Piscirickettsia salmonis* Using MALDI-TOF Mass Spectrometry

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Piscirickettsia salmonis is a gram-negative, intracellular bacterium and the causative agent of piscirickettsiosis or salmonid rickettsial septicemia (SRS). Isolation of *P. salmonis* has significant implications to the salmonid aquaculture industry worldwide and is of particular concern in the Chilean Atlantic salmon aquaculture industry. Current diagnostic techniques are laborious and expensive requiring isolation on a specific culture medium, blood agar supplemented with cysteine and glucose. There are no routine biochemical bacteriological methods for the identification of the bacterium because of its fastidious nature and slow growth rate. Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) is an easy and rapid method for identifying bacteria, and its use for aquatic bacterial organisms has been reported. The commercial MALDI-TOF reference library does not contain an entry for *P. salmonis*. The primary objective of this study is to generate Main Spectral Profiles (MSP) using isolates that have been confirmed by gene sequencing. *P. salmonis* isolates representing different genotypes and isolated from different geographic regions will be included in the study. Once MSPs have been developed, they will be validated using a subset of the development isolates, as well as clinical isolates from current cases. Successful development of MSPs for *P. salmonis* will provide a cost-effective and rapid test for the diagnosis of SRS for diagnosticians, research scientists, and aquaculture veterinarians.

Conference Session Designation: (Bacteriology / Mycology)
Preferred Presentation Format: (Oral)

Attenuation of an Unencapsulated *Streptococcus agalactiae* Mutant in Tilapia (*Oreochromis* sp.) Model of Infection

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Streptococcosis is a disease with major health and economic impacts on the tilapia (*Oreochromis* sp.) industry worldwide. *Streptococcus agalactiae* (also referred to as group B Streptococcus [GBS]) is a zoonotic bacterium that infects a wide range of fish species in fresh and marine water. There is abundant information supporting the use and economic soundness of vaccination in aquaculture. Oral vaccines specifically target the intestinal mucosa. Compared to injection methods, oral vaccine delivery is simple, cost-effective, induces minimal stress and side effects, and suitable for mass immunization of fish of all sizes. The use of live modified or attenuated vaccines provides one of the greatest potentials for mucosal vaccines in aquaculture. One of the many advantages of live attenuated vaccines is the strong induction of humoral and cell mediated immune response. Capsule is one of the most important virulence factor in GBS. Its presence reduces GBS ability to entrance in host. For this reason, GBS unencapsulated mutant could be useful to increase uptake and immunogenicity of GBS. The main goal of this study was to investigate the attenuation of a GBS capsule-defective mutant, ΔCPS-SaTiBe08-18, using a tilapia model of infection. Nile tilapia fingerlings were inoculated intragastric with 10^7colony forming units (CFU)/fish of GBS wild-type (WT) strain, 10^7CFU/fish of ΔCPS-SaTiBe08-18 or sterile broth and monitored for a seven-day period. Dead and moribund fish were necropsied and organs collected for histopathological analysis. No mortality was observed in fish inoculated with broth or mutant strain; however, mild granulomatous splenitis and epicarditis with intracellular bacteria in macrophages was observed in one out of eight surviving fish infected with mutant. Fish inoculated with the wild-type strain presented 30% mortality after 7 days. All surviving fish inoculated with WT presented severe systemic granulomatous splenitis, hepatitis, nephritis, meningoencephalitis and choroiditis. Our data suggest that even though the acapsulated mutant is attenuated, it is still able to cross the gut epithelium and access internal organs, warranting future studies investigating its potential as a life attenuated vaccine.

Conference Session Designation: (Bacteriology / Mycology)
Presentation Format: (Oral)

Characterization of *Streptococcus Iniae* Isolates from Diseased Wild and Farmed Fish Across the North American Continent

Taylor I. Heckman[1*], Matt J. Griffin[2], Juan Alberto Morales[3], Elias Barquero Calvo[4], Fernanda de Alexandre Sebastião[1], Adrian Lopez Porras[3], Xindy Víquez-Rodríguez[4], Cynthia Ware[2], Esteban Soto[1]

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Streptococcus iniae is a Gram-positive, zoonotic bacterium known to infect a wide variety of farmed and wild fish species worldwide. The high mortality rates in fish cause significant economic losses in the aquaculture industry and can also have environmental and cultural impacts when causing disease in wild fish. As an emerging pathogen of global significance, understanding the coalescing factors that contribute to the pathogenesis of piscine streptococcosis is crucial for developing strategies to control infections. Intraspecific antigenic and genetic variability of *S. iniae* has made developing vaccines a challenge; particularly in areas where genetic and antigenic diversity of locally endemic *S. iniae* is unknown. This study genetically and phenotypically characterized novel isolates of *S. iniae* taken from diseased wild and farmed fish from North America, Central America and the Caribbean. Repetitive sequence mediated PCR fingerprinting placed isolates in four distinct clusters, with marine isolates forming a geno-group distinct from freshwater isolates. Heparinized whole blood from rainbow trout *Oncorhynchus mykiss* and the endothelial *Oreochromis mossambicus* bulbus arteriosus cell line were used to investigate the persistence and virulence of representative isolates from each genogroup using *in vitro* assays. *In vivo* challenges using the Nile tilapia *Oreochromis niloticus* model were also used to evaluate virulence using intra-gastric and intra-muscular routes of infection. Isolates showed significant differences in virulence and persistence, with some correlation to genogroup, establishing a basis for further work uncovering genetic factors leading to increased pathogenicity.

Conference Session Designation: (Bacteriology / Mycology)
Presentation Format: (Oral)
Student Presentation: (Yes)

Tuesday September 4th – Archibald / Campbell
Sea Lice 1 & 2
Moderator – Mark Fast (Atlantic Veterinary College / UPEI)

9:30 AM	Sea Lice 1	Nowak - Amoebic Gill Disease – An Emerging Disease in Mariculture?
9:45 AM		Jorgensen - Interactions Between the Skin Parasite *Ichthyophthirius multifiliis* and a Fish Host *Danio rerio*
10:00 AM		Dalvin - Morphological and Immunological Changes in Rainbow Trout (*Oncorhynchus mykiss*) Skin in Response to Salmon Louse (*Lepeophtheirus salmonis*) Infection
10:15 AM		Overgard - Do *Lepeophtheirus salmonis* Rhabdoviruses (LSRVs) Dampen the Local Skin Inflammatory Response in Atlantic Salmon *Salmo salar*?
10:30 AM		**Refreshments**
10:45 AM	Sea Lice 2	Poley - Excretory and Secretory Factors of the Salmon Louse: Genomic Characterization, Effects on Feeding, and Impacts on Host Immunity
11:00 AM		Carvalho - Functional Feeds Impact Molecular Responses of Atlantic Salmon (*Salmo salar*) to Co-Infection with *Lepeophtheirus salmonis* and Infectious Salmon Anemia Virus
11:15 AM		Skugor - The Effect of the *Caligus rogercresseyi* Parasite Burden on the Progression of Co-Infection With the Intracellular Bacterium *Piscirickettsia salmonis*
11:30 AM		Kvamme - Salmon Lice as an Environmental Indicator for Management of Norwegian Salmon Farming – An Overview
11:45 AM		Are Hamre - Development of the Salmon Louse *Lepeophtheirus salmonis* Parasitic Stages in Temperatures Ranging From 3°C to 24°C.

Amoebic Gill Disease – An Emerging Disease in Mariculture ?

Barbara F. Nowak

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Amoebic gill disease (AGD) was first reported 30 years ago from two salmonid species, Atlantic salmon in Tasmania, Australia and coho salmon in Washington State USA, farmed in marine environment. The causative organism, *Neoparamoeba perurans*, was described more recently and Koch's postulates have been fulfilled even later due to initial difficulties with culturing the causative agent *Neoparamoeba perurans*. Clinical AGD has been observed in cultured fish in fourteen countries across six continents. Atlantic salmon is the main commercial species affected, but the disease has been also seen in cleaner fish and other fish species farmed in the marine environment. While immune response of salmon affected by AGD has been studies, there is no vaccine available. This presentation reviews our current knowledge of this disease, in particular its emergence in mariculture.

This presentation summarises our current knowledge about the known host range, characteristics of the parasite, case definition and host-parasite relationship. While there has been a lot of progress in our understanding of this disease some fundamental questions still remain unanswered. The emergence of AGD appears to coincide with high sea surface temperature and with the intensification of mariculture. In contrast to the situation in Tasmania, in Europe AGD is one of many gill conditions affecting Atlantic salmon farmed in sea cages. This means that a new approach is needed for holistic management of gill health in farmed Atlantic salmon.

Conference Session Designation: (Ectoparasites / Sea Lice)
Presentation Format: (Oral)

Interactions Between the Skin Parasite *Ichthyophthirius Multifiliis* and a Fish Host *Danio Rerio*

Louise von Gersdorff Jørgensen

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The parasite Ichthyophthirius multifiliis is the causative agent of white spot disease and a major problem for the aquaculture and ornamental fish industry. It infects skin and gills of freshwater fish and cause high mortality during outbreaks. The zebrafish has become an important model to study a wide spectrum of vertebrate biological processes and has proven especially valuable within developmental biology and genetics. I have used the zebrafish as a model to study immunological responses during infections with Ichthyophthirius multifiliis. Using adult transgenic zebrafish with Green Fluorescent Protein (GFP)-tagged neutrophils a setup was for the first time developed completely immobilizing the tail fin of the live fish. Utilising this setup, the behaviour of neutrophils during a parasite assault was examined with a confocal microscope. This model offers an unprecedented real time view into the interactions between the parasites and the neutrophils at the single cell level. The neutrophil population dynamics were also investigated and within the first day of the parasite infection, the number of neutrophils in the tail fin increased four-fold. However, during the following two days, the number of neutrophils declined even though the size of the parasites increased and the damage to the fish intensified. Video-recordings of the interface between the parasites and the neutrophils at the single cell level revealed how the parasites have a way of evading and fighting the immune system of the host. Using zebrafish as a tool to investigate cellular immunity has expanded our knowledge on this host/parasite relationship and with the many accessible transgenic lines there is potential for new discoveries.

Conference Session Designation: (Ectoparasites)
Presentation Format: (Oral)

Morphological and Immunological Changes in Rainbow Trout (*Oncorhynchus Mykiss*) Skin in Response to Salmon Louse (*Lepeophtheirus Salmonis*) Infection

Sussie Dalvin[1,2*], Louise v G Jørgensen[3], Per W Kania[3], Kurt Buchmann[3], Frank Nilsen[4], Aina C Øvergård[4]

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Infestations with the salmon louse (*Lepeophtheirus salmonis*) is a challenge in North Atlantic salmonid aquaculture industry, where both Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) are farmed. The salmon louse damages the skin of the host by feeding on skin and mucus. In addition to these mechanical damages and resulting host responses, there is also evidence, mainly from Atlantic salmon, indicating that salmon lice can reduce the immune response of the fish. The mechanisms and timing of such a process is however yet to be elucidated. Here we have infected rainbow trout with salmon lice and followed development from attached copepodids and subsequent stages leading to mobile adult lice The objective of this study was: 1) to describe the morphological changes in fish skin at infestation sites, 2) to compare transcriptomic changes in fish skin in uninfected and infected individuals (both at and away from the site of attachment) and 3) to localize immune cells at the infestation site using immunohistochemistry. Our results indicate large morphological changes in the skin of the fish during maturation of the sea lice as well as associated transcriptomic changes and occurrence of immune cells in the skin. The outcome of this work will enhance our understanding of the interaction between salmon louse and salmonid hosts.

Conference Session Designation: (Parasitology Sea Lice – Ectoparasites)
Presentation Format: (Oral)

Do *Lepeophtheirus Salmonis* Rhabdoviruses (Lsrvs) Dampen the Local Skin Inflammatory Response in Atlantic Salmon *Salmo Salar* ?

Aina-Cathrine Øvergård*, Lars A. Hamre, Sindre Grotmol, Heidi Kongshaug, and Frank Nilsen.

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Rhabdoviruses are a family of enveloped negative-sense single-stranded RNA virus infecting a variety of hosts. Recently, it has been shown that the salmon louse (*Lepeophtheirus salmonis*) is commonly infected by one or two vertically transmitted *L. salmonis* rhabdoviruses (LsRVs). Their prevalence was close to 100 % along the Norwegian cost, and it is challenging to obtain material for studies on host impact and infection routes due to the present lack of suitable cell lines to propagate these viruses. Hence, virus free louse strains were established from virus infected lice carrying both LsRVs by treating them with N protein dsRNA twice during development. We could then analyze how these viruses transmit among lice, whether the viruses affect louse biology and study the interaction between the lice and the salmon host. The viruses had limited effect on louse survival, developmental rate and fecundity. The LsRVs were present in the louse salivary glands, and interestingly, LsRV free lice induced a higher local skin expression of IL1β and IL8 than the LsRV infected lice. The inflammatory response is important for louse clearance, and the present results suggest that the LsRVs can be beneficial for the louse by dampening inflammation. However; it is not known whether this is a direct modulatory effect of secreted virions, or if virus replication is altering the level of louse salivary gland proteins.

Conference Session Designation: (Parasitology Sea Lice – Ectoparasites)
Presentation Format: **(** Oral)

Excretory and Secretory Factors of the Salmon Louse: Genomic Characterization, Effects on Feeding, and Impacts on Host Immunity

Jordan D. Poley[1*], Laura M. Braden[1] Dylan Michaud[1], Aina-Cathrine Øvergård[2], Heidi Kongshaug[2], Sussie Dalvin[2], Simon R. M. Jones[3], Mark D. Fast[1]

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Virulence is a property of the host-parasite (HP) relationship that can be measured by the consequent effects on host fitness and parasite success. With respect to the salmon louse the HP relationship involves the feeding response by the louse and the subsequent immune response by the salmon. In resistant species (e.g., coho salmon) the host response dominates, while in susceptible species (e.g., Atlantic salmon) the feeding/parasitic response generally suppresses the host response. This is achieved by producing immunosuppressive molecules that directly affect the ability of the hosts immune system to recognize the attached parasite while facilitating nutrient acquisition (i.e., virulence factors, VFs). Here, we have employed a multi-pronged approach to describe a group of putative VFs in the salmon louse. A cluster of VF genes associated with feeding were identified using correlation analysis of gene expression across six different studies. These VFs were annotated by predicting ORFs, adding accessions from UniProt, GenBank, and LiceBase, applying conserved protein domain layouts (ranges + accessions), determining the presence of signal peptides, and determining orthologs to known virulence factors in *Ixodes* spp. Using LC-MS/MS we identified 18 virulence-associated proteins in the secretions of *L. salmonis*, 9 of which were concordant with putative VFs in the transcriptome. We then characterized the pattern of expression of a select group of VFs (*hypodermin-B*, *carboxypeptidase B*, *cathepsin L*, *legumain*, and *neprilysin*) during feeding on resistant or susceptible hosts by qPCR. *In situ* localization and RNA interference experiments indicate these molecules may be important mediators of the HP interaction.

Conference Session Designation: (Sea Lice / Ectoparasites)
Presentation Format: (Oral)

Functional Feeds Impact Molecular Responses of Atlantic Salmon (*Salmo Salar*) to Co Infection with *Lepeophtheirus Salmonis* and Infectious Salmon Anemia Virus.

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Infectious salmon anemia virus (ISAv) is a highly contagious aquatic pathogen that causes considerable economic losses in farmed Atlantic salmon (*Salmo salar*). Sea lice (*Lepeophtheirus salmonis*) infestation also represents a major challenge in the salmonid farming industry and various treatment protocols have been put in place to manage and prevent sea lice outbreaks at farm sites. Immunostimulant additives to functional diets aid in strengthening the host immune response and are becoming an integral part of disease management practices in aquaculture. This study aims to characterize biomarkers and molecular responses in Atlantic salmon during a co- infection with *L. salmonis* and ISAv during administration of four blinded functional feed diets. A highly virulent ISAv strain was IP injected into donor fish which were cohabited (ca. 10-15% of tank density) 10 days after injection with experimental tanks (4 tanks per feed group) to achieve peak shedding rates at time of stocking. By 46 days post infection, mean lice abundance ranged from 6.1 – 9.2 lice per fish, with two diets showing significantly lower sea lice infections (ca. 27-29% reduction). However, under co-infection with ISAv, the diets with lowest louse abundance had the poorest survival, 37-46%, and highest viral load. Host transcriptional responses to infection were assessed in multiple tissues and across low, medium and high lice counts and viral loads. Biomarkers associated with functional feed background and infection level were identified and their significance will be discussed.

Conference Session Designation: (Co-Infections / Sea Lice)
Presentation Format: (Oral)

The Effect of the *Caligus Rogercresseyi* Parasite Burden on the Progression of Co- Infection with the Intracellular Bacterium *Piscirickettsia Salmonis*

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In salmonid aquaculture worldwide, co-infection by multiple species of parasites and microbial pathogens is the norm rather than the exception. Yet, not much is known about the burden of coinfection on fish health and the differences between infections by single pathogen species and coinfection. The aims of the study were to identify gene expression profiles specific for the co-infection of ectoparasitic *Caligus rogercresseyi* and intracellular bacterium *Piscirickettsia salmonis*, the causative agent of SRS, and delineate the effect of the parasite burden on the progression and severity of the ensuing condition.

Atlantic salmon were infected with 15 (Low) and 50 (High) number of *C. rogercresseyi* infective copepodids, and half of the fish from each group was I.P. injected with the LF89 strain of *P. salmonis* 16 days post-infection (dpi) with *C. rogercresseyi*. In addition, the study included non-infected fish and one group infected only with *P. salmonis*.

Lice numbers were counted at three time points (13, 28 and 36 dpi), and mortalities were followed until 36 dpi. The average number of lice was 11 in the Low and 31 in the High group at 13 dpi while the numbers rose to 81 and 136 by 36 dpi as a consequence of lice becoming sexually mature and reproducing in our tank system. Mortalities started at day 11 post *P. salmonis* injections, and the group co-infected with 50 copepodids and bacterium showed highest number of dead fish by 36 dpi.

Profiling of plasma revealed the effect of the treatments on urea and iron levels, with most pronounced differences seen in the High co-infected group. Results from microarray profiling of head kidney samples taken prior to lice infection, 13 dpi and at two time points during the co-infection (28 and 36 dpi) will be discussed.

Conference Session Designation: (Co-infection / Sea Lice)
Presentation Format: (Oral)

Salmon Lice as an Environmental Indicator for Management of Norwegian Salmon Farming – An Overview

Bjørn Olav Kvamme*, Lars Asplin, Pål Arne Bjørn, Sussie Dalvin, Kristine Marit Elvik, Ingrid Johnsen, Rune Nilsen, Mari Myksvoll, Anne Sandvik, Rosa Maria Serra-Llinares, Rasmus Skern-Mauritzen, Terje Svåsand, Geir Lasse Taranger, Bjørn Ådlandsvik and Ørjan Karlsen.

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Norway is the largest producer of farmed salmon in the world, with an annual production of more than 1.2 million tons. The growth of the salmon farming in Norway may continue, but only if the environmental impact of the industry can be considered sustainable. At present, the mortality salmon lice inflict on wild salmonids are the only indicator chosen to indicate this impact. As such, the salmon louse may have a substantial impact on the billion-dollar salmon farming industry in Norway. Recently a novel management system for the fish farming industry was ratified, dividing the Norwegian coast into 13 production areas where high levels of sea lice would induce reduction of production in the entire area, whereas low lice levels would induce growth in production. This system relies on tools and results from the Institute of Marine Research (IMR), varying from field surveillance of sea lice infestations on wild salmonids and salmon louse biology to modelling of salmon louse dispersal and infestation levels.

Traditionally, salmon lice on wild salmonid populations has been monitored by a large scale surveillance program along the coast of Norway. This has provided data on salmon louse levels on wild salmonids in areas with and without salmon farming, effect of fallowing and protected areas, and salmon louse induced mortality on wild salmonids. However, as it is not possible to survey the entire Norwegian coast, a model system describing the dispersal of salmon louse has been developed. This modelling system has been used to divide the coast into semi-isolated production areas, describe the dispersal of salmon louse from all fish farms, an operational near real time dispersal model of louse, create salmon migration models as well as infestation models. Here a brief overview of the Norwegian management system will be presented together with results originating from IMR during the development of the system.

Conference Session Designation: (Parasitology Sea Lice – Ectoparasites)
Presentation Format: (Oral)

Development of the Salmon Louse *Lepeophtheirus Salmonis* Parasitic Stages in Temperatures Ranging from 3° C to 24° C.

By Lars Are Hamre*[1], Samantha Bui[2], Frode Oppedal[2], Rasmus Skern-Mauritzen[3] and Sussie Dalvin[3]

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The development rate of the salmon louse *Lepeophtheirus salmonis* is greatly influenced by sea water temperature. This study describes how the growth rate of *L. salmonis* change with temperature and identify the extreme high and low temperatures at which development to adult is compromised. Atlantic salmon was infected with copepodids and development was monitored in 8 temperature groups spanning from 3°C-24°C until the lice were adult. Development was severely compromised at 3°C and 24°C, while the lice developed normally without severe mortality in the range 6°C-21°C. At 6 °C most female lice had become adults at 67 days post infection, or c. 400 daydegrees, but at 21°C development was significantly quicker and most females were adults after 14 days, at only c. 300 daydegrees. The lice developed almost five times faster at 21°C than at 6°C and required c. 25% fewer daydegrees to become adult. Body size also decreased significantly while temperature increased. After infection the lice grow through five stages before reaching the adult stage: the copepodid stage, two chalimus stages and two preadult stages, all of which, with a few exceptions, appeared to last approximately equally long for males and females respectively. Thus, a simple model describing the mean daily growth rate (stages pr. day) as a function of temperature was made for each sex. The relationship between mean daily growth rate and temperature was best described by a second order polynomial. The term relative age is introduced and used to describe the pattern of development in terms of percent of total development time to adult, and applied to calculate the timing of developmental events as a function of temperature.

Conference Session Designation: (Parasitology Sea Lice – Ectoparasites)
Presentation Format: **(** Oral)

Tuesday September 4th – Archibald / Campbell
Sea Lice 3
Moderator – Mark Fast (Atlantic Veterinary College / UPEI)

1:15 PM	Sea Lice 3	Bui - Effect of Lice Prevention Technologies on Salmon Welfare and Infection Status
1:30 PM		Elghafghuf - Quantifying and Modelling the Impact of the Influx of Sea Lice From Neighbouring Farms in Grand Manan, New Brunswick
1:45 PM		**Nilsen - Production of Atlantic Salmon (*Salmo salar*) in Closed Confinement Systems (CCS)**
2:00 PM		Kattambally - Marine Fishes From Kerala Coast, India Are the Potential Hosts for Caligids (Caligidae: Copepoda)

Effect of Lice Prevention Technologies on Salmon Welfare and Infection Status

Samantha Bui [1]*, Henrik Trengereid[2], Lars Stien[1] and Frode Oppedal[1]

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The impact of sea lice (*Lepeophtheirus salmonis* and *Caligus* spp.) on the salmon aquaculture industry extends to the environmental, welfare, and economic burdens associated with their treatment and control. With the increasing pressure to suppress sea lice levels in salmon farms, many farmers will consider implementing multiple prevention methods throughout a production cycle, and possibly even use methods simultaneously to maximise their integrated pest management (IPM) strategy. However, often new cage technologies have not been thoroughly verified in their efficiency, or impact on fish welfare and behaviour. We aimed to determine whether certain technologies could have a cumulative effect on preventing sea lice infestations or welfare status, at a commercial scale. Four prevention approaches were tested: the use of cleaner fish, the provision of feed that has been enhanced with functional ingredients, the implementation of a deep light source in combination with deep feeding zones, and the use of a non-permeable lice skirt.

A commercial-scale study was conducted over 14 months, following the welfare and infection status of salmon held in sea cages with increasing cumulative prevention/control methods (in 4 treatment groups). All cages were sampled every 3-4 weeks, with detailed welfare assessments and lice counts recorded. Welfare was determined using the Salmon Welfare Index Model which uses ratings of factors such as skin, fin, eyes and gill condition, condition factor, presence of wounds, and parasite load, to calculate an Overall Welfare Index. This value allows direct comparison of welfare status across treatment groups. Vertical swimming behaviour of the school and environmental conditions was monitored throughout.

Overall welfare status fluctuated with season, however cages with the addition of deep lights + deep feeding, and lice skirts, demonstrated a slightly better welfare score over the 14 months compared to the groups without. The treatment group with all technologies generally supressed new infestations more than those groups with fewer technologies. During the winter peak of infestation pressure, cages with all technologies acquired approximately 50% fewer lice than the others. However, the lower new infestations over the 14 months did not translate to fewer delousing events, indicating a possible negative interaction between the efficiency of cleaner fish and the deep lights + deep feeding or skirts.

Industry decisions on integrated pest management should incorporate scientific evidence on new technology efficiencies and welfare impact, to create optimal solutions that consider fish health and welfare.

Conference Session Designation: (Parasitology Sea Lice – Ectoparasites)
Presentation Format: (Oral or Poster)

Quantifying, and Modelling the Impact of, the Influx of Sea Lice from Neighbouring Farms in Grand Manan, New Brunswick

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Sea lice infestation is a major challenge for salmon aquaculturists in Canada and worldwide. The parasites cause welfare problems for farmed salmon and lead to economic losses on farms. We here quantify the infestation pressure from sea lice on farms in Grand Manan using four different methods, and estimate the effects of these pressures on farm-level sea lice abundance using a multivariate state-space model. The state-space model includes, in addition to the internal and external infestation pressure variables, seawater temperature, bath and in-feed treatments as covariates. In the analysis, we allow for the effects of internal and external infestation pressures to be estimated either as a common parameter across all production cycles or as different parameters (i.e., each production cycle has its own parameters). Other variable effects in the model are estimated as common parameters across production cycles. The performance of methods for quantifying infestation pressure and models within the same method are compared based on the sample-size corrected Akaike Information Criterion (AICc).
The results showed that the model with common internal and external infestation pressures across all production cycles had the best fit. Furthermore, methods of quantifying infestation pressure that take into account water temperature and development times of the pre-infective stages of lice had better model fits.

Conference Session Designation: (Parasitology Sea Lice)
Presentation Format: (Oral)

Production of Atlantic Salmon (*Salmo Salar*) in Closed Confinement Systems (CCS)

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There is increasing concern about Norwegian salmon farming and the possible environmental impacts from sea lice, escaped fish and release of toxic chemicals and organic emissions to the coastal waters. Closed containment systems (CCS) have the potential to eliminate the problems with salmon lice (*Lepeophtheirus salmonis*) and to reduce escapes and emissions. From May 2012 to May 2017 we monitored post-smolt (90 – 1000 g) in 30 closed cages and 9 open reference cages. We report the effect on salmon lice, growth rates (SGR/TGC), feed conversion ratio (FCR), crude mortality rates, cause specific mortality rates and mortality patterns. We also describe the specific mortality causes, identified pathogens and the most important risk factors.

Conference Session Designation: (Parasitology Sea Lice – Ectoparasites)
Presentation Format: (Oral)
Student Presentation: (Yes)

Marine fishes from Kerala coast, India are the potential hosts for Caligids (Caligidae:Copepoda)

Rijin Kattambally[2], Drisya Othayothkannoth[2], Nikhila Reshmi Mundavalappil[1], Amrutha Vani Sahadevan[2] and Sudha Kappalli [1*]

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Caligids, generally called as sea lice comprising over 30 genera and 465 species causing severe economic losses to fin-fish aquaculture. The present paper reports the massive infection of caligids on the marine fishes of Kerala coast, India. 24 species of caligids from 6 genera including *Caligus, Hermilius, Synestius, Euryphorus, Caligodes, Parapetalus* were recovered from 21 species of fishes belonging to 13 families (Scombridae, Coryphaenidae, Carangidae, Stromateidae, Priacanthidae, Polynemidae, Cichlidae, Scatophagidae, Trichiuridae, Mugilidae, Arridae, Belonidae, Rachycentridae). The maximum species of caligids were recovered from the fish family Scombridae. The genus *Caligus* dominates with 18 species. All recovered caligids showed wide variation in their prevalence, intensity, host specificity, site specificity and microhabitat preference. The highest prevalence (71.4%) was shown by Caligus *rotundigenitalis* infecting the fish *Scatophagus argus*. The maximum intensity (5.3) was shown by *Euryphorus nordmanni* infecting the fish *Coryphaena hippurus*. The further aspects of host fish-caligid interaction are also discussed.

Conference Session Designation: (General session-Parasitology Sea Lice-Ectoparasites)
Presentation Format: (Oral)

Tuesday September 4th – Archibald / Campbell
Cleaner Fish Disease 1 & 2
Moderator – Gustavo Ramirez-Paredes (Ridgeway Biologicals)

2:15 PM	Cleaner Fish 1	Gulla - Infectious Diseases of Cleaner Fish in Norway
2:30 PM		**Scholz - Cleaner Fish: Emerging Diseases and Biosecurity Implications**
2:45 PM		Midtlyng - "Cleanerfish Bank" and "Kindergartens" – New Management Tactics to Maintain Control of Salmon Lice Without Chemotherapeutic or Handling Interventions
3:00 PM		**Refreshments**
3:15 PM	Cleaner Fish 2	Sandlund - Screening for Pathogens in Wild Goldsinny Wrasse (*Ctenolabrus rupestris*), an Important Cleaner Fish in Norwegian Aquaculture
3:30 PM		**Chakraborty - Infection Model Development and Immunization of Lumpfish (*Cyclopterus lumpus*) Against *Aeromonas salmonicida***
3:45 PM		Ramirez-Paredes - Efficacy of Multivalent Autogenous Vaccines Against Atypical Furunculosis and Vibriosis in Scottish Ballan Wrasse (*Labrus bergylta*)
4:00 PM		Powell - Amoebic Gill Disease in Ballan Wrasse (*Labrus bergylta*) Juveniles and Its Control by UV Irradiation.
4:15 PM		**Papadopoulou - Bath Challenge Model Against Atypical *Aeromonas salmonicida* in Farmed Ballan Wrasse (L. *Bergylta*)**
4:30 PM		**Buba - Phenotypic and Genotypic Characterisation of Atypical *Aeromonas salmonicida* in Ballan Wrasse (*Labrus bergylta,* Ascanius 1767)**
4:45 PM		O'Brien - Pathobiology of an *Exophiala* sp. Disease Event From Aquaculture Reared Lumpfish (*Cyclopterus lumpus*) in Newfoundland and Labrador, Canada
5:00 PM		**Scholz - Experimental Investigations Into Ranavirus (Iridoviridae) Infections in Lumpfish (*Cyclopterus lumpus*).**

Infectious Diseases of Cleaner Fish in Norway

Snorre Gulla * and Duncan J. Colquhoun

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The use of cleaner fish has expanded dramatically in Norwegian salmon farming in recent years due to increasing chemoterapeutant resistance in salmon lice. While wild-caught wrasse species dominated initially, cleaner fish used in Norway today predominantly consist of farmed lumpsucker, which tolerate lower water temperatures. In 2017, just over 50 million cleaner fish were used (official statistics) in Norway, and estimates for 2018 range around 60 million. These high numbers reflect the short life expectancy of cleaner fish after stocking in salmon farms.

A large proportion of cleaner fish losses are related to infectious diseases. While bacterial diseases are known to play a leading role, the significance of viral infections (such as Lumpfish Flavivirus) and the various parasites infecting these fish species remains to be established. Bacterial pathogens of cleaner fish regularly detected in Norway include 'atypical' *Aeromonas salmonicida*, *Pasteurella* sp., *Pseudomonas anguilliseptica*, *Moritella viscosa*, *Tenacibaculum* spp. and *Vibrio anguillarum*/*ordalii*, in addition to a range of other *Vibrio* species. *A. salmonicida* subsp. *salmonicida* and *Paramoeba perurans*, both serious pathogens of Atlantic salmon, have also been sporadically detected in cleaner fish. While vaccination of farmed Norwegian cleaner fish against some bacterial pathogens may have contributed towards some mitigation of losses, much optimisation work remains in terms of cleaner fish vaccinology.

The high and relatively rapid mortalities experienced amongst cleaner fish today undoubtedly represent a major animal welfare concern, raising a legitimate question mark over the ethicality of current cleaner fish practices. Current and historic trends from cleaner fish diagnostics and research performed at the Norwegian Veterinary Institute will be presented, with a focus on relevant bacterial pathogens.

Conference Session Designation: (Cleaner Fish Diseases)
Presentation Format: (Oral)

Cleaner Fish: Emerging Diseases and Biosecurity Implications.

Felix L Scholz[1,2*], Hamish D Rodger[1], Ian O'Connor[2], Luca Mirimin[2], Neil M Ruane[3], Mar Marcos-López[1], Susie O Mitchell[1] and Eugene MacCarthy[2]

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Sea lice, *Lepeophtheirus salmonis*, are endemic in European Atlantic salmon farming, limiting the growth of the industry and compromising its sustainability in some regions. Chemical treatments are expensive, their use is in part restricted by legislation and resistances are emerging in sea lice, making the development of non-medicinal solutions for sea lice control a priority for the industry. One approach that has increased in recent years is the stocking of lumpfish (*Cyclopterus lumpus*) and wrasse species (*Labridae*) as cleaner fish. Wrasse species are mostly wild caught while lumpfish are farmed using wild caught broodstock. Mortalities of cleaner fish at sea have been high, partly unexplained and largely attributed to infectious diseases. To expand our limited knowledge on pathogens of cleaner fish and potential biosecurity risks to cohabited Atlantic salmon, the health status of Irish cleaner fish populations at sea and in hatcheries was monitored over the course of three years. Multiple pathogens identified in this study were not previously known to infect cleaner fish species and findings included significant pathogens of Atlantic salmon, such as *Neoparamoeba perurans* for which interspecies transmission between lumpfish and salmon has been proven. Three pathogens of salmon were described in cleaner fish species for the first time: piscine myocarditis virus (PMCV) in wrasse and *Piscirickettsia salmonis* and *Exophiala salmonis* in lumpfish. The first case of microsporidiosis due to *Tetramicra brevifilum*, a significant pathogen of turbot (*Scophthalmos maximus*), was described in lumpfish. A new species of ranavirus, which has been isolated from clinically healthy lumpfish in Iceland, Scotland and the Faroe Islands, was isolated from lumpfish fry experiencing mortality. The findings of this study indicate potential biosecurity risks associated with the use of cleaner fish with implications for practices such as reuse of cleaner fish, moving them between pens and the use of wild caught stock. An overview of emerging pathogens of concern will be presented and implications discussed.

This research is funded by the Irish Research Council (Employment Based Postgraduate Program), FishVet Group Ireland and in part by Bord Iascaigh Mhara.

Conference Session Designation: (Cleaner Fish Diseases)
Presentation Format: (Oral)
Student Presentation: (Yes)

“ Cleanerfish Bank ” and “ Kindergartens ” – New Management Tactics to Maintain Control of Salmon Lice Without Chemotherapeutic or Handling Interventions.

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Until quite recently, successful control of salmon louse (*Lepeophtheirus salmonis*) infestations in Norwegian farmed salmon became increasingly difficult due to development of multi-resistance to the active ingredients used in licensed medicines. The critical situation stimulated rapid development and use of various non-medicinal methods for removing lice, including equipment for mechanical brushing, use of low-pressure washing, short-term warm water exposure, or use of wellboats for long-term freshwater baths. However, all these methods require rather extensive handling operations that cause considerable stress to the fish, resultant mortality, and high labor and equipment costs. Aquaculture veterinarians and their clients in mid-Norway have, therefore, developed a number of new tactical means for use in salmon louse control, that require neither the use of chemotherapy nor handling.

A “cleanerfish bank” means a repository of juvenile cleanerfish on-site, where these fish are fed and cared for while awaiting deployment into farmed salmon cages. This allows for flexibility in delivery time and number relative to salmon sea transfer and the most reproductive season of the lice and enables maximally flexible reaction to increasing numbers of mobile and mature salmon lice by rapidly moving the waiting cleanerfish into cages where and when the need for their workforce is most urgent.

“Kindergartens” are sea transfer sites situated so that smolts and post-smolts are maximally sheltered from copepodid challenge. Typically, the sites are placed in fjords with significant freshwater influx in spring and early summer, or with minimal surface currents likely to carry louse larvae from upstream areas with high density of pre-harvest size salmon. “Kindergarten” sites are typically used for spring entry smolts for 6-8 months while the total biomass is low. The fish will normally be moved to sites with larger carrying capacity and currents carrying infective louse larvae in late autumn (S1 smolts) or late spring (S0 smolts).
Recent field results from mid-Norway will be presented to illustrate how the louse levels on farmed salmon have been gradually and substantially reduced. In our opinion, this is in large parts due to employment of the new fish health management tools described.

Conference Session Designation: (Aquatic Animal Health Management)
Presentation Format: (Oral)

Screening for Pathogens in Wild Goldsinny Wrasse (*Ctenolabrus rupestris*), an Important Cleaner Fish in Norwegian Aquaculture

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The development of resistance of salmon lice (*Lepeophtheirus salmonis*) towards various chemical treatments calls for alternative delousing methods in salmonid aquaculture. The use of cleaner fish as biological control of sealice in Norwegian salmonid farming has increased steadily over the last decade. Different species of wrasse and lumpfish (*Cyclopterus lumpus*) are used. All lumpfish and some ballan wrasse are farmed, but most wrasse is wild caught and goldsinny wrasse (*Ctenolabrus rupestris*) is the most important species.
The loss of wrasse in net pens may be high, from escapes or disease and mortality.
In 2017 almost 28 million wild caught wrasses were used as cleaner fish in Norway. Large quantities of fish are transported between different regions in the country and approximately 1 million were imported from Sweden. The health status of such wild caught fish is mostly unknown, so there is a potential for the wrasse to act as disease vectors. Currently, we gathered information on this, and one approach is the screening wild wrasse for pathogens in the recipient areas. These areas had high densities of fish farms using high amounts of imported wrasse. Fish from other areas with lower densities of aquaculture farms were also sampled. One aim was to better understand the potential risk for disease transfer from wild wrasse to salmon and rainbow trout in the net pens (e.g. VHSV). The main aim was to reveal evidence for any past introductions, from quantitative (prevalence) and qualitative studies (genotypes).
In 2017, almost 1000 goldshinny wrasse were examined for the presence of various pathogens. The results showed a high prevalence of Nucleosporidae gen. sp. in the gills and a more variable presence of *Aeromonas salmonicida* ssp. in the kidney and Betanodavirus in brain. VHSV was not detected.

Conference Session Designation: (Cleaner Fish Diseases)
Presentation Format: **(** Oral)

Infection Model development and Immunization of Lumpfish (*Cyclopterus lumpus*) Against *Aeromonas salmonicida*

Setu Chakraborty[1*], Trung Cao[1], Hajarooba Gnanagobal[1], Ahmed Hossain[1], Danny Boyce[2], and Javier Santander[1]

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One of the major current health challenges for the aquaculture industry is sea-lice (*Lepeophtheirus salmonis*) infestation. Lumpfish (*Cyclopterus lumpus*), a native fish to Canada, is a biological delousing agent. The use of cleaner fish is attractive because they can reduce the use of chemo therapeutants and, in addition, it is less stressful to the fish. The number of cleaner fish used by the salmon farming industry has increased exponentially since 2008, and it is estimated that 50 million lumpfish will be required by 2020. The cleaner fish delouse the salmon skin by eating the parasite, but also ingest other potential pathogens that may be transmitted by sea lice. Cleaner fish pathogens post sea transfer are currently a major global challenge, especially bacterial infections caused by *Aeromonas salmonicida* among other pathogens. In this study, we followed the *A. salmonicida* infection in lumpfish to establish a vaccine challenge model. Groups of 120 fish where intraperitoneally (i.p.) injected with different doses of *A. salmonicida* ranges from 10^1 to 10^5 cells per fish. Samples of blood, head-kidney, spleen, and liver were collected at different time points. *A. salmonicida* was detected after 5 days post-infection in the head kidney and later in the rest of the tissues. *A. salmonicida* killed lumpfish in a dose-dependent fashion and the lethal dose 50 (LD_{50}) was estimated at 10^2 CFU/ml. Also, we evaluate *A. salmonicida* iron uptake outer membrane proteins (IROMPs), outer membrane proteins (OMPs), *Aeromonas salmonicida* bacterin grown under iron-limited conditions as a vaccine and compared to a commercial vaccine preparation. Groups of 100 fish where intraperitoneally (i.p.) immunized and boost 4 weeks post prime-immunization. Samples of blood, head-kidney, spleen, and liver were collected at different time points. Twenty-one weeks post prime-immunization the fish were i.p. challenged with a high dose of the *A. salmonicida* (10^7 cells per fish) to evaluate vaccine efficacy. We found that fish immunized with *A. salmonicida* OMPs develop a toxic shock-like after boost immunization. Challenge assays showed that the different vaccine formulations conferee similar levels of protection.

Conference Session Designation: (Immunology Vaccines / Cleaner Fish Diseases)
Presentation Format: (Oral)
Student Presentation: (Yes)

Efficacy of Multivalent Autogenous Vaccines Against Atypical Furunculosis and Vibriosis in Scottish Ballan Wrasse (*Labrus bergylta*)

J Gustavo Ramirez-Paredes[1,2*], Athina Papadopoulou[1], Sean J Monaghan[1], Rimmer G[3], Louise Smith[3], Tim Wallis[2], Carolina Gutierrez[4], David Cockerill[4], Andrew Davie[1], David Verner-Jeffreys[3], Herve Migaud[1], Alexandra Adams[1]

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Recent bacteriological surveys of the Scottish ballan wrasse industry have indicated that atypical *Aeromonas salmonicida* (a*As*) and *Vibrionaceae*-related bacteria are predominant diagnostic findings during natural outbreaks of disease. Some of these bacteria are very likely to have a role in the high mortalities experienced on production sites, and so hatcheries are at present vaccinating with autogenous vaccines containing antigens derived from a wide range of these putative pathogens. The use of these broad spectrum autogenous vaccines has proven to effective controlling mortalities in the field. However information regarding the level of protection conferred per component and the virulence of the strains used as antigens remains to be elucidated. In this study, infections via intra peritoneal injection were performed to investigate the virulence of three subtypes of a*As* and three isolates of *Vibrio splendidus* (*Vs*) in pre-deployment wrasse. While the experimental infections with a*As* successfully reproduced the clinical presentation atypical furunculosis, the i.p. injection of 10^9 to 10^{10} cfu/fish of *Vs* did not cause significant mortalities or clinical signs of vibriosis over a period of 7 days. An *in vivo* challenge model was established with a*As* and used to assess the efficacy of a commercial autogenous vaccine. Specific antibody responses to a*As* and bacterial loads of *aAs* in vaccinated and challenged fish were analysed by ELISA and qPCR as correlates of protection. The vaccine provided 79% and 20% relative percent survival (RPS) against experimental homologous challenge with $1x10^7$ and $1x10^8$ cfu a*As vapA* V B4 / fish, respectively, and 95% and 91% RPS against $1x10^6$ and $1x10^7$ cfu a*As vapA* V B2 / fish, respectively after an immunisation period of 700 degree days.

Conference Session Designation: (Immunology-Vaccines or Cleaner Fish)
Presentation Format: (Oral)

Amoebic Gill Disease in Ballan Wrasse (*Labrus Bergylta*) Juveniles and its Control by UV Irradiation.

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Amoebic gill disease caused by *Neoparmaoeba perurans* continues to be a significant challenge for the producers of cleaner fish (such as ballan wrasse *Labrus bergylta*, lumpfish *Cyclopterus lumpus*) in land-based pump-ashore hatcheries as well as in open sea cages. Since the produced fish are destined for stocking with highly AGD-susceptible Atlantic salmon as a biological control of sea lice, it remains imperative that they are not potential vectors of AGD in the sea cage environment. *Neoparamoeba perurans* re-isolated from fish after a challenge with a clonal strain, were maintained in liquid culture. Amoebae were exposed to ultraviolet radiation or ozone at different doses for varying periods of time. The subsequent morphology and growth characteristics of the amoebae cultures was monitored using MPN methods. UV appeared to have significant effects on inhibiting growth at most of the doseages used, although amoebae survived. In a laboratory challenge study, ballan wrasse juveniles were exposed to 100 cells L^{-1} of *Neoparamoeba perurans* trophozoites either irradiated with 0, 2 or 20 mJ cm^{-2} UV. Over the subsequent 6 week period, AGD developed only in the 0 UV group. AGD-affected wrasse developed epithelial hyperplasia, characteristic of AGD, in individual filaments with large numbers of unaffected filaments exhibiting a normal respiratory epithelium. In the ballan wrasse, hyperplasia associated with AGD lesions were characterized by increased numbers of mucous cells and an infiltration of eosinophilic granule cells. These studies suggest that UV irradiation of water at doses exceeding 2 mJ cm^{-2} is sufficient to inhibit infection of ballan wrasse with *Neoparamoeba perurans* and the subsequent development of AGD.

Conference Session Designation: (Gill Health or Cleaner Fish Heath)
Presentation Format: **(** Oral)

Bath Challenge Model Against Atypical Aeromonas Salmonicida in Farmed Ballan Wrasse (*L. Bergylta*)

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Ballan wrasse (*Labrus bergylta*) is commercially farmed and deployed as cleaner fish in Atlantic salmon cages as an environmentally friendly approach to delousing. Atypical *Aeromonas salmonicida;* a*As*, representing an important bacterial pathogen of *L. bergylta*, and other potential pathogens were isolated during outbreaks at hatcheries and/or cage sites in Scotland between 2016 and 2017. The pathogenicity and virulence of these routinely recovered bacteria including one isolate from Norway were assessed on juvenile (approx. 2 g) farmed Ballan wrasse by bath exposure, which enabled the development of a bath challenge model against a*As*. Juvenile Ballan wrasse (n= 50) were exposed to four bacteria species – a*As*, *Aliivibrio salmonicida*, *Photobacterium indicum* and *Vibrio anguillarum*; Scottish and Norwegian isolates – two strains of each species in duplicate (8 groups x 2 = 16; 2 controls; 18 tanks total) at an OD 1.0 (10^5 – 10^7 cfu/ml bacterial strain dependant) in 5 l sea water (33ppt) for 4 h at 15°C in static conditions. Duplicate groups of control fish were exposed to sterile sea water. The fish were then split into 18 tanks and monitored for up to 22 days. Moribund and diseased fish were examined for gross pathological changes and samples were taken for bacteriology, histopathology and molecular assessment. Ballan wrasse juveniles were susceptible to a*As* which was in contrast with the Vibrionaceae tested in this study. Notably, differential virulence was observed for a*As* subtype V - sub pulsotypes. Greater cumulative mortalities of 52 and 60 % were recorded when fish were challenged with a*As* sub pulsotypes B2 in contrast to 20% for B4. Furthermore, 4% mortality was observed for a Scottish isolate of *V. anguillarum;* while no mortalities were recorded for a Norwegian isolate of *V. anguillarum* or *Allivibrio salmonicida* and *P. indicum*. To our knowledge this is the first report of a successful a*As* bath challenge model for juvenile Ballan wrasse. This study provides an important foundation for future studies on vaccine efficacy, protection and immunocompetence at this developmental stage.

Program Session Designation: (Cleaner Fish Diseases)
Presentation Format: (Oral)
Student Presentaton: (Yes)

Phenotypic and Genotypic Characterisation of Atypical *Aeromonas Salmonicida* in Ballan Wrasse (*Labrus Bergylta*, Ascanius 1767)

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Cleaner fish, Ballan wrasse (*Labrus bergylta*) and lumpsucker (*Cyclopterus lumpus*) are used as an alternative approach for effective removal of sea lice from Atlantic salmon in aquaculture. Although both species are susceptible to infection by atypical *Aeromonas salmonicida* (a*As*), little is known about the diversity of a*As*. The aim of this study was to characterise 87 a*As* isolates from cleaner fish in Scotland and to compare these to a*As* from other fish species. Phenotypic characterisation of the a*As* isolates was initially performed to compare 35 representative isolates using conventional bacterial identification methods and a profiling system (BIOLOG GEN III) composed of 94 biochemical assays. Genotyping methods based on a PCR assay for the *virulence array protein* gene (*vapA*; A-layer), macro-restriction analysis using pulsed-field gel electrophoresis (PFGE) and plasmid sequencing was also conducted on the isolates. Phenotypically, the a*As* isolates resembled translucent, circular, convex colonies with or without brown diffusible pigmentation and were found to be non-motile, oxidase positive and Gram-negative coccobacilli or short rods under microscopic examination. The BIOLOG GEN III panel showed variability in 22 biochemical tests, of which two tests separated the UK a*As* isolates into two groups according to the *vapA* type; a minor group A (*vapA* type VI; n= 12 isolates) associated with both wrasse and lumpsucker fish species and a major group B (*vapA* type V; n= 75) confined to the wrasse host. Band analysis of the PFGE profiles revealed 10 pulsotypes from six sites in Scotland that also clustered according to the two *vapA* types. The major PFGE cluster B could be further sub-divided into nine pulsotypes (B1 - B9), with B2 pulsotype being predominant (n= 56) at four different sites. Plasmid profiling indicated the existence of multiple plasmids ranging in size from 5 to 155 kb, forming three plasmid groups according to sequence analysis. Homology to known plasmids present in typical strains of *A. salmonicida* was observed within the groups. The sequence data also indicated in five a*As* isolates exhibiting reduced antibiotic susceptibility, the presence of multi-drug resistance elements on plasmids capable of conjugal transfer. Together these findings help identify the prevalent strains of a*As* isolates causing disease in farmed wrasse in Scotland limiting cleaner fish deployment, and inform fish health management and future control measures.

Conference Session Designation: (Cleaner Fish Session)
Presentation Format: (Poster)
Student Presentation: (Yes)

Pathobiology of an *Exophiala* Sp. Disease Outbreak from Aquaculture Reared Lumpfish (*Cyclopterus lumpus*) in Newfoundland & Labrador, Canada

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Cleaner fish are used worldwide as a tool in an Integrated Pest Management Plan to mitigate sea lice in salmonid aquaculture. Lumpfish (*Cyclopterus lumpus*) and Cunners (*Tautogolabrus adspersus*) are used in salmonid aquaculture as cleaner fish. Lumpfish are favoured in the Newfoundland & Labrador aquaculture industry due to the short growing season. Lumpfish grown in an aquaculture setting reach sexual maturation in about 2 years and the males have a characteristic colouring allowing for easy identification. The females are stripped, eggs collected and the milt harvested post-mortem. The eggs are placed in a single layer on a specialized grid surface to enable the egg mass to be formed. By ~300°C degree days the eggs will hatch. Currently cleaner fish in NL are being vaccinated with a dip vaccine containing the antigens *Vibrio anguillarum* and *Vibrio ordalli*.

The current case study describes a population of 2015 year class Lumpfish that were hatched and raised at a land based sea water research facility in Newfoundland and Labrador. In January 2018, the population of lumpfish presented with clinical signs described as darkened skin lesions. The clinical signs consisted of systemic disease resulting in darkened and necrotic gills as well as dark internal organs, such as the heart. This population of fish were diagnosed with a fungal infection, identified as *Exophiala* spp. This mycotic class of organisms are ubiquitous in the soil and water and can affect finfish by causing local invasion or systemic disease. Diagnosis was initially determined by routine histopathology, with the aid of PAS staining to assist in identifying septate fungal hyphae in the affected organs and tissues. Subsequent confirmation was completed by culturing the infective isolate and submitting samples to a Canadian reference lab for morphologic and genomic identification. This talk will outline the epidemiology and pathology of the mycotic infection.

Conference Session Designation: (Cleaner Fish Diseases)
Presentation Format: **(** Oral)

Experimental Investigations Into Ranavirus (*Iridoviridae*) Infections in Lumpfish (*Cyclopterus lumpus*)

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A ranavirus (*Iridoviridae*), closely related to the notifiable epizootic haematopoietic necrosis virus (EHNV), has been repeatedly isolated from lumpfish (*Cyclopterus lumpus*). Isolates from Scotland, Iceland and the Faroe Islands were not associated with clinical disease. In Ireland the virus was isolated from lumpfish fry experiencing high mortality, but to date the virus has not been proven to be the aetiological agent of the disease. However, histopathology was indicative of viral aetiology and no other pathogens were identified using histology, bacteriology or parasitology. Several ranavirus species can cause severe systemic disease in fish and show a low host specificity, raising concerns about potential biosecurity risks posed to cohabited Atlantic salmon (*Salmo salar*). Challenge trials were conducted to evaluate the virulence of the virus to lumpfish and Atlantic salmon. Initially, sea transfer size lumpfish and lumpfish fry were challenged by immersion, Atlantic salmon smolts were challenged by immersion and intra peritoneal (IP) injection with the Irish isolate. Infection was demonstrated in fry but results were considered inconclusive and a second trial was set up using a cohabitation model. In this model, lumpfish fry were injected with Irish, Icelandic and Faroese strains of the virus and cohabited with naïve lumpfish. Atlantic salmon juveniles were IP injected with the Irish isolate without cohabitation of naïve fish. This challenge model demonstrated replication of the virus in the lumpfish, horizontal transmission of the virus and reduced survival in the IP injected lumpfish. A ranavirus qPCR assay was used to monitor the viral load in shedders and cohabitants at set time points and in mortalities. Results will be presented.

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Conference Session Designation: (Cleaner Fish Diseases)
Presentation Format: (Oral)
Student Presentation: (Yes)

Tuesday Sepember 4th – Langeve / Cartier
Zebrafish / Lab Animal Health
Moderator – Michael Kent (Oregon State University)

9:30 AM	Zebrafish / Lab Animal	Kent - Overview of Diseases of Zebrafish in Research Facilities
9:45 AM		Farmer - Challenges and Opportunities for Management of Disease Control and Biosecurity in Biomedical Zebrafish Facilities
10:00 AM		Sanders - Overview of Impacts of Common Zebrafish Pathogens
10:15 AM		Murray - Reversibility of Proliferative Thyroid Lesions Induced by Iodine Deficiency in a Laboratory Zebrafish Colony

Overview of Diseases of Zebrafish in Research Facilities

Michael L Kent[1,2 *] and Katrina Murray[2]

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The use of zebrafish *Danio rerio* as an in vivo model in biomedical research has expanded at an incredible rate over the last few decades. Originally it was mostly used as model in developmental genetics, but now is widely used in a variety of research areas that utilize adult zebrafish as research endpoints. Concurrently, the importance of acute diseases that cause high mortality, as well as those that are chronic or subclinical have become more important. The Zebrafish International Resource Center has been providing a diagnostic service to the research community since 1999, in which histopathology is our primary diagnostic tool. We have evaluated over 17,000 fish from laboratories around the world, and the following summarizes the patterns of infections and diseases that we have seen. *Pseudoloma neurophilia* is the most common pathogen, infecting the central nervous system in about 50% of the facilities, with about 10% prevalence in number of fish examined. Other common diseases and pathogens are as follows: Mycobacteriosis, caused by *M. chelonae*, *M. marinum* or *M. haemophilum*, *Pseudocapiillaria tomentosa*, *Myxidium streisingeri* and transmissible intestinal neoplasms associated with a *Mycoplasma* sp. *Edwardsiella ictaluri* is uncommon, but has caused severe disease. Other very common neoplasms are seminomas and ultimobranchial tumors. Common water quality related diseases include gas supersaturation and idiopathic gill diseases.

Conference Session Designation: (Zebra Fish or Lab Animal Medicine)
Presentation Format: (Oral)

Challenges and Opportunities for Management of Disease Control and Biosecurity in Biomedical Zebrafish Facilities

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The presence of zebrafish (*Danio rerio*) as an animal model for human diseases has grown tremendously in recent years, and they now are the second most commonly used research species in Great Britain (Home Office annual statistics for 2015). A literature review suggests that more than 5 million zebrafish are used yearly in research performed at more than 3,250 institutions in over 100 countries. Zebrafish are a popular model for a variety of reasons, including that they are genetically similar to humans, are more easily and economically housed and maintained than many other animals, and are robust breeders. Efficient genetic manipulation of zebrafish is possible using several approaches. In addition, zebrafish are a valuable model for pharmacology and toxicology studies as a given test substance may be added to their tank water, reducing a need for more invasive animal manipulations like injection or gavage. However, the rapid expansion of this animal model has led to challenges in facility disease control and biosecurity. When zebrafish were first used in biomedical research, there were no commercial vendors providing pathogen defined zebrafish. While there are now vendors who provide specific pathogen-free zebrafish and health testing of zebrafish on a fee for service basis, some institutions still do not routinely test their colonies or share this information when sharing fish. Because many facilities now operate as centralized resources, it has become critical that zebrafish facility personnel and scientists understand which diseases must be carefully excluded to reduce negative research results in particular studies. This talk will focus on the diseases that are most important to exclude from research colonies, the advantages and disadvantages of available testing methods for performing health surveillance, and characteristics of a robust biosecurity program. The use of these approaches is crucial to ensure the quality of research produced using this animal model and to reduce variation between research done at different institutions and laboratories.

Conference Session Designation: (Zebrafish / Lab Animal Medicine)
Presentation Format: (Oral)

Overview of Impacts of Common Zebrafish Pathogens

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Zebrafish used in laboratory research can harbor pathogens such as mycobacteria, microsporidia and helminths. Some of these infections can be clinical and lead to significant mortalities. However, subclinical infections can impact experimental endpoints, resulting in non- protocol induced variation. As the use of zebrafish has rapidly expanded to include a wide range of studies involving immune function, microbiome composition, and behavior, it is important to understand how subclinical infections by these pathogens can impact experimental results. The results of several experiments will be presented demonstrating the impacts of the most common pathogens, Pseudoloma neurophilia, Mycobacterium chelonae, and Pseudocapillaria tomentosa, on several experimental endpoints including fecundity, immune function, microbiome, and behavior.

Conference Session Designation: (Zebrafish / Lab Animal Medicine)
Presentation Format: (Oral)

Reversibility of Proliferative Thyroid Lesions Induced by Iodine Deficiency in a Laboratory Zebrafish Colony

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Zebrafish (*Danio rerio*) in a large laboratory facility experienced a widespread occurrence of red nodular lesions that were located predominantly in the ventral mandibular region. This facility housed approximately 37,000 fish distributed among 2040 tanks, of which, 220 tanks (~10%) were overtly affected. Among affected tanks, approximately 25% of the fish had externally visible masses. The masses were observed in wild-type, mutant, and transgenic lines, without apparent predilection for sex, researcher, or room location, and lesion occurrence was not associated with increased morbidity or mortality. Initially, twelve fish with visible masses were submitted to the Zebrafish International Resource Center (ZIRC, University of Oregon, Eugene, OR) diagnostic service for histopathologic processing and evaluation. Following humane sacrifice, fish were preserved as whole body specimens in Dietrich's fixative, processed routinely for paraffin embedding, sectioned in the parasagittal plane, and stained with H&E. All twelve fish were determined consequently to have proliferative thyroid lesions, in pharyngeal and/or ectopic locations, which were diagnosed as follicular cell hyperplasia, adenoma, or carcinoma in accordance with published morphologic criteria. Although salt had been used previously to maintain low levels of salinity within the containment systems, the thyroid lesions regressed dramatically throughout affected populations following transition to a brand of salt that contained a higher iodine content. Within five months the thyroid masses were no longer grossly visible, and eleven months after the salt change, there was no macroscopic or microscopic evidence of thyroid proliferative disease based on repeated diagnostic sampling. These findings suggest that at least in zebrafish, both hyperplastic and neoplastic thyroid proliferation may be hormone dependent, and such dependency may persist even following full lesion development. In addition, these results underscore the adaptive ability of zebrafish to absorb iodine from water and food, which has implications for the current campaign to standardize diets and include minimum environmental parameter recommendations in zebrafish publications.

Conference Session Designation: (Zebrafish / Lab Animal Medicine)
Presentation Format: (Oral)

Tuesday September 4th – Langeve / Cartier
Invertebrate and Shellfish Diseases 1
Moderator – Roxanna Smolowitz (Roger Williams University)

10:45 AM	Invert/Shellfish 1	**Atherley - Microsporidiosis in the Caribbean Spiny Lobster: A Rare Infection**
11:00 AM		**Elliott - First Detection of *Panulirus argus* Virus 1 (PAV1) by PCR in Spotted Spiny Lobsters (*Panulirus guttatus*) Held in Captivity**
11:15 AM		Hawke - An Update on White Spot Syndrome Virus Disease in Louisiana Red Swamp Crawfish *Procambarus clarkii*
11:30 AM		Battison - Observation of Epithelial and Endothelial Intracytoplasmic and Hepatopancreatic Intranuclear Viral Particles in Snow Crab *Chionoecetes opilio* From the Gulf of St. Lawrence and the Scotian Shelf
11:45 AM		Mahadevan - Previously Undescribed Histopathology Findings From Research Investigating Poor Post-Capture Survivability in Australian Southern Rock Lobster (*Jasus edwardsii*)
12:00 PM		**Lunch**

Microsporidiosis in the Caribbean Spiny Lobster: a Rare Infection

Nicole A. M. Atherley*, Michelle Dennis and Mark A. Freeman

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The Caribbean spiny lobster, *Panulirus argus*, is important to the economy of several countries in the Caribbean region, including Saint Kitts and Nevis. According to the IUCN red list, the *P. argus* population is currently decreasing and is believed to be exploited throughout its geographical range. As a result, it is important to investigate the factors that would affect population health and consequently a safe commercial yield. Although few lobster diseases and parasites have been reported, surveillance is important since existing diseases may affect yield and marketability of this species. Microsporidians comprise a group of spore-forming, unicellular organisms, related to the fungi, which are obligate intra-cellular parasites. These parasites have been known to infect a range of organisms from commercial insect colonies to fish and numerous mammals. This includes several species causing disease in crustaceans such as crabs, shrimp and lobster. Clinical signs of microsporidiosis in Crustacea are generally characterized by a change in the muscle from translucent to white/opaque, resembling cooked meat.

Lobsters were collected from fishermen during the period July 2017 to January 2018 as part of a PhD project entitled 'The biology, ecology and diseases of *Panulirus argus*'. This project seeks to examine the population dynamics and health together with novel ageing studies of lobsters from St Kitts. During necropsies, abnormal skeletal muscle and myoliquefaction was observed in one lobster. Wet-mounts confirmed the presence of microsporidian spores. Samples of muscle, eyestalk, ovary, gill and heart tissue were placed in Davidson's fixative for histopathological analysis. DNA was extracted from muscle samples and subsequent PCR reactions allowed for the amplification of partial sequences for the ribosomal RNA gene. Thereafter, database searches were used to identify the closest known microsporidian sequences.

Based on necropsies and the histopathological analysis of one hundred and fifteen lobsters, one lobster was infected with microsporidian spores; therefore, the prevalence of this parasite in Saint Kitts is only 0.87%. Although this parasite is seemingly not prevalent in wild lobster populations, there is a concern this could change with the potential development for aquaculture of the spiny lobster in the Eastern Caribbean region. The intensive systems generally used in lobster aquaculture may lead to higher levels of infection in the cages or tanks, which will likely reduce commercial yields and possibly impact local wild populations, as high numbers of resilient spores would be produced.

Screening of the lobster population in Saint Kitts is ongoing and will continue for the next two years as part of this PhD study. A literature review of previously published work revealed that the incidence of microsporidiosis in spiny lobster populations is rare and has up to now been restricted to regions around Florida. However, our DNA data indicates that this is the same microsporidian parasite, which clearly has a more widespread distribution than previously thought. These results highlight its possible geographical expansion and this is the first report of this microsporidian in the Eastern Caribbean region.

Conference Session Designation: (Shellfish)
Presentation Format: (Oral)
Student Presentation: (Yes)

First Detection of *Panulirus Argus* Virus 1 (Pav1) By PCR in Spotted Spiny Lobsters *(Panulirus Guttatus)* Held in Captivity

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Panulirus argus Virus 1 (PaV1) is the first naturally occurring pathogenic virus described in Caribbean spiny lobsters (*Panulirus argus*). The virus has been reported throughout the Caribbean sea and is typically lethal to infected juvenile lobsters within weeks to months. Previously, PaV1 infection has not been detected in other decapods that co-occur with *P. argus*, including the spotted spiny lobster (*Panulirus guttatus*); however, these studies primarily used histological evaluation, and PCR was not performed. In 2016, 14 Caribbean spiny lobsters (*P. argus*) and 5 spotted spiny lobsters (*P. guttatus*) were collected off of Summerland Key, Florida to supplement the resident population at the Aquarium of the Americas in New Orleans, Louisiana. The lobsters were transported and placed in quarantine tanks at Audubon's Aquatic Center housed at the Freeport McMoran Audubon Species Survival Center. After 5 months lobsters began to show clinical signs of lethargy and dying in the molt. Tissues were submitted to the Louisiana Animal Disease Diagnostic Laboratory (LADDL) at the Louisiana State University School of Veterinary Medicine for necropsy. Initial histopathological investigation revealed intranuclear inclusion bodies in cells of the exoskeletal membrane, indicating a viral infection. Differential diagnoses included White Spot Syndrome Virus (WSSV), a devastating disease of shrimp and crawfish also known to infect lobsters, and PaV1. Real time qPCR was performed on samples of haemolymph and tissues from both species of lobster for WSSV at LADDL and for PaV1 at the University of Florida; samples were negative for WSSV, but positive for PaV1. Tissues from the deceased spotted spiny lobsters were too severely autolyzed for critical histologic diagnostic evaluation. Remaining Caribbean spiny lobsters were euthanized in 10% MgCl in 100% ETOH and tissues were fixed in Davidson's fixative, gluteraldehyde, and 80% ETOH. Transmission electron microscopy (TEM) was performed to further characterize the viral infection. The most significant lesions were found in the hepatopancreas, where the virus is known to infect fixed phagocytes and circulating hemocytes including hyalinocytes and semi-granulocytes. TEM revealed that viral inclusions were localized exclusively in the cells underlying the hepatopancreatic epithelium of the hepatopancreatic tubules. This case demonstrates that PaV1 is a continuing threat to populations of Caribbean spiny lobsters held in captivity, as well as their co-habitant, the spotted spiny lobster (*P. guttatus*).

Conference Session Designation: (Invertebrate and Shellfish Disease)
Presentation Format: (Oral)
Student Presentation: (Yes)

An Update on White Spot Syndrome Virus Disease in Louisiana Red Swamp Crayfish *Procambarus clarkia*

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The red swamp crawfish *Procambarus clarkii* is the most economically important aquaculture species grown in Louisiana. In 2016 the farm gate value of crawfish aquaculture was approximately $196 million, with an additional added value of $128 million from processing and marketing. There are 1500 farm operations and approximately 220,000 acres of ponds many of which are in rotation with rice production. The crawfish harvesting season extends from November to June most years. White spot syndrome virus WSSV was discovered in the Louisiana crawfish industry in 2007 from specimens submitted to the Aquatic Disease Section of the Louisiana Animal Disease Diagnostic Laboratory at the LSU School of Veterinary Medicine. A surveillance project conducted by USDA/APHIS followed with detection of the virus by real time PCR in 60% of 184 sites sampled from 18 parishes across the state. Although its occurrence was widespread, losses over the next nine years were apparently not significant to the industry. However, mortalities in 2017 and 2018 have been much more severe causing many farmers to suspend production early. Clinical signs of WSSV in crawfish are lethargy, high mortality rates of large crawfish in the ponds, dead and dying large crawfish in traps with young active crawfish in the pond, and drastic drops in catch rate. White spots are typically not seen in the carapace. There are many unanswered questions and unidentified predisposing factors that trigger high mortality rates in crawfish ponds. For this reason we propose to engage in future research to evaluate effects of size, age, dissolved oxygen concentrations, and water temperature on susceptibility of red swamp crawfish to WSSV. We will utilize methods of viral quantification and experimental infection developed previously in our labs at LSU.

Conference Session Designation: (Virology or Invertebrate and Shellfish Disease)
Presentation Format: (Oral)

Observation of Epithelial and Endothelial Intracytoplasmic and Hepatopancreatic Intranuclear Viral Particles in Snow Crab *Chionoecetes Opilio* from the Gulf of St. Lawrence and the Scotian Shelf

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Viral inclusions were observed during review of haematoxylin and eosin stained histologic sections of gill and hepatopancreas tissues from snow crab (*Chionoecetes opilio)* as part of a study to collect biological baseline information on the species. Tissues were collected from crabs at three sampling stations in the southern Gulf of St. Lawrence (Margaree NS, Cheticamp NS and Grand Riviere QC) and one on the Scotian Shelf (Louisbourg NS). Intracytoplasmic coarsely granular eosinophilic to amphophilic inclusions were observed in 18/446 crabs where gill tissue was examined. Inclusions were most conspicuous in the gill epithelium but were also noted in endothelium of the gill and hepatopancreas. Transmission electron microscopy revealed numerous hexagonal viral particles (~ 70 – 80 nm in diameter) often packed in honeycomb-like arrangements in the cytoplasm of affected cells. The intracytoplasmic particles were observed more often in samples from the Cheticamp NS and Margaree NS sampling stations. Large basophilic to amphophilic homogenous intranuclear inclusions were observed nearly completely filling the nuclei of hepatopancreas B-cells in 6/1113 crabs – four from the Louisbourg NS, one from the Margaree NS, and one from the Grande Rivière QC sampling station. Transmission electron microscopy images suggested closely packed linear aggregates of viral particles in the nucleus. Co-infections were not detected.

Conference Session Designation: (Invertebrate and Shellfish Disease)
Presentation Format: (Oral)

Previously Undescribed Histopathology Findings from Research Investigating Poor Post - Capture Survivability in Australian Southern Rock Lobster (*Jasus Edwardsii)*.

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During 2017 the wild catch Southern Rock Lobster (SRL) industry initiated research into improving post-capture survivability and in particular the possible effects of Australia's changing environment on lobster health. The research was divided into three areas of investigations; SRL physiology, epidemiology of reduced survival and pathology associated with mortality events. As part of the pathology component multiple SRL samples from mortality, reduced survival events and health surveillance sampling were submitted in by processors across Australia for diagnostic evaluation. Histopathology findings were detailed for organs systems that showed consistent pathology from all accessions (ie: gills, hepatopancreas, nerves, reserve cell tissue and antennal glands). A range of degenerative and inflammatory lesions were noted in antennal glands with some extensive destruction of the gland tissue associated with high levels of intra-lesionary bacteria. Three other significant histological changes were observed. Two of which have not previously been noted for SRL (ie: mineralised inclusions, nuclear basophilic inclusions). Additionally there was repeat observations of cytoplasmic eosinophilic inclusions which have been previously described. These inclusion changes were noted in 66% of the animals examined and in the majority with apparently normal antennal gland tissue. This presentation will discuss the general histopathological findings noted in 2017 and focus particularly on changes observed in the antennal gland of SRL that prior to this study appear to have not been described or published.

Conference Session Designation: **(** Invertebrate and Shellfish Disease)
Presentation Format: **(** Oral)

Tuesday September 4th – Langeve / Cartier
Invertebrate and Shellfish Diseases 2
Moderator – Roxanna Smolowitz (Roger Williams University)

1:15 PM	Invert / Shellfish 2	Smolowitz - Hemocytic Neoplasia of Hard Clams (*Mercenaria mercenaria*), an Emerging Neoplastic Disease?
1:30 PM		Ferguson - A Case Report and Statewide Surveillance of "Weak Meat" Condition of Alaska Weathervane Scallops *Patinopecten* caurinus Associated With a Recently Identified Pathogenic Apicomplexan Parasite
1:45 PM		Waller - The Unknown State of Freshwater Mussel Health and Disease
2:00 PM		Gustafson - A Birds-Eye View of Shellfish Health: Advances in Regional Management in the Eastern USA
2:15 AM		Kane - Visual Keys Support Oyster Health Monitoring and Oyster Reef Restoration Efforts
2:30 AM		**Kantzow - Conditioning Pacific Oyster *Crassostrea gigas* Spat for Improved Survival of Ostreid Herpesvirus – 1 (Oshv-1) by Controlled Infection**
2:45 AM		Corbeil - Differentially Expressed Genes in Haliotis Iris (*Paua abalone*) Associated With Haliotid Herpesvirus Challenge.

Hemocytic Neoplasia of Hard Clams (*Mercenaria mercenaria*), An Emerging Neoplastic Disease?

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In 2009, a new lethal disease was identified by Smolowitz and Murphy in hard clams (*Mercenaria mercenaria*), cultured in Wellfleet, Massachusetts, U.S., that caused high mortality. Annual monitoring of hard clams since 2009 has shown a continued high prevalence of the disease associated with significant mortality in aquacultured hard clams. Hard clams originating from multiple hatcheries are affected by the neoplastic disease indicating it is not genetic in origin and is not associated with a specific broodstock but instead provides strong evidence it is a contagious disease. Histologically, large neoplastic cells circulate in the open vascular system of the hard clams causing restriction of the hemolymph circulation in the vascular system with obturation of vessels, eventual loss of normal hemocytes, and loss of the tissue functions. The similarity of the disease to disseminated neoplasia in soft shell clams is striking. Recently evidence has strongly suggest a neoplastic cell is the infective agent in the soft shell clam disease. Identification of the cause of disease in hard clams is ongoing. The disease in hard clams has become a major concern for Wellfleet aquaculturists and the potential for spread to hard clams in other water bodies is a strong possibility.

Conference Session Designation: (Invertebrate and Shellfish Disease)
Presentation Format: (Oral)

A Case Report and Statewide Surveillance of "Weak Meat" Condition of Alaska Weathervane Scallops, *Patinopecten Caurinus*, Associated with a Recently Identified Pathogenic Apicomplexan Parasite

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Weathervane scallops, *Patinopecten caurinus*, are the largest scallop species in the world and they are distributed from Northern California to the Pribilof Islands of Alaska. They are only commercially fished in Alaska and the fishery is considered to be well managed, but there has been a recent decline in scallop catches in some management areas. In the Kamishak Bay fishing District during the 2002 season there was a dramatic decline in Catch Per Unit Effort and an unprecedented incidence of detected "clappers", dead scallops with valves connected but lacking soft tissues. Additionally, fishermen and other stakeholders have encountered scallops with abnormal adductor muscles that have been colloquially named "weak meat". The muscle of affected scallops have brownish coloration, stringy texture and will occasionally either slip off the shell with the viscera or tear apart during the shucking process. Brenner et al. (2012) examined the quality of scallop weak meat using chemical and physical parameters and concluded that nutritional stress was likely involved. A somewhat similar syndrome in sea scallops, *Placopecten magellanicus*, described as "gray meat", has been documented in the eastern U.S. and Canada, which was recently linked to an apicomplexan parasite (Inglis et al., 2016). This is the same parasite that was responsible for the collapse of Icelandic scallops, *Chlamys islandica*, and initial phylogenetic studies placed it within the family Aggregatidae (Kristmundsson et al., 2015). More recently it was identified as *Merocystis kathae* that sexually matures in common whelks, *Buccinum undatum* (unpublished data). In January 2015, fishermen reported high numbers of weak meat in their catch from the Bering Sea and samples were subsequently submitted to the Alaska Department of Fish and Game for diagnostic examination. Histopathology revealed that the poor muscle integrity was likely due to a severe apicomplexan parasite infection. Due to the reduced quality and marketability of scallops with this condition and the potential association with poor survival, we conducted a statewide surveillance study of 180 scallops from subareas within each of the three major geographically broad scallop beds in Alaska. All beds were infected and the highest infection intensities occurred near Dutch Harbor and Southwest Kodiak. A representative set of samples tested by PCR indicated this to be the same parasite as the one that infects and causes disease in other scallop species in the Atlantic Ocean. Currently there is no clear management approach for mitigating this disease.

Conference Session Designation: **(** Invertebrate and Shellfish Disease)
Presentation Format: **(** Oral)

The Unknown State of Freshwater Mussel Health and Disease

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Freshwater mussels (Unionacea) are in decline worldwide, with causes attributed to factors such as habitat degradation, pollution, and invasive species, among others. However, these purported causes cannot fully explain the enigmatic decline and large-scale die-offs of mollusks that have occurred in relatively "healthy" streams across a wide geographic region–from the Southeast to the Pacific Northwest in the U.S. and Sweden to France in Europe. The role of the microbiota and pathogens in the health of freshwater mussels has been understudied and as a result, there are few reference data to compare the "normal" microbiota of healthy to "stressed" or dying mussels. Captive propagation and stocking programs for freshwater mollusk restoration have expanded across the globe. There are no standard diagnostic protocols to assess the health and disease status of cultured, stocked or wild mussels. Continued introduction of nonindigenous species, changing climate, and high-density propagation present risks for outbreaks of opportunistic and new emergent diseases in freshwater mussel populations.

A workshop on Freshwater Mollusk Health and Disease, sponsored by the Freshwater Mollusk Conservation Society, was held in March 2018 as a first step towards advancing an initiative on this topic. The Workshop advocated for inclusion of freshwater mollusks in the One Health concept (recognition that the health of people is connected to the health of animals and the environment) and for integrating multidisciplinary technologies with traditional diagnostic tools to assess the health status of mollusk populations. This strategy is being demonstrated in response to a chronic mussel-die off occurring in the Clinch River in the southeastern U.S. The Clinch River contains one of the premier freshwater mussel communities in the country and includes 29 threatened and endangered species. Samples of mussels were collected from affected and unaffected reaches of the river in 2017. A metagenomic approach is being used to characterize and compare viral and bacterial communities among mussel populations and will be combined with histopathologic evaluation and other approaches. The project is one of the first comprehensive assessments of an on-going mortality event and will provide information not only on potential causal factors, but also on "best practices" for addressing such events. Given changing environments around the world, there is a global need for such a framework.

Conference Session Designation: **(** Invertebrate and Shellfish Disease)
Presentation Format: **(** Oral)

A Birds-Eye View of Shellfish Health: Advances in Regional Management in the Eastern USA

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Shellfish health management is typically structured along jurisdictional boundaries. Fourteen states, many reliant on inter-state trade of larvae or spat, rear molluscan shellfish in the East Coast region alone. While growth has been rapid to keep pace with public demand, pathogens - and, by association, their management - have the potential to derail both population health and industry vitality. In fact, both movement of pathogens with trade, as well as redundancy or delays in regulatory permitting of trade, can have unintended consequences. Typically, trade (or movement) decisions are made at the state, or sometimes even more local, level. Regulators are often constrained to operate on local (fragmented) knowledge, and, because mollusc disease risk and status do not adhere to jurisdictional boundaries, the industry may face redundant testing, conflicting demands, or delayed decisions. As a response to this conundrum, a consortium of academic, regulatory and industry representatives have been working to advance shellfish health management along the East Coast of the United States through partnerships in information and decision support. Central to this effort are mechanisms to enhance information exchange. Recent focus has been on the design of a shared, interactive, database for the storage and retrieval of regional health information that crosses jurisdictional and organizational bounds. We describe the importance of rapid and accurate information exchange for this growing industry, and the strategy underway to meet that goal.

Conference Session Designation: (Bivlave or Shellfish Diseases)
Presentation Format: (Oral)

Visual Keys Support Oyster Health Monitoring and Oyster Reef Restoration Efforts

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Environmental and health observations are an integral component of monitoring oyster reefs restoration projects. We developed and applied visual keys to facilitate consistency in ranking severity scores for stained *Perkinsus marinu*s hypnospores, empirical meat ranks (volume within the left shell, plumpness, translucency), and severity of boring shell parasites affecting Eastern oyster, *Crassostrea virginica*. Measurement of shell parasite prevalence and severity for *Polydora websteri* (polychaete), *Diplothyra smithii* (clam) and *Cliona celata* (sponge) is important since these organisms excavate shell matrix throughout the life of the oyster (and in the case of *Cliona*, throughout the life of the shell). Further, these parasites reduce shell density and dramatically increase surface area of the shell exposed to the environment. Higher salinity conditions foster the severity of these shell parasites, and shells with extensive excavation and high surface area may dissolve more rapidly in saltwater, break down into shell fragments, and destabilize reef structure in the long term. Visual keys that support these metrics will be demonstrated and their utility for training new investigators and providing intra- and inter-laboratory quality control for diagnostic health assessments will be discussed. Consistency in reporting health metrics as part of oyster resource monitoring has become increasingly important as restoration efforts with limited resources focus more on sustainable, measureable outcomes. This project was supported in part through the National Fish and Wildlife Foundation Gulf Environmental Benefit Fund, Florida Fish and Wildlife Conservation Commission, the University of Florida Institute for Food and Agricultural Science (IFAS), and the Florida Sea Grant Program.

Conference Session Designation: (Invert & Shellfish Disease)
Presentation Format: (Oral)

Conditioning Pacific Oyster (*Crassostrea Gigas*) Spat for Improved Survival of *Ostreid Herpesvirus – 1* (Oshv-1) by Controlled Infection

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The high mortality and economic loss in farmed Pacific oysters (*Crassostrea gigas*) caused by the virulent genotypes of *Ostreid herpesvirus - 1* (OsHV-1) has spurred research into strategies to mitigate the impact of the disease. Both elevated water temperature and the absence of previous exposure to OsHV-1 increase the level of mortality from infection with OsHV-1. Infection at 18°C can result in infection, but occurrence of mortality is dose dependent. The pathogenesis at 22°C is more rapid and results in greater mortality. The present study evaluated the effect of water temperature and pre-exposure to OsHV-1 on oyster survival following a second exposure to OsHV-1 in a controlled laboratory experiment. It was conducted with 6 month old commercial triploid Pacific oyster spat in a physical containment level 2 aquatic animal facility. Oysters were first exposed at a water temperature of either 18°C or 22°C with either OsHV-1 or a negative control inoculum. Surviving oysters were then maintained at 18°C or 22°C, or the water temperature was increased from 18°C to 22°C for the second exposure to OsHV-1. Mortality in the 10 days following the second exposure at 22°C was 10% and 24% for those pre-exposed at 18°C with OsHV-1 and the negative inoculum respectively with a hazard ratio (HR) of 0.22 (95%CI: 0.1 – 0.8). Mortality was 34% and 40% for oysters pre-exposed at 22°C with OsHV-1 and the negative inoculum respectively, HR: 0.7 (95%CI: 0.3 – 1.9). The concentration of OsHV-1 DNA at the time of death or end of the trial was assessed using a linear mixed model which indicated a significant interaction between the pre-challenge water temperature and the inoculum ($P<0.001$). This study determined that pre-exposure to OsHV- 1 can infer greater survival on re-exposure to OsHV-1. This confirms field observations that oysters which have survived an OsHV-1 outbreak are more likely to survive further outbreaks and controls for confounding factors reflecting changes in the host and environment which can impact disease outcome. A pre-challenge with OsHV-1 presents a possible method of reducing mortality from OsHV-1 epizootics in farmed Pacific oyster populations.

Conference Session Designation: **(** Invertebrate and Shellfish Disease)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Differentially Expressed Genes in *Haliotis Iris* (Paua Abalone) Associated with Haliotid Herpesvirus Challenge.

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The natural resistance of New Zealand paua abalone (*Haliotis iris*) to infection by Haliotid herpesvirus 1 (HaHV) and to the disease abalone viral ganglioneuritis (AVG) was investigated using high throughput RNA-Seq. HaHV-infected paua up-regulated broad classes of genes that contained chitin-binding peritrophin-A domains, which may indicate the production of a defensive "peritrophic matrix", as seen in other molluscs and insects. The paua also up-regulated VAP-1, an important adhesion molecule for lymphocytes in mammals, and CHIT-1, an immunologically important gene in mammalian immune systems. Moreover, several blood coagulation pathways were dysregulated in the paua, possibly indicating viral modulation. We also saw several indications that neurological tissues were affected by HaHV, including the dysregulation of B4GALNT, GM2 ganglioside, neuroligin-4 and the Notch signalling pathway. This research may support the development of an AVG resistant breeding program in disease susceptible Australian abalone or the development of molecular therapeutics useful to control and/or manage virus outbreaks in Australian abalone culture.

Conference Session Designation: (Invertebrate and Shellfish Disease)
Presentation Format: (Oral)

Tuesday September 4th – Langeve / Cartier
Aquatic Epidemiology I
Moderator – Ian Gardner (Atlantic Veterinary College / UPEI)

3:15 PM	Aquatic Epidemiology I	Laurin - Monitoring Data From Farmed Salmon *Salmo salar* and *Oncorhynchus* spp. In British Columbia, Canada, From 2011–2013
3:30 PM		Marty - Trends in Disease Prevalence Among Regulatory Audit Samples of Farmed Atlantic Salmon in British Columbia, Canada: 1990–2017
3:45 PM		**Burnett - Drivers of Spatio-Temporal Variability in Sea Lice Connectivity Among Salmon Farms in the Broughton Archipelago, British Columbia**
4:00 PM		McEwan - Agent-Based Modelling as a Tool for Exploring Integrated Pest Management in Aquaculture
4:15 PM		**Jia - Literature Review of Historical Information on Pathogen Occurrence Among Wild Salmonids in British Columbia, Canada**
4:30 pm		Ferguson - An Epidemiological Model of Virus Transmission in Salmonid Fishes of the Columbia River Basin
4:45 PM		Thakur - Infectious Agent Detections in Archived Sockeye Salmon (*Oncorhynchus nerka*) Samples From British Columbia (1985–1994)
5:00 PM		Sigurðardóttir - A Survey of Three Viruses in Wild and Cultured Salmon in Iceland

Monitoring Data from Farmed Salmon (*Salmo Salar* and *Oncorhynchus* Spp.) in British Columbia, Canada, from 2011-2013

Emilie L. Laurin[1*], Diana Jaramillo[1], Raphaël Vanderstichel[1], Hugh Ferguson[2], Karia H. Kaukinen[3], Angela. D. Schulze[3], Ian R. Keith[4], Ian A. Gardner[1], and Kristina M. Miller[3,5]

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The purpose of this study was to describe spatial and temporal patterns of endemic and new infectious agents and histopathologically-identified lesions in dead-and-dying farmed non-native Atlantic (AS) and native Pacific (PS) salmon in British Columbia, Canada, between 2011 and 2013. Novel high-throughput molecular testing and blinded histopathological examination of tissues were used to evaluate these patterns in fish-level analyses. Twenty-five of 45 infectious agents were detected, and 87% of 897 total fish tested had mixed detections, with up to nine agents in a single fish, and a higher agent diversity in PS than AS. Most frequently detected agents were the parasite *Desmozoon lepeophtherii* (*D.lep*) in farmed AS (88%), and the bacterium *Candidatus Branchiomonas cysticola* (*Ca.B.cys*) in farmed PS (89%). Overall, 92% of AS and 88% of PS had some histopathological change, mostly of mild to moderate severity, with renal interstitial hyperplasia as the most frequent change (AS: 33%; PS: 48%). Spatial patterns were statistically significant for five agents in PS *versus* AS in southwest Vancouver Island, Sunshine Coast, and Discovery Islands. Statistically significant temporal patterns were detected for three agents each in AS and PS, with only *D.lep* common for both. Importantly, infectious salmon anemia virus, salmonid herpesvirus, salmon alphavirus, and infectious pancreatic necrosis virus were not detected. The majority of agents detected on BC salmon farms were known to be endemic, but new findings include the marine detections of some infectious agents reported to only cause freshwater or hatchery-based diseases (*Flavobacterium psychrophilum* and *Ichthyophthirius multifiliis*). The results of this descriptive study provide the proportion of positive test results in sampled dead-and-dying farmed AS and PS, and temporal-spatial information on both agent and lesion detection, targeting areas of interest and concern to researchers, regulators, and aquaculture industry veterinarians for future population-based analyses.

Conference Session Designation: **(** Aquatic Epidemiology)
Presentation Format: (Oral)

Trends in Disease Prevalence Among Regulatory Audit Samples of Farmed Atlantic Salmon in British Columbia, Canada: 1990 – 2017

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Since 1990, government regulators have systematically assessed disease among Atlantic salmon (*Salmon salar*) farmed in marine net pens in British Columbia (BC), Canada. The first diagnostic assessment by BC included a total of 215 Atlantic salmon sampled from 1990 – 1992. The second assessment initiated by BC in the early 2000s continues today as the BC Fish Health Auditing and Surveillance Program administered by Fisheries and Oceans Canada (DFO).

Program staff sample moribund and recently dead Atlantic salmon cultured in marine net pens. Audited farms are selected to be representative of the number of farms in each region of the province. Annual sample size from 2006 – 2009 and 2014 – 2017—the years with the most complete data—ranges from 470 – 784 fish. Since 2006, diagnostic analysis to determine cause of death includes gross and microscopic lesions (up to 9 organs for histopathology), aerobic bacterial culture of kidney, and pooled PCR tests for *Piscirickettsia salmonis*, endemic viruses IHNV and VHSV, and exotic viruses IPNV and ISAV. Since 2014, exotic virus testing includes SAV, and fish with idiopathic cardiomyopathy are tested for PMCV. Trends based on sample prevalence of fish with a lesion/pathogen diagnosed as cause of death:

1. *Renibacterium salmoninarum* declined from 45% (1990 – 1992) to 6.8 – 9.4% (2006 – 2009), and further to 1.4 – 3.4% from 2014 to 2017;
2. *Piscirickettsia salmonis* affected 1 – 2% of samples each year since 2006 except for a 2-year outbreak peaking at 13% in 2015 and decreasing to 7.4% in 2016;
3. Idiopathic cardiomyopathy ranged from 1 – 3% through all years studied, 1990 – 2017.

For VHSV, the proportion of PCR+ sample pools declined from 3.1 – 9.7% (2006 – 2009) to 0.6 – 2.5% (2014 – 2017). During the reported years, *Paramoeba perurans* was first diagnosed by histopathology in 2014, and infection prevalence ranged from 0.3 – 2.2% through 2017. Severe cases of idiopathic meningoencephalitis affected 0.3 – 1.1% of sampled fish from 2007 – 2009 and 2014 – 2017. All samples were PCR-negative for IHNV, IPNV, ISAV, SAV, and PMCV.

Each year (2006 – 2009 and 2014 – 2017), 6.1 – 19% of Audit Program Atlantic salmon were diagnosed with an infectious disease that (i) could potentially affect wild Pacific salmon and (ii) was not primarily from an environmental source. The other 81 – 94% of the sampled fish had no evidence of these diseases. Epidemiologic principles inform us that diseases that do not spread widely among concentrated farm fish are less likely to spread among dispersed wild fish.

Conference Session Designation: **(** Aquatic Epidemiology)
Presentation Format: **(** Oral)

Drivers of Spatio -Temporal Variability in Sea Lice Connectivity Among Salmon Farms in the Broughton Archipelago, British Columbia

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Connectivity of aquaculture sites from the perspective of disease transmission is determined by a combination of hydrodynamic circulation and the biology of the organisms involved. For example, viral disease connectivity will likely be driven almost entirely by the underlying circulation. However, living infectious agents (such as sea lice copepods) have maturation, mortality, limited energy reserves, and behaviours (such as swimming avoidance or seeking behaviours) that can impact connectivity of farms. Here we analyze drivers of the temporal variability of an infectious agent network of farms that was determined from a coupled biological-physical particle-tracking model. The infectious agent whose biology we modelled was the sea louse. The simulation mimicked conditions from March- July, 2009 (150 days in total), in the Broughton Archipelago, British Columbia, Canada. Temporal analyses indicated large changes in the strength of connectivity of infectious agent networks, with large peaks in connectivity lasting for around 5 days at three specific points in the simulation. The peaks are largely driven by the biology of the organism, rather than the underlying circulation model, highlighting the importance of including a biological model in particle tracking simulations used to determine connectivity. The main drivers of the strength of connectivity include the temperature and salinity the particles were exposed to, which was driven in large part by the freshet run-off from the many rivers surrounding the BA. Additionally, several sub-networks of farm-to-farm connectivity emerged, each with distinctive space-time connectivity characteristics. Our results suggest that typical measures of connectivity, such as seaway distance, may not accurately capture the reality of high temporal variability and non-intuitive spatial relationships when the infectious agent has complex biology, such as is the case for sea lice.

Conference Session Designation: (Aquatic Epidemiology)
Presentation Format: (Oral)
Student Presentation: (Yes)

Agent-Based Modelling as a Tool for Exploring Integrated Pest Management in Aquaculture

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In aquaculture, there is an increasing move towards Integrated Pest Management (IPM) strategies. Previously, there was a heavy reliance on chemical treatments, whereas now physical, biological, and management controls are also being used. There are several motivating factors, such as increasing resistance to the chemical treatments, and public perception of the industry. The problem now is that farmers are faced with many options regarding treatments – chemical, physical, biological, management – and their combinations, with little guidance on how to choose the best strategy in any given situation. Real-world testing is not feasible as each treatment choice typically involves a large commitment of time and money. Testing more than a very few combinations would be a daunting and expensive task.

Our solution is to use computer simulation modelling. Modelling allows relatively cheap and quick exploration of a variety of treatment strategies. It also allows detailed tracking of information that is not easily obtained in the real world, such as genetic resistance. In particular, we use agent-based models (ABM). ABMs model each individual (agent) with simple parameters and behaviour. Complex patterns emerge from populations of agents interacting with each other. The advantages of ABMs in this context are extensibility – individual agents are extensible to capture new behaviour, and new types of agents are easily added – and agent sensing / memory can be used to make individual contextual changes.

In this presentation we describe our use of an ABM to explore IPM in the context of controlling sea lice on Atlantic salmon farms. We have used the ABM to explore the evolution of chemical resistance in the presence of wild salmon populations, using different strategies for using chemical treatments, and the efficacy of cleaner fish in different environments. These projects all relied on the unique strengths of ABMs in terms of extensibility and individual context.

Conference Session Designation: **(** Aquatic Epidemiology)
Presentation Format: **(** Oral)

Literature Review of Historical Information on Pathogen Occurrence Among Wild Salmonids in British Columbia, Canada

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Maintaining healthy populations of wild salmonids in British Columbia is a high priority for numerous stakeholders including Fisheries and Oceans, Canada, the Pacific Salmon Foundation, First Nations, and NGOs. Farming of salmon in the same waters is a controversial topic, and strengthened control of infectious diseases is needed to minimize spread of important infectious agents from farmed to wild salmonids and wild to farmed salmon. The dynamics of wild salmon populations and complex interconnectivity of potential hazards have been regarded as challenging due to knowledge gaps and uncertainty of existing information. However, as stated in a recently published the Scottish historical review, the pathogens were detected in wild marine fish caught remotely from aquaculture sites, and hence limited evidence for clinical disease in wild fish due to the pathogens. During a recent workshop held in June 2018, various stakeholders agreed that wild salmonid dynamics in the region is a complicated issue with concurrent biological, environmental, social, and cultural ramifications. There is a need to reach mutual-understanding on host-pathogen-environmental interactions among the wild fish population and to shape the roles from diverse factors within the ecosystem. In this context, historical information on the spatiotemporal occurrence of diseases in wild finfish is necessary to inform discussions about their risk at a population level. Here we conducted a literature review of published journal and grey literatures for pathogens likely to occur in the wild finfish population in British Columbia. The objective of the literature review is to establish a transparent reporting process for historical information and also bias reduction through interrogating the databases that have reported the health events of wild fish in selected study areas. We started with a primary screening and identified literatures relevant to the infection of the 9 pathogens among the 5 species of Pacific salmon in freshwater and ocean environment in the province. Next, we appraised the quality and validity of the studies or documented information based on research designs, their implementation, statistical methods and their appropriateness of answering research questions. We then compiled the baseline occurrence of the 9 pathogens among salmonids with the application of multivariate information visualization through data mining techniques. In the last step, we synthesized snapshots of information on aspects other than health status alone, including spatial-temporal dynamics of stock assessment of wild fish, human activities impacting on both freshwater and seawater habitats, coastal environmental conditions, and meteorological indices. Thus we summarized what has been already known and what is still unknown about the challenging topic. This literature review will demonstrate a transparent way of contrasting the potential divergence of literature resources and provide new insights for the future research methods to provide an improved understanding the complexity of the marine ecosystem and interactions that occur within.

Conference Session Designation: (Aquatic Epidemiology)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

An Epidemiological Model of Virus Transmission in Salmonid Fishes of the Columbia River Basin

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We have developed a dynamic epidemiological model informed by records of viral presence and genotypes to evaluate potential transmission routes maintaining a viral pathogen in economically and culturally important anadromous fish populations. In the Columbia River Basin, infectious hematopoietic necrosis virus (IHNV) causes severe disease, predominantly in juvenile steelhead trout (*Oncorhynchus mykiss*) and less frequently in Chinook salmon (*O. tshawytscha*). Mortality events following IHNV infection can be devastating for individual hatchery programs. Despite extensive surveillance efforts, there are questions about how viral transmission is maintained. Modeling this system offers important insights into disease transmission in natural aquatic systems, as well as about the data requirements for generating accurate estimates. We simulated six scenarios in which testing rates and the relative importance of different transmission routes varied. The simulations demonstrated that the model accurately identified routes of transmission and inferred infection probabilities accurately when there was testing of all cohort-sites. When testing records were incomplete, the model accurately inferred which transmission routes exposed particular cohort-sites but generated biased infection probabilities given exposure. After validating the model and generating guidelines for result interpretation, we applied the model to data from 14 annual cohorts (2000–2013) at 24 focal sites in a sub-region of the Columbia River Basin, the lower Columbia River (LCR). We demonstrate that exposure to IHNV via the return migration of adult fish is an important route for maintaining IHNV in the LCR sub-region, and the probability of infection following this exposure was relatively high at 0.16. Although only 1% of cohort-sites experienced self-exposure by infected juvenile fish, this transmission route had the greatest probability of infection (0.22). Increased use of secure water supplies and continued use of biosecurity protocols may reduce IHNV transmission from adult fish and juvenile fish within the site, respectively, to juvenile salmonids at hatcheries. Models and conclusions from this study are potentially relevant to understanding the relative importance of transmission routes for other important aquatic pathogens in salmonids, including the agents of bacterial kidney disease and coldwater disease, and the approach may be useful for other pathogens and hosts in other regions.

Conference Session Designation: (Aquatic Epidemiology)
Presentation Format: (Oral)

Infectious agent detections in archived Sockeye salmon (*Oncorhynchus nerka*) samples from British Columbia (1985-1994)

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Commercial Atlantic salmon aquaculture expanded into British Columbia (BC) Canada between 1989-1993, concurrent with initial declines in productivity and abundance of Sockeye salmon stocks in southern BC. In response to concerns that novel infectious agents were introduced through movement of eggs during the development of Atlantic salmon aquaculture, we undertook a study to estimate the prevalence of infectious agents in archived historical return-migrating Sockeye salmon, spanning the period before and during aquaculture expansion in BC (mid-1980s to 1990s). Of 45 infectious agents assessed with molecular assays across 652 samples, 23 (7 bacterial, 2 viral and 12 parasitic) were detected in liver tissue across six regions in BC. Prevalence ranged from 0.5 to 83.3% and varied significantly by region and year. Agent diversity ranged from 0 to 12 per fish, median 4, with lowest diversity observed in fish from the Transboundary and Central Coast. Agents known to be endemic and associated with mortality in Sockeye salmon in BC, such as *Flavobacterium psychrophilum*, Infectious hematopoetic necrosis virus, *Ceratonova shasta*, and *P. minibicornis*, were commonly observed. Others, such as *Kudoa thyrsites* and *Piscirikettsia salmonis*, which have impacted BC salmon aquaculture, were also detected in the mid 1980s, suggesting they were likely present historically. Surprisingly, infectious agents described only recently in BC salmon, *Ca. Branchiomonas cysticola*, *Parvicapsula pseudobranchicola*, and *Paranucleospora theridion*, and common in salmon aquaculture in BC and Norway, were also detected, indicating their potential presence prior to the expansion of the aquaculture industry. Only two agents currently common in BC salmon aquaculture, gill chlamydia and Piscine orthoreovirus, detected in recently sampled liver tissue, were not detected in historic Sockeye. In general, our data suggest that agent distributions have not substantively changed since the expansion of the salmon aquaculture industry.

Conference Session Designation: (Aquatic Epidemiology)
Presentation Format: **(** Oral)

A Survey Of Three Viruses In Wild And Cultured Salmon In Iceland

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With increasing culture in net pens around Iceland, the need for information on the status regarding pathogens that are common in aquaculture around the North-Atlantic Ocean is obvious. The aim of the survey was to screen groups of wild and cultured Atlantic salmon for three viruses. These are infectious salmon anemia virus (ISAV), piscine myocarditis virus (PMCV) and piscine reovirus (PRV).

The survey groups included juveniles and adult fish and were divided into three categories: salmon from wild origin, offspring of an old stock of sea-ranching brood fish and cultured salmon. Tissue samples from individual fish were placed in RLT-buffer. RNA was isolated and used for RT-qPCR virus assays. PRV positive samples were sequenced.

All samples tested were negative for PMCV and ISAV. PRV was detected in all groups except one. In the wild fish category, PRV frequency ranged between 0-100%, while in the sea-ranching and cultured category it was 95-100%. The distribution of cycle threshold values varied in the groups, representing variable levels of virus in the samples. The lowest levels of virus were observed in the wild fish.

The results show that PRV is widespread in Atlantic salmon in Iceland. Similar observations have been reported in surveys elsewhere. Sequences obtained were identical within each group of salmon but there were differences between the salmon groups. The sequences showed similarity to PRV isolates from Norway and Canada and were classified as genotype 1a.

Conference Session Designation: (Aquatic epidemiology Session)
Presentation Format: (Oral)

Tuesday September 4th – Tilly / Tupper
World Aquatic Veterinary Medical Association
Moderator – A. David Scarfe (University of Pretoria)

9:30 AM	WAVMA	Dhar - The University of Arizona, Aquaculture Pathology Laboratory: A Worldwide Resource for Diagnostic Services and Collaborative Research to Shrimp Aquaculture Industry
10:00 AM		**Llano - Parasitic Survey on Captive, Wild and Reintroduced Sirenians (*Thrichechus inunguis* and *Trichechus manatus*) in Brazil**
10:30 AM		**Refreshments**
10:45 AM	WAVMA	Scarfe - Ensuring a Well-trained Aquatic Veterinary Workforce: Past, Present and Future Initiatives
11:15 AM		Miller-Morgan - Defining the Practice of Aquatic Veterinary Medicine – A Unique Approach for Establishing Day-1 Competencies
11:45 AM		Panel / Audience Discussion - Optimal Approaches for Ensuring a Well-Educated Aquatic Veterinary Workforce – Meeting the Needs of the Profession & Clients Served
12:15 PM		**Lunch**

The University of Arizona, Aquaculture Pathology Laboratory: A Worldwide Resource for Diagnostic Services and Collaborative Research to the Shrimp Aquaculture Industry

Arun K. Dhar*, Brenda L. Noble, Fernando Aranguren Caro, Michelle Garfias, Jasmine D. Millabas, Kevin M. Gee, L. Siddhartha Kanrar, Hung N. Mai, Roberto C. Flores, Paul J. Schofield, and Tanner J. Padilla.

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Aquaculture Pathology Laboratory (APL) in The University of Arizona, Tucson, Arizona is an OIE-Reference Laboratory of Crustacean Diseases. The laboratory is also an USDA Reference Laboratory of Crustacean Diseases and an ISO 17025 accredited laboratory. The APL has two laboratories under its operation. The disease diagnostic laboratory is located in the main campus of The University of Arizona and the wet lab is located in the West Campus Agricultural Center (APL-WCAC) of the university. APL's missions are: (a) provide disease diagnostic services to shrimp industry, (b) provide educational and training services to researchers and professionals from public and private institutions, non-governmental organizations, (c) conduct inter- laboratory calibration test, also called proficiency test or ring test, (d) carry out shrimp disease challenge studies, testing therapeutics, feed & feed additives, and (e) carry our basic research in shrimp virology, microbiology & genomics. Several major diseases that have impacted and continue to impact shrimp farming worldwide were discovered in APL, and method developed for experimental challenge and their detection by histopathology and molecular tools. In 2017 alone, APL has provided disease diagnostic services to clients from industry and academia from 36 countries around the world. As of 2018, APL has conducted 28 trainings in shrimp disease diagnostics through a summer course called "Shrimp Pathology Short Course" that has trained almost 1500 researchers and professionals engaged in shrimp diseases around the world. The "Shrimp Pathology Short Course" provides a unique platform for researchers from around the world to interact and foster collaborations. In addition, APL has conducted numerous trainings in shrimp disease diagnostics in countries in Latin America and Asia. The APL-WCAC research facility is conveniently located over 200 miles from the nearest ocean which has proven to be the ideal place to study shrimp and other crustacean pathogens as well as other shellfish and fish pathogens without the risk of introduction into their natural habitat. Since its inception 28 years ago, the APL-WCAC lab has helped to develop pathogen resistant stocks, develop experimental challenge methods for multiple shrimp pathogens which aid in the selection of genetically superior and disease resistant stocks, tested numerous products against viral and bacterial pathogens, run primary quarantine to aid in the diversity of the gene pool in shrimp captive breeding programs, and studied new and emerging pathogens in live animals. The presentation will provide an overview of accomplishments and a role this iconic laboratory has played over the past four decades in making shrimp industry sustainable worldwide.

Conference Session Designation: (World Aquatic Veterinary Medical Association)
Presentation Format: (Oral)

Parasitic Survey on Captive, Wild and Reintroduced Sirenians of the Species *Trichechus inunguis* and *Trichechus manatus* in Brazil: Preliminary Results

Horwald A. B. Llano[1], Daniella C. R. Oliveira[1*], Gláucia P. Sousa[1,2], Heloíse Z. Polato[3], Lilia R. A. Ferreira[4], Aline R. Souza[5], Stella M. Lazzarini[5], Iran C. Normande[2], Fernanda L. N. Attademo[2], Fábia O. Luna[2], Lara B. Keid[3], Rodrigo M. Soares[1]

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Sirenians, as *Trichechus inunguis* and *Trichechus manatus*, are aquatic mammals vulnerable to extinction for which better knowledge regarding the health condition of their population is urgent for conservation purposes. Both species figure on National Action Plans for conservation that relies, among other measures, on the rehabilitation and reintroduction of specimens into the wild. Prior to the release of rehabilitated manatees into the wild, the health assessment is necessary and is based only on serological tests for *Brucella*, *Toxoplasma* and *Leptospira* infection, as well as on coproparasitological analysis, but proper characterization of the parasites usually is not conducted. In view of the gap on parasitic information regarding these species, the goal of this study is to survey parasites from fecal samples of Brazilian manatees in order to perform future molecular characterization of the findings to apply the information on these species management. Fresh fecal samples were collected in 2016 and 2017 from 29 captive *Trichechus inunguis* from Aquatic Mammals Preservation and Research Center, Eletrobras Amazonas Energia, Amazonas State, Brazil and from three *Trichechus manatus manatus* (two natives, and one reintroduced) from the area of environmental protection of Costa dos Corais, Alagoas State, Brazil by *Instituto Chico Mendes para Conservação da Biodiversidade*. The samples were stored at 4° C in 2.5% potassium dichromate solution (1:2). The centrifugal flotation method using Sheather's sugar solution and the formalin-ether sedimentation technique were conducted on the 32 stool samples followed by direct examination. Eggs of helminths were observed in 6 samples (19%) and protozoa cysts/oocysts in 29 samples (91%). Ten samples (31%) had protozoa cysts/oocysts and helminths concomitantly. Among the protozoa positive samples, 26 had *Eimeria* sp. oocysts (80%) and six had *Giardia* sp cysts (19%).

Within the *Giardia* sp. positive samples, all also had *Eimeria* sp. oocysts, and only one had helminths eggs concomitantly (3%). All *T. m. manatus* had coccidian oocysts; one reintroduced and one native specimens had *Eimeria* sp., and a native one was also positive to *Giardia* sp. Five *T. inunguis* had helminth eggs (16%), and five of them had Cyathostominae infective larvae (16%). This constitutes the first report of Strongylida in manatees. The preliminary results presented here reinforce the urgent need of long-term parasitic surveys on wild, captive and reintroduced manatees. This study will be further enriched by molecular characterization of parasites in order to assess the involvement of potential zoonotic agents and to better know the parasitic fauna that infect the studied hosts. Although the impact of our results on the conservation strategies and management in captivity of manatees is still unknown, this study will help to assess the risk of releasing the animals back to their natural habitat as well as to orient sirenians´ management actions.

Conference Session Designation: (Aquatic Mammals / WAVMA)
Presentation Format: (Oral)
Student Presentation: (Yes)

Ensuring a Well-trained Aquatic Veterinary Workforce: Past, Present and Future

A. David Scarfe*[1,2], Timothy Miller-Morgan[3], Dušan Palić[4], Howard K. H. Wong[5], Gillian D. Taylor[1], Aimee Reed[6] & Sophie St. Hilaire[5]

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With increasing impacts of diseases on aquaculture production in all countries, the need for a well-trained aquatic veterinary workforce (including veterinarians and para-veterinarians) has become a global imperative. Numerous educational efforts are underway ensure that sufficient numbers are available to support aquaculture industries, producers, governmental agencies and a myriad of supporting industries that provide services or products to prevent, control or eradicate diseases. Without this infrastructure, sustainable and economically viable aquaculture will simply not thrive. A number of International and National veterinary organizations have, or are developing processes to determine, evaluate, harmonize and accredit veterinary education throughout the world, the educational needs within veterinary degree-earning curricular, and extracurricular continuing education and professional development (CEPD) programs, to ensure an adequate veterinary workforce to meet contemporary and societal needs. These include, but are not limited to, the World Veterinary Association (WVA), the Council on International Veterinary Medical Education, the World Organization for Animal Health (OIE), the North American Veterinary Medical Education Consortium (NAVMEC), the Federation of Veterinarians of Europe (FVE). Although organizations all address aquatic veterinary education to some degree, two are focusing on ensuring aquatic are addressed in veterinary curricular and CEPD programs – the World Aquatic Veterinary Medical Association (WAVMA), and the International Partnership on Aquatic Veterinary Education (i-PAVE). The WAVMA Aquatic Veterinary Certification (CertAqV) Program used to certify veterinarians who have Day-1 competency to practice aquatic veterinary medicine (equivalent to competencies required of individual receiving a veterinary degree), has identified 9 core subjects. To build on WAVMA and other veterinary organization's efforts, i-PAVE has embarked on a multi-year project to verify and validate that the aquatic veterinary knowledge, skills and experience (KSEs) identified by WAVMA, actually fulfill the needs of veterinarians actively practicing aquatic veterinary medicine in any country, using a DACUM (**D**eveloping **A** **C**urricul**UM**) process in workshops. These workshops in N. and S. America, Europe, Africa, and the Asia-Pacific are then followed by a survey of every veterinary school in each region, to determine which of the core aquatic veterinary subjects are actually covered in veterinary school curricula, or veterinary CEPD programs.

Conference Session requested: (WAVMA)
Presentation Format: **(** Oral)

Defining the Practice of Aquatic Veterinary Medicine – A Unique Approach for Establishing Day-1 Competencies

Timothy Miller-Morgan*[,1], A. David Scarfe[2,3], Dušan Palić[4], Howard K. H. Wong[5], Gillian D. Taylor[2], Aimee Reed[6] & Sophie St. Hilaire[5]

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A model curriculum has yet to be developed for the field of aquatic veterinary medicine we have utilized a unique approach to veterinary curricular development that seeks input from practicing full-time aquatic veterinarians identify the key Day-1 knowledge, skills and experiences that would be expected of new graduates entering the field of aquatic veterinary medicine. We have utilized a process called DACUM (Developing a Curriculum), a process that has proven time and again to be very effective, relatively quick, and a low-cost approach to accurately developing occupational standards for any job or occupational area. Because of its low cost and effectiveness, it has been and continues to be used by educators and trainers in over 40 countries (Adams, et al, 2015).

The DACUM process for job/occupational analysis involves a panel of 5-12 expert workers – the men and women with reputations for being "the best" at their jobs. Whether at the skilled, technical, supervisory, or professional level, these workers explain exactly what they do that allows them to be successful. We involved a committee of 6 aquatic veterinarians who are actively engaged in private aquatic veterinary medicine practice. They participated in a 3-day workshop during which they completed an occupational analysis that identified key General Areas of Competence (GAC), and the essential competencies (essential knowledge and skills) within each GAC, necessary to practice competent aquatic veterinary medicine (Adams, et al, 2015). The resulting occupational analysis identified 18 General Areas of Competence and 189 individual competencies essential for the Day-1 practitioner of aquatic veterinary medicine.

Finally, small groups of 3-5 aquatic veterinary practitioners from different global regions will be used to validate the findings in a series of online or face-to-face validation workshops, relative to the needs for aquatic veterinary education in the Americas, Europe, Africa and the Asia-Pacific. Their job will be to ensure that significant GAC's have not been omitted and to weight each competency in relation to its GAC. The resulting occupational analysis will serve as the basis evaluating whether existing veterinary curricula adequately cover sufficient information to prepare a veterinarian to practice aquatic veterinary medicine.

Conference Session Designation: (WAVMA)
Presentation Format: (Oral)

Tuesday September 4th – Tilly / Tupper
World Aquatic Veterinary Medical Association
Moderator – A. David Scarfe (University of Pretoria)

1:15 PM	WAVMA	**Parker-Graham - Pharmacokinetics of a Single Dose of Danofloxacin Administered Intramuscularly in Koi *Cyprinus carpio***
1:45 PM		Pulver - Past, Present, and Future Perspectives on Fish Drug Development
2:15 PM		**Parker-Graham - Effect of Anesthetic Time and Concentration on Blood Gasses, Acid-Base Status and Electrolytes in Koi (*Cyprinus carpio*) Anesthetized with Buffered Tricaine Methanosulfonate (MS-222)**
2:45 PM		Refreshments
3:15 PM	WAVMA	**Parker-Graham - Treatment of Severe Fishing Line Entanglement Injuries in a Free-Ranging Canada Goose, *Branta canadensis***
3:45 PM		Hickey - Size of Release? Time of release? What About Health Status at Release?
4:15 PM		Spark - Sector Specific Biosecurity Plans: Development and Implementation
4:45 PM		Nietlisbach - Tilapia Health on Wisconsin's Aquaponics Farms

Pharmacokinetics of a Single Dose of Danofloxacin Administered Intramuscularly in Koi (*Cyprinus Carpio*)

Christine A. Parker-Graham[1]*, Wesley C. Siniard[2], Matthew F. Sheley[3], Barbara A. Byrne[4], Heather K. Knych[5], and Esteban Soto[1]

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Despite the frequency of antimicrobial use in pet fish there remains a paucity of pharmacokinetic studies critically evaluating commonly-employed drugs. Danofloxacin, a synthetic fluoroquinolone antimicrobial, is frequently employed as a first line treatment against infectious skin disease and septicemia in fish. For this study we evaluated culture and minimum inhibitory concentration (MIC) data (n=204) from ornamental fish presented to the Veterinary Medical Teaching Hospital at UC Davis and found that Aeromonas spp, Vibrio spp., and Pseudomonas spp. were the most commonly isolated pathogens from skin and posterior kidney. The MIC90 of danofloxacin for these pathogens was 1 µg/ml, which was equivalent or lower than the MIC90 of oxytetracycline, ceftiofur, and enrofloxacin on the same sensitivity panels.

A single 10 mg/kg dose of danofloxacin (Advocin, 180 mg/ml injectable solution, Zoetis Laboratories, Parsippany, New Jersey, USA) was administered to adult koi intramuscularly (IM). Fish were sampled at each time point: 0.25, 0.5, 0.75, 1, 4, 12, 24, 72, 96, 120, and 144 hours post-injection. Whole blood was drawn antemortem and heparinized plasma was obtained from these samples; fish were euthanized and samples of liver, spleen, gill, anterior kidney, posterior kidney, skin and muscle, and scales were collected. Plasma and tissue concentrations were determined by a validated liquid chromatography- mass spectrometry method and non-compartmental pharmacokinetic analysis was performed for plasma. Subsamples of examined tissues of each fish sampled at 144 hours were evaluated via histopathology.

Peak plasma danofloxacin concentration of 8.31 µg/ml was reached 0.75 hours post-injection. The plasma elimination half-life was 15 hours and danofloxacin was detected in some tissues for at least 144 hours. Danofloxacin sustained concentrations greater than 1 µg/ml in all examined tissues except scales for at least 96 hours post-injection. Histopathology at 144 hours was unremarkable. This study shows that danofloxacin at a dose of 10 mg/kg administered IM reaches therapeutic concentrations rapidly in plasma and tissues and maintains concentrations greater than the observed MIC90 for commonly isolated aquatic bacterial pathogens for at least 96 hours in koi housed at 18°C.

Conference session designation: (American Association of Fish Veterinarians)
Presentation format: (Oral)
Student presentation: (Yes)

Past, Present, and Future Perspectives on Fish Drug Development

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The Food and Drugs Administration (FDA) is the government agency responsible for approving aquaculture drugs in the United States. The first drug application specifically for fish was approved in 1967 (sulfamerazine). Over fifty years later, the current products approved for fish include only nine active ingredients. There are 15 different products approved and marketed for 26 different indications. The number of drugs approved for fish is significantly lower than the number approved for the major animal species. This is largely due to the relatively small market for aquaculture drugs in the U.S. and the corresponding lack of financial incentive for companies to invest in aquaculture products. Steps were taken to address the issue of incentives with the passage of the Minor Use and Minor Species Animal Health Act of 2004; however, some challenges remain.

CVM is supportive of drug development for minor species, including fish. For example, CVM has conducted studies to support drug approval projects, provides a FDA liaison to The Minor Use Animal Drug Program (formerly USDA National Research Support Project #7), and has utilized alternative approaches to meeting the drug approval requirements. CVM also supports the aquaculture industries by meeting regularly with public partners and impacted groups, continuing to attend and present at meetings where fish drug development is discussed, and conducting outreach to better understand regulations and policies.

While there has been significant progress over the past fifteen years in making more drugs legally available for aquaculture, there is still a need to complete drug approval projects to provide for additional approved drugs and indications, and to identify where else drugs are needed. For example, only one drug compound (formalin) is approved to treat parasitic diseases, yet parasite infestations are a major cause of fish disease. The development of marine aquaculture in the U.S. will require new approved drugs for the treatment of sea lice and other diseases of marine fish. Looking forward, drug development efforts will face additional challenges in responding to industry transformations (e.g. new species, the use of recirculating aquaculture systems), emerging diseases, and the need for new antimicrobial products.

Opportunities are abundant for pharmaceutical companies, researchers, veterinarians, and hatcheries to contribute to the development of new animal drugs to meet these needs. Aquaculture drug development is challenging for a myriad of reasons; however, CVM continues to encourage the industry to communicate with us to find solutions to protect human and animal health.

Conference Session Designation: (American Association of Fish Veterinarians)
Presentation Format: (Oral)

Effect of Anesthetic Time and Concentration on Blood Gases, Acid-Base Status, and Electrolytes in Koi (*Cyprinus Carpio*) Anesthetized with Buffered Tricaine Methanesulfonate (MS-222)

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Anesthesia is commonly employed in aquatic medicine to reduce the stress of handling on fish and to facilitate physical exams, diagnostics, and surgical interventions. Tricaine methanesulfonate (MS-222) is the most commonly used anesthetic for fish and is currently the only anesthetic approved by the United States Food and Drug Administration, Center for Veterinary Medicine (FDA-CVM) for food-producing fish. Despite the frequency of anesthetic procedures in fish, anesthetic monitoring remains rudimentary in many facilities. This study evaluated the impact on blood gases, acid-base balance, and electrolytes in koi (*Cyprinus carpio*) anesthetized with two different concentrations of buffered MS-222: 100 mg/L and 150 mg/L. Blood samples from 25 fish per anesthetic treatment group were collected after five and twenty minutes of anesthetic immersion and analyzed on the pHOx Ultra table-top blood gas analyzer (Nova Biomedical, Waltham, Massachusetts 02454-9141, USA).

All but one fish from the 150 mg/L group recovered uneventfully from anesthesia and all koi were sufficiently anesthetized to facilitate handling and venipuncture. Results showed significant increases in pCO2 ($p=0.006$) and hyperglycemia ($p=<0.0001$) with both increasing anesthetic concentration and increasing time under anesthesia. There was a significant decrease in pO2 with increased anesthetic time ($p=0.021$), independent of anesthetic concentration. There were several electrolyte changes observed as well with both increasing anesthetic time and concentration. Despite the changes all electrolytes except potassium remained within published reference ranges for koi; potassium showed a significant decrease in concentration associated with increasing anesthetic time and increasing anesthetic concentration. Plasma lactate concentrations were not significantly different across the study groups, suggesting that koi maintained adequate perfusion throughout the duration of the study period. The results of this study indicate that buffered MS- 222 at 100 mg/L and 150 mg/L are safe anesthetic concentrations for koi undergoing minimally- invasive diagnostics; however, koi anesthetized with MS-222 at a concentration of 150 mg/L experienced more significant changes in blood gases, acid-base balance, and electrolyte concentrations, including hypercapnia, hypoxemia, and hyperglycemia and may require more careful monitoring to avoid physiologic imbalances while under anesthesia.

Conference session designation: (WAVMA)
Presentation format: **(** Oral)
Student presentation: **(** Yes)

Treatment of Severe Fishing Line Entanglement Injuries in a Free-Ranging Canada Goose (*Branta Canadensis*)

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Injuries caused by discarded fishing gear are commonly encountered in wildlife medicine; monofilament lines, discarded nets, lead sinkers, and hooks are pervasive environmental threats to aquatic birds. Cellulitis, ischemia, osteomyelitis, and loss of limb function are common sequelae to fishing line entanglement injuries. This case describes a Canada goose (*Branta canadensis*) that was presented for fishing line entanglement; several feet of monofilament fishing line were tangled around the bird's feet and legs, causing severe constrictive injuries to both pelvic limbs. On physical exam deep soft tissue wounds with purulent discharge and exposure of the tarsometatarsi were appreciated bilaterally. Both feet appeared to have deep pain sensation but the goose demonstrated profound proprioceptive deficits in both feet and was ventrally recumbent. Radiographs were notable for cortical bone erosion on the lateral aspects of both tarsometatarsi and severe soft tissue swelling proximal and distal to the constrictive lesions.

Supportive care was initiated including subcutaneous fluids with B complex vitamins, meloxicam, tramadol, and ceftiofur. Surgical debridement of the wounds revealed that the combined flexor and extensor tendons were intact; the wounds were dressed with topical Manuka honey and any constrictive scabs that formed in the wound bed were debrided for the next week. The legs were treated globally with cold laser therapy. After a week of intensive wound care the soft tissue trauma was healing well but the goose continued to be ventrally recumbent and had profound proprioceptive deficits in both feet. A pair of orthopedic splints were created for the goose to hold the feet in a normal digitigrade stance and physical therapy was initiated, including walking the goose with the orthopedic boots in the place and swimming the goose daily. After three days of exercise with the boots in place the goose began to place the right foot normally. Nineteen days after intake the goose had sufficient proprioceptive placement of both feet and was able to walk normally without the corrective splints and was discharged to a wildlife rehabilitator for pre-release conditioning. The goose was released back to the wild with apparently normal proprioception and musculoskeletal function of both legs twenty-nine days after initial intake. This case highlights a constellation of possibilities for treatment of fishing line entanglement wounds that are low in cost and readily accessible to practitioners.

Conference session designation: **(** World Aquatic Veterinary Medical Association)
Presentation format: **(** Oral)
Student presentation: **(** Yes)

Number Released? Size at Release? Time of Release? What About Health Status At Release?

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For many fish hatchery enhancement programs, there are three major metrics to measure production success: number released, size at release, and time of release. One metric that is often overlooked and undervalued is fish health status at release. Pre-release examinations should always include review of mortality records and inspection of all containers of fish to be released. Other aspects of a pre-release examination may include necropsy and disease surveillance testing, including testing lots of fish to determine pathogen prevalence.

Even when pre-release health checks are incorporated into a program, they may be viewed as an impediment as opposed to an important indicator of the overall viability and performance of an enhancement program. First and foremost, pre-release health checks protect both hatchery fish and wild fish from serious diseases. Second, hatchery programs that release unhealthy fish or fish experiencing an ongoing epizootic is a practice that may have significant, and often massively underestimated, negative impacts on an enhancement program. Finally, repeated fish health problems apparent at a pre-release fish health examination may suggest that other metrics of success--number released, size at release, and time of release--are unreasonable expectations for that hatchery program. For example, fish that are experiencing mortality from furunculosis secondary to the stress of being held past smolting might benefit from being released earlier; or for fish experiencing a bacterial coldwater disease outbreak due to high densities, reduction in number of fish produced at a facility may result in improved health and ultimately release numbers.

Just performing pre-release examinations is not enough. It is critical for fish veterinarians to get involved in management meetings where the other metrics of success--number released, size at release, and time of release--are decided, because they have critical information to help inform the decision making process.

Conference Session Designation: (AAFV / WAVMA)
Presentation Format: (Oral)

Sector Specific Biosecurity Plans: Development and Implementation

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Worldwide, there is increasing risk of significant known and unknown aquatic animal diseases emerging and spreading. Although Australia has a relatively favorable aquatic animal health status, two of Australia's highest priority aquatic diseases, abalone viral ganglioneuritis (AVG) and Pacific Oyster Mortality Syndrome (POMS), have caused substantial economic impacts in Australia's seafood industries and now present trade barriers for movement of livestock. Despite the abalone and oyster industries and relevant jurisdictions have implemented a range of measures to mitigate these significant disease risks, both industry sectors were lacking a nationally consistent, agreed approach to auditable biosecurity to facilitate trade.

The Commonwealth Department of Agriculture and Water Resources (DAWR) and the Fisheries Research and Development Corporation (FRDC) consequently jointly funded a national project to deliver sector-specific biosecurity plans (templates and guidance documents) for the abalone and oyster industries. These will become an essential component of health accreditation programs and import protocols to facilitate safe trade in aquatic animals.

The effective implementation and operation of good on-farm biosecurity provides improved business security through reducing risks to production additional to enhanced market access. Unnecessary costs and production losses can be avoided by good biosecurity especially when disease can be prevented from entering the farm. The ultimate aim is a more profitable, secure and resilient aquaculture industry.

Biosecurity plans describe the systems necessary to protect a farm from diseases. The development of farm biosecurity plans involves the identification of relevant risks (or routes of disease introduction and spread); implementation of appropriate risk mitigation measures; and the development and maintenance of supporting documentation. Although every farm is unique, there are common risk pathways (e.g. animals, people, equipment, water, feed and waste) and effective risk mitigation measures are shared by farms within a sector. These common risks and mitigation measures are the basis on which these plans have been developed in consultation with industry.

This project was completed in 2017 and as a result all South Australian land based abalone farms have audited biosecurity plans in place as of early 2018 with significant progress being made with oyster hatcheries.

Conference Session Designation: **(** Aquatic Animal Health Management)
Presentation Format: **(** Oral)

Tilapia Health *Oreochromis niloticus* on Wisconsin's Aquaponics Farms

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Aquaponics is a sustainable method of farming that merges recirculating aquaculture systems and hydroponics by feeding plants with nitrogenous waste products produced by fish. Its purported attributes include significantly less water usage, highly efficient space utilization, and reduced pollutant-rich effluent compared to traditional aquaculture and agriculture systems. Aquaponics also allows local produce to be grown year-round, even in harsh climates.

Aquaponics has become increasingly popular across the country in recent years, and especially in Wisconsin. Despite the number of aquaponics farms in the state, very little is known about the health status of fish in these systems. This is possibly due to the fact that, despite being integral to the system, fish only account for about 10% the profits. The goal of this project was to investigate disease issues present on Wisconsin aquaponics farms, specifically focusing on Nile Tilapia (*Oreochromis niloticus)*. Tilapia are hardy, productive, and are often mistakenly billed as a "disease-resistant fish," making them the most popular choice for new aquaponics farmers in Wisconsin.

We found that aquaponics farms do indeed struggle with fish health problems. We found *Trichodina sp.* and monogenean trematodes (*Gyrodactylus sp.* and *Dactylogyrus sp.)* on every farm we visited. Some farms had particularly high numbers of external parasites, yet few fish exhibited clinical signs of infestation. But, these parasites could easily become a health problem if the fish experience a stressful and immunosuppressive event. Some farms also experienced bacterial infection outbreaks. Piscine francisellosis (*Francisella noatunensis*), one of the major emerging bacterial diseases of cultured tilapia, was diagnosed on one farm. We also investigated a severe, acute mortality event, which was diagnosed as a particularly virulent strain of *Aeromonas veronii,* serovar *sobria.*

A better understanding of the health status of fish on aquaponics farms needs to be studied further. Doing so will give the veterinary community a better idea of how we can be of service to the growing aquaponics industry.

Conference Session Designation: (WAVMA)
Presentation Format: (Oral

Keynote Presentation

Wednesday Morning September 5rd

Weapons of Micro-Destruction: An Interdisciplinary Approach to Understanding a Parasitic Cnidarian

Jerri L. Bartholomew

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Myxozoa are now understood to be an ancient, diverse group of endoparasitic Cnidaria. On their route to parasitism, myxozoans have become morphologically and genetically simple, losing many traits of their free-living relatives. While most cnidarians have life cycles that alternate between a medusa and a polyp, myxozoans alternate between two different spore stages (e.g. myxospore and actinospore) that develop in two different hosts (e.g. fish and aquatic annelid. Myxozoans have retained the phylum-defining stinging organelles known as cnidae or polar capsules, but we are only just beginning to understand what features have been lost or modified to suit their parasitic needs.

Free-living Cnidaria like corals, jellyfish and anemones, use their stinging capsules for prey capture or defense, but in Myxozoa these capsules have the essential function of initiating the host infection process. To better understand the evolutionary relationships between myxozoan polar capsules and cnidae in free-living species, we examined the structure and firing behavior of capsules in 4 myxozoans, including *Myxobolus cerebralis*, the causative agent of whirling disease in trout, and *Ceratonova shasta*, the cause of enteronecrosis in salmonids. Electron microscopy and high speed video analyses showed that the three *Myxobolus* species had highly elastic polar tubules, which is a property unknown in free-living cnidarians. By dye-labeling the polar capsules prior to firing, we discovered that two of the species could release their entire capsule content, which suggested that cytotoxic or proteolytic compounds may be present in the capsule, and are injected into the host to facilitate infection. Moreover, while free-living cnidarians inject toxins through the tip of the tubule, we identified pores along parts of tubules of *Myxobolus* species, and showed that the tip of the tubules was sealed. In contrast, *C. shasta* and *M. cerebralis* tubules had no openings at all and no apparent delivery of capsule content, thus are likely used simply to anchor the spore to the host without any toxin injection.

To examine functions of polar capsules at a proteomic level, we isolated *C. shasta* capsules using a dielectrophoresis-based microfluidic chip platform. In comparison to jellyfish, sea anemone and *Hydra*, which share 49 protein domains, only 27 domains were shared between these Cnidaria and *C. shasta*. While *C. shasta* capsules have retained typical structural and housekeeping proteins found in free-living Cnidaria, they have lost toxin-like proteins, which supports the structural evidence of the video analysis. Our findings indicate that although polar capsules and nematocysts are homologous organelles, myxozoan capsules have more functions

than previously assumed. Understanding these mechanisms may provide a means to prevent myxozoan infection to fish by interfering with the initial stages of the infection process.

The ultimate function of myxozoan cnidae is to sense and respond to proximity with the host, then fire the tubule within a fraction of a second, to anchor the spore to the host. This process initiates the infection process, but must be followed by invasion of the parasite sporoplasm into the host tissues, which requires motility. We used *C. shasta* as a model to study motility because we have a reference transcriptome and different parasite genotypes with varying degrees of virulence. Developmental stages of *C. shasta* showed a variety of locomotion mechanisms, using amoeboid movement, filopodia, lamellipodia and blebbing to migrate between host cells. We mined the *C. shasta* reference transcriptome for motility genes that may serve as virulence factors. Here we looked at genes that may be involved in the actomyosin machinery of the cell, in cell adhesion, and in cell motility regulation. The more virulent genotype had increased levels of adhesion factors that connect the parasite cytoskeleton with the host extracellular matrix, which suggests that these components are virulence factors of the parasite. In addition, examination of the transcriptome for proteases shows that proteases were expressed in the developmental stages and these may also function as virulence factors, facilitating parasite invasion.

Our changing vision of what myxozoans are has been informed by engaging researchers in other scientific fields, but we can also learn something by stepping outside our scientific silos to gain perspectives and inspiration from other disciplines. To explore how the humanities contributes, or enhances, our understanding and appreciation of our science, I will weave in my own artistic interpretations as well as collaborations with poets, artists and musicians.

Wednesday September 5th – Gray / Palmer / Pope Ballroom
Myxozoa 1 & 2
Moderators - Jerri Bartholomew, Stephen Atkinson (Oregon State University)

9:30 AM	Myxozoa 1	**Stillwell - Myxozoan Parasites of the Western Mosquitofish, *Gambusia affinis***
9:45 AM		**Naldoni - Ultrastructure, Histology and Phylogeny of *Henneguya* spp. Parasites of *Pseudoplatystoma* spp. Taken from the Brazilian Amazon**
10:00 AM		Kaur - Evidence of Species Complexes in the Genus *Thelohanellus* (Cnidaria: Myxosporea) Infecting Cyprinid Carps from the Indian Subcontinent
10:15 AM		Freeman - Studies of Systemic *Myxidium giardi* Infections in Icelandic Eels Identifies an Overlooked Clade of Myxosporeans
10:30 AM		**Refreshments**
10:45 AM	Myxozoa 2	Picard-Sánchez - Immune Players in Acquired Protection to *Enteromyxum leei* (Myxozoa) in Gilthead Sea Bream, *Sparus aurata* (Teleostei: Perciformes)
11:00 AM		Saleh - Kinetics of Local and Systemic Immune Cell Responses in Whirling Disease Infection and Resistance in Rainbow Trout
11:15 AM		Kumar - Identification of Differentially Expressed Kidney Genes During Proliferative Kidney Disease and *In Vivo* Induced Genes of *Tetracapsuloides bryosalmonae* (Myxozoa) in Brown Trout
11:30 AM		Gu - Nematocysts Arise Autogenously and Dominate the Cnidarian Adaptation Via Massive Decentralized Modification
11:45 AM		Atkinson - Incapacitated by Salt? *In Vitro* Discharge Tests of Myxozoan Parasite Infection Mechanisms

Myxozoan Parasites of the Western Mosquitofish, *Gambusia affinis*

Justin M. Stilwell[1*], Thomas G. Rosser[2], John Leary[1], Charles C. Mischke[3], Alvin C. Camus[1], Matt Griffin[2,3]

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The objective of this study was to provide updated histologic and molecular characterization of infections by the myxozoan parasites *Henneguya gambusi* and *Myxobolus pharyngeus* in western mosquitofish from experimental channel catfish, *Ictalurus punctatus*, ponds in the Mississippi Delta. Three hundred and fifty-one fish were processed routinely, stained with hematoxylin and eosin, and examined by light microscopy for the presence of myxozoans. PCR was performed on DNA extracted from fresh, formalin-fixed, and/or ethanol-fixed, paraffin-embedded tissues to facilitate sequencing of the 18S ribosomal RNA gene of each myxozoan. Myxozoal stages were isolated from formalin and ethanol fixed paraffin embedded tissue sections using laser capture microdissection (LCM). Histologic examination revealed scale pockets throughout the dermis distended by plasmodia of *H. gambusi* that contained various developmental stages of the parasite. Plasmodia of *M. pharyngeus* were embedded within the epithelium lining the branchial cavity. Three additional myxozoans were identified including a coelozoic species within the gall bladder, a *Myxobilatus*-type myxozoan in the renal tubules, and a *Myxobolus*-type myxozoan within cartilage of the head and multiple fins. Pathologic changes caused by the various parasites were minimal, with the exception of the *Myxobolus*-type, which caused cartilage lysis and necrosis. Sequencing of the 18S rRNA gene revealed that *H. gambusi* and the cartilage *Myxobolus*-type were less than 90% similar at the nucleotide level to any other myxozoan. The nucleotide sequence from the renal *Myxobilatus*-type was 100% similar to a triactinomyxon-type actinospore isolated from the oligochaete worm *Dero digitata*, which molecularly confirms the life cycle for this novel *Myxobilatus* species. At present, the complete life cycle of only one other *Myxobilatus* sp., *M. gasterostei*, reported from the ureters of the three-spine stickleback is known. This species also utilizes an oligochaete (*Nais communis)* as its definitive host. In all, we report the histological and molecular characterization of five different myxozoan parasites in the western mosquitofish and the life cycle of a novel *Myxobilatus* sp.

Conference Session Designation: (Parasitology Myxozoa)
Presentation Format: (Oral)
Student Presentation: (Yes

Ultrastructure, Histology and Phylogeny Of *Henneguya* Spp. Parasite of *Pseudoplatystoma* Spp. Taken From the Brazilian Amazon

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The Amazon basin drains about 6.5 million km^2 and is a complex ecosystem consisting of highly diverse environments, providing a favorable habitat for exceptional fish biodiversity, with approximately 5000 fish species. Many of these fish species are economically important for commercial, sport fishing and aquarism, and other species have fish farming potential. Among the fish that are economically important for extractive fishing and farming are species of the Pimelodidae family. The present study provides ultrastructural, histological and phylogenetic analyses of *Henneguya* spp., parasites of *Pseudoplatystoma tigrinum,* popularly known as "caparari" (English name - Tiger surubim) and *Pseudoplatystoma punctifer,* popularly known as "surubim" (English name - Spotted tiger shovelnose catfish), caught in the Tapajós river, Pará state, Brazilian Amazon. These fish species were parasitised by distinct *Henneguya* spp. and the histological analysis revealed that the plasmodia of both *Henneguya* species developed in the sub-epithelial connective tissue of the gill filaments. Ultrastructural analysis showed that the plasmodial wall had some pinocytotic canals connecting the outside of the plasmodia to the ectoplasm zone and delicate projections towards the host tissue. A layer of fibrous material was found in the periphery of the plasmodia. Some mitochondrias were observed in the ectoplasm, while generative cells and several developmental stages of sporogenesis were seen in the inner layers. Both species had the plasmodial wall surrounded by a fibroblast layer. Molecular analysis based on ssrDNA from the spores of *Henneguya* sp. parasite of *P. tigrinum* resulted in a 1936 bp sequence that did not match any myxosporean species sequences available in GenBank, and the blast analysis showed *H. maculosus* Carriero et al. 2013, as the closest relative, with 93% of similarity. The sequencing of the ssrDNA from the spores of *Henneguya* sp. parasite of *P. punctifer* resulted in a 1922 bp sequence that showed 99% of similarity to *H. eirase* Naldoni et al. 2011, and were here considered co-specifics. In the phylogenetic analysis, using only myxosporean species parasites of siluriforms, *Henneguya* spp. focus of this study clustered in a sub-clade composed by other *Henneguya* species parasites of gills of South American pimelodid fish. This is the first report of myxozoans infecting *P. punctifer* and *P. tigrinum.*

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Conference Session Designation: (Parasitology Myxozoa)
Presentation Format: (Oral)
Student Presentation: (Yes)

Evidence of Species Complexes in the Genus Thelohanellus (Cnidaria: Myxosporea) Infecting Cyprinid Carps from Indian Subcontinent

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Out of the total 2600 myxozoan species reported so far, Thelohanellus Kudo, 1933 is the second most prevalent genus comprising of about 150 species after the genus Myxobolus Butschli, 1882 infecting both freshwater and marine fishes. In general, these are mostly histozoic (within the tissues) and sometimes coelozoic (in body cavities). The present paper deals with the study of species complex among the member species of Thelohanellus genus from the Indian subcontinent infecting gills, fins and muscles of cyprinid carps. 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.

Conference Session Designation: (Myxozoa)
Presentation Format: (Oral)

Studies of Systemic *Myxidium giardi* Infections in Icelandic Eels Identifies an Overlooked Clade of Myxosporeans

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The myxosporean *Myxidium giardi* was described in 1906 infecting the kidney of the European eel, having spindle-shaped myxospores and terminal sub-spherical polar capsules. Since then, numerous anguillid eels globally have been documented to have similar *Myxidium* infections. Many of these have been identified using the morphological features of myxospores or by the location of infection in the host, and some have been subsequently synonymized with *M. giardi*. Therefore, it is not clear whether *M. giardi* is a widely distributed parasite, infecting numerous species of eels, in multiple organs, or whether some infections represent other, morphologically similar but different species of myxosporeans. The aim of the present study was to assess the status of *M. giardi*-like infections in Icelandic eels, and identify any similar myxosporeans infecting the related Pacific tarpon, *Megalops cyprinoides*, from Southeast Asia. Myxosporeans were identified using spore morphology and molecular techniques in order to evaluate the diversity present.

The morphological measurements of the myxospores from Icelandic eels was not significantly different between sites of infection in the host fish, but the spores from the Pacific tarpon were noticeably smaller. However, the SSU rDNA sequences from the different tissues locations in eels, were all very distinct, with percentage similarities ranging from 92.93% to as low as 89.8%, with the sequence from tarpon being even more dissimilar. Molecular phylogenies consistently placed these sequences together in a clade that is strongly associated with the *Myxidium* clade *sensu stricto*.

Our results demonstrate that there is not a single species of *Myxidium* causing systemic infections in eels from Iceland; there are three confirmed species, one of which probably represents *M. giardi*, as it infects the kidney. Additional species probably exist that infect different tissues and the site of infection in the host fish appears to be an important diagnostic feature for members of this clade. Myxospore morphology is generally conserved in the clade, although actual spore dimensions can vary between some species. Myxosporeans from this clade are currently only known to infect fishes from the Elopomorpha.

Conference Session Designation: (Parasitology Myxozoa)
Presentation Format: (Oral)

Immune Players in Acquired Protection to *Enteromyxum Leei* (Myxozoa) in Gilthead Sea Bream, *Sparus Aurata* (Teleostei: Perciformes)

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The intestinal myxosporean parasite *Enteromyxum leei* causes chronic catarrhal enteritis in gilthead sea bream (GSB, *Sparus aurata*) leading to intestinal dysfunction, poor growth, and higher susceptibility to handling and stress. It entails severe economic losses to the aquaculture sector. Previous observations in our lab showed that fish that recovered from an *E. leei* infection did not get infected upon re-exposure, hinting towards the possibility of protective immunization of GSB against *E. leei*. To study this in more detail, we re-challenged putative "resistant" (R) GSB that recovered from this myxozoan infection, by exposure to *E. leei* effluent infected water. Another group of naïve (N) GSB (never exposed to the parasite) was also challenged. Both fish groups were sampled at 0, 61 and 86 days post-exposure (p.e.) and different specific and non-specific humoral factors were measured in serum, such as the total peroxidase activity, total IgM and IgT (by ELISA), and the presence and quantity of specific antibodies against the parasite by immunohistochemistry (IHC). The expression of a panel of immune-related genes was analysed in head kidney, anterior and posterior intestines of N and R fish after 86 days p.e. At this final sampling point, 83.3% of N fish *vs* 0% of R fish tested positive for *E. leei* by histology. The total peroxidase activity decreased with the progression of the infection in both groups, but only significantly in R fish, probably due to a higher consumption of this enzyme to fight the parasite. Total IgT and IgM levels did not significantly differ between groups. However, IHC evidenced that R fish had a higher initial level of specific anti-*E. leei* IgM than N fish. The PCR array showed a differential response between N and R fish and among tissues. R fish had significant up-regulation of *IgM*, *IgT*, *il10* and *gzmA* and down-regulation of *il1β* in anterior intestine. Complement (*c3* and *fcl*) and the anti-protease *α2m* were significantly higher expressed in R posterior intestine and head kidney. However, the anti-protease *lcpI* was down-regulated in all tissues of R fish when compared to N. The higher initial pool of specific circulating antibodies against the parasite, together with the higher expression of Igs in anterior intestine, the higher cytotoxic activity in the whole target organ and the systemic increase in complement (lectin pathway) seem to be crucial to resist *E. leei* re-infection. Hence, this acquired protective immunity sets the grounds for the development of a vaccine or the production of recombinant antibodies against *E. leei*.

This study was supported by EU H2020 program and by the Spanish Ministry of Economy and Competitiveness through ParaFishControl (634429) and AGL-2013-48560-R research projects, respectively. This publication reflects the views only of the authors, and the European Commission cannot be held responsible for any use which may be made of the information contained therein. MCP was contracted under CSIC PIE project no. 201740E013 and IE was recipient of APOSTD/2016/037 grant by the "Generalitat Valenciana".

Conference Session Designation: (Parasitology Myxozoa)
Presentation Format: **(** Oral)

Kinetics of Local And Systemic Immune Cell Responses in Whirling Disease Infection and Resistance in Rainbow Trout

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Whirling disease caused by the myxozoan parasite *Myxobolus cerebralis* infects several salmonid fish. The disease is responsible for high mortalities in rainbow trout (*Oncorhynchus mykiss*) hatcheries and natural populations. While substantial investigations has provided insight into disease pathology and host invasion, the cellular responses and the leukocyte kinetics underlying fish resistance or susceptibility remain elusive. To elucidate how resistant and susceptible rainbow trout strains respond to early invasion, we used a well-established model of whirling disease infection to demonstrate the kinetics of the mucosal and systemic immune responses in two rainbow trout strains, a susceptible American (T) and a more resistant German (H). In the course of three weeks following exposure, leukocyte kinetics was monitored by flow cytometry in caudal fin, head kidney and spleen. For the analysis of the leukocyte composition, cells were stained with the monoclonal antibodies with known specificity for distinct subpopulations of rainbow trout leukocytes. Experiments indicated increases of T cells B cells and myeloid cells in the caudal fin of the susceptible strain at 2, 4, 8, 12 or 24 h followed by subsequent time-dependent percent suppressions after 2, 4, 8, 14 or 21 d of exposure compared to non-infected control fish. In spleen and head kidney, decreases of myeloid cells, B cells, and T cells were observed at almost all time points. On the other hand, in the resistant strain, except for percent suppressions of B cells, T cells and myeloid cells at early time point after 2, 4, 8 or 12 h as well as after 1d for T cells in caudal fin, time-dependent percent increases were observed in caudal fin, spleen and head kidney after 1, 2, 4, 8, 14 or 21 d of exposure compared to non-infected control fish. These findings highlight the significance of effective local and systemic immune reaction and indicate proper activation of B cells, T cells and myeloid cells with less T cell responses at earlz time point at the site of infection is critical for host resistance during *M. cerebralis* infection. Alteration of the leukocyte populations with early augmented local cellular responses followed by later declines leads to host immune suppression and supports parasite invasion and survival. The present study provides an initial view into the cellular basis underlying immune response and resistance of rainbow trout to the whirling disease parasite and helps us to elucidate the mechanisms that underlie the variation in resistance to whirling disease infection.

Conference Session Designation: (Parasitology Myxozoa)
Presentation Format: **(** Oral)

Identification of Differentially Expressed Kidney Genes During Proliferative Kidney Disease And *In Vivo* Induced Genes of *Tetracapsuloides Bryosalmonae* (Myxozoa) in Brown Trout

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Tetracapsuloides bryosalmonae is a myxozoan parasite and the causative agent of proliferative kidney disease (PKD) in salmonids. PKD significantly affects both farmed and wild salmonids in Europe and North America, causing significant economic losses and endangers wild fish population. The life cycle of *T. bryosalmonae* requires two hosts: an invertebrate freshwater bryozoan, *Fredericella sultana* and a vertebrate salmonid, brown trout. Here, we identify differentially expressed kidney genes of brown trout during proliferative kidney disease and discuss the research plan to identify *in vivo* induced genes of *T. bryosalmonae* during the course of infection. Fish were exposed to the spores of *T. bryosalmonae*, released from infected *F. sultana* colonies. Fish were sampled, including blood at different time points. Infection in individual kidney and blood was confirmed by quantitative real-time PCR. The cDNA of kidneys of infected and non-infected brown trout were hybridized and compared by suppressive subtractive hybridization technique. Afterward, transcripts were transformed, validated and functionally analyzed. The identified transcripts function in various processes, including cell stress, cell growth, signal transduction, antigen processing and presentation in the kidney of brown trout during proliferative kidney disease. The results from this study contribute to the understanding of kidney response during *T. bryosalmonae* development. Additionally, further work is in progress to identify *in vivo* induced genes of *T. bryosalmonae* in infected brown trout using *in vivo* induced antigen technology. The identification of the molecules related to the pathogenic mechanisms would improve our understanding of *T. bryosalmonae* infection and support the development of therapeutic and vaccine applications to prevent myxozoan infection in salmonids.

Conference Session Designation: (Parasitology / Myxozoa)
Presentation Format: (Oral)

Nematocysts Arise Autogenously and Dominate the Cnidarian Adaptation via Massive Decentralizaed Modification

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Extrusion apparatus, which has been observed in microsporidians, dinoflagellates, ciliates, cnidarians and myxozoans, is a primary example of a complex apparatus whose origins and evolutionary history have proven difficult to reconstruct. Among the extrusion-apparatus-bearing organisms, myxozoans represent a major lineage of metazoan parasites with extremely simplified morphology and structure. Recent phylogenomic evidence suggests an evolutionary origin of myxozoan within cnidaria and for a long time the homology between cnidarian nematocysts and myxozoan polar capsules is the key to indicating the close relationship of the two lineages. However, in contrast to extensively studied nematocysts, the myxozoan polar capsules still remains poorly characterized. To gain better understanding of the structure, function of this myxozoan-specific organelle, to straightforwardly re-evaluate the relationship between myxozoans and cnidarians, and to explore the origin and evolution of extrusion apparatus, here we present the first proteome map of polar capsules from myxozoans. Major components of polar capsules isolated from three myxobolids, *Myxobolus honghuensis*, *Myxobolus wulii* and *Thelohanellus kitauei*, were characterized by using our newly developed reference species-specific proteome called Myxozoan Comprehensive Proteomic Identification Datasets (MCPID), which are derived from deep transcriptome profile and partial genome sequencing data. The MCPID facilitated the proteomic analysis by identifying 19.1%-43.8% more proteins with a maximum 84.6% of database size reduction, finally enabling the identification of 1111, 490 and 597 polar capsule proteins (PCPs) in *M. honghuensis*, *M. wulii and T. kitauei* respectively. Comparative proteomics show that the polar capsule proteomes are highly elaborate and include novel structural proteins and venom proteins. Extending the comparison wihtin all available nematocyst proteomes enables us to inspect key questions in the assumptions about evolutionary scenario of nematocyst: how it evolved within the cnidarians and its relationship with organism evolution.

Conference Session Designation: Parasitology Myxozoa
Presentation Format: Oral Presentation
Student Presentation: Not Student Presentation

Incapacitated by Salt ? *In-Vitro* Discharge Tests of Myxozoan Parasite Infection Mechanisms

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Myxozans are widespread, speciose parasites of freshwater and marine fishes. They are now categorized as Cnidarians, alongside free-living species of corals, anemones and jellyfish. All Cnidarians have nematocysts: complex stinging organelles that discharge a tubule for defense, prey capture or, in the case of myxozoans, for initiating infection. Myxozoan spores detect the host, then fire their tubules to bring host epidermis into close contact, so the motile parasite sporoplasm can enter and begin to replicate. Given that nematocyst firing, sporoplasm activation and movement are essential first steps of the myxozoan infection process, can different compounds interfere with these mechanisms? We aimed to identify ions or molecules that either promote or block nematocyst firing and sporoplasm motility, and thereby gain insight into the sensory and mechanistic functions of myxozoan spores, and reveal paths to therapeutants.

We designed an *in-vitro* assay using spores from two myxozoans, *Myxobolus cerebralis* and *Ceratonova shasta*. We hypothesized that changes in external ion concentrations may block sensory receptors, and enhance or diminish the osmotic gradient which drives tubule discharge and sporoplasm release. Fresh spores were examined under a microscope to determine the proportion of 'intact' spores (nematocysts not discharged, sporoplasms present at the apex of the spore) at the start of the test, then how this changed both after addition of the test compound, then after addition of rainbow trout mucus as a proxy for host contact. We subsampled spore aliquots after each stage of the testing, and fixed these in 5% buffered formalin, to facilitate counting after the tests.. We tested chlorides of Na^{+}, Ca^{2+}, Mg^{2+}, Fe^{2+}, Gd^{3+}, all of which can affect nematocysts of free-living cnidarians. We determined fixation of tested spores in formalin was an excellent way to stabilize test subsamples without additional firing or migration. We found that Ca^{2+} and Mg^{2+} promoted tubule pre-firing. Na^{+} promoted premature sporoplasm migration, but increased tubule firing sensitivity to fish mucus. Ca^{2+} and Gd^{3+} both reduced sporoplasm migration. We will use these findings both for *in-vivo* infection experiments with host fish, and for searches of the parasite transcriptomes for gene pathways that may be linked to sensory or nematocyst functions.

Conference Session Designation: (Parasitology Myxozoa)
Presentation Format: (Oral)

Wednesday September 5th – Gray / Palmer / Pope Ballroom
Myxozoa 3 & 4
Moderators - **Graham Rosser** (Mississippi State University) **Sascha Hallett** (Oregon State University)

1:45 PM	Myxozoa 3	Alexander - Predicting Myxozoan Disease Dynamics in The Context of Climate Change using a Model Ensemble
2:00 PM		Hallett - Mitigating Enteronecrosis in Klamath River Salmon with Managed Flow Events
2:15 PM		Griffin - Monoculture of Hybrid Catfish Can Limit Proliferative Gill Disease Caused by *Henneguya ictaluri* (Myxozoa: Myxobolidae) in Catfish Aquaculture Ponds
2:30 PM		**Barrett - RNA-seq Analysis of the Early Immune Response to the Parasite *Ceratonova shasta* in Resistant and Susceptible Lines of Steelhead**
2:45 PM		Marshall - Measures of Prevalence and Genetic Diversity of *Kudoa thyrsites* Infections Within Farmed Atlantic Salmon (*Salmo Salar* L.) Suggest That New Infections After 2000 Degree Days Are an Exception
3:00 PM		**Refreshments**
3:15 PM	Myxozoa 4	Kristmundsson - The Role of Proliferative Kidney Disease (PKD) in the Severe Decline of Arctic Charr, *Salvelinus* Lake Ellidavatn, Iceland
3:30 PM		Bailey - Black and White With Shades of Grey: Exploring Tolerance and Resistance Concepts and the Immune Response of Brown Trout During Proliferative Kidney Disease (PKD) Infection
3:45 PM		Nelson - Renal Myxozoanosis in Salmonids in the Western United States
4:00 PM		Lovy - Myxozoan Parasites as They Correlate with Life History in Anadromous River Herring

Predicting Myxozoan Disease Dynamics in the Context of Climate Change Using a Model Ensemble

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Abstract Climate change has been linked with changes in the dynamics of infectious diseases in aquatic systems. Climate related shifts in water temperatures and precipitation patterns will have significant effects on the myxozoan disease dynamics, but predicting the magnitude and direction of specific responses is challenging. We present an overview of myxozoan disease dynamics illustrated with data from salmonid ceratomyxosis in the Klamath River CA, USA. Using a model ensemble we predicted host and parasite dynamics under future climate scenarios (hot/dry-cold/wet). We used data from hydraulic and water temperature models, predictive statistical models, and empirical data to parameterize an epidemiological model. Epidemiological model outputs were compared to observations from the Klamath River collected from 2006 to 2017. The majority of climate scenario predictions were similar to values measured in years having high disease risk for salmonids. This result suggests *C. shasta*-induced mortality will likely remain high and could increase in Klamath River salmonids, making the recovery and management of salmon even more challenging.

Conference Session Designation: (Aquatic Epidemiology or Climate Change)
Presentation Format: (Oral)

Mitigating Enteronecrosis in Klamath River Salmon with Managed Flow Events

Sascha L Hallett*, Ryan B Craig, Stephen D Atkinson, Julie D Alexander, Richard A Holt and Jerri L Bartholomew

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Ceratonova shasta causes enteronecrosis in juvenile salmon in the Pacific Northwest of North America, and is associated with population-level impacts in the Klamath River. Transmission occurs through waterborne stages: actinospores released from polychaete worms develop into myxospores in salmonid fishes. To better understand host/parasite dynamics in this regulated system and inform model development and management actions, we contribute to a parasite monitoring program that includes molecular quantification (qPCR) of parasite DNA in both river water samples and outmigrating juvenile salmonids. During salmonid outmigration (April through June), sampling occurs weekly and data are shared semi-real-time to inform management actions, such as modification of the river flow through water release from the lowermost dam. Two different managed flow events have occurred, which we monitored to evaluate their impact on disease in juvenile salmonids.

The 2013 Biological Opinion (NMFS & USFWS) includes the management action that if waterborne levels of *C. shasta* exceed 5 spores/L of genotype II and water temperature exceeds 16°C, a 'pulse flow' would be released. In May 2014, our data showed that parasite levels were over this threshold, and thus triggered a pulse flow: dam release ramped up from 1140 cfs to 1910 cfs, then back down after 12 hours. Before, during and after the flow event, we monitored parasite levels in river water samples, infection in sentinel fish and sampled polychaetes. We found that parasite levels decreased during the flow event, and mortality was lower in one of four groups of fish exposed during the flow; polychaetes were absent from fine substrate after the flow.

In 2017, the trigger conditions for a managed flow were modified by a US Court Order. An 'emergency dilution flow' is now required if either 5 spores/L is detected (of any *C. shasta* genotype at any index site) or prevalence of infection in juvenile salmonids exceeds 20%. In May 2018, the infection threshold was surpassed and thus the flow was triggered: dam releases were ramped up from 1300 cfs to 3130 cfs, and remained at 3000 cfs for 12 days before ramping back down to base flow levels of ~1100 cfs. Again, we measured effects of the flow event on parasite levels, infection in sentinel fish and polychaete abundance. We will present results from the 2018 flow event at the meeting.

Conference Session Designation: (Aquatic Animal Health Management)
Presentation Format: (Oral)

Monoculture of Hybrid Catfish Can Limit Proliferative Gill Disease Caused by *Henneguya Ictaluri* (Myxozoa: Myxobolidae) in Catfish Aquaculture Ponds

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Catfish aquaculture is the largest foodfish aquaculture industry in the United States and a vital economic component of several southern states. Recent industry trends have led to increased production of channel ♀ (*Ictalurus punctatus*) x ♂ blue (*I. furcatus*) hybrid catfish to take advantage of more favorable production characteristics. As a result, hybrids are estimated to comprise 40%-50% of total catfish production. Proliferative gill disease (PGD) caused by the myxozoan parasite *Henneguya ictaluri* is the most prevalent parasitic disease in Mississippi catfish aquaculture. Known colloquially as "Hamburger gill," PGD accounts for 10-30% of annual disease case submissions to the Aquatic Research and Diagnostic Laboratory of the Thad Cochran National Warmwater Aquaculture Center in Stoneville, MS. In channel and hybrid catfish, continuous exposure to the actinospore stage of the parasite life cycle triggers a severe inflammatory response at the gills, leading to impaired osmoregulatory and respiratory function, resulting in reduced feeding activity and in severe outbreaks mortality can reach 100%. The static, earthen bottom ponds of catfish aquaculture, and the close proximity of fish and oligochaete hosts, provides an ideal environment for the propagation of myxozoan life cycles. Control measures to reduce incidence of PGD have been largely unsuccessful, however, several controlled studies have revealed an arrested development of *H. ictaluri* in hybrid catfish. While hybrids demonstrate an inflammatory response comparable to channels during acute stages of infection, research indicates significantly less *H. ictaluri* DNA present in hybrid tissues across the developmental timeline and mature *Henneguya* spp. myxospores in these studies, while abundant in channel catfish, are almost non-existent in hybrids. To evaluate the impacts of these findings at the pond level, 18, 1-acre ponds were stocked with channels (n=9) or hybrids (n=9) and maintained as monoculture systems for 3 years, with harvest and understocking performed as warranted. Water samples were collected monthly for eDNA analysis, with additional samples collected in April and May when PGD is most prevalent. In addition, sentinel fish exposures were performed in April and May to estimate PGD severity in naïve fish stocked into these systems. While no differences were observed in the first year, by the second year of the study, *H. ictaluri* DNA and lesion scores in hybrid systems were significantly reduced, and PGD lesions in sentinel fish placed in hybrid ponds were negligible. These results suggest hybrid monoculture can significantly reduce the burden of PGD on catfish aquaculture in the southeastern US.

Conference Session Designation: (Myxozoa)
Presentation Format: (Oral)

RNA-Seq Analysis of The Early Immune Response to the Parasite *Ceratonova Shasta* in Resistant and Susceptible Lines of Steelhead

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Ceratonova shasta is virulent myxozoan parasite of salmonid fish in the Pacific Northwest of North America. It is a significant cause of mortality in out-migrating juveniles and has been associated with pre-spawn mortality of returning adults. *C. shasta* attaches to the gills and replicates in the gill blood vessels before migrating to the intestine. Successful establishment in the intestine results in hemorrhaging and necrosis of the tissue leading to mortality of the host and release of myxospores. Fish resistant to *C. shasta* appear to limit parasite establishment in the intestine via an effective immune response. However, the mechanisms at work in the early stages of infection remain unclear. Here, we investigated this early immune response by exposing resistant and susceptible stocks of steelhead (*Oncorhynchus mykiss*) to *C. shasta* and collected gill tissue at 1 day post exposure (dpe) and intestine at 7, 14 and 21 dpe, along with additional tissue samples for histology. qPCR results indicate that the resistant fish prevent parasite establishment in the intestine while the susceptible fish fail to limit establishment or proliferation. RNA-seq was conducted on gill tissue from 1 dpe and intestinal tissue from 7 dpe for both susceptible and resistant fish, along with their respective controls. The results indicate a more severe reaction to the initial infection in susceptible fish and a failure to recognize parasite establishment in the intestine. This is the first study to employ RNA-seq in order to profile the transcriptional response to this important parasite. Our results support the hypothesis that resistance to *C. shasta* occurs before the parasite reaches the intestine and will inform future studies investigating the specific mechanism of resistance.

Conference Session Designation: (Genomic Applications in Aquatic Animal Health)
Presentation Format: (Oral)
Student Presentation: (Yes)

Measures of Prevalence and Genetic Diversity of *Kudoa Thyrsites* (Myxozoa) Infections Within Farmed Atlantic Salmon (*Salmo Salar* L.) Suggest That New Infections After 2000 Degree Days Exposure are an Exception.

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Kudoa thyrsites causes post-harvest myoliquefaction or 'soft flesh' in a variety of marine fishes. In farmed Atlantic salmon, intracellular cysts form within skeletal muscle 1500-2000 degree days (DD) post exposure with highest prevalence usually occurring between 2000-3000 DD. In some regions of British Columbia (BC), Canada, infection intensities can negatively affect up to 4-7% of fillets, thus impacting the competitiveness of BC's farms. Mitigation strategies for farms located in areas known to have higher infection risk involve rearing smolts at nursery farms located in low risk regions prior to transfer to the higher risk recipient grow out farms. Under these conditions, soft flesh after harvest is reduced to near that expected of fish reared entirely under low risk conditions, suggesting that fish may be only be vulnerable to infection during the beginning of their seawater exposure.

We measured the genetic diversity of *K. thyrsites* from populations of infected fish to look for evidence of new infections after 2000 DD chronic exposure. Within fish haplotype diversity was also measured through comparisons of discrete tissue samples. Under conditions of chronic high risk exposure, we tested 12 fish each at 2000, 3000, and 4000 DD, assuming that under continuous infection haplotype diversity would increase. To identify the origin of infection in transferred fish, we compared haplotype diversity of *K. thyrsites* in three populations of fish: 1) 20 fish reared entirely at a low risk farm; 2) 20 fish reared entirely at a high risk farm; and 3) 10 fish transferred from the low risk donor farm to a high risk recipient farm after 2000 DD. Haplotype counts and frequencies were calculated from 39 alleles from 891 sequences amplified from clone libraries produced from infected flesh. We also used PCR and histology to measure change in infection prevalence in four unrelated populations transferred after 2000 DD.

Under chronic high exposure, diversity and haplotype counts did not increase with time and low haplotype counts of one to five within individual fish suggested that new parasites were unlikely to invade the flesh of previously infected fish. In transferred fish, high levels of genetic variability precluded identity of the origin of infections; however, prevalence monitoring indicated that increases in infection prevalence following transfer varied between 0 and 50%, depending on the population. Matching haplotype identities between different tissue samples within the same fish support a model of mixed infection but with few individual parasites successfully clonally amplifying within the infected fish --thus emphasizing the importance of understanding host parasite dynamics during early stages of infection. Such information could be applied to current mitigation practices in order to improve the efficiency or perhaps mimic the effects of fish transfers.

Conference Session Designation: **(** Parasitology Myxozoa)
Presentation Format: **(** Oral)

The Role of Proliferative Kidney Disease (PKD) in the Severe Decline of Arctic Charr, Salvelinus Alpinus, in Lake Ellidavatn, Iceland

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Proliferative kidney disease (PKD) is a widespread temperature dependent disease in salmonids caused by a myxozoan parasite, *Tetracapsuloides bryosalmonae (T.b.)*. During the last decades, the effect of PKD on wild populations of salmonids has received renewed interest. Research from Norway, Switzerland, USA and Iceland, suggest that it is an emerging disease and most likely associated with global warming. Over the last two decades, the population of Arctic charr in Lake Ellidavatn in SW Iceland has experienced a severe and unexplained decline. In 2008, PKD was reported for the first time in Iceland, from Arctic charr in the above mentioned lake. Since then, fish populations in the lake have been regularly monitored with regard to diseases.
Results show that *T.b.* infections in Arctic charr in Lake Ellidavatn are highly prevalent, regardless of the water temperature during the summer. However, the prevalence and severity of clinical signs of PKD vary considerably between years; being common and severe during warmer summers. Available long term data on water temperature and catch figures (catch per unit effort –CPUE) over the years 1989-2017, as well as data from the annual PKD monitoring in the years 2008-2017, allowed us to perform a retrospective, prospective analysis on the severity of PKD, twenty years prior to its initial identification. The results show that the main factors influencing the progress of PKD is the water temperature in August and the total number of consecutive days, during the summer, the water temperature exceeds 12°C. A statistically significant negative relationship was observed between the severity of PKD and CPUE during the years 1989-2017.
Generally, Arctic charr have been poorly studied with regard to PKD. However, available data suggests that it is extremely susceptible to infection and shows clinical signs of PKD at lower water temperatures than other common salmonid species. The psychrophilic nature of Arctic charr may be one explanation for this phenomenon. In addition to fighting infections, suboptimal temperatures for this arctic fish species cause physiological stress, which is likely to lower their resistance and further increase the severity of the disease. Our results strongly indicate that PKD is a major factor in the severe reduction experienced in the Arctic charr populations in Lake Ellidavatn, Iceland. Numerous other Arctic charr populations in Iceland have also greatly declined over recent decades. In the light of these results from Lake Ellidavatn, it is reasonable to assume that PKD might also be a contributing factor to the declines observed in other lakes.

Black and White With Shades of Grey: Exploring Tolerance and Resistance Concepts and the Immune Response of Brown Trout During Proliferative Kidney Disease (PKD) Infection

Christyn Bailey *, Nicole Strepparava, Thomas Wahli and Helmut Segner

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In Europe proliferative kidney disease (PKD) of salmonids is an emerging disease of economic and environmental concern. PKD is caused by the myxozoan parasite *Tetracapsuloides bryosalmonae*. In evolutionary ecology once, infected organisms may protect themselves against parasitic infection either by reducing parasite burden (resistance) or the damage caused by parasites in spite of high pathogen burdens (tolerance). However, little is understood concerning resistance and tolerance concepts and their application in fish host-parasite interactions. While the main aim of the present study is to describe the brown trout host immune response after exposure to the parasite *T. bryosalmonae*, we also took an evolutionary ecology perspective and explored salmonid patterns of tolerance and resistance during infection, comparing them in multiple species (the native brown trout and the farmed non-native rainbow trout). Regarding, the brown trout host immune response at day 40 and 50 post-exposure IgM sec and Blimp1 were strongly upregulated, whereas Pax5 was downregulated. Hence, the combinatorial signature of the B cell molecules at these time points indicates plasma blast/plasma cell phenotype. In addition, expression of Blimp1 was also strongly correlated with parasite development. While all Th1-like transcripts measured in the study were elevated at day 50 post-exposure. Concerning tolerance / resistance patterns species-specific differences were seen in resistance with brown trout conferring relative resistance but only rainbow trout were able to confer absolute resistance resulting in a different evolutionary outcome for each salmonid species, in that rainbow trout can clear the infection and in brown trout the parasite persists i.e. the stable co-evolved host-parasite system. Further exploration of tolerance/resistance and an association of immune mechanisms with such concepts opens an additional gateway for interpreting fish host-parasite interactions.

Conference Session Designation: (Immunology General)
Presentation Format: (Oral)

Renal Myxozoanosis in Salmonids in the Western United States

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Renal myxozoanosis occurs in many farmed and wild salmonids in the Western United States, and several species are implicated with varying pathogenicity and clinical significance. This retrospective study of diagnostic cases seen at the Washington Animal Disease Diagnostic Laboratory (WADDL) during the last 10 years includes mountain whitefish, rainbow trout, and salmon species. Myxozoan genera diagnosed include *Tetracapsuloides*, *Parvicapsula*, and a species newly identified at WADDL. Infections range from subclinical with primarily intratubular involvement to clinically significant with significant epithelial necrosis and/or interstitial inflammation. While *Tetracapsuloides* has only non-sporogonic stages within lesions, most renal myxozoan infections have intraluminal sporogonic stages.

Conference Session Designation: (Myxozoan Parasitology)
Presentation Format: (Oral)

Myxozoan Parasites as They Correlate with Life History in Anadromous River Herring

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River herring are made up of two species including the alewife *Alosa pseudoharengus* and the blueback herring *Alosa aestivalis*. Both are considered threatened and declining species due to a number of anthropogenic factors including habitat fragmentation from dams, historical overfishing, an increase in predation, and other habitat alterations resulting from coastal development. Though these factors have directly impacted populations throughout their range, less is known of the infectious agents and potential disease impacts in these threatened populations. In New Jersey, sampling for river herring has been done annually to survey young-of-the-year (YOY) and adult fish to better understand their population levels. From sampling conducted between 2014-2018, we describe three myxozoan parasites in river herring which correlate to particular life history stages of the fish. In YOY fish two myxozoan species were detected, including *Myxobolus mauriensis* and a *Kudoa* spp. According to sequences of the 18S rDNA, *M. mauriensis* was most closely related to other marine myxobolids with tropism for cartilage, with closest identity of 83% to *Myxobolus groenlandicus*. Histology suggested this species to be pathogenic in young fish, causing lysis and breaks in the rib bones associated with chondritis and myositis. Lesions extended to the skin causing dermatitis and extracorporeal release of spores. The *Kudoa* spp. also occurred in YOY fish indicating that infection with this parasite occurred within the river environment prior to out-migration into the ocean. Little to no host response was associated with *Kudoa* in the muscle. Genetic analysis was conducted to understand the relationship of this *Kudoa* spp. to others found in the marine environment. Adult river herring returning to spawn in their natal rivers were infected with myxozoan developmental stages located beneath the renal tubular epithelium. Sequence analysis of the 18S rDNA indicated this to be a species closely related to the genus *Ortholinea*. Though this myxozoan was found at a high prevalence in river herring, the lack of host changes associated with infection suggested this to be a non-pathogenic species. It is believed that this myxozoan is associated with fish during their spawning migration, since when adult non-spawning river herring were examined from the ocean, this myxozoan was not detected. The association of these infections with specific stages in the fish's life history provides insights into environments in which the intermediate hosts for these myxozoan parasites likely exist. Further research is required to identify the intermediate hosts for these myxozoans and to fully understand risk factors for infection.

Conference Session Designation: (Myxozoa,)
Presentation Format: (Oral)

Keynote Presentation

Wednesday Afternoon September 5rd

Nano-Evolution: Balancing Safety and Applications of Nanotechnology in Aquatic Systems

Tara Sabo-Attwood

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The development of synthetic nanomaterials over the past decade has led to the expansion of innovative uses and applications of such materials in the field of aquatic science and industry. Nanomaterials by definition, are typically composed of small (nano)particles of 1 – 100 nm in at least one dimension. Synthesizing nanoparticles on such a small size scale significantly changes their physiochemical properties as compared to their bulk counterpart materials which are highly desired for applications such as water disinfection and pathogen removal. For example, aquaculture is the fastest growing food-producing sector however, the presence of waterborne pathogens in high-density fish farming operations is a primary cause for aquaculture crop loss, globally. Therefore, providing safe and pathogen free water for this important food industry is essential to increase production and to ensure future food security. But with growing utility and use of nanomaterials in much desired and needed applications with little regulation, innovative physiochemical properties associated with nanomaterials may also produce unintended or unexpected consequences to exposed organisms and ecosystems. Based on these notions, the focus of this talk is to describe the current state of nanomaterial applications specifically to the aquatic and aquaculture fields, highlighting the need for safety assessments that consider a 'safety by design' paradigm. The talk will be grounded in case studies that showcase several cutting edge approaches to water disinfection in tandem with safety assessments. Current gaps in the field, from both a basic and applied research viewpoint and education and training considerations for future incorporation of nanomaterials in aquatic industries will be presented.

Wednesday September 5th – Tilly / Tupper
Diseases of Wildfish 1 & 2
Moderator - Roland Cusack (Nova Scotia Department of Fisheries & Aquaculture)

9:30 AM	Diseases of Wildfish 1	de Jourdan - Histopathological Liver Changes and Additional Findings in Inland Silverside *Menidia beryllina* Exposed to Individual Polycyclic Aromatic Hydrocarbons
9:45 AM		**Lynn - Observations of Increasing Prevalence and Intensity of the Fluvial Ectoparasite *Argulus canadensis* on Migrating Outer Bay of Fundy Atlantic salmon.**
10:00 AM		**Cook - Dermal Injuries, Disease, and Immune Responses in Wild-Caught Pacific Salmon**
10:15 AM		**Chapman - Temperature Influences Post-Release Condition and Disease Progression in Adult Atlantic Salmon**
10:30 AM		**Refreshments**
10:45 AM	Diseases of Wildfish 2	Teffer - Incorporating Multiple Infections and Cumulative Stressors in Evaluations of Disease Development in Wild Fish
11:00 AM		**Soto-Davila - Atlantic Cod (*Gadus morhua*) Primary Macrophages Response to *Aeromonas salmonicida* Infection**
11:15 AM		Becker - An Epidemiologic Model of Koi Herpesvirus (KHV) Biocontrol for Carp in Australia
11:30 AM		Borucinska - Selected Biomarkers of Health and Water Quality in Dogfish Sharks *Mustelus canis* from the Long Island Sound in 2000 and 2017 Cohorts

Histopathological Liver Changes and Additional Findings in Inland Silverside *Menidia Beryllina* Exposed to Individual Polycyclic Aromatic Hydrocarbons

Ben de Jourdan[1], William Stubblefied[2], Jerry Heidel[3], Jeff Wolf[4] and Mike Kent[5]

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Histopathology provides links between toxicological effects observed in laboratory studies and responses at the cellular and tissue level, and thus may elucidate health impacts of contaminants that may be related to survivability. However, a limitation of histopathology is that it requires trained individuals to make proper diagnostic evaluations, and the interpretation of borderline pathological changes is to some degree subjective. Here we evaluated over 1,000 Inland silverside (*Menidia beryllina*) exposed to dissolved concentrations of single polycyclic aromatic hydrocarbons (PAHs). The primary endpoints were morbidity, growth, and histopathological changes. The liver was the only organ that exhibited exposure-related histopathologic changes. Two liver findings consistently observed in hepatocytes of exposed fish were increases in nuclear pleomorphism and increases in lipid, but not glycogen vacuolation. For certain PAHs, increases in nuclear pleomorphism were strongly correlated with increased mortality. Given the potential subjectivity of histopathological interpretations, two of the authors with extensive experience in fish histopathology evaluated all the histological slides independently so that the degree of consistency between the pathologists could be compared. There was a high degree of scoring consistency between the two pathologists that was statistically significant. The results of this study demonstrated that histopathology interpretations of fish tissues are repeatable when trained pathologists adhere to specific diagnostic criteria.

Conference Session Designation: (Toxicology / Tox Path)
Presentation Format: (Oral)

Observations Of Increasing Prevalence And Intensity Of The Fluvial Ectoparasite *Argulus Canadensis* On Migrating Outer Bay Of Fundy Atlantic Salmon

Tyler Lynn[1*], Stephanie Ratelle[2], Ross Jones[2], Becky Graham[3], John Whitelaw[3], Leroy Anderson[3], Sarah Tuziak[2], Aaron Frenette[1] and Michael Duffy[1]

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Parasites and diseases are a high concern threat affecting Outer Bay of Fundy (OBoF) Atlantic salmon, a species not listed federally under the Species at Risk Act, but assessed as endangered in Canada (COSEWIC, 2010). OBoF Atlantic salmon were examined for ectoparasites at two sites near Fredericton, NB; the Nashwaak River Counting Fence (NRCF), and the Mactaquac Generating Station (MGS). The MGS is a 55-metre high hydroelectric dam located in the main stem of the SJR. The NRCF is located downstream of the dam and ~25 km from the main SJR stem. Both sites are located ~150 km from the estuary at the Bay of Fundy. Salmon are subject to observation of ectoparasites at each location. Ectoparasites collected 2013-2017 were distinguished morphologically with >99.9% (n = 4301) identified as *Argulus canadensis* (Branchiura), and <0.1% (n = 3) identified as the sea "louse", *Lepeophtheirus salmonis* (Copepoda). *Argulus* spp. infect fishes from freshwater, marine, and estuarine waters and so it is unknown where migrating OBoF salmon acquire *A. canadensis*. However, 90-100% of adult *A. canadensis* survive off their host (*in vitro*) for 60 hours in freshwater or estuarine conditions (17 ppt) whereas 100% of parasites die within 48 hours in seawater (34 ppt). Furthermore, additional *in vitro* studies reveal that *A. canadensis* eggs do not develop in seawater (34 ppt), while 59.3% (38-78%) develop to metanauplii in estuarine conditions (17 ppt) and 95.7% (95-96%) develop to metanauplii in freshwater. Whereas 58% (48.8-73.4%) of eggs hatched to metanauplii in freshwater, only 8.7% (3-16%) hatched in estuarine water. These results suggest that *A. canadensis* is transmitted to salmon exclusively during their migration within the SJR system. This parasite is known from the SJR system prior to completion of the Mactaquac dam in 1968 but was first observed on OBoF salmon at the dam in the mid-1990's. The year 2017 marks the highest prevalence of infection observed to date at both sites, with 91.8% and 81.2% of migrating salmon infected at the MGS and NRCF sites, respectively. We see a dramatic increase in the proportion of fish with high intensity infections (>50 parasites/fish) at Mactaquac from 2009 (1.5%) to 2017 (49.4%). Conversely, the proportion of infected fish with high intensity infections (>50 parasites/fish) remains static at the NRCF during this same time period (0-4%) suggesting differences in parasite transmission associated with fish migration to these two sites. The reason for the increase in ectoparasite prevalence and intensity remains unknown, but is of interest given tissue damage observed at the site of infection on some individuals and the critically low Atlantic salmon numbers.

Conference Session Designation: **(** Diseases of Wild Fin-Fish)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Dermal Injuries, Disease, and Immune Responses in Wild-Caught Pacific Salmon

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Commercial fisheries targeting wild Pacific salmon capture a mixture of co-migrating species, and those of conservation concern must be released. Among released fish, injury severity has been identified as an important predictor of mortality. Typically capture-induced injuries affect the dermis (e.g. skin, scale and mucous loss, net abrasions). Not only are the mucus and scales the primary line of defense against invading pathogens, but the stress of capture may also influence subsequent immune function, potentially interacting with physical injury to accelerate vulnerability to disease. Working alongside commercial Pacific salmon fisheries, non-lethal gill samples were collected from chum salmon, a species commonly released from these operations. Using HT-qPCR on the Fluidigm Biomark Dynamic Array™ microfluidics platform, fish were screened for the presence and relative abundance of 44 microparasite taxa identified as potentially infectious agents in the region. In addition, biomarkers of salmon immune function were examined to identify changes in expression profiles associated with infection dynamics. Severity of dermal injuries was estimated in captured fish and we conducted at-sea holding studies for up to 10 days to monitor changes in microparasite communities and immune response. The Fluidigm Biomark platform has been used with success to monitor the health of wild pacific salmon in BC. Here we expand on these efforts to understand how the latent effects of a fisheries interaction may influence disease progression and host immune function post-release.

Conference Session Designation: (Diseases of Wild Fin-Fish)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Temperature Influences Post-Release Condition and Disease Progression in Adult Atlantic Salmon

Jacqueline M. Chapman[1]*, William M. Twardek[1], Robert J. Lennox[1], Ian Flemming[2], Martha Robertson[3], Kristi M. Miller[4], Steven J. Cooke[1].

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Catch-and-release is a common practice in recreational fisheries, so understanding how fisheries interactions – including exhaustive exercise, air exposure, and handling – impacts post-release condition and survival is necessary for developing appropriate management strategies and inform angler best practices. The impacts of fisheries stress may vary with changing environmental conditions, such as increasing temperature. Here, an in-river holding study was used to understand how exercise, air exposure, and handling in warm and cool waters influence the post-release health and condition of wild Atlantic salmon. In the Campbellton River, Newfoundland, adult salmon migrating back to fresh water were collected at a counting fence and subject to experimental exhaustive exercise, air exposure, and handling, biopsied for gill and blood, then placed in an in-river holding pen for monitoring. RNA extracted from gill biopsies were used to screen for pathogen loads and Atlantic salmon immune and osmoregulatory gene expression. By combining in-situ fisheries simulations and gene expression technologies, we explore the relationship between fisheries related stressors, salmon condition, microbial pathogen productivity, and post-release survival.

Conference Session Designation: (Diseases of Wild Fin-Fish)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Incorporating Multiple Infections and Cumulative Stressors in Evaluations of Disease Development in Wild Fish

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As constituents of aquatic ecosystems, infectious agents inherently influence the survival of wild fish. However, empirical evidence demonstrating links between infections and mortality of wild fish is scare, especially regarding multiple infections and stressors. We conducted a series of laboratory and field studies to characterize the disease-associated mechanisms of early mortality of adult Pacific salmon (*Oncorhynchus* spp.) during freshwater migration in the context of cumulative stressors (high river temperature, fishery non-retention). Individuals were collected prior to or following river entry and transported to cool or warm freshwater tanks, or radio-tagged and released to evaluate migration behavior. A subset was also exposed to a fishery non-retention treatment. Held fish were biopsied weekly while tagged fish were biopsied at release. Physiology, immune activity and multiple infections were measured using high-throughput qPCR of gill tissue and chemical analysis of blood. Ecologically relevant high temperatures increased mortality and infection development and reduced the capacity of individuals, especially females, to resolve stress. Fishery stress also reduced survival but was context-dependent and mortality was generally delayed by more than a week. Fish with heavy infections migrated faster in the river but had reduced migration success, while gillnetting and air exposure reduced migration rates. River exposure was associated with more severe infections and increased mortality relative to fish that bypassed the lower river (marine-collected), supporting a causal influence of river-derived infections in early mortality. Our results suggest that multiple infections influence adult Pacific salmon survival in fresh water by affecting host physiological and behavioral responses and overall resilience to cumulative stressors.

Conference Session Designation: (Diseases of Wild Fin-Fish)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Atlantic Cod (*Gadus morhua*) Primary Macrophages Response to *Aeromonas salmonicida* Infection

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In contrast to other teleost, Atlantic cod (*Gadus morhua*) has an expanded repertoire of MHC-I components, but lacks the MHC-II and CD4, which are essential for antibodies production and prevention of infectious diseases. The mechanisms underlying fights against bacterial infections in *G. morhua* are not understood. *Aeromonas salmonicida* subsp *salmonicida* is a recurrent infection in cultured and wild fish, and has been reported in Atlantic cod. Macrophages are some of the first responders to bacterial infection and the link between innate and adaptive immune response. Here, we evaluated the viability, production of reactive oxygen species (ROS), cell morphology, and gene expression of cod primary macrophages in response to *A. salmonicida* infection. We found that *A. salmonicida* infects cod macrophages without killing the cod cells in contrast to *Salmo salar* infected macrophages. Cod infected macrophages upregulated key genes involved in the inflammatory responses (IL-1β, IL-8, IL-10, MHC-I, LECT-II, G-type L) and bacterial pathogen recognition (BPI/LBP). These results suggest that *A. salmonicida* trigger immune mechanisms that allow cod infected macrophage survive during infection in contrast to *S. salar* infected macrophages.

Conference Session Designation: (Diseases of Wild Fin-Fish)
Presentation Format: (Oral)
Student Presentation: (Yes)

An Epidemiologic Model of Koi Herpesvirus (KHV) Biocontrol for Carp in Australia

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Native to parts of Eastern Europe and central Asia, the common carp (*Cyprinus carpio*) is the third most farmed aquatic species in the world. In 2015, 4.3 million tonnes were produced, representing 8.3% of global farmed fish production. Despite their popularity for farming and recreational fishing, carp are considered an important invasive species in North America and Australia. Except for the Northern Territory, carp are established throughout Australia and numerous large populations are found in south-eastern Australia. Despite several introductions in Australia from the 1850s onwards, it was the introduction of the 'Boolara' strain in the early 1960s that led to a dramatic invasion of this pest species. Carp dominate fish communities in some areas of Australia's largest river catchment, the Murray-Darling Basin, comprising of 80 to 90% of the biomass. In 2016, the Australian Government announced the National Carp Control Plan to undertake research and stakeholder consultation to develop a plan for the potential release of *Cyprinus herpesvirus 3* (CyHV-3) to control carp populations.

Since emerging in 1997, koi herpesvirus disease (KHVD) has caused high mortality in common carp affecting all age classes of both wild and farmed fish. KHVD is notifiable to the OIE. Natural infections with CyHV-3 have only been detected in common carp, and varieties (e.g. koi carp). The disease is characterised by irregular patches on the skin and severe gill necrosis and inflammation. CyHV-3 infections occur in water temperatures between 16 to 28°C with optimal transmission and development of viremia between 22 to 24°C. Surviving carp develop anti-CyHV-3 antibodies and may have enhanced resistance to the disease, but may also become persistent carriers and shed virus. KHVD is exotic to Australia as no outbreaks have been recorded.

The purpose of this paper was to review the current knowledge of transmission factors for CyHV-3 and discuss the potential for recurring epidemic-level mortality events in carp found in the Murray-Darling Basin. Case studies will be presented comparing KHVD outbreaks in wild and farmed carp in Japan and *Epizootic haematopoietic necrosis virus* (EHNV) outbreaks in Australia. First emerging in the early 1980s, EHNV is only found in Australia and infections with this virus are notifiable to the OIE. Clinical outbreaks of EHN have only been observed in the introduced species, redfin perch and rainbow trout. EHNV has spread through several catchment areas in the Murray-Darling Basin with the last recorded outbreak in 2010. Host and environmental transmission factors for EHNV will be compared with CyHV-3. The model from this related virus will inform the potential impact of CyHV-3 as a biocontrol for carp.

Conference session: **(** Diseases of Wild Fish)
Presentation format: (Oral)

Selected Biomarkers of Health and Water Quality in Dogfish Sharks *Mustelus Canis* from the Long Island Sound in 2000 and 2017 Cohorts.

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The aim of this pilot study was to compare selected biomarkers of fish health and environmental quality in Long Island Sound in the western Atlantic. We examined 50 smooth dogfish sharks, *Mustelus canis,* common predatory bottom dwellers in the Long Island Sound. The fish were collected in June of 2000 (30 fish) and 2017 (20 fish). Autopsy was performed following cervical dislocation. All macroscopic abnormalities were noted and fork length, body weight, and liver weight were taken. In addition, gut content samples were collected aseptically from the 2017 fish for future microbiome analysis. Standardized to size and location, sections from gonads and liver were collected for histopathology. After routine processing, tissues were stained with hematoxylin and eosin and examined by bright-field microscopy. Additional staining was done as needed and included PAS, PTAH, Pearl's and Fontana Masson's. Morphometric analysis of hepatic melanomacrophages (MMC) was done using SPOT software. The studied biomarkers included condition factor (CF), hepatosomatic index (HIS), the numbers of MMC and % hepatic surface covered by MMC, levels of follicular atresia, and histopathology of liver and gonads. Apparent differences in the biomarkers between the two years of collection were noted in regards to CF, HIS and MMC and were suggestive of declining fish condition and/or water quality. The microbial gut community will be characterized using 16S rRNA gene sequencing on an Illumina platform from the 2017 cohort in order to compare gut flora with fish health and water quality. Although the small sample size precludes general conclusions, the data are inviting more studies to validate our findings and to continue to monitor trends in water quality in the Sound.

Conference session designation: (Diseases of Wild Fin-Fish and Shellfish)
Presentation format: (Oral)

Wednesday September 5th – Tilly / Tupper
Aquatic Epidemiology 2
Moderator - Ian Gardner (Atlantic Veterinary College - UPEI)

1:45 PM	Aquatic Epidemiology 2	Gautam - A GIS-based Multi-Criteria Analysis Framework to Inform Risk-based Surveillance of Wild Aquatic Animals in Freshwater System
2:00 PM		Jung-Schroers – Epidemiological Study on the Occurrence and the Pathogenicity of the Carp Edema Virus (CEV) in Fish in Germany
2:15 PM		Laurin – Guidelines for Pooling Samples for use in Surveillance Testing of Infectious Diseases in Aquatic Animals
2:30 PM		**Lopez-Porras – A Molecular Survey of Bacterial Fish Pathogens in Nile Tilapia *Oreochromis niloticus* Hatcheries in Costa Rica**
2:45 PM		**Patanasatienkul – Mathematical Modeling to Optimize Mitigation Strategies against *Ciona intestinalis* in Mussel Production**

A GIS-Based Multi-Criteria Analysis Framework to Inform Risk-Based Surveillance of Wild Aquatic Animals in Freshwater System

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Surveillance is necessary to establish zones of disease presence and/or absence. Risk-based surveillance is a type of surveillance that helps in effective allocation of resources, and increases the probability of disease detection. This increased likelihood of detection is achieved by focusing surveillance effort on populations and areas at greater risk of disease introduction and establishment. The identification of high risk populations and areas can, however be a challenge, particularly in natural aquatic systems. Such challenges may include the distribution of susceptible host population(s) in an environment consisting of thousands of water bodies. To simplify this complexity, a multi-criteria analytical tool evaluating the likelihood of pathogen entry from an infected source population to a population of interest was developed using four possible pathways of pathogen transfer. These four pathways of pathogen transfer were: (i) hydrological connection, (ii) anthropogenic movement of live animals, (iii) anthropogenic movement of eggs and/or germplasm, and (iv) vectors and fomites. Each of these pathways received a likelihood score between 0 and 1 depending on specific criteria. The relative importance of each of the pathways was also evaluated by weighting them. The weights were established by expert consultation. The population of interest was defined as any secondary watershed that did not have a known health status. The health status of the source population was defined as either infection free (0), unknown (0.5), suspect infected (0.75) or confirmed infected (1). Using the appropriate combination of the relative weight and likelihood score of each pathway, and the source population health status, pathway specific likelihood scores were calculated for all secondary watersheds. A total likelihood score of pathogen entry was then determined by adding the likelihood scores of all the pathways. Using this, the likelihood of exposure and potential infection was determined by considering the distribution of susceptible species and environmental conditions. The likelihood score for entry, exposure and infection were used to risk-categorize secondary watersheds in Canada and represented visually as risk maps, using Geographic Information System (GIS).The GIS maps can be used to identify and spatially visualize high risk areas and can be used as decision support tools to determine sampling locations for designing risk-based surveillance in aquatic animals. This model is presented using Whirling Disease and spring viraemia of carp in Canadian waters as examples.

Conference Session Designation: (Aquatic Epidemiology)
Presentation Format: (Oral)

Epidemiological Study on The Occurrence and the Pathogenicity of the Carp Edema Virus (CEV) in Fish in Germany

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Koi sleepy disease (KSD) caused by infections with the carp edema virus (CEV) seems to pose a potential risk to carp aquaculture and koi trade. During the years 2015 and 2016 an epidemiological study on the occurrence of CEV in fish in Germany was performed. In total 421 gill samples were analyzed. Most of these samples were taken from common carp or koi carp, only a few samples were taken from additional fish species that were kept together with carp. In 194 samples CEV genome fragments were detected. Most detections, in total 179, were made in samples from koi carp (*Cyprinus carpio*), in 61 samples of common carp (*Cyprinus carpio*) CEV was detected and in 1-2 samples each of *Ctenopharyngodon idella*, *Esox lucius*, *Gymnocephalus cernua*, *Perca fluviatilis*, *Sander lucioperca* genome fragments of CEV were found in low amounts (1.10E+00 – 1.19E+03). Highest amounts of viral DNA were detected in samples of koi carp (1.00E+00 – 4.82E+06) and common carp (1.00E+00 – 4.03E+06). Sequencing of the DNA fragments revealed that there are at least two different genogroups of the virus are present and that almost all isolates detected in common carp are belonging to genogroup 1 whereas almost all isolates detected in koi carp are belonging to genogroup IIa.

Characteristic symptoms for an infection with CEV were enophthalmus, anorexia, gill necrosis, gill swelling and lethargic behavior. Mostly in spring, between May and July, CEV was detected. In koi carp disease outbreaks due to CEV were mostly seen when the water temperature was between 17-18°C, whereas in common carp at water temperatures between 9-13°C CEV was detected most frequently.

In 46.66% of samples taken from clinically healthy koi or carp from retailers, CEV was detected. Taken all samples from clinically healthy koi and carp, CEV could also be detected, but only in 26.32% of all examined fish. Therefore purchasing new fish from retailers might be one risk factor for the introduction of CEV in a pond. In common carp more frequently diseases signs and mortalities were recorded compared to koi carp. The probability of losses of more than 50% in a system was around 5 times higher in common carp aquaculture than in facilities for koi carp. Fish health services should therefore be aware of the presence of CEV which may result in high losses in carp aquaculture and testing of koi and carp for CEV should become part of fish disease surveillance programs of national and regional fish disease laboratories.

Conference Session Designation: (Aquatic Epidemiology)
Presentation Format: **(** Oral)

Guidelines for Pooling Samples for Use in Surveillance Testing of Infectious Diseases in Aquatic Animals

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Specimens from multiple animals may be pooled and tested to reduce costs of surveillance for infectious agents in aquatic animal populations. The primary advantage of pooling is to provide better population coverage when prevalence is low (<10%), increasing the likelihood of including at least one infected animal in a pooled sample. However, critical questions still need to be addressed relating to the effects of pooling on diagnostic sensitivity of a test used for a surveillance system supporting claims of disease freedom. Unfortunately, many of the pooling recommendations in the 2017 OIE Manual of Diagnostic Tests for Aquatic Animals are incomplete and not supported by peer-reviewed studies. No clear patterns were evident for pooling methods and characteristics from our systematic review of peer-reviewed aquatic diagnostic accuracy studies (DAS) using pooled animals (only 9 DAS with surveillance purposes out of 73 papers were identified). Therefore, the purpose of our study was to discuss pooling and interpretation of pooled sensitivity in DAS with surveillance purposes. A practical flowchart of pooling guidelines was developed that would be useful for peer-reviewed journals and for research institutions studying the comparative accuracy of individual and pooled tests for surveillance of infectious diseases of aquatic animals.

Conference Session Designation: **(** Diagnostics & Quality Assurance)
Presentation Format: (Oral)

A Molecular Survey of Bacterial Fish Pathogens in Nile Tilapia (*Oreochromis niloticus*) Hatcheries in Costa Rica

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Streptococcus spp., *Edwardsiella* spp. and *Francisella noatunensis* subsp. *orientalis* (Fno) are some of the most important fish pathogens affecting global tilapia (*Oreochromis* spp.) aquaculture. In Costa Rica, the aquaculture industry is dominated by fresh-water cultured Nile tilapia (*Oreochromis niloticus*), which is cultured in all seven provinces. At present, very little is known regarding the diversity of fish pathogens present in these systems and definitive diagnoses of agents associated with disease outbreaks are rare. To evaluate the prevalence of common pathogens within these systems, this study employed multiplex real-time PCR assays targeting several bacterial pathogens as a diagnostic and surveillance tool. In 2017, seven different tilapia hatcheries were visited, and 350 fingerlings were subjected to a complete necropsy and molecular diagnosis. Fish presenting with gross signs of disease were subjected to histological and microbiological analysis. For the first time, *Edwardsiella anguillarum* was recovered and molecularly confirmed from diseased tilapia in Costa Rica. Additionally, *Francisella noatunensis* subsp. *orientalis* was identified in a region of Costa Rica it had not been previously reported.

Conference Session Designation: **(** Aquatic Epidemiology)
Presentation Format: (Oral)
Student Presentation: (Yes)

Mathematical Modeling to Optimize Mitigation Strategies Against *Ciona intestinalis* in Mussel Production

Thitiwan Patanasatienkul *, Crawford W. Revie, Jeff Davidson, and Javier Sanchez

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Over the past two decades, the Prince Edward Island (PEI) mussel industry has been challenged with the infestation of invasive tunicate species, which foul mussel socks and culture gear, causing significant economic losses to the industry due to added production costs of biofouling control. Field experiments to find suitable mitigation strategies require considerable time and are resource intensive. We applied a mathematical model to assess several control strategies against *Ciona intestinalis* populations in PEI. A temperature dependent compartmental model incorporating environmental carrying capacity was used to model the total abundance of *C. intestinalis*. A mitigation strategy was defined as a combination of timing and frequency of treatments. Various strategies were explored to obtain the combination that maximized the difference in predicted abundances between the untreated and the different mitigation strategies. Treatment frequency was allowed to vary between one to four times over a given production year. The model was assessed under baseline conditions, which mimicked water temperatures from Georgetown Harbour, PEI, in 2008, and under scenarios that reflected prolonged summer or warm spring temperatures. Furthermore, the sensitivity of the model to variations in presumed treatment efficacy was evaluated. The use of all four available treatments, starting around the first week of July and correctly timed thereafter, provided the most effective strategy, assuming the baseline temperature scenario. However, the effectiveness of this mitigation strategy depended on temperature conditions. The mathematical model developed in this study allows decision makers to optimize different strategies to control the abundance of *C. intestinalis* in mussel production areas under different environmental conditions. In addition, the modeling framework developed can be adapted to simulate similar ectoparasitic infestation in aquatic environments.

Conference Session Designation: **(** Aquatic Epidemiology)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Wednesday September 5th – Tilly / Tupper
Antibiotic Use / Pharmacology
Moderator - Patricia Gaunt (Mississippi State University)

3:15 PM	**Antibiotic Use / Pharmacology**	Gaunt – Benefits vs.Costs of Antibiotic Medicated Feed Use vs. Unmedicated Feed Use During Bacterial Outbreaks in Pond-Reared Warmwater Fish
3:30 PM		Blair – USFWS Aquatic Animal Drug Approval Partnership Program
3:45 PM		Geiseker – Setting Epidemiological Cutoff Values for Monitoring Antibiotic Resistance Oof *Aeromonas hydrophila* Isolates Collected from Fish
4:00 PM		Oyebanji – Knowledge of Antibiotic Resistance Among Fish Farmers in Oyo Town, Nigeria
4:15 PM		**Pravdova – Association Between Pharmaceuticals and Parasite Infection in Natural Brown Trout (*Salmo trutta*) Populations**
4:30 pm		Sidhu – Comparative Pharmacokinetics of Oxytetracycline in Tilapia *Oreochromis* spp. Maintained at Three Different Salinities
4:45 PM		Karadzovska – Effect of Medicated Feeding Period on the Efficacy of a Potential Sea Lousicide Containing Lufenuron
5:00 PM		Zargar – The Effect of *Echinacea purpuria* and *Cinnamomum verum* on Some Immune Parameters of Rainbow Trout (*Oncorhynchus mykiss*)

Benefits Vs. Costs of Antibioitc Medicated Feed Use Vs.Withholding Feed During Bacterial Outbreaks in Pond-Reared Warmwater Fish

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Bacterial disease outbreaks in pond-reared fish are frequently experienced during growing season when fish are being fed to achieve maximal growth rate by fall. When there is detection of disease associated with a bacteria susceptible to antibiotics, frequently farmers will treat with medicated feed. This presentation will assess the benefits vs. costs of treating with medicated feed, withholding feed, and unmedicated feed for control of mortality associated with bacteria in pond-reared fish.

Early signs of bacterial disease in fish can range from anorexia to morbidity such as erratic swimming to acute mortality. When the fish are diagnosed, the farmer must decide on treatment options. Should he use medicated feed containing an antibiotic that the bacteria are susceptible to? Should he feed unmedicated feed to the appetent fish so that he will have fewer, but larger fingerlings by fall? The advantages and disadvantages of these strategies will be explored in this presentation. A partial budget analysis will be performed to explore the economic effects of specific medicated and therapeutic treatments.

Conference Session Designation: (Antibiotics)
Presentation Format: (Oral)

USFWS Aquatic Animal Drug Approval Partnership Program

Marilyn "Guppy" Blair[1*], Bonnie Johnson[1], Niccole Wandelear[1], Molly Bowman[1]

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The mission of the U.S. Fish and Wildlife's (USFWS) Aquatic Animal Drug Approval Partnership Program (AADAP) is to obtain U.S. Food and Drug Administration (FDA) approval of safe and effective new medications for use in aquaculture and fisheries management. As the only program of its kind in the U.S., the AADAP team works with other Federal, State, Tribal, University, and private sector partners from across the country to administer the National Investigational New Animal Drug (INAD) Program, conduct research to support New Animal Drug Approvals (NADAs), and provide for drug and chemical use information dissemination. This presentation will give an overview of the drug approval process as well as an update of the current status of drugs approved by FDA or currently available under an INAD exemption for aquaculture in the U.S.

Conference Session Designation: (Aquatic Animal Health Management)
Presentation Format: (Oral)

Setting Epidemiological Cutoff Values for Monitoring Antibiotic Resistance of *Aeromonas hydrophila* Isolates Collected from Fish

Charles M Gieseker[1*], Patricia S Gaunt[2], John P Hawke[3], Tina C Crosby[1], Nicholas R Hasbrouck[1], Cynthia B Stine[1], Christopher Grim[4]

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Antimicrobial resistance is a major public health issue that has created concerns about the use of antibiotics in aquaculture. Therefore, laboratories need standard methods and criteria called epidemiological cutoff values (ECVs, a.k.a. ECOFFs) to monitor for the development of resistance. ECVs are a critical part of standard susceptibility tests as they are used to interpret if an isolate has lost susceptibility to an antibiotic. To create ECVs for the pathogen *Aeromonas hydrophila*, we gathered 286 isolates from various fish health laboratories and confirmed the isolates identity with *rpoD* and/or *gyrB* gene sequencing. One hundred four isolates were confirmed as *A. hydrophila*. Using Clinical Laboratory Standard Institute (CLSI) guidelines, we tested the susceptibility of these isolates with standard minimal inhibitory concentration (MIC) and zone of inhibition (ZOI) testing against eight antibiotics: erythromycin, florfenicol, gentamicin, oxytetracycline, enrofloxacin, oxolinic acid, ormetoprim / sulfadimethoxine, and trimethoprim / sulfamethoxazole. We then analyzed frequency distributions for each antibiotic to estimate a cutoff value which separates the wild-type isolates without resistance from the non-wild-type isolates that have developed resistance. We determined a cutoff value for six of the eight antibiotics tested. No ECV was estimated for the erythromycin ZOI due to excessive intra-laboratory variation. ECVs were not estimated for the two potentiated sulfonamides since the potentiator could mask sulfonamide resistance. The ECVs proposed from this study are being reviewed by CLSI to be included in a guideline for standard testing of aquatic bacteria. If approved, the ECVs will be included in the next revision of the guideline. Standard test methods and interpretive criteria allow for effective surveillance of antibiotic resistance, promoting judicious use of antibiotics that farmers need for managing the health of their fish.

Conference Session Designation: **(** Antibiotic Use / Pharmacology)
Presentation Format: (Oral)

Knowledge of Antibiotic Resistance Among Fish Farmers in Oyo Town, Nigeria

Bukola, O. Oyebanji

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Antibiotic resistance is one of the biggest threats to global health, food security, and development today. This survey was carried out to test the knowledge of antibiotics use and antibiotic resistance of fish farmers in Oyo state, Nigeria. Thirty-four fish farmers were interviewed with the aid of a structured questionnaire. The data generated were analyzed using SPSS data package software version 14 for descriptive statistics. Rates were computed and the results were tested for responses using correlation analysis. 71% of the respondents were male while 29% were females. Majority (44.12%) of the farmers were between the age range of 41-50 years, and 58.8% were married. 44.2% of the respondents had high school certificate as the highest level of education attained while 5.88% had no formal education.64.7% were into small scale production, while 5.88% were into large scale production. 79.4% said it was easy for them to have access to antibiotics, 14.9% said it was very easy for them to have access to antibiotics, 2.99% said they find it difficult.55.8% got their antibiotics from their farm consultant, 17.65% from mobile salesman and 26.47% from shop. Oxytetracycline was the most used antibiotics followed by streptomycin while penicillin was the least used. 76.4% of the respondents disagreed that antibiotic resistance is a problem in Nigeria while 47.1% did not agree that the issue could affect them.64.7% did not agree resistance could spread from person to person. In conclusion, there is a need for concerted effort to educate the fish farmers in this region on use of and resistance to antibiotics. There should also be restriction on access to antibiotics and standard treatment guidelines to control antibiotics use.

Conference Session Designation: (Antibiotic Use / Pharmacology)
Presentation Format: (Oral)

Association Between Pharmaceuticals and Parasite Infection in Natural Brown Trout (*Salmo Trutta*) Populations

Markéta Pravdová[1,2]*, Markéta Ondračková[1], Jitka Kolářová[3], Kateřina Grabicová[3], Tomáš Randák[3] and Pavel Jurajda[1]

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Contaminants in the natural environment are known to affect aquatic biota. In recent years, the role of pharmaceutical contaminants has grown in importance due to the ever increasing amounts released into the environment and their biological activity. Sewage treatment plants represent one of the most important sources of such contaminants. In addition to direct negative impacts on fish health, pollutants can also have a more indirect impact on another natural stressor, parasites. High pollutant concentrations can lead to a lowered immune response, resulting in higher parasitic infection. On the other hand, pollutants can also negatively affect free living parasite stages, thereby reducing parasite abundance. In this study, I assess the association between pharmaceuticals and parasite infection in a natural brown trout (*Salmo trutta* m. *fario*) population. Fish were obtained from the Zivny stream (Czech Republic) upstream (control) and downstream (polluted) of a sewage treatment plant known to release pharmaceuticals. Fish length and condition parameters (condition factor, hepatosomatic index, spleen somatic index, gonadosomatic index) did not differ between sites. Of the 79 pharmaceuticals measured, 42 were detected in fish tissue, with highest concentrations found in liver, followed by kidney and brain. Antibiotics and antidepressants dominated at both localities, with concentrations significantly higher at the downstream polluted locality (together with beta-blockers). CNS stimulants showed similar concentrations at both sites. There was a negative relationship between overall pharmaceutical load and fish condition factor and hepatosomatic index at the polluted locality, while parasite abundance increased with overall pharmaceutical load at both localities. Fish were infected by four parasite species: two monogeneans *Gyrodactylus derjavinoides* and *Gyrodactylus truttae*, one nematode *Salmonema ephemeridarum* and one trematode *Crepidostomum metoecus*. Ectoparasite abundance was higher at the polluted site, while endoparasite abundance was higher at the control site. The higher number of gyrodactylids at the polluted site probably reflects an increased parasite reproduction rate under stressful conditions. As macrozoobenthos density (including the intermediate hosts of the target parasites) was higher at the polluted locality, we hypothesise that the decrease in endoparasites potentially resulted from as yet unidentified negative effects of pharmaceutical contaminants on the parasite's free living stages.

Conference Session Designation: (Parasitology General)
Presentation Format: (Oral)
Student Presentation: (Yes)

Comparative Pharmacokinetics of Oxytetracycline in Tilapia (*Oreochromis spp.)* Maintained at Three Different Salinities

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The pharmacokinetics of a drug in fish can be affected by environmental factors such as temperature, pH, and water salinity in which the animals are maintained. This can affect the plasma concentrations and clearance rates of drugs used in fish, and as a result can affect the withholding times of treated fish used for human food. The purpose of this study was to generate pharmacokinetic data on oxytetracycline (OTC) in tilapia (*Oreochromis sp*p.) maintained in three different salinity environments. Juvenile tilapia (mean weight 122±0.9 gm) were divided into three groups (n =138) and acclimated to and maintained in three separate recirculation systems with three different aquatic environments: freshwater (0 ppt salinity), brackish water (15 ppt salinity) and salt water (30 ppt salinity). Water quality parameters (temperature, ammonia, nitrites, nitrates, pH and salinity) were monitored on a daily basis and a standard pelleted tilapia feed was provided to the fish at a daily rate of 3% body weight. Fish were fasted 24h prior to oral gavage of OTC (Bio-Mycin 200) at a dose rate of 50mg/kg. At 0 time (control), 6 fish from each group were sedated with buffered MS-222, bled from the caudal tail vessels and then euthanized. After OTC administration to the remaining fish, blood samples from six fish in each group were collected at 23 additional time points (0.25, 0.5, 1, 2, 4, 6, 9, 12, 24 h, and 2, 4, 6, 8, 10, 12, 14, 18, 22, 26, 30, 34, 38 and 42 days). Blood samples were placed in individual plasma separator tubes containing lithium heparin (BD Microtainer), centrifuged at 3000 x g for 10 minutes and the plasma samples separated and frozen at -80^{0}C until analysis. Oxytetracycline was extracted from fish plasma by Solid Phase Extraction (SPE) and an ultra-high-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method was used to determine OTC concentrations. Pharmacokinetic data were analyzed using a non-compartment method. The mean plasma peak levels of OTC were 1.221±0.124, 1.343±0.212 and 1.220±0.257 µg/ml in the freshwater, brackish and salt water tilapia, respectively. Plasma concentrations in salt water tilapia were lower than freshwater and brackish water fish at all time points after C_{max}. The T_{max} was 6h in salt water tilapia compared to 8h and 12h in freshwater and brackish water tilapia, respectively. The $AUC_{0\infty}$ in salt water tilapia (55.5h.µg/ml) was ~3 times lower than the values obtained for freshwater (165.3h.µg/ml) and BW (144.6h.µg/ml) tilapia. The terminal half-lives of OTC in freshwater, brackish water and salt water tilapia were 176h, 154h and 69.3h, respectively. This study suggests that in tilapia, the pharmacokinetics of OTC differs with water salinity conditions as the drug clearance rates in freshwater (0.293L/h/kg) were remarkably lower than in salt water (0.878L/h/kg). This is the first study reporting OTC pharmacokinetic differences between freshwater, brackish water and salt water in the same species of fish.

Conference Session Designation: (Antibiotic Use / Pharmacology)
Presentation Format: (Oral)

Effect of Medicated Feeding Period on the Efficacy of a Potential Sea Lousicide Containing Lufenuron

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The proposed product, code-named AH-2178, contains 10% lufenuron and is dosed *via* medicated feed to young Atlantic salmon in freshwater for a target 7 days while fish are at the hatchery. The total dose to be offered is 35 mg/kg. Field studies in several countries indicated that erratic fish feeding behavior in certain situations may require extended feeding periods (up to 14 days) to ensure the dose is accepted. A large-scale field study was therefore conducted at a commercial fish farm in Canada to confirm that drug uptake and/or efficacy were not affected by increasing the feeding period while maintaining the total dose at 35 mg/kg. Approximately 422,000 salmon were included as either treated groups with dosing regimens of 7, 10 or 14 days (noting a feeding error saw the 7-day group fed for 8 days) or as untreated controls. Once medicated feeding was finished the salmon were transferred to the marine site where regular louse counts were undertaken until loss of protection was determined by comparison of treated groups to untreated controls. Fish from each group were sacrificed and fillets analyzed for lufenuron resides at 3 days post-treatment (DPT) and the end of the study (251 DPT). Statistical differences in lufenuron concentrations in fillet between the treatment groups were analyzed using analysis of variance, using the Tukey's method of adjustment of the p-values. There were no statistically significant differences in lufenuron concentrations between the dosing regimens of AH-2178 at 3 or 251 DPT. The duration of efficacy against *Lepeophtheirus salmonis* for each regimen was determined to be 172–186 days (parasite stage dependent; efficacy, >95%, was still evident at 228 days although the result could not be used in the statistical analysis due to reduced louse burdens on controls). Duration of efficacy against *Caligus elongatus* couldn't be estimated for the same reason. The administration of 10% lufenuron in medicated feed at 35 mg/kg over 7–14 days was well tolerated and the dosing period had no impact on efficacy.

Conference Session Designation: (Parasitology Sea Lice – Ectoparasites)
Presentation Format: (Oral)

The Effect of *Echinacea Purpuria* and *Cinnamomum Verum* on Some Immune Parameters of Rainbow Trout (*Oncorhynchus Mykiss*)

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The aim of this study was to investigate the effect of purple coneflower (Echinacea) and cinnamon extracts alone and together on some immune parameters of rainbow trout for 60 days rearing period. Nine hundred specimens (39.5 ± 0.5 g) were randomly allocated into 18 fiber glass tank (4100 L) at a density of 50 fish per tank (150 fish per treatment). Fish in the first and second groups were fed diet supplemented with echinacea (1 and 1.5 gram per kilogram feed). The third and fourth groups were fed diet supplemented with cinnamon (1 and 1.5 gram per kilogram feed). The fifth group were fed diet supplemented with echinacea and cinnamon together (1 gram per kilogram feed from each one) and the last group fed basal diet. The leukocyte counts, serum lysozyme and complement, serum biochemical factors, total IgM were measured in fifteen days interval till the end of the experiment. The results revealed that feeding trout with 1.5 g kg-1 echinacea remarkably elevated the immune parameters tested ($P<0.05$). Group fed cinnamon also showed increased immune parameters compared to control group; however, the difference was not significant ($P>0.05$). The results also indicated that there is no synergistic effect between echinacea and cinnamon extracts on immune parameters of rainbow trout.

Conference Session Designation: (Pharmacology / Medicine / Immunology)
Presentation Format: **(** Oral)

Wednesday September 5th – Archibald / Campbell
Ornamental and Aquarium Medicine 1 & 2
Moderator - Johnny Shelley (USDA – Agricultural Research Service)

9:30 AM	**Ornamentals 1**	Shelley - Edwardsiellosis in Ornamental Fish
9:45 AM		Smith - An Outbreak of *Cryptobia iubilans* in a Captive Population of Mayan Cichlids *Cichlasoma urophthalmus*
10:00 AM		Kim - Use of the Microalgae *Phaeodactylum tricornutum* for the Remedy of Monogenean Infections: Treatment of *Gyrodactylus turnulli* in guppies
10:15 AM		Miller-Morgan - Ensuring Health Throughout the Supply Chain: Developing a Supply-Chain Health Management Training Program for a Wild-Caught Aquarium Fishery in Brazil – A Case Study
10:30 AM		**Refreshments**
10:45 AM	**Ornamentals 2**	**Scherbatskoy - Finding Nemo's Picornavirus**
11:00 AM		Adamek - Is there a Difference in Virulence Between Carp Edema Virus from Different Genogroups?
11:15 AM		**Koda - Phylogenomic Characterization of Megalocytiviruses in Archived Ornamental Fish Samples**
11:30 AM		**Munday - The Effects of Venting, Transport, and Holding Methods on Yellow Tang (*Zebrasoma flavescens*) Health in the Marine Ornamental Aquarium Fish Trade**

Edwardsiellosis in Ornamental Fish

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Historically *Edwardsiella ictaluri* has been primarily associated with Channel Catfish, *Ictalurus punctatus*, aquaculture and the food fish industry. It is the causative agent of enteric septicemia of catfish (ESC) and in the United States is the most economically important infectious disease in farm-raised catfish. Once considered a host-specific pathogen of catfish species in the US, it has since been isolated from non-ictalurid species in natural or experimental infections from the US and internationally. *Edwardsiella ictaluri* appears to have a history within the ornamental fish industry as well, with sporadic reports in the 1980s. In 2013, Hawke *et al.* described a new strain of *E. ictaluri* as an emerging pathogen of zebrafish based on eight cases from five states that were submitted to the Zebrafish International Resource Center (ZIRC) and the Aquatic Disease Section of the Louisiana Animal Disease Diagnostic Laboratory (LADDL) between 2011 and 2012. The fish exhibited exophthalmia along with hemorrhage in the skin around the eyes, operculum, base of fins and the abdomen. Additionally, they had swollen abdomens due to ascites and they displayed neurologic swimming behaviors such as spinning, spiraling and lethargy. The zebrafish strain of *E. ictaluri* is believed to be unique compared to the catfish strain and the tilapia strain described by Soto *et al.* in 2013. Since 2011, the species that the zebrafish strain has been known to infect has expanded to include all the varieties of *D. rerio*, as well as the Blue Danio (*Danio kerri*), the Leopard Danio (*Danio frankei*) and the Giant Danio (*Devario aequipinnatus*). With the expansion of susceptible species, *E. ictaluri* is now recognized as an important pathogen of zebrafish and control methods are under investigation. Future works with the zebrafish strain of *E. ictaluri* will focus on comparing it against the channel catfish strain at the molecular level and the serological level for common and unique features related to virulence and infection. Additionally, the effectiveness of different disease management strategies will be investigated to help the ornamental fish industry combat the spread of the zebrafish strain of *E. ictaluri*.

Conference Session Designation: **(** Ornamentals and Aquarium Medicine)
Presentation Format: **(** Oral)

An Outbreak of *Cryptobia Iubilans* in a Captive Population of Mayan Cichlids (*Cichlasoma Urophthalmus*)

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Significant mortality was observed in a captive population of juvenile (~3 month old) Mayan cichlids (*Cichlasoma urophthalmus*) over a two-week period of time. The tanks with the Mayan cichlids were part of the multi-tank recirculation system (50 tanks x 15 gal) with mechanical and biological filters which also had a 10-20% water exchange per day with municipal water that was treated with carbon filters for dechlorination. Clinical signs prior to death included a distended abdomen, loss of equilibrium and erratic swimming behavior. During this time the fish were treated with chlortetracycline (50-100 mg/L) bath for 10 days and then a penicillin/streptomycin bath (0.5 mg/L) for several days. Despite these treatments, an increasing number of fish continued to demonstrate clinical signs and experience mortality. Other adult cichlid (e.g. red head, *Cichlasoma synspilum* and midas, *Amphilophus citrinellum*) and non-cichlid fish (e.g. zebrafish, pacu, and arowana) in separate tanks of the same recirculation system did not experience any clinical signs or mortality. Four morbid fish were humanely euthanized with buffered MS-222 and a complete necropsy of each fish performed. Samples of representative tissues were preserved in 10% neutral buffered formalin, trimmed and submitted to the Virginia-Maryland College of Veterinary Medicine for histopathology. In all four fish, there were multiple discrete granulomas within the wall of the stomach. In addition, there were a number of extraluminal granulomas surrounding the stomach that extended into the coelomic cavity of several fish. A small number of flagellates were observed in the lumen of the stomach. All granulomas were acid-fast negative suggesting they were not caused by *Mycobacterium* spp. There was also diffuse degeneration and necrosis of both the tubules and glomeruli of the kidney from which *Citrobacter freundii* was isolated. All other tissues examined from these fish appeared normal. Based on the limited location (i.e. primarily stomach) of the granulomas, lack of acid-fast staining of the granulomas, the presence of flagellates within the lumen of the stomach and the limited pathology observed in other organs, a diagnosis of cryptobiosis caused by the protozoan parasite, *Cryptobia iubilans* was made. The Mayan cichlid (*C. urophthalmus*) represents a new host species for *Cryptobia iubilans*.

Conference Session Designation: (Ornamental / Aquarium)
Presentation Format: (Oral)

Use of the Microalgae *Phaeodactylum Tricornutum* for the Remedy of Monogenean Infections: Treatment of *Gyrodactylus Turnbulli* in Guppies

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Parasitic diseases are a major constraint to sustainable aquaculture production and product trade. Monogenean infestations in an intensive aquaculture system reach epizootic levels due to their direct life cycle and unnaturally high densities of the host fish. Traditional chemical treatments pose associated problems, mainly human health-related risks, but also environmental concerns and resistance development. The common treatment against monogenean parasites, organophosphates, pose a risk of neural disorders in humans and this treatment was therefore recently banned in many countries.

In recent years regulations on the use of chemicals in aquaculture is becoming more stringent thus there is a growing trend and need on developing natural, therapeutants. *Phaeodactylum tricornutum* is a diatom microalga for which anti-bacterial effect against numerous bacterial species had been demonstrated. The current work aimed to test the potential of *P. tricornutum* as a treatment against a monogenean parasite of fish. *Gyrodactylus turnbulli*, monogenean infecting guppies were selected as a model for this study. An extract was prepared from *P. tricornutum* biomass using different solvents, including ethanol at different concentrations and ethyl acetate, with and without subsequent sonication and addition of a 1% of a food-grade detergent.

For *in vitro* analysis of the extracts, tail clips with up to 30 parasites were collected from heavily infected guppies and distributed between wells of 24 well plates. The effect of the extracts on *G. turnbulli* detachment from the tail fin and mortality of the parasite were analyzed by direct microscopic observation. Parasites on the tail fin clip of infected guppies were continuously observed after the addition of microalgal extracts, and time to detachment and death were recorded.

We present our findings on the efficiency of using the ethanolic extract of *P. tricornutum* against *G. turnbulli* at both *in vitro* and *in vivo* trails. At a concentration of 5 ppt, complete detachment and 97% mortality were achieved within 240 min, as analyzed *in vitro*. In a preliminary immersion treatment trial with infected guppies, the extract was effective at a concentration of 2.5 ppt, eliminating any evident parasites from the guppy tail fin within 24 h.

Results suggest that *P. tricornutum* - based preparations are potential natural therapeutants against monogenean infection in fish. Further research is focused on increasing the efficacy of the extract and evaluating its potential as a therapeutant against monogenean infection in guppies as well as additional monogenean species parasitizing fish.

Conference Session Designation: (Parasitology or Ornamentals & Aquarium Medicine)
Presentation Format: (Oral)

Ensuring Health Throughout the Supply Chain: Developing a Supply-Chain Health Management Training Program for a Wild-Caught Aquarium Fishery in Brazil – A Case Study.

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The aquarium fishery is the principal subsistence activity for the riverine communities in the municipality of Barcelos (Amazonas state, Brazil). For more than 25 years, Project Piaba has been working with the aquarium fishery of the Rio Negro. Very early on it was discovered that the capture of many of these species was not only sustainable, but it was the principal driver for creating value for the environment. Every year a small group of international fish health specialists, trade stakeholders, public aquarium biologists, and fish enthusiasts participate in an annual expedition to Barcelos and the fishing grounds. The outcomes of this program have led to a much better understanding of the role of this fishery and project members are helping the fishery adapt to changes in global markets.

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

Project Piaba partnered with the Aquatic Animal Health Program (AAHP) to initiate a project to identify the key factors impacting fish health throughout the chain-of–custody for the Rio Negro aquarium fishery. Utilizing this information, the AAHP team developed a train-the-trainer program that would train and provide local biologists to act as trainers and consultants for the Rio Negro fishers, transit station managers and exporters as they worked to improve the health and quality of their collected fish. The trainers are fisheries biologists and aquaculture specialists selected based upon their relationships with the local communities and their knowledge of the fishery and local environment. Once trained these trainers began offering training and consultation to local fishers and facility managers throughout the supply chain addressing health management techniques that would lead to improved fish health and quality. We will discuss the development and implementation of this program and some of the early outcomes.

Conference Session Designation: (Ornamental and Aquarium Medicine)
Presentation Format: (Oral)

Finding Nemo's Picornavirus

Elizabeth C. Scherbatskoy[1*], Kuttichantran Subramaniam[1], Lowia Al-Hussinee[1], Kamonchai Imnoi[1], Samantha A. Koda[1], Patrick M. Thompson[2], Deborah B. Pouder[3], Roy P.E. Yanong[3], Jeffrey C. Wolf[4], Vsevolod L. Popov[5], Terry Fei Fan Ng[6] and Thomas B. Waltzek[1]

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Over the last decade, a number of aquaculture facilities have suffered significant mortality events in their clownfish (*Amphiprion ocellaris* and *A. percula*). Clinical signs of disease include darkened body coloration, increased gilling, reduced body condition, and abnormal positioning in the water column. Diseased clownfish were processed for parasitologic, bacteriologic, histopathologic, and virologic diagnostic testing. No significant parasite burdens were detected, and while bacteria were isolated from some of the fish, they appeared to be more consistent with a secondary infection. Histopathological examination revealed prominent single cell necrosis and mild inflammation of the mucosal epithelium within the branchial cavity, pharynx, esophagus, and/or stomach. Homogenates from pooled external and internal tissues were inoculated onto striped snakehead (SSN-1) cells, resulting in complete lysis in the initial infection and upon subsequent passages. Transmission electron microscopy of infected SSN-1 cells revealed small (28-30 nm), naked, icosahedral particles within the cytoplasm, occasionally arranged in paracrystalline arrays, consistent with the ultrastructure of a picornavirus. The virus was concentrated by ultracentrifugation prior to RNA extraction, cDNA library generation, and sequencing using an Illumina MiSeq sequencer. Sequencing recovered the full genome of a novel picornavirus most closely related to those recently described from other fish hosts including common carp (*Cyprinus carpio*), eel (*Anguilla anguilla*), bluegill (*Lepomis macrochirus*), and fathead minnow (*Pimephales promelas*). Future challenge studies are planned to elucidate the clinical significance of this picornavirus in clownfish. Disease progression will be assessed by regularly sampling fish over the study period to assess gross and microscopic lesions (histopathology and *in situ* hybridization) as well as viral load (virus isolation and RT-qPCR) within external and internal tissues.

Conference Session Designation: **(** Ornamentals and Aquarium Medicine)
Presentation Format: **(** Oral)
Student Presentation: **(** Yes)

Is There a Difference in Virulence Between Carp Edema Virus from Different Genogroups ?

Mikolaj Adamek[1*], Verena Jung-Schroers[1], Felix Teitge[1], Max Heling[1], Veronika Piackova[2], David Gela[2], Martin Kocour[2], Jerzy Adamek[3], Sven Bergmann[4], Dieter Steinhagen[1]

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Koi sleepy disease (KSD) caused by infections with the carp edema virus (CEV) seems to pose a potential risk to carp aquaculture and koi trade. Sequence comparisons of virus isolates infecting fish in several European countries revealed the existence of two or three distinct genogroups. The genogroup I is predominantly found in cultured common carp while genogroup IIa is mostly associated with infections in koi. Our studies on imported koi suggest that genogroup IIa is constantly spread by koi trade form Japan. Viruses from genogroup IIa can be present in common carp and viruses from genogroup I in koi, but when detected, then in very low copy numbers. Therefore infection experiments with virus from these two genogroups of CEV were used to evaluate possible differences in infection biology of the virus while several carp strains were used to study influences of the genetic background of carp on their susceptibility to the infection. In an infection experiment with CEV from genogroup I Amur wild carp (AS) was less susceptible to the infection than Prerov scale carp (PS) or koi. When CEV from genogroup IIa was used all common carps (AS, PS, Rop) were far more resistant to the infection than koi. Analyses of behavioural, histopathological and molecular indicators of infection revealed differences in the virulence of the two CEV genogroups. Viruses showed higher virulence towards the same fish group as the donor fish (koi or in common carp) inducing rapid onset of KSD. The results from the study show that resistance to CEV infection is largely dependent on the genetic background of the carp. Furthermore significant differences in virulence and genetics of CEV genogroups rise questions about the geographical distribution of the genogroups and a better separation of these viruses by nomenclature.

Conference Session Designation: (Virology)
Presentation Format: (Oral)

Phylogenomic Characterization of Megalocytiviruses in Archived Ornamental Fish Samples

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The subfamily *Alphairidovirinae*, within the family *Iridoviridae*, includes three genera of double-stranded DNA viruses that infect poikilothermic vertebrates (e.g. fish, amphibians, and reptiles). The *Alphairidovirnae* genus *Megalocytivirus* (MCV) have been reported to infect >125 ornamental and food fish species in both freshwater and marine environments around the globe. Phylogenetic characterization of MCVs based on the conserved major capsid protein (MCP) supports three major genotypes: infectious spleen and kidney necrosis virus (ISKNV), red seabream iridovirus (RSIV), and turbot reddish body iridovirus (TRBIV). Recently, these three MCV genotypes have each been further subdivided into two clades based on phylogenetic analyses of the MCP. Using a pan-MCV primer set that targets the MCV myristylated membrane protein, archived samples of South American cichlids (keyhole cichlid *Cleithracara maronii* and oscar *Astronotus ocellatus*), three spot gourami *Trichopodus trichopterus*, blue chromis *Chromis cyanea*, and clownfish *Amphiprion ocellaris* from various facilities in the United States tested positive. Sanger sequencing of the purified PCR products revealed that the South American cichlid samples were infected with a TRBIV Clade 2, while the blue chromis and clownfish were both found to be infected with RSIV Clade 1. Histopathological examination of these cases revealed cytoplasmic basophilic inclusions compatible with those induced by MCVs within a variety of tissues, including hematopoietic tissues such as the spleen and anterior kidney. Transmission electron microscopy for the three spot gourami and blue chromis cases allowed identification of unenveloped, hexagonal virus particles with electron dense cores within the cytoplasm of infected cells, consistent with previous reports of the ultrastructure of MCV virus particles. Detection and molecular characterization of MCVs in freshwater and marine ornamental fishes traded in North America expands the known host range and genetic diversity of MCVs circulating in the region.

Conference Session Designation: (Ornamentals and Aquarium Medicine)
Presentation Format: (Oral)
Student Presentation: (Yes)

The Effects of Venting, Transport, and Holding Methods on Yellow Tang (*Zebrasoma Flavescens*) Health in the Marine Ornamental Aquarium Fish Trade

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Each year, 14-30 million fish are removed from coral reefs and enter the aquarium trade supply chain. Our objective was to examine this supply chain and determine how capture, transport, and holding methods affect fish health and mortality. To simulate the supply chain this, yellow tang fish (*Zebrasoma flavescens*) were captured from the wild offshore of the west coast of the island of Hawaii, USA, held in an active aquarium fish export facility for 4 days, transported by air to Portland, OR, and finally to the Hatfield Marine Science Center in Newport, OR, USA and held for 6 months. Plasma cortisol concentrations were used as a proxy for stress, and were determined when fish were first brought to the export facility, immediately before air transport, and after 6 months in captivity. Baseline cortisol was determined by collecting blood from fish on SCUBA. Reflex Action Mortality Predictors were also used to determine if reflex impairment could predict later mortality or health problems in fish. Water quality was monitored and compared between 3 active export facilities. Plasma cortisol decreased while fish were in the export facility (from day 0 to 4), and decreased to baseline concentrations after 6 months in captivity. Because all fish survived, we were unable to predict mortality using RAMP scoring. Our work demonstrates that fish plasma cortisol returns to baseline after fish are held in captivity, suggesting that captive fish held in an appropriate environment are able to acclimate to a captivity and are not stressed.

Conference Session Designation: (Ornamental Diseases)
Presentation Format: (Oral)
Student Presentation: (Yes)

Wednesday September 5th – Archibald / Campbell
Nutrition and Fish Health
Moderator - Andre Dumas (Center for Aquaculture Technologies)

1:45 PM	Nutrition and Fish Health	da Silva - Health Modulation through Nutrition
2:00 PM		Galagarza - Uncovering the Effects of a Dietary Supplementation of *Bacillus subtilis* Strains for Improved Fish Health
2:15 PM		Jakob - Functional Diet for the Control of *Piscirickettsia salmonis*
2:30 PM		Papanna - A Pathology Case Study of Cultured *Pagrus pagrus* in the Mediterranean-Ionian Sea of Greece
2:45 PM		Trullas - Effect of Jerusalem Artichoke-Prebiotic Supplemented Diets on Growth Performance and the Expression of Antioxidant Related Genes in Juvenile Red Tilapia.

Health Modulation Through Nutrition

Polyana F. da Silva*, Julia Mullins, Carlos Zarza, Linda Jensen, Charles McGurk

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The aquaculture industry's major economic losses have to date, been primarily due to disease, which remains a key constraint to its continued growth. Although the development of efficacious vaccines has mitigated several bacterial and viral syndromes, such an approach has not proven successful in countering several key fish pathogens, whereas crustaceans do not even possess the immune pathways necessary for traditional vaccination approaches. Also, there is still heavy reliance on the administration of therapeutic agents, with ongrowing concern for the antimicrobial resistance against the limited portfolio of chemotherapeutants currently authorised. Consequently, there has been increased focus on holistic integrated management programmes, within which the benefits of high quality functional feeds become more apparent.

It is widely recognised that nutritional modulation can have a profound effect on the overall performance of fish, and that certain feed additives, besides satisfying the dietary nutrient requirements for maximum growth, can reduce the impacts of pathogenic diseases by supporting inherent immune defences, countering oxidative stress while also limiting pathogen replication and shedding while supporting recovery from infections. Consequently, functional nutrition has become established as a core component of best practice for health and welfare control in aquaculture globally.

Skretting's Aquaculture Research Centre has been focused on the development and validation of functional nutrition strategies for more than 30 years. The world's first commercial fish health diet "RESPONS" was successfully launched by Skretting Norway in 1992, with continued research since then, leading to a range of diets to mitigate negative health impacts in farmed fish and shrimp.

The effects and potential role of Skretting's functional feeds on the overall health performance of fish and shrimp constitue a major topic of this presentation.

Conference Session Designation: (Nutrition and Fish health)
Presentation Format: (Oral)

Uncovering the Effects of a Dietary Supplementation of *Bacillus subtilis* Strains for Improved Fish Health

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Aquaculture is one of the fastest growing food-producing sectors, with potential to address the future concerns of global food insecurity. Despite its major contributions to total fish production, the intensification and rapid expansion of aquaculture continue to be constrained by bacterial diseases, resulting in major economic losses to the industry. Given that traditional methods of antibiotic use remain controversial in the modern era, probiotics have been explored as an alternative method for both improved animal health and disease protection. The field of probiosis in aquaculture has advanced in recent years, but much remains to be revealed in terms of dosage, specific strains characterization, and mode of action of the biological agent.

To contribute to the body knowledge on application of probiotics for improved fish health, our laboratory has investigated the supplementation of direct feeding of spores of the strains of *Bacillus subtilis* O14VRQ and NZ86. The studies have consisted in dietary supplementation of the two strains in four different species of fish, including Pacific white shrimp (*Penaeus vannamei*), striped catfish (*Pangasius hypophthalmus*), Nile tilapia (*Oreochromis niloticus*), and giant pangasius (*P. sanitwongsei*). The work in Pacific white shrimp and striped catfish showed that, after challenge with either *Aeromonas hydrophila* or *Edwardsiella ictaluri*, mortalities and number of observed clinical signs of infection were significantly decreased ($p<0.05$). These results revealed the potential of both strains of *B. subtilis* to enhance disease resistance. Additionally, the study in tilapia confirmed stimulation of innate immunity by both bacilli strains, as a plausible mode of action. The work in giant pangasius, which is currently ongoing, is aimed to further validate the effects of strain NZ86 in the innate immunity of the host.

All together, these findings enhance the knowledge on the applicability of these two strains to help alleviate the problems of disease in aquaculture, which is vital for the continued growth of fish farming to achieve a healthier foodfish supply.

Conference Session Designation: (Nutrition and Fish Health)
Presentation Format: (Oral)

Functional Diet for the Control of *Piscirickettsia Salmonis*

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Bacterial diseases remain a problem in the aquaculture industry and although against some of them successful vaccine strategies have been implemented like *Aeromonas salmonicida* and *Vibrio spp*, others are more complicated to manage. Especially intracellular bacteria like *Piscirickettsia salmonis* and *Renibacterium salmoninarum* still require the use of antibiotics. For the Chilean salmon industry the control and management of *P.salmonis*, causative agent of SRS, is of major importance. The development of a functional diet to reduce the severity of the disease and therefore reduce the use of antibiotics is an important part of an integrated disease management strategy. Health diets have been assessed in the past against a number of viral pathogens including piscine reovirus (PRV) as well as salmon alpha virus (SAV). Optimization of dietary protein: dietary energy ratios was thought to be an important component of the improved protection observed. The aim of the study was to develop a dietary formulation that can significantly reduce the mortality in Atlantic salmon challenged with *P. salmonis*. The three test formulations contained optimized raw materials, varying digestible protein and digestible energy concentrations, as well as a mix of nucleotides and peptidoglycan as functional compounds. A cohabitation challenge using *P. salmonis* was conducted under controlled conditions at the Cargill Innovation Center at Colaco, Chile. A cohabitation infection model was used to allow for a more natural disease progression. Each diet group was evaluated in 4 replicates containing 120 fish each. The main outcome was survival and growth, however samples were taken for histopathology as well as gene expression. Results showed >30% increase in relative percent survival for the test diets compared to the control diet, as well as a significant increase in growth.

Conference Session Designation: (Nutrition and Fish Health)
Presentation format: (Oral)

A Pathology Case Study of Cultured *Pagrus pagrus* in the Mediterranean-Ionian Sea of Greece

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In recent years the farming of *Pagrus pagrus* in the Mediterranean is emerging as a new species of aquaculture-diversification under commercial on-growing, besides the traditionally cultured sea bass and sea bream. The currently adopted *Pagrus pagrus* species for farming in the Mediterranean appears to be a more desired species than the previously existed *Pagrus pagrus* species two decades ago, which has now disappeared and is non-existant in aquaculture. The presently adopted species of *Pagrus pagrus* grows faster and aquires a good colour pattern similar to any known brightly coloured Pagrus or red fish species. Further the morphological features of the currently farmed *Pagrus pagrus* seems to resemble that of Pagrus major cultured in Japan at present, both in terms of colour and shape making it more attractive for the farmers interested in diversification. For this reason its taxonomical status to be called as *Pagrus pagrus* synonymous with the old species is called in to question and there are proposals to review the taxonomic position of the current species and its name as *Pagrus pagrus*.

This conference presentation, describes a Pathology case study centered on the new *Pagrus pagrus* species cultured under commercial production conditions in Mediterranean Ionian sea based on new commercial fish feeds that are being tried and developed. Hatchery produced juveniles have been cultured since 2010, under commercial conditions fed on commercial feed pellets. While the production of the species on a small scale was appreciably good and encouraging in 2011, the fish stocked in 2011 and 2012 developed characteristic pathologies in the visceral organs in the size groups of around 200 to 500 gram fish. The clinical pathological picture was more drastic in the bigger size groups than the smaller size groups. While the liver spleen and the kidney were most affected in terms of pathological manifestations, other organ pathologies were also evident in the affected fish. This presentation will describe the findings of this case study and will highlight the potential causes for these pathological and histological changes observed.

Conference Session Designation: (Nutrition and Fish Health)
Presentation Format: (Oral)

Effect of Jerusalem Artichoke-Prebiotic Supplemented Diets on Growth Performance and the Expression of Antioxidant Related Genes in Juvenile Red Tilapia.

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An experiment was conducted to investigate the effect of Jerusalem artichoke-supplemented diet on the growth performance and the expression of antioxidant related genes in juvenile Red Tilapia. Red tilapia fish (average body weight of 14.08±0.53 g) were fed with basal (control, C), 5.0 g kg^{-1} JA-supplemented (5 JA), and 10.0 g kg^{-1} JA-supplemented (10 JA) for 4 weeks. Weight of the fish were measured at the beginning and end of the experiment. After feeding 4 weeks, the liver tissue were randomly collected. The results revealed that the growth performance the WG, SGR and ADG of fish fed with the 5 JA and 10 JA diets were significantly ($P < 0.05$) higher than for fish fed the control diet. The prebiotic diet (5 JA and 10 JA) showed significantly increase the expression of the *gpx1* gene (1.94- and 1.57-fold) and *gst* gene (3.53- and 4.2-fold) in Red tilapia. Moreover, the gene expression profiles of *gr* gene revealed that the fish fed the prebiotic diet (10 JA) showed significantly up-regulated by 3.17 fold. The expression analysis of *cat* and *sod* of fish fed the prebiotic diet (5 JA and 10 JA) were higher ($P > 0.05$) than those fed the control diet but were not statistically significant. Our study indicated that the Jerusalem Artichoke supplemented diets enhanced growth performance and, the expression of antioxidant related genes in juvenile Red Tilapia.

Conference Session Designation: (Nutritional and Fish Health)
Presentation Format: (Oral)

Wednesday September 5th – Archibald / Campbell
Emergent Disease 1
Moderator - Al Camus (University of Georgia)

3:15 PM	**Emergent Disease I**	Camus - Pathology of Emergent Infectious Diseases in the Ornamental Fish Industry
3:30 PM		Becker - Risks to Australia's Biosecurity from the trade of Ornamental Fish
3:45 PM		Forwood - ~~Border Control – Stopping the Spread of Emerging Diseases~~ **(WITHDRAWN)**
4:00 PM		Surachetpong - Implementation of Biosecurity to Limit the Spreading of Emerging Diseases in Tilapia Farms
4:15 PM		Soto - *Erysipelothrix rhusiopathiae* spaB, an Emerging Pathogen of Cultured Barramundi, *Lates calcarifer*
4:30 pm		**Stilwell - First Detection of *Erysipelothrix* sp. Infection in Western Mosquitofish, *Gambusia affinis*, from Channel Catifsh, *Ictalurus punctatus*, Ponds in Mississippi**
4:45 PM		**Armwood - Molecular Characterization and Histopathology of *Edwardsiella anguillarum* Infections in Nile Tilapia (*Oreochromis niloticus*)**
5:00 PM		Griffin - *Edwardsiella piscicida*, an Emergent Pathogen in Farmed Channel ♀, *Ictalurus punctatus* x Blue ♂, *Ictalurus furcatus* Hybrid Catfish Cultured in Mississippi

Pathology of Emegent Infectious Diseases in the Ornamental Fish Industry

Alvin C. Camus[1*], Abigail R. Armwood

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International trade in ornamental fish, is a multibillion dollar industry that contributes significantly to the local economies of several countries. The United States and the European Union represent the world's two largest import markets. Involving both capture and culture fisheries, less than 10% of marine ornamental fish are captive bred, while a larger proportion of freshwater fish are cultured. The industry is multifaceted, involving local collectors, producers, and dealers, exporters and importers, wholesalers, retailers, and shippers. Although import/export regulations have increased at various international, national, and state levels, health surveillance and biosecurity measures are often limited. Lack of disease monitoring coupled with the inherently stressful nature of capture, holding, and shipment favor the development and dissemination of infectious disease. This report characterizes disease outbreaks diagnosed since 2010 by the University of Georgia's Aquatic Pathology Service, involving four pathogens emerging in the ornamental fish trade. The agents include *Francisella noatunenis* subsp. *orientalis*, *spa*C-type *Erysipelothrix* sp., megalocytivirus, and betanodavirus. Franscisellosis was diagnosed in four groups of damselfish (*Chromis viridis*, *Chrysiptera springeri*) or fairy wrasse (*Cirrhilabrus* spp.) species. Characterized by disseminated granulomas and granulomatous inflammation, lesions are typified by macrophages containing the small, gram-negative coccobacilli within intracytoplasmic vacuoles. An *Erysipelothrix* sp., distinct from *Erysipelothrix rhusiopathiae*, was isolated in several diseased tetra (Family *Characidae*) species with necrotizing dermatitis and myositis. Acute lesions contained massive numbers of gram-positive bacterial rods that have distinct tropism for connective tissues. Six cases involving freshwater (*Trichopodus leeri*, *Mesonauta festivus*, Family *Cichlidae*), brackish water (*Toxotes chatareus*) and saltwater (*Pomacanthus xanthometopon*) fish species had histologic features of megalocytivirus, Family *Iridoviridae*, infection characterized by cytomegalic inclusion bodies, typically adjacent to vascular lumens, in multiple organs. Betanodavirus infections caused two outbreaks in species of anthias (*Pseudoanthias* spp.) fish. Histologically, vacuolar degeneration was present in retinal lesions and brains. The ongoing emergence of pathogens in the ornamental fish trade emphasizes the need for routine surveillance and trained professionals to recognize their diagnostic features, as well as pursue appropriate confirmatory testing.

Conference Session Designation: (Ornamental Diseases)
Presentation Format: (Oral)

Risks to Australia's Biosecurity from the Trade of Ornamental Fish

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The ornamental fish industry presents a high risk to Australia for introducing exotic aquatic pathogens of international significance with several documented occurrences. Notably, these include the megalocytivirus, *Infectious spleen, and kidney necrosis virus* (ISKNV), cyprinid herpesvirus 2 (CyHV-2) and *Edwardsiella ictaluri*, with the latter two now considered endemic in some wild fish populations. Nearly 18 million ornamental fish are imported annually to Australia under a policy based on an Import Risk Analysis published in 1999. Recently, there has been particular interest in the risk associated with imported ornamental fish infected with the megalocytiviruses, ISKNV and red sea bream iridoviurs (RSIV). The objective of this project was to determine if aquatic pathogens of potential biosecurity concern are entering Australia through the trade in ornamental fish.

Repeated cross sectional surveys were undertaken in imported freshwater and marine ornamental fish under quarantine prior to entry into Australia. They were tested for the presence of nationally listed aquatic viral and bacterial pathogens and to identify external and internal parasite assemblages. A design prevalence of 2% to 10% was used depending on specific pathogen and diagnostic test. Fish hosts were prioritized based on prior knowledge of infection with the listed pathogens, volumes of importation to Australia and current import conditions.

Testing was completed on 62 populations of fish representing 12 consignments received from five different countries. We detected viruses of biosecurity concern, including ISKNV-like megalocytiviruses and viral nervous necrosis viruses (NNV). About 52% (24/46) of the populations tested for ISKNV were positive, which included five species of marine fish. NNV was detected in 13% (3/23) of marine fish, with all positive populations received from Indonesia. There was no evidence of koi herpesvirus (CyHV-3), spring viremia of carp virus (SVCV), viral hemorrhagic septicemia virus (VHSV), *Aeromonas salmonicida* or *Edwardsiella ictaluri*. The parasite assemblages found on pre-import ornamental fish were diverse and abundant. Despite the import conditions requiring freedom, many fish, in particular goldfish (*Carassius auratus*) from several countries were heavily infected with freshwater dactylogyrid gill trematodes.

The risk imported ornamental fish present to Australian aquatic animal industries and natural resources was high with respect to megalocytiviruses and parasitic agents. Recommendations to support revision to Australia's national biosecurity policy were made so that appropriate regulations can be put in place to manage the risk.

Conference session: (Emergent Diseases)
Presentation format: **(** Oral)

Border Control – Stopping the Spread of Emerging Diseases (WITHDRAWN)

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Biosecurity has played a critical role in reducing risk and placing Australia as one of the few countries in the world to remain free from the world's most severe agricultural diseases. While our geographical isolation has played a key role in maintaining this status, the objective is now to maintain freedom from these diseases in an environment of increasing global trade while meeting our international trade agreement obligations.

To reduce the risk of introducing an exotic disease with imported goods, countries often undertake formal risk assessments as prescribed by the OIE, from which appropriate risk management measures are developed and applied by the importing country, to reduce the biosecurity risk with the trade in aquatic animals and their products to an acceptable level. Risk assessments are complex and resource intensive and can only account for the diseases known at the time of the assessment. Unless new scientific evidence is acquired these risk assessments are often not revised due to the resources required to undertake such reviews.

Emerging animal diseases are a considerable risk for regulators, because a risk assessment is a snapshot of the perceived risks at a point in time, based on available knowledge and research and emerging diseases are often unknown and not considered, or there is limited scientific information available when developing the assessment. Therefore, the development of suitable risk management measures may over time lose their efficacy for maintaining the appropriate level of protection for the safe trade of susceptible species. The development of suitable risk management measures requires vigilance in the pursuit of new and emerging scientific evidence, and the regulated review of risk assessments as appropriate.

One challenge in developing suitable risk management measures for an emerging disease is the variability among trading partners of their willingness or capacity to conduct the necessary research and effectively communicate findings with other trading partners. Effective communication between trading partners on emerging diseases will facilitate importing countries to better evaluate the efficacy of their risk assessments, and if necessary, undertake the formal review and revise risk management measures accordingly. This can also be mutually beneficial for all parties with the potential for collaborative research between trading partners and the sharing of intellect acquired.

Improved communication between trading partners can not only strengthen the disease status of the importing country, viability of susceptible domestic industries and protect the environment, but also limit the geographical spread of the emerging disease and reduce the global spread and impact of the disease. Australia's approach to managing the emergence of dwarf gourami iridovirus and related viruses in freshwater ornamental finfish will be discussed.

Conference Session Designation: (Emergent Disease)
Presentation Format: (Oral)

Implementation of Biosecurity to Limit the Spreading of Emerging Diseases in Tilapia Farms

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Biosecurity is a standard practice that aims to limit the introduction and spread of pathogen in the production environment. For tilapia production, recent emerging of viruses and bacterial diseases such as Tilapia Lake Virus (TiLV) and *Streptococcus agalactiae* have been reported in Nile tilapia and red hybrid tilapia in different parts of the world. These emerging pathogens associate with high mortality of 80-90% within 1-2 weeks after the disease has been observed. Importantly, the pathogens may spread horizontal and/or vertical with infected fish. For example, a recent study by our laboratory suggested that TiLV could be detected in the mucus of moribund tilapia and that infected virus could spread through fish mucus until 12 days post infection. Therefore, removing of moribund and dead fish will reduce the risk of disease transmission and prevent the spread of pathogens in the farm and region. To limit the catastrophic loss of infectious diseases, implementation of biosecurity and control measures should be applied at the farm, regional, national and international levels. Such concepts that could be employed including the screen of live fish and broodstock using PCR or real-time PCR, restricting fish movement, applying disinfectants as a standard practice, fry vaccination, and eliminating potential vectors. Overall, applying a standard of biosecurity plan and control measures at the farm and national level should limit the sources of disease outbreak.

Conference Session Designation: (Emergent Disease)
Presentation format: (Oral)

Erysipelothrix rhusiopathiae spaB*, an emerging pathogen of cultured barramundi, *Lates calcarifer

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Members of the genus *Erysipelothrix* have recently been described as emergent pathogens of cultured Australian eels, *Anguilla reinhardtii* (Steindachner, 1867) and *A. australis* (Richardson, 1841) in Australia, as well as several ornamental centrarchid and cyprinid species in the USA. Since 2013 *E. rhusiopathiae* has been reported from outbreaks of disease in barramundi, *Lates calcarifer*, cultured in North America. Eight *E. rhusiopathiae* isolates were recovered from diseased fish during different outbreaks. The *E. rhusiopathiae* isolates from barramundi were compared phenotypically and genetically to *Erysipelothrix* sp. *spaC* isolates recently characterized from ornamental fish and *E. rhusiopathiae* recovered from aquatic and terrestrial animals from multiple facilities. All barramundi isolates were PCR positive for surface protective antigen type B (*spaB*). Additionally, isolates from clinically affected barramundi had ≥ 99.7% sequence similarity among concatenated MLST gene sequences, indicating a high degree of genetic homogeneity. These isolates were > 99% similar to other *spaB* positive isolates, consistent with findings for other *spa* types. While concatenated MLST sequences demonstrated >99% similarity within *spa* groups, *spaA* and *spaB* isolates shared <98% similarity between them, and <90% similarity to *spaC* isolates. Experimental immersion challenges in tiger barbs, *Puntigrus tetrazona* were attempted in efforts to fulfill Koch's postulates. Tiger barbs were exposed to 5 x 10^7 CFU/mL *E. rhusiopathiae spaB* for 1 hour at 26 C. Within 5 days of challenge, 85% of the exposed tiger barbs died, with the first mortality observed 3 d post-challenge. *Erysipelothrix rhusiopathiae spaB* was re-isolated from moribund fish. This study supports previous work citing the genetic variability of *Erysipelothrix* spp. spa types and the emergence of members of the genus *Erysipelothrix* as nascent fish pathogens.

Conference session designation: (Emerging Diseases)
Presentation format: (Oral)

First Detection of *Erysipelothrix* sp. Infection in Western Mosquitofish, *Gambusia affinis*, from Channel Catfish, *Ictalurus punctatus*, ponds in Mississippi

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Native and introduced fish species can serve as reservoirs for multiple pathogens in cultured fishes, such as the channel catfish. Three hundred and fifty-one western mosquitofish collected in commercial catfish ponds in the Mississippi Delta were surveyed histologically for pathogens using light microscopy. Following routine processing, sectioning, and H&E staining, a number of disease agents were detected, including five myxozoan species in various tissues, intestinal acanthocephalans, and branchial epitheliocystis inclusions. In eight fish, numerous, ill-defined, basophilic colonies of short, slender, Gram-positive, rods lined connective tissues and basement membranes of the skin, skeletal muscle, bone, pharynx, intestines, bile ducts, kidneys, and nasal mucosa. Lesions consistent with descriptions of *Erysipelothrix* sp. infection in tropical fish species. The diagnosis was confirmed molecularly by excising bacterial colonies from formalin fixed paraffin embedded tissue sections using laser capture microdissection (LCM). Sequencing of the 16s, gyrase B (gyrB), and surface protective antigen (spa) genes identified the bacteria as an *Erysipelothrix* sp. Spa C type, with 91% and 99% sequence identity to *E. rhusiopathiae* at the gyrB and 16s gene sites, respectively. The bacteria groups phylogenetically with other recently characterized *Erysipelothrix* sp. isolates believed to represent a novel species within the genus *Erysipelothrix*. *Erysipelothrix* sp. has caused significant mortality in cultured characin and cyprinid species and represents an emerging disease in the ornamental fish industry. To the authors' knowledge, this represents the first report of *Erysipelothrix* sp. infection in a poeciliid fish. Susceptibility of other fish species, including the channel catfish, is largely unknown. Due to the significance of commercial catfish aquaculture in the southeastern United States and the presence of mosquitofish in ponds, experimental immersion and injection challenges were initiated. Results were not available at the time of submission, but will be presented during the meeting presentation.

Conference Session Designation: (Emergent Disease)
Presentation Format: (Oral)
Student Presentation: (Yes)

Molecular Characterization and Histopathology of *Edwardsiella anguillarm* Infections in Nile Tilapia (*Oreochromis niloticus*)

Abigail Armwood[1*], Alvin Camus[1], Adrian Lopez-Porras[2], Matt Griffin[2], and Esteban Soto[3].

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The genus *Edwardsiella*, family Enterobacteriaceae, contains the well-known fish pathogens, *E. ictaluri* and *E. tarda*, as well as two recently described species, *E. piscicida* and *E. anguillarum*, which affect various cultured fish species worldwide. Distinction between species is complicated by similar phenotypic characteristics and similarities in the 16S rRNA gene sequence, highlighting the need for more resolute methods of identification. *Edwardsiella anguillarum* was originally described in 2015 from cultured eel species in China. In 2017, mortalities of 10 to 30% occurred for two months in Nile tilapia (*Oreochromis niloticus*) fry and fingerlings in an aquaculture facility in Central America. Clinical signs were limited to erratic swimming, exophthalmia, and progressive lethargy. Bacteria cultured from affected fish were consistently identified as *E. anguillarum* using an *Edwardsiella* spp. quantitative multiplex PCR, *E. anguillarum* specific end-point PCR, as well as ~1800 bp of the *gyrB* and ~500 bp of the *sodB* gene sequences. The isolates were found to be largely homogenous by repetitive sequence mediated (rep) PCR using the ERIC and BOX primer sets. Additional ancillary diagnostics, including testing for tilapia lake virus and *Francisella noatunensis* subsp. *orientalis*, were negative. Microscopic examination of whole tilapia fingerlings revealed disseminated, mixed, multifocal to coalescing sheets of granulomatous inflammation, dominated by epithelioid macrophages, and discrete granulomas. Lesions often contained large central regions of necrotic debris and numerous 3 to 5 µm, intra- and extracellular Gram-negative bacilli. The most severely affected tissues included the spleen, anterior kidney, and posterior kidney. In multiple fish, additional lesions were present in the ocular choroid rete, gill, pseudobranch, heart, swim bladder, liver, gastrointestinal wall, and skeletal muscle. Severe involvement of the spinal cord and lateral ventricles of the brain were present in several fish and may account for signs of erratic swimming. Findings suggest that *E. anguillarum* may be an emerging pathogen in the aquaculture industry with an expanding host range. Additional work is required to identify overall prevalence of infection and susceptible species.

Conference Session Designation: (Emergent Disease)
Presentation Format: (Oral)
Student Presentation: (Yes)

Edwardsiella Piscicida, an Emergent Pathogen in Farmed Channel ♀, *Ictalurus Punctatus* X Blue ♂, *Ictalurus Furcatus* Hybrid Catfish Cultured in Mississippi

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Catfish aquaculture is the largest foodfish aquaculture industry in the United States and a vital economic component of several southern states. Recent industry trends have led to increased production of channel ♀ (*Ictalurus punctatus*) x ♂ blue (*I. furcatus*) hybrid catfish to take advantage of more favorable production characteristics. As a result, hybrid utilization is estimated to comprise 40%-50% of total catfish production. There is a trend towards increased incidence and prevalence of *Edwardsiella piscicida*-septicemia in US catfish aquaculture, particularly in hybrid catfish. From 2013-2017, a total of 3,242 disease case submissions were submitted to the Aquatic Research and Diagnostic Laboratory (ARDL) at the Thad Cochran National Warmwater Aquaculture Center in Stoneville, MS. Of these, 1,400 (43.2%) were hybrids. *Edwardsiella piscicida* was suspected in 138 (4.3%) cases, the majority of which (89.1%) were from hybrid catfish. A molecular survey of these isolates confirmed the majority (92.0%) to be *E. piscicida*. Furthermore, cases of *E. piscicida* from hybrids submitted to the ARDL, and the Aquatic Diagnostic Laboratory of the Mississippi State University College of Veterinary Medicine in Starkville, MS, were documented for gross lesions and histological analysis. Grossly, *E. piscicida* presents with small dermal ulcerations, a raised fluid-filled cranial mid-line lesion that is frequently ulcerated, hemorrhage in the gills, exophthalmia, and abdominal distension. Internally, lesions include splenomegaly, straw-colored ascites, renomegaly and occasionally hemorrhagic intestines. Histopathological examination is in agreement with gross observations and infected fish repeatedly demonstrate a mononuclear meningoencephalitis, hemorrhagic branchitis, splenitis, ulcerative dermatitis, granulomatous interstitial nephritis and hepatitis coupled with a hemorrhagic enteritis.

Conference Session Designation: **(** Emergent Disease)
Presentation Format: **(** Oral)

Wednesday September 5th – Langevin / Cartier
Virology 1 & 2
Moderator - Tom Waltzek (University of Florida)

9:30 AM	Virology 1	Waltzek - Expansion of the Mimivirus Host Range From Microbes to Vertebrates
9:45 AM		**Koda - Repeated Detections of Red Seabream Iridovirus in Florida Pompano Maricultured in the Caribbean Sea**
10:00 AM		Watlzek - Phylogenomic Characterization of Carp Edema Virus
10:15 AM		Lovy - Carp Edema Virus Associated with Natural Mortality of Wild Carp in New Jersey
10:30 AM		**Refreshments**
10:45 AM	Virology 2	**Sriwanayos - Phylogenomic Characterization of Ranaviruses Detected in Fish and Amphibians in Thailand**
11:00 AM		**Walker - Phylogenomic Characterization of Acipenserid Herpesvirus 1 in Lake Sturgeon (*Acipenser fulvescens*)**
11:15 AM		Subramaniam - Phylogenomic Characterization of Squamate Erythrocytic Iridoviruses
11:30 AM		**Haggard - Genomic Characterization of Percid Herpesvirus 1 Associated with Epidermal Hyperplasia in Walleye *Sander vitreus***
11:45 AM		Waltzek - Genomic Characterization of the First Fish Bunyaviruses through Next-Generation Sequencing

Expansion of the Mimivirus Host Range from Microbes to Vertebrates

Thomas Waltzek[1*], Kuttichantran Subramaniam[1], Gavin Wilkie[2], and Andrew Davison[2]

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Mimiviruses are ubiquitous among varied aquatic habitats where they infect unicellular eukaryotes including phagocytic flagellates and amoebae. Mounting evidence suggests that mimi-like viruses also infect diverse marine algae and perhaps even metazoans including reef-building corals and sponges. Here, for the first time, we characterize a new branch of mimiviruses responsible for lethal diseases in critically endangered sturgeon, expanding their host range from aquatic microbes to vertebrates, potentially spanning three eukaryotic supergroups. Purified viral DNA from the White Sturgeon Mimivirus (WSMV) was used to generate a DNA library for sequencing on an Illumina MiSeq sequencer. The resulting sequence reads were trimmed and assembled using multiple assembly softwares. The complete genome (427,714 bp) was recovered and is predicted to encode 365 open reading frames within the unique region and inverted terminal repeats. The success of mimiviruses, as evident by their abundance across varied hosts and environments, may be linked to their extraordinary genomic complexity and plasticity. We found that sturgeon mimiviruses, contrasted against mimiviruses that infect microbes lacking an acquired immunity, carry a repertoire of immune evasion genes likely pirated from their vertebrate hosts. Accumulating evidence would suggest that we are only now realizing the influence of mimiviruses and other giant viruses have on ocean biogeochemical cycling and eukaryotic biodiversity through a combination of bottom-up and top-down mechanisms.

Conference Session Designation: (Virology)
Presentation Format: **(** Oral)

Repeated Detections of Red Seabream Iridovirus in Florida Pompano Maricultured in the Caribbean Sea

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Megalocytiviruses (MCVs) are finfish pathogens negatively impacting ornamental and food fish aquaculture around the world. Within the genus *Megalocytivirus*, *Infectious spleen and kidney necrosis virus* (ISKNV) is the only recognized species. ISKNV is subdivided into three genotypes: ISKNV, red seabream iridovirus (RSIV), and turbot reddish body iridovirus (TRBIV). Red seabream iridoviral disease (RSIVD), caused by genotypes ISKNV and RSIV, is listed as a disease reportable to the World Organization for Animal Health (OIE). RSIV was first reported in 1990 from Shikoku Island, Japan in cultured Red Seabream (*Pagrus major*). Since then, there have been repeated cases of RSIVD in Asian maricultured species. More recently, RSIVD has been reported multiple times in Florida Pompano (*Trachinotus carolinus*) reared in net pens in the Caribbean Sea. Histopathological examination of affected fish revealed microscopic lesions typical of MCV, including cytoplasmic basophilic inclusions in various internal organs. From outbreaks that occurred in 2010 and 2014, we sequenced the full MCV genomes from infected internal tissues using an Illumina MiSeq sequencer. Maximum Likelihood phylogenomic analyses based on full genomic alignments of the pompano and other previously sequenced MCVs revealed the pompano MCV is supported within the RSIV genotype. Partial amplification and sequencing of the MCV myristylated membrane protein gene from archived formalin-fixed paraffin-embedded tissue sections, displaying the aforementioned microscopic lesions, revealed previous outbreaks in Caribbean maricultured pompano were also due to the RSIV genotype. Although these cases are the first detections of RSIV in maricultured Florida Pompano, RSIV has previously been reported in Snubnose Pompano (*Trachinotus blochii*) maricultured in Japan. These cases extend the known geographic range of RSIV into the Caribbean and suggest further investigation is needed to determine the risk RSIV poses to the mariculture of Florida pompano.

Conference Session Designation: (Virology)
Presentation Format: (Oral)
Student Presentation: (Yes)

Phylogenomic Characterization of Carp Edema Virus

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Double-stranded DNA viruses (dsDNA) infect a wide range of homeothermic and poikilothermic vertebrates. However, only the dsDNA families *Alloherpesviridae* and *Iridoviridae* are well studied among poikilothermic vertebrates (e.g. fish, amphibians, and reptiles). Herein, we report the phylogenomic characterization of a fish poxvirus, carp edema virus (CEV) that infects common carp (*Cyprinus carpio*) varieties including koi. CEV is a globally emerging virus that has negatively impacted facilities rearing common carp for food, sport, and recreation. In this study, we built a DNA library from CEV infected gill tissue DNA derived from an outbreak that occurred in wild common carp in New Jersey in 2017. Sequencing of the library was performed on an Illumina MiSeq sequencer and the resulting data trimmed and assembled using multiple assembly softwares. The nearly full genome (>450,000 bp) was recovered including the inverted terminal repeats. Ultrastructural examination revealed abundant large spheroid particles within the cytoplasm of gill epithelial cells consistent with previous studies. The mature CEV virion appears to possess a single lateral body, similar to previous reports of fish poxviruses in Atlantic salmon (*Salmo salar*) and ayu (*Plecoglossus altivelis*). Maximum Likelihood phylogenetic analysis based on the concatenated amino acid sequences of seven conserved poxvirus proteins revealed that CEV is the sister taxon to the salmon gill poxvirus and together they form the most basal branch of the *Chordopoxvirinae*. The genetic distinctness of the fish poxviruses argues that they represent a new genus within the *Chordopovirinae* that we suggest could be named Piscipoxvirus. However, completion of the CEV genome annotation is needed to determine whether the fish poxviruses share a suite of derived genomic features that support the creation of the proposed genus.

Conference Session Designation: (Virology)
Presentation Format: **(** Oral)

Carp Edema Virus Associated with Natural Mortality of Wild Carp in New Jersey

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In North America, carp edema virus (CEV), the cause of koi sleepy disease, has previously been limited to sporadic detections in koi imports as early as 1996, though not documented in wild carp populations. In May 2017, mass mortality of adult wild carp occurred in Mill Pond, Bergen County, NJ, USA. During the mortality fish showed signs of severe lethargy, often resting on the bottom of the pond. In a sampled moribund fish, histologic lesions were limited to the gill only. Microscopic lesions included diffuse lamellar fusion with extensive cell death suggestive of apoptosis seen as pyknosis, cytoplasmic condensation, and formation of apoptotic bodies. Transmission electron microscopy identified immature and mature pox-like virions consistent with CEV within gill epithelial cells. Amplification and sequencing of the CEV partial 4a gene sequence from the gill revealed this to belong to genogroup I, closely related to European viral strains associated with pond-farmed carp for food. Though this case marks the first documentation of CEV in a wild carp population in North America, it is possible that it was previously overlooked due to the lack of available diagnostic tests for this virus. The finding of CEV in wild carp emphasizes the need for strict biosecurity for hobby and commercial koi operators as transmission could be perpetuated through a "spillover and spillback" mechanism between wild carp and the commercial koi trade. Following the mortality in Mill Pond a follow-up survey was conducted in October 2017 to determine if the virus persisted in the surviving population. A total of 31 adult fish were captured by electrofishing and lethally sampled to screen their gills for CEV using a specific quantitative real-time PCR assay. The testing did not detect the virus in any of these collected samples, supporting that the virus is unlikely to persist in the gills following an epizootic, as has been previously reported.

Conference Session Designation: (Virology / Diseases of Wild Fin-Fish)
Presentation Format: (Oral)

Phylogenomic Characterization of Ranaviruses Detected in Fish and Amphibians in Thailand

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Ranaviruses are emerging pathogens associated with epizootics in farmed and wild poikilothermic vertebrates (e.g. fish, amphibians, and reptiles) worldwide over the past two decades. In this study, we describe the full genomes of seven ranaviruses, each isolated from one of the following species: marbled sleeper goby (*Oxyeleotris marmorata*); goldfish (*Carassius auratus*); guppy (*Poecilia reticulata*); tiger frog (*Hoplobatrachus tigerinus*); Asian grass frog (*Fejervarya limnocharis*); and two from East Asian bullfrog (*H. rugulosus*) in Thailand. The full genomes of the fish and amphibian isolates were sequenced using an Illumina MiSeq sequencer. The nucleotide (nt) sequences of the major capsid protein (MCP) from the Thai isolates compared to a Chinese isolate from tiger frog were highly similar (99.8-100% nt identity). Comparison of the MCP sequences from the seven Thai isolates to 22 other fully sequenced ranaviruses, recovered from Genbank, displayed a lower nt sequence identity ranging from 93.1-98.9%. Phylogenomic analysis based on the concatenated locally collinear blocks alignment, generated using Mauve 2.4, for 29 fully sequenced ranaviruses revealed that these eight Asian isolates, including the Chinese isolate, formed a well-supported monophyletic group referred to as tiger frog virus (TFV) clade. Our findings confirm the international movement of TFVs among Asian cultured fish and amphibians. Biosecurity measures are needed to ensure TFV does not continue to spread throughout Southeast Asia and between this region and other parts of the world.

Conference Session Designation: (Virology)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Phylogenomic Characterization of Acipenserid Herpesvirus 1 in Lake Sturgeon (*Acipenser fulvescens*)

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Acipenserid herpesvirus 1 (AciHV1) was first isolated from moribund farmed juvenile white sturgeon (ws; *Acipenser transmontanus*) in California and later in Europe on an Italian farm rearing ws. Fish infected with this virus (AciHV1-ws) presented with focal white cutaneous plaques that upon histopathological examination revealed keratinocyte swelling and hyperplasia. In spring 2017, two wild, adult lake sturgeon (ls; *A. fulvescens*) captured from the Wolf River, WI, presented with cutaneous lesions similar to those previously reported in farmed ws in California and Europe. Biopsies were obtained for histopathologic evaluation and molecular diagnostic testing. Microscopic examination of the cutaneous lesions in these two ls revealed hyperplasia and hydropic change of keratinocytes consistent with previous cases of AciHV1-ws disease. A degenerate PCR targeting the DNA-dependent DNA polymerase (pol) of large DNA viruses generated the expected 500 bp amplicons from both skin samples. Sanger sequencing of the purified PCR products followed by BLAST analyses using the National Center for Biotechnology Information non-redundant nucleotide and protein databases confirmed the presence of an alloherpesvirus closely related to AciHV1-ws in both ls samples (AciHV1-ls). A DNA library was prepared from the DNA extracted from biopsied skin lesions and sequenced using a v3 chemistry 600 cycle kit on an Illumina MiSeq sequencer. The *de novo* assembly of 6,477,748 paired-end reads using the SPAdes genome assembler recovered a large Alloherpesvirus contig that was extended and joined to other contigs manually by PCR and Sanger sequencing, resulting in the complete AciHV1-ls genome sequence (201,788 bp). Maximum Likelihood phylogenetic analysis based on the concatenated amino acid alignments of the partial pol and exon two of the terminase (term) genes revealed that AciHV1-ls branches as the sister group to AciHV1-ws. The AciHV1-ls and AciHV1-ws amino acid sequences of the partial pol and term amino acid sequences were 93.1 and 100% identical, respectively. This study provides the first complete AciHV1 genome sequence and expands the host range of this virus to include lake sturgeon.

Conference Session Designation: **(** Virology)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Phylogenomic Characterization of Squamate Erythrocytic Iridoviruses

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Erythrocytic iridoviruses (EIV) have been documented in squamates within the families Gekkonidae, Phyllodactylidae, Scincidae, Cordylidae, Lacertidae, Pythonidae, Colubridae, Viperidae, Varanidae, Iguanidae, Phrynosomatidae, Agamidae, and Chamaeleonidae. Interestingly, similar viral agents have also been reported in more than 20 species of anadromous and marine fishes throughout the Atlantic and Pacific Oceans, as well as amphibians. However, the phylogenetic relationship of these viruses to other iridoviruses remains unclear to date. In this study, we compared the light microscopic abnormalities of infected cells, the ultrastructural morphology and phylogenetic relationship of EIVs to other iridoviruses. Recently, EIVs were partially characterized in a wild Peninsula ribbon snake (*Thamnophis sauritus sackenii*) and captive bred inland bearded dragons (*Pogona vitticeps*). The Peninsula ribbon snake displayed two types of cytoplasmic inclusions in erythrocytes, polychromasia, anisocytosis, and hypochromasia, while the erythrocytes of the bearded dragon exhibited prominent blue-staining inclusions within normal appearing erythrocytes. Cytoplasmic inclusion bodies within erythrocytes of the Peninsula ribbon snake and bearded dragons examined by transmission electron microscopy revealed cytoplasmic icosahedral particles morphologically consistent with iridoviruses. The complete genome of the EIV from Peninsula ribbon snake (*Thamnophis sauritus sackenii*; TsEIV) comprises 111,413 bp nucleotides which encodes 115 potential open reading frames. Maximum Likelihood phylogenetic analysis based on 19 conserved genes revealed the squamate EIVs form a well-supported clade distinct from other established iridovirus genera, and likely represent founding members of a novel genus. We propose the genus Hemocytivirus for this new clade of iridoviruses to reflect their predilection for red blood cells.

Conference Session Designation: (Virology)
Presentation Format: **(** Oral)

Genomic Characterization of Percid Herpesvirus 1 Associated with Epidermal Hyperplasia in Walleye (*Sander vitreus*)

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Percid herpesvirus 1 (PeHV1), known informally as walleye herpesvirus, was first reported in walleye (*Sander vitreus*) in 1971 during a spawning event in the Bad Carrot River, Canada and subsequently, in the Northern United States. Infected adults displayed cutaneous whitish plaques during the spring spawning season. Genetic data confirming PeHV1 as a member of the family *Alloherpesviridae* (i.e. fish and amphibian herpesviruses) is lacking. In this study, a Canadian PeHV1 isolate was propagated on the walleye ovary (WO) cell line and infected WO cells were examined by transmission electron microscopy. As expected for a herpesvirus, enveloped virus particles with hexagonal nucleocapsids were observed within the cytoplasm of infected WO cells. DNA was extracted from infected WO cell culture supernatant and used to build a DNA library for sequencing on an Illumina MiSeq sequencer. The 13,099,218 paired-end reads were assembled *de novo* in SPAdes resulting in two herpesviral contigs that were joined manually by PCR and Sanger sequencing. The complete PeHV1 genome was determined to be 127,290 bp encoding 86 putative proteins including those conserved in all fish herpesviruses. Maximum Likelihood phylogenetic analysis based on the concatenated partial DNA-dependent DNA polymerase (pol) and second exon of the terminase (term) gene sequences (249 amino acid characters including gaps) revealed PeHV1 forms a novel branch between the alloherpesvirus genera *Ictalurivirus* and *Salmonivirus*. The genetic analysis of the partial PeHV1 pol (151 amino acid characters including gaps) and term (98 amino acid characters including gaps) sequences ranged from 34.6-72% and 35.9-77.2% identities to other alloherpesviruses, respectively. Our study provides the first sequence data supporting PeHV1 as a novel species in the family *Alloherpesviridae*. Challenge studies are planned to confirm PeHV1 is the causative agent of the observed cutaneous disease in adult walleye.

Conference Session Designation: **(** Virology)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Genomic Characterization of the First Fish Bunyaviruses through Next-Generation Sequencing

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Members of the family *Bunyaviridae* are a diverse assemblage of negative-sense single-stranded RNA viruses. Bunyaviruses (BVs) typically replicate alternatively in arthropods and vertebrates with disease most often observed only in the vertebrate host. The family includes five genera: orthobunyaviruses vectored through mosquitoes, ticks, and flies; nairoviruses and phleboviruses vectored through ticks; tospoviruses vectored through thrips; and hantaviruses vectored through rodents. Here we report the first genomic characterization of piscine BVs isolated from goldfish, largemouth bass, and white sucker. Phylogenetic analyses revealed piscine BVs represent a new branch within the family. Future studies are planned to understand the clinical significance of these piscine BVs.

Conference Session Designation: (Virology)
Presentation Format: **(** Oral)

Wednesday September 5th – Langevin / Cartier
Toxicology / Pathology
Moderator - Dave Groman (Aquatic Diagnostic Services – AVC / UPEI)

1:45 PM	Toxicology / Pathology	Casanova - Biomarkers for Effects of Assessment of Specific Marine Life in the Grand Banks of Newfoundland and Labrador
2:00 PM		Gonzalez - Effects of Glyphosate on Plasma and Brain Cholinesterase in Finfish of Importance in Colombia
2:15 PM		Nwamba - Effect of Propanil on Biochemical, Haematological and Oxidative Stress Parameters of *Clarias gariepinus* Juveniles. - No abstract submitted
2:30 PM		**Omovwohwovie - Haematological Effect of Iron and Lead on *Clariasgariepinus* Juveniles**
2:45 PM		Primus - Initial Findings from an Aquatic Ecosystem Health Study Site

Biomarkers for Effects Assessment of Specific Marine Life in The Grand Banks of Newfoundland And Labrador

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A biomarker is a measurable biological state or response of a species that provides information about the condition of a species and its surrounding environment. The biomarker concept can include animal condition indices, observations on gross pathology, organ histopathology, select biochemical responses and xenobiotic metabolites. There is limited information available to assess the baseline health of marine organisms that exist on the Grand Banks. Furthermore, there is uncertainty surrounding which species' biomarkers can be reliably used as effective assessment tools in characterizing and monitoring the Grand Banks ecosystem. Increasing knowledge in this area would improve the understanding of the current condition of a variety of species, as well as the natural variation present within these species and their surrounding environment. This improved understanding would increase our ability to detect natural variation from the effects and impacts of an environmental incident.

Our project followed a model similar to that of studies ongoing in the Gulf of Mexico that apply a number of biochemical and other tools to perform investigations on ecosystem health as well as recommendations made by agencies such as the International Commission for the Exploration of the Sea, on the need for the development and expansion of the role and use of biomarkers in monitoring and assessment studies. This multi-year project focused on measuring a battery of biomarkers on marine species of commercial and/or ecological importance to Newfoundland and Labrador such as snow crab, Icelandic scallop, sea urchin, Northern shrimp Atlantic cod, sand lance, redfish and yellowtail flounder. The overall objective of the study was to develop a set of biomarkers that could be reliably applied as part of offshore petroleum environmental effects assessments in response to incidents that could occur in Newfoundland and Labrador. Final results of the project will be presented.

Conference Session Designation: (Toxicology / Tox Path)
Presentation Format: **(** Oral)

Effects of Glyphosate on Plasma and Brain Cholinesterase in Finfish of Importance in Colombia

Jaime F. González[*1], Diana M. Ochoa[1], Javier F. Borbón[1], Carlos A. González[1], Dora Figueredo[1], Marisol Ramírez[1], Carmen H. Moreno[2], Pilar C. Rojas[2], Alejandra Jiménez[2]

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Glyphosate is widely used in Colombia to control illegal crops such as poppy and coca plants as well as weeds of edible crops. Acute (96-h), glyphosate exposures (as Roundup®) in different finfish species of importance in Colombia determined from mild to severe central nervous system (CNS) effects and changes in plasma and brain cholinesterases (AChE, BChE). Ghost fish (*Apteronotus albifrons*), an electric fish of the Orinoco and Amazonas Rivers, exposed to 0, 10 and 90 ppm Roundup® (v/v) (n=27) displayed a higher plasma AChE activity (nmols/ml/min) (anova, $p<0.05$) at 90 ppm Roundup® (112.8 ± 46) as compared to controls (65.0 ± 25.6) and 10 ppm (67.4 ± 26.4). These changes were accompanied by mild CNS signs. Juveniles of red tilapia (*Oreochromis* sp.) (n=36) exposed to 0, 1, 5, 15, 45 and 90 ppm Roundup® (v/v) showed significant increases in both plasma AChE and BChE at the two highest concentrations (anova, $p<0.05$) along with severe CNS symptoms in comparison to controls or low-concentration exposures; whereas in Nile tilapia (*Oreochromis niloticus*) juveniles (n=12), exposed to 0 and 15 ppm Roundup®, there was a low AChE plasma activity in glyphosate-exposed fish (171.7 ±34.2) as compared to controls (334.5 ± 56.8) (T-test, $p<0.05$). Interestingly, AChE activity returned to normal baseline levels in tilapias that had been exposed to Roundup® after 10 days of suspending the exposure. Bocachico (*Prochilodus magdalenae*) (n=12) and yamú (*Brycon amazonicus*) (n=18), two indigenous fish species of Colombia, showed reduced brain AChE (nmols/min/mg protein) at 10 ppm (bocachico 1.8 ± 0.6, yamú 7.2 ± 1.8) as compared to controls (bocachico 47.0 ± 4.5, yamú 160.2 ± 13.7) whereas bocachico increased ACHE at 30 ppm Roundup® (113.0 ± 8.0) as compared to controls and 10 ppm. A mechanistic approach to explain interactions between this herbicide and the cholinesterases enzymes remained unknown in our investigations as well as likely ecological implications on fish behavior or interrelations amongst fish in natural bodies of water due to the presence of the herbicide as a contaminant and the effects on cholinesterase activity.

Conference Session Designation: (Latin American Fish Health)
Presentation Format: (Oral)

Haematological Effect of Iron and Lead on *Clariasgariepinus* Juveniles After 15 Days

Omovwohwovie Erhowose Emmanuel[1], Ekokotu P. A[2] and Arnold E.[2]

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Laboratory study was undertaken to evaluate some physical and haematological changes resulting from the exposure of freshwater fish Clariasgariepinus to sub lethal concentrations ($0.1mg^{-1}$ and $0.4mg^{-1}$ of ion (fe) chloride, and ($0.1mg^{-1}$ and $0.4mg^{-1}$ of lead (Pb) chloride) in the water for a period of 15 days. Five(5) groups of twenty fishes each were subjected to serial dilutions of the stock solution of iron (fe) 0(control), $0.1mg^{-1}$ and $0.4mg^{-1}$ and lead (Pb) 0(control), $0.1mg^{-1}$ and $0.4mg^{-1}$ in a large plastic bowl of 60 litres capacity for 15days at the end , blood sample were taken from the control and experimental fish. Blood was assayed for selected haematological parameters (haematocrit, haemaglobin, red blood cells counts, white blood cell counts, differential white blood cell counts, erythrocyte sedimentation rate, and total plasma protein and plasma glucose concentration). The derived haematological indices of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were calculated. $0.1mg^{-1}$ and $0.4mg^{-1}$ of lead (Pb) when compare to control. There is no significant difference on differential white blood cell count in iron (fe) concentration except Neutrophill and lymphocytes and there is a decrease in red and white blood cells on different concentrations of lead (Pb) $0.1mg^{-1}$, $0.4mg^{-1}$ and iron(fe) $0.1mg^{-1}$, $0.4mg^{-1}$ treatment when compare to their control. In conclusion, the changes observed indicate the haematological parameters can be used as an indicator of iron and lead related stress in fish on exposed to elevated iron and lead levels.

Conference Session Designation: (Toxicology / Pathology)
Presntation Format: (Oral)

Initial Findings From an Aquatic Ecosystem Health Study Site

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Water quality is a critical concern globally and anthropogenic water pollution can have highly detrimental effects on public health, animals, and the environment. Methodologies enabling accurate evaluation and management of aquatic environments is central to ensuring continued resilience of these systems. We have begun to develop a study site and refine methodologies to evaluate the health of freshwater aquatic systems from an ecosystem health perspective. Our current study uses contaminant data and indicators of fish health to evaluate ecosystem health in a group of freshwater lakes in northeastern Minnesota. In our first field season, we gathered data from 18 lakes in the region which we classified as either undeveloped, developed, or discharge-related depending on the relative amount of anthropogenic influence. Water, sediment, and fish tissue from each lake was tested for over 180 contaminants that include heavy metals, endocrine disrupting compounds, industrial and agricultural by-products, and pharmaceuticals. The number of contaminants detected in any one sample range from 1 to 84. We also collected data from each site that may be used as an indicator of fish health. Specifically, we performed a quantitative analysis of several ectoparasites, and conducted a fish health index based on the appearance of gross abnormalities of several organ systems. Up to 20 fish of each of two species – either walleye and yellow perch or lake trout and cisco – were evaluated at each site. This work will shed light on the complex dynamics of this system and serve as a baseline for future studies focused on refining tools and approaches used to evaluate aquatic ecosystems health.

Conference Session Designation: (Toxicology / Pathology)
Presentation Format: (Oral)

Wednesday September 5th – Langevin / Cartier
Virology 3
Moderator - Mark Polinski (Dept of Fisheries & Oceans Canada)

3:15 PM	Virology 3	Polinski - Piscine Orthoreovirus Infection Dynamics and Host Interactions Depend on the Strain of Atlantic Salmon Infected
3:30 PM		Wessel - PRV1: Virulence Differences in Atlantic Salmon
3:45 PM		Markussen - Analyses of Genome Sequences and Protein Structure of Strains of Piscine Orthoreovirus (PRV1) with Putative Different Virulence in Atlantic Salmon (*Salmo salar*)
4:00 PM		Siah - Genetic Diversity of Piscine Orthoreovirus 1 Across Geographic and Host Ranges: A Phylogenomic and Historical Analysis
4:15 PM		Gagne - A Survey of Piscine Reovirus (PRV) in Atlantic Canada
4:30 pm		**Vendramin - Piscine Orthoreovirus-3 (PRV-3), a New Pathogen for Farmed Rainbow Trout**
4:45 PM		Di Cicco - The Same Strain of Piscine Orthoreovirus (PRV-1) is Involved with the Development of Different, but Related, Diseases in Atlantic and Pacific Salmon in British Columbia
5:00 PM		**Zhang - Does Piscine Orthoreovirus (PRV) Harm the Respiratory Capability of Infected Atlantic Salmon (*Salmo* salar) Smolts? An Assessment that uses Physiology to Characterize Phenotype**

Piscine Orthoreovirus Infection Dynamics and Host Interactions Depend on The Strain of Atlantic Salmon Infected

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Piscine orthoreovirus (PRV) is the most recently identified member of the orthoreovirus genus. It has a pervasive and global distribution in salmonid species that encompasses both wild and farmed populations. Like many reoviruses, the virulence of PRV appears to be generally low; however, in an aquaculture setting this virus can be the aetiology of disease. Specifically, a variant known as PRV-1 from Norway has been demonstrated by *in vivo* experimentation to cause heart and skeletal muscle inflammation (HSMI) in Mowi strain Atlantic salmon. This disease is currently one of the most impactful transmissible diseases affecting Atlantic salmon production in Norway. Interestingly, experimental challenge studies with PRV from Pacific Canada (a subtype of PRV-1) have routinely caused extreme viremia in Pacific adapted Mowi-McConnell Atlantic salmon but without noteworthy pathology or other manifestations. The disparity for disease outcome between these two regions appears to be linked with host recognition of the virus, and could be a result of virus and/or host specific factors. Here we present our current research into determining if host-specific factors may contribute to the development of PRV associated disease such as HSMI. This work identified that identical challenge of two discrete strains of Atlantic salmon from Canada yielded strikingly different PRV infection dynamics and host responsiveness, suggesting that PRV-associated disease is at least in part conditional on genotypic factors specific to the host organism.

Conference Session Designation: (Virology)
Presentation Format: (Oral)

PRV1: Virulence differences in Atlantic salmon

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Piscine orthoreovirus 1 (PRV1) is a ubiquitous virus in farmed Atlantic salmon (*Salmo salar*) in Norway, and has been shown to be the etiological agent of Heart and skeletal muscle inflammation (HSMI). HSMI is a prevalent disease in Norway; however, PRV1 is also present in apparently healthy Norwegian salmon. Furthermore, in BC, Canada, the virus is prevalent but the presence of HSMI is less evident. Earlier experimental transmission studies in BC using PRV1-containing material showed that PRV1 from BC was transmissible to but failed to induce HSMI in Atlantic salmon. It is apparent that the development of disease is complex, involving viral, host and environment factors. Studies that are able to separate out the impact of the different factors are highly warranted. Recently, we were able to purify PRV1 from blood which enables more standardized studies comparing putative virulence differences between virus strains. In the present study, we compared three strains of PRV1. This included two Norwegian strains; one originating from a severe HSMI outbreak in 2012 while the second strain was revived from archived material dating back to 1988 approximately 10 years before HSMI appeared in farmed salmon in Norway. In addition, a BC strain not associated with HSMI was included. The three different strains were propagated in Atlantic salmon and heparinized blood was collected at peak of infection and used as source for PRV1 purification. Finally, the purified virus was inspected by electron microscopy to confirm presence of virus particles and the batch was quantified by absolute quantification RT-PCR. Atlantic salmon were challenged by ip injection using equal amounts of the three virus strains. Results from the study will be presented, including analysis of viral load, hemoglobin concentration and histopathological lesions.

Conference Session Designation: (Virology)
Presentation Format: **(** Oral)

Analyses of Genome Sequences and Protein Structure of Strains of *Piscine Orthoreovirus* (Prv1) with Putative Different Virulence in Atlantic Salmon (*Salmo Salar*)

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Piscine orthoreovirus 1 (PRV1) causes heart and skeletal muscle inflammation (HSMI) in farmed Atlantic salmon (*Salmo salar*) in Norway. The virus is widespread, especially in the marine production phase, but is increasingly also found in the fresh water phase. PRV1 is present in farmed populations with or without a history of HSMI. The virus is also present in migrating wild Atlantic salmon, but at a lower prevalence.

PRV1 replicates in various cell types, and the erythrocyte is the major target cell early in the infection cycle, but the virus also replicates in various cell types including myocytes and macrophage-like cells, including melanomacrophages. The ability of a virus to cause disease is directly related to its target cells.

In lack of susceptible cell lines or functional reverse genetics, virulence studies are performed by experimental infection trials in the target species. In this study, the genome sequences and protein structures of strains of PRV1 with putative different virulence in Atlantic salmon, including a revived Norwegian isolate from 1988, i.e. from more than ten years before HSMI was observed, were compared. Additional information of the strains, like HSMI or no disease record in the population were obtained. The results indicate that the historical Norwegian PRV1 strain represents a precursor form, for several genomic segments, for current virulent strains causing HSMI in Norway, and for the BC strain. Available full genome data suggest higher genome diversity among Norwegian PRV strains, particularly for the S1 genomic segment (σ3 protein). Substitutions in BC strains for this segment have been found in Norwegian wild salmon. The segmented genome of PRV makes gene segment reassortment a likely evolutionary mechanism. We cannot yet firmly link single gene segments or amino acid motifs to virulence. However, the high number of amino acid substitutions make the two gene segments encoding σ3, p13 and µ1 prime candidates. An association between genomic segment linkage and virulence should not be ruled out.

Conference Session Designation: (Virology / PRV)
Presentation Format: **(** Oral)

Genetic Diversity of Piscine Orthoreovirus 1 Across Geographic and Host Ranges: A Phylogenomic and Historical Analysis

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Piscine orthoreovirus (PRV) is a double-stranded, non-enveloped RNA virus which is found globally in farmed and wild salmonids. Currently, there are three genogroups that have been identified for PRV: PRV1 which infects Atlantic Salmon, Pacific salmon, Trout and some non-salmonid finfish; PRV2 which infects Coho Salmon in Japan; and PRV3 which infects Rainbow and Brown Trout in Europe. Each genetic subtype of PRV has a 10 segmented genome consisting of 3 large (L1, L2, L3), 3 medium (M1, M2, M3) and 4 small (S1, S2, S3, S4) segments. Due to its sequence variability, segment S1 has been commonly used to infer phylogenetic relationships and has demonstrated two main sub-genotypes within PRV1: PRV1a and 1b. However, although highly variable, segment S1 alone does not always provide enough genetic variation to discriminate between closely related PRV genotypes.

We have now begun using full genome sequencing to determine the genetic diversity of PRV1 across broad geographic and host ranges. Our goal is to develop information and tools to better study the epidemiology of PRV1 in the North Pacific. To this end we have sequenced PRV1 genomes from different species of salmonids collected in Eastern and Western North America and compared these using a phylo-dynamic analysis alongside publically available PRV1 genome sequences from Norway, Chile and Canada.

Our preliminary phylogenetic analysis of concatenated genome sequences shows that PRV1 from the West Coast of North America clusters separately with high bootstrap credibility from PRV1 from Eastern Canada, Norway and Chile regardless of host species. Within this cluster, a monophyletic group was suggested for PRV1 from farmed Atlantic Salmon. Interestingly, PRV1 from Eastern Canada which forms a separate monophyletic group clusters separately from PRV1 from Chile and Norway. Phylo-dynamic analysis of these data along with historical records of fish movements within and between countries will inform hypotheses of how PRV1 spread among regions.

Conference Session Designation: (Virology)
Presentation Format: (Oral)

A Survey of Piscine Reovirus (PRV) in Atlantic Canada

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The piscine reovirus (PRV) is a recently identified virus (Palacios et al 2010) that has been linked to Heart and Skeletal Muscle Inflammation (HSMI) in Atlantic salmon (Wessel et al 2017). PRV is often detected without symptoms of HSMI (Garver et al 2016) and viral culture cannot be done. Although the host range of this virus appears primarily restricted to salmonids, it has been occasionally detected in a few non-salmonid species such as capelin *Mallotus villosus*, Atlantic horse mackerel *Trachurus trachurus*, Atlantic herring *Clupea harengus*, and great silver smelt *Argentina silus* (Wiik-Nielsen et al 2012).

In Norway, high loads of PRV have been suggested as a requirement for the development of HSMI in Atlantic salmon. In western North America (N-A), PRV is detected in both wild Pacific salmon and farmed Atlantic salmon, with a high prevalence in farmed fish (Marty et al 2015). In N-A, observation of lesions typical of HSMI by histology has only recently been made in one BC farm infected with PRV, with lesions more pronounced in individuals with higher load of PRV. No typical clinical signs associated to HSMI (i.e. change in behaviour, mortalities, etc.) were observed in parallel (DiCicco et al 2017).

Determination of the PRV situation on the Eastern coast of N-A is ongoing, as part of collaboration with the industry initiated in 2016. Work completed in the past 2 years include a PRV survey in wild fish, hatcheries and sea farms. Prevalence, sequencing of PRV and phylogeny, and *in vivo* challenge results will be presented.

Conference Session Designation: (Virology)
Presentation Format: (Oral)

Piscine Orthoreovirus-3 (PRV-3), a New Pathogen for Farmed Rainbow Trout

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Piscine orthoreovirus – PRV have emerged as relevant pathogens for salmonid aquaculture worldwide. Currently three different subtypes with specific host are described for this viral species.

PRV-1 is the causative agent of heart and skeletal muscle inflammation (HSMI) in Atlantic salmon and and is associated with jaundice syndrome in farmed Chinook salmon

PRV-2 causes erythrocytic inclusion body syndrome (EIBS) in Coho salmon.

- PRV-3 causes heart pathology resembling HSMI in rainbow trout.

PRV-3 was firstly discovered in 2013 in Norway during disease outbreaks affecting farmed rainbow trout. A first series of experimental trials conducted in a joint project involving DTU, NVI and NMBU were performed to assess its pathogenicity and pathogenesis in *O. mykiss* and *S. salar*. The Norwegian PRV-3 isolate has been further characterized analyzing its genome and antigenic features. An experimental infection study with purified virus demonstrated that PRV-3 infects rainbow trout and induces pathological heart lesions similar to HSMI, and thus fulfill Koch's postulates. Furthermore, the infection upregulates IFN production, and induces specific antibody response in later phases. In late 2017 the presence of PRV-3 was also reported in different countries in Europe including Scotland, Germany, France, Italy and Denmark. Interestingly, these viral isolates appear to be genetically distinct from the Norwegian isolate leading to proposition of two separate clades within PRV-3 viral type (PRV-3a and PRV-3b).

In Denmark the virus has been associated with severe disease outbreaks in recirculating aquaculture systems. Clinical signs are represented by reduced appetite followed by uncoordinated swimming behavior and increased mortality; necropsy findings include severe anemia and ascites. Such outbreaks are complex disease cases where different bacterial (including *Flavobacterium psychrophilum* and *Renibacterium salmoninarum*) and viral pathogens (IPNV) are present at the farm. Notably PRV-3 load increases in the target organs (heart, spleen) before the clinical disease appear, whereas the other pathogens are not detected in a systematic pattern. In 2018 in cooperation with the Danish Aquaculture industry a project mapping the prevalence of PRV-3 in the country, investigating its virulence and the risk for vertical transmission, was funded and initiated. An overview of the results will be presented.

Conference Session Designation: (Virology - PRV)
Presentation Format: **(** Oral)
Student Presentation: **(** Yes)

The Same Strain of *Piscine Orthoreovirus* (PRV-1) is Involved with the Development of Different, but Related, Diseases in Atlantic and Pacific Salmon in British Columbia

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Piscine orthoreovirus Strain PRV-1 is the causative agent of heart and skeletal muscle inflammation (HSMI) in Atlantic salmon (*Salmo salar*). Given its high prevalence in net pen salmon, debate has arisen on whether PRV poses a risk to migratory salmon, especially in British Columbia (BC) where commercially important wild Pacific salmon are in decline. Various strains of PRV have been associated with diseases in Pacific salmon, including erythrocytic inclusion body syndrome (EIBS), HSMI-like disease, and jaundice/anemia in Japan, Norway, Chile and Canada. We examine the developmental pathway of HSMI and jaundice/anemia associated with PRV-1 in farmed Atlantic and Chinook (*Oncorhynchus tshawytscha*) salmon in BC, respectively. In situ hybridization localized PRV-1 within developing lesions in both diseases. The two diseases showed dissimilar pathological pathways, with inflammatory lesions in heart and skeletal muscle in Atlantic salmon, and degenerative-necrotic lesions in kidney and liver in Chinook salmon, plausibly explained by differences in PRV load tolerance in red blood cells. Viral genome sequencing revealed no consistent differences in PRV-1 variants intimately involved in the development of both diseases, suggesting that migratory Chinook salmon may be at more than a minimal risk of disease from exposure to the high levels of PRV occurring on salmon farms.

Conference session designation: (Virology)
Presentation format: (Oral)

Does Piscine Orthoreovirus (PRV) Harm the Respiratory Capability of Infected Atlantic Salmon (*Salmo Salar*) Smolts? An Assessment that uses Physiology to Characterize Phenotype.

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Piscine orthoreovirus (PRV) replicates in red blood cells (RBC) and is the etiologic agent of the heart and skeletal muscle inflammation (HSMI) of Atlantic salmon. Consequently, PRV clearly has a potential to impact cardiac pumping, oxygen binding or the oxygen consumption in skeletal muscle, each of which independently or collectively could result in impaired oxygen uptake in normoxia and escalated harm in hypoxia. Hence, we applied an integrated respiratory assessment paradigm (IRAP) to test if experimentally induced infection with a Pacific strain of PRV would physiologically compromise the *in vivo* respiratory capability of Atlantic salmon smolts domesticated on the Pacific coast (Mowi-McConnell) over a 21-week period post-injection. The IRAP assessments were time-matched with assessments of cardiac histopathology, oxygen carrying capacity of RBC and viral load (as determined by qPCR) to provide a broad assessment of harm, which were all intended to coincide with early infection (week one), peak viral load (week 3), highest prevalence of physiological symptoms (week 9), late persistence (week 18), and respiratory response following a hypoxic challenge (week 21). IRAP assessments revealed that saline-injected control fish did change their respiratory physiology over time. However, time-matched comparison of the blood-injected control (BC) fish and the PRV-injected fish revealed no appreciable and sustained differences in respiratory capability over the first 18 weeks of PRV infection, despite the generation of substantial viremia and a significantly elevated presence of minor heart inflammation in PRV-infected fish. Normal oxygen affinity and maximal oxygen carrying capacity of RBC were also maintained. Thus, fish that were infected with high loads of PRV maintained their respiratory performance in normoxia and hypoxia over the first 18 weeks of infection. At week 18, the fish administered a hypoxia challenge (O_2 reduced to 15 % sat.) were physiologically reassessed at week 21. While PRV infected fish had a 12% lower absolute aerobic scope (AAS) at week 21 than BC fish, this was in part because BC fish increased their AAS by 7% post hypoxia. Therefore, PRV infection and a high PRV load alone had a negligible effect on respiratory capability of hosts in normoxia and hypoxia, but in conjunction with hypoxia reduced its aerobic phenotypic plasticity. In summary, despite the presence of severe PRV viremia and mild heart inflammation, functional harm at RBC, anatomical harm at heart and physiological harm at whole-animal have been demonstrated to be absent in Pacific adapted Mowi-McConnell Atlantic salmon. Consequently, these results question the suitability of using PRV load as the sole predictor of harm to PRV-infected Atlantic salmon.

Conference Session Designation: (Virology)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Keynote Presentation

Wednesday ISAAH Banquet September 5rd

Dietary Modulation of Risk in Salmon Farming Operations

Adel El Mowafi *, Terje Utle and Simon Wadsworth

Cargill Innovation Center, Dirdal, Norway

A number of challenges remain inherent to salmon farming operations globally. Up to 20% of all salmon transferred to sea are lost prior to harvest. This correlates to over 53 million fish in Norway alone, representing $2 billion in lost revenue. Risks may be mitigated by a range of factors such as management, vaccination, genetic selection as well as the use of medicines. The use of diet has also been increasingly effective and can provide an important additional tool to reduce risk and improve performance of operations.

Keynote Presentation

Thursday September 6rd

" Without Good Fish Health – No Blue Revolution "

Alf-Helge Aarskog. CEO

Marine Harvest ASA, Sandviksboder 77AB, 5035 Bergen, Norway alf.helge.aarskog@marineharvest.com

Marine Harvest is the fourth biggest seafood company in the world and the world's largest salmon producer. Marine Harvest operates in 24 countries and farm salmon I Chile, Canada, Scotland, Ireland, Faroe Island and Norway. Salmon from Marine Harvest can be bought in more than 80 countries in the world. The salmon farming industry and Marine Harvest has over the years grown significantly, the CAGR is 7 % over the past 20 years, but with reduced growth in the past few years. Salmon farming is in most areas where it is done a highly regulated industry, but the reason for the reduced growth is more linked to a more challenging environment regarding fish health.

Food from the ocean is a limited source of nutrition's, in 2017 the ocean supplied only 5 % of the proteins consumed by the world's population. Fish farming is an old form of producing food and in China it started more than 2000 years ago. Farming the oceans is a newer industry and started in Norway in the mid 1960's. Marine Harvest is a pioneer in this industry and has its roots back to 1964 in Norway. Today farmed salmon is still a minor supplier of food with a share of 4,2% all seafood, but the most industrialized of any specie being farmed. The potential in the industry is much larger and market for the product has been steadily growing worldwide.

The biggest challenges to increase output from salmon farms are fish health related. In most countries the single biggest challenge is linked to a parasite called in general called "salmon louse". The salmon louse is natural to wild salmon, but has an excellent environment in farms with access to plenty fuel number of hosts. The parasite in small numbers will not kill the host, but in large numbers it has the potential to create major damages to the salmon and invite other diseases in, because the immune system can be badly damaged. Many treatments against salmon lice have been developed, but the common denominator is that the parasite is able to build resistance to almost all of them. Marine Harvest has a broad approach in the war against this parasite, including everything from cleaner fish, to very sophisticated ways of cleaning, and thus removing the lice. Freshwater and other means are also developed, and a well thought through lice strategy developed over the past few years. Still this is the industry single biggest issue and the one that is limiting our goal of producing more healthy food from farming of salmon. The solution is maybe not developed yet, but new genetic tools show significant potentials, and even more can be done through good cooperation between farmers and a well thought through production plan involving size of smolt and a coordinated pest management treatment regime.

If we are to be successful with our blue revolution and supply more energy efficient and healthy food from the ocean, it is essential to find solutions for the salmon louse. In the same time, we should continue good R&D in terms of further improving fish health in general. Good examples from the past with excellent development of vaccines against bacterial diseases and newer solutions towards virus diseases like IPN or IHN give me good hope that solutions will be found towards todays and future challenges regarding fish health, securing the population more food from the oceans, produced in the best possible way. Combining traditional fish health work with nutritional requirements and with help from DNA based solutions will be essential.

Thursday September 6th – Gray / Palmer / Pope Ballroom
Virology 4 & 5
Moderators – Suja Aarattuthodiyil (Mississippi State Univ.) Nick Phelps (Univ. of Minnesota)

9:30 AM	Virology 4	Gorgoglione - Differential effects of VHSV and IHNV genes on the modulation of the host innate immune response
9:45 AM		Emmenegger - Virulence of Spring Viremia of Carp Virus (SVCV) Strains in Two Koi Stocks
10:00 AM		Kvamme - Susceptibility of Sea Trout (*Salmo trutta*) to Important Viral Pathogens (SAV3 and PRV1)
10:15 AM		**Wei - Persistence of Cyprinid Herpesvirus 2 in Asymptomatic Goldfish Surviving in Experimental Infection**
10:30 AM		**Refreshments**
10:45 AM	Virology 5	Al-Hussinee - Partial Validation of a Taqman Real-Time Quantitative PCR for the Detection of Tilapia Lake Virus
11:00 AM		Kim - Development of a New Real-Time RT-PCR Using Peptide Nucleic Acid (PNA) Probes for Detection and Genotyping of VHSV
11:15 AM		Raissy - Molecular Identification of Viral Hemorrhagic Septicemia Virus (VHSV) in Rainbow Trout, Iran
11:30 AM		Roberts - Hydrodynamic Dispersion Model of Ostreid Herpesvirus to Improve Surveillance and Emergency Responses
11:45 AM		**Open**

Differential Effects of VHSV and IHNV Genes on the Modulation of the Host Innate Immune Response

Bartolomeo Gorgoglione * & Douglas W. Leaman

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Viral Haemorrhagic Septicaemia virus (VHSV) and Infectious Haematopoietic Necrosis virus (IHNV) are highly contagious pathogenic Novirhabdoviruses (Fam. Rhabdoviridae) affecting a wide range of fish species, both causing OIE notifiable diseases. VHSV genotype -IVb is now endemic in the Laurentian Great Lakes region. IHNV is not yet reported in the Great Lakes area, but is of high concern due to the economic importance of salmonids in the American Northwest. This study analysed the relative capacities of the individual IHNV and VHSV genes to interfere with the general host cell transcription and explores the action of IHNV genes on regulation of the Type I IFN pathway.
An efficient cell transfection protocol was optimised, including the use of new transfection reagents, for EPC, BF-2, RTG-2 and RTgill-W1 cell lines, dramatically increasing their transient transfection efficiency. Single VHSV and IHNV genes, including N, P, M, G (structural) and Nv (non-structural), were cloned into expression plasmids. To assess their impact on the cell general transcription, single VHSV and IHNV genes were co-transfected with specific luciferase constructs with a modified Actin promoter (pCAG) and a luciferase reporter assay was performed at 24 (exclusively for EPC) or 48 hours post transfection (hpt). For both viruses, P and G showed mild suppression of transcriptional potential, whereas M was confirmed as a potent suppressor of the host transcriptional activity in all cell lines tested. In contrast, the Nv gene showed a consistent stimulation of host transcription (from 2 to 8 fold-increase). IHNV-N gene showed a potent anti-host effect in BF-2, RTG-2 and RTgill-W1, but not in EPC, nor for VHSV-N. To characterise the impact of IHNV genes on the innate antiviral response, single genes were co-transfected with specific luciferase constructs with promoters for rainbow trout (*Oncorhynchus mykiss*) Type I IFN, MX-1 and IFITM1. The basal IFN production was stimulated by co-transfecting MAVS, and the luciferase reporter assay was performed at 72 hpt. As for their effect on the general transcription, P and G showed minor effects, N and M showed consistently strong suppression of transcription. IHNV-Nv consistently showed a strong stimulatory effect in all cell lines tested. The precise action of Nv gene on the viral replication is still unclear, although previous studies available in literature identified its action in subverting the innate immune recognition. In our study, we have instead recorded a consistently marked positive effect of Nv, comparatively assessed for either VHSV and IHNV, in boosting the cell general transcription and the IFN pathway in four distinct cell lines. This study provides novel insights on the viral regulators of the innate signalling, helpful to identify potential key targets to design more efficient vaccination strategies.

Conference Session Designation:	Virology
Presentation Format:	Oral
Student Presentation:	NO

Virulence of Spring Viremia of Carp Virus (SVCV) Strains in Two Koi Stocks

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Spring viremia of carp virus (SVCV, species *Carp sprivivirus*), is considered one of the most lethal freshwater pathogens of cyprinid fish. Common carp *Cyprinus carpio carpio* and koi *C. carpio koi* are the most susceptible host fish species. The virus was formally described in the 1960's after outbreaks occurred in carp species on the European continent, but detections of SVCV have now also been reported in Asia, Middle East, South America, and North America. The virus genome contains five genes (N, M, P, G, and L) and phylogenetic analyses of the P and G genes separates SVCV isolates into four genogroups (Ia, Ib, Ic, and Id). In this study we compare the virulence of eight SVCV strains including representatives of each genogroup. These were tested in two koi breeds, long fin semi-scaled (Shusui) and short-fin fully scaled (Taisho Sanke). Koi fry challenged by immersion, with isolates from USA, China, and Europe, were observed for mortality and assessed for infection. Cumulative mortality ranged from 4 – 82% and 0 – 94% in the Shusui and Sanke koi breeds respectively. Each virus strain had similar levels of virulence (high, moderate, or low) in both of the koi breeds with the exception of the SVCV Fijan (genotype Id) isolate. Analysis of the virulence patterns and infection levels of the tested strains is ongoing. To our knowledge this is the first side-by-side virulence comparison of SVCV strains representing each genogroup in an infected primary host species.

Conference Designation: (Virology)
Presentation Format: (Oral)

Susceptibility of Sea Trout (*Salmo Trutta*) to Important Viral Pathogens (SAV3 and PRV1)

Bjørn Olav Kvamme[1,*], Anne Vossgård[1,2], Egil Karlsbakk[1,2], Sonal Patel[1,3], Ingrid Fiksdal[1], Maria Dahle[4], Håkon Berg-Rolness[1], Stig Mæhle[1], Joachim Nordbø[1] and Abdullah Madhun[1].

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Both PD and HSMI constitutes major disease problems in Norwegian Atlantic salmon (*Salmo salar*) farms. As open cages are the dominating production method there is a high probability of release of virus (e.g. SAV3, PRV1) to the environment. Wild fish may be exposed, contract infections and possibly develop disease. Diseased fish are particularly prone to predation, so there may be a potential for negative population levels effects. Due to the increased public concern about environmental impact of diseases in fish farming it is crucial to fill knowledge-gaps to support management decisions.

Due to its coastal residence, sea trout is at increased risk of exposure to, and infection by, disease agents released from farmed fish, when compared to wild Atlantic salmon. Despite this, surveys conducted annually by the Institute of Marine Research (IMR) has not detected elevated prevalence of these viruses in wild trout or salmon collected from areas of intense farming or many disease outbreaks.

Two important questions are to what extent sea trout is susceptible to the same pathogens as salmon, and if there are differences in susceptibility between different lifestages of these species to important pathogens found in aquaculture.

In order to address these questions, we have initiated a series of disease challenge experiments. Here we present the results of four challenge experiments, carried out in the disease challenge facilities of IMR, studying the susceptibility and time course of infection of SAV3 and PRV1 in sea trout and salmon post-smolts and fry. Viral load over time for each of the viruses was quantified using qPCR, and development of disease was evaluated by histology. The results suggest that both sea trout post smolts and fry are less susceptible to both PRV and SAV than salmon, and that the time course of the infections are different in these hosts. These results corroborate the findings of the wild fish surveys, and supports the conclusions in the IMR risk assessments for the environmental effects of Norwegian aquqaculture. The results are important for the evaluation of risk of population reducing effects on wild fish associated with the presence of SAV and PRV in farmed fish. It also demonstrates the complementary role of wild fish surveys and controlled disease challenge experiments.

Conference Session Designation: (Virology)
Presentation Format: (Oral)

Persistence of Cyprinid Herpesvirus 2 in Asymptomatic Goldfish Surviving in Experimental Infection

Chang Wei [1*], Hayato Iida[1], QiuYuan Chuah [1], Mikio Tanaka[2], Goshi Kato[1] and Motohiko Sano[1]

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Cyprinid herpesvirus 2 (CyHV-2) is the causative agent of herpesviral hematopoietic necrosis in goldfish *Carassius auratus* and Prussian carp *C. auratus gibelio*. Apparent healthy virus-carrier can be suspected as a source of the infection. In this study, we investigated virus persistence in the goldfish experimentally infected with CyHV-2. The virus DNA in the organs including spleen, kidney, heart, brain, gills and fin were monitored in fish groups reared constantly at a virus-permissive temperature (28°C). Cumulative mortality of the fish group was 89% in 2 weeks, and the spleen and kidney of the survivors showed high detection rate of virus DNA at 2 months after infection. We also monitored virus DNA in survivors, which had been treated with non-permissive water temperature (34°C) for 4 days initiated at 24, 48 and 72 h after virus infection and were subsequently reared at 25°C to make the fish with different virus loads. The results showed that DNA-positive rates in the organs were high in severely infected fish (72 h-group) even at 30 days after infection and the spleen and kidney showed commonly positive by PCR in all the groups. In addition, some organs dissected from the four of five asymptomatic survivors at 5 months after virus infection, where the virus DNA was negative by PCR initially, turned positive after being incubated *in vitro* in a medium for 5 days. The spleen, kidney and heart showed highest virus detection rates. By inoculation of the homogenate of the PCR-positive kidney tissue after being incubated *in vitro*, one of three fish died due to the virus infection. These results suggest that the hematopoietic tissues, spleen and kidney, can be the most potential persistent site of CyHV-2 and asymptomatic healthy surviving fish can be the potential source of the infection.

Conference Session Designation: (Virology)
Presentation Format: (Oral)
Student Presentation: (Yes)

Partial Validation of a Taqman Real-Time Quantitative PCR for the Detection of Tilapia Lake Virus

Lowia Al-Hussinee[1*], Kuttichantran Subramaniam[1], Mohammad Shamim Ahasan[1,2], Bill Keleher[3] and Thomas B. Waltzek[1]

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Since 2014, tilapia lake virus (TiLV) has emerged as a significant threat to tilapia production in Asia, Africa, and South America. Clinical signs associated with TiLV infections include lethargy, anorexia, and swimming at the surface away from schooling tankmates. Infected fish display gross lesions including gill pallor, exophthalmia, body discoloration (darkening), scale protrusion and loss, and ascites. The most common microscopic lesions associated with TiLV infections include a syncytial hepatitis and an encephalitis. To date, TiLV has not yet been detected in North America and there are no surveillance programs in the United States or Canada due to the unavailability of validated diagnostic assays. However, producers in the United States are increasingly being asked to ensure tilapia exports are TiLV-free. To fill this industry need, we developed a TaqMan real-time PCR assay targeting a conserved region within segment 9 of the TiLV genome. A series of experiments using a serially diluted standard revealed high assay analytical sensitivity and efficiency. The assay did not amplify other fish orthomyxoviruses including infectious salmon anemia virus and an uncharacterized orthomyxovirus isolated from koi. The reported TiLV qPCR assay is not only critical to ensure US producers continue to be able to export tilapia, but could also serve as an integral part of a coordinated US surveillance program aimed at protecting the naïve US tilapia industry from this globally emerging virus.

Conference Session Designation: (Virology)
Presentation Format: (Oral)

Development of a New Real-Time RT-PCR Using Peptide Nucleic Acid (PNA) Probes For Detection and Genotyping of VHSV

Hyoung Jun Kim[1*], Argelia Cuenca[2] and Niels Jørgen Olesen[2]

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Viral haemorrhagic septicaemia (VHS) is one of the most serious viral diseases in salmonid and olive flounder farms. The causative agent VHS virus (VHSV) is classified into four genotypes, I-IV, based on the sequence analysis of the N, G and NV-genes, respectively. Among the various diagnostic methods, gene detection by real-time reverse transcription PCR method (RT-qPCR) based on TaqMan-probe is a stable, rapid, specific and highly sensitive method for viral gene detection. However, the method can only check the amplification curves and Ct values. Peptide nucleic acid (PNA) is artificially synthesized DNA analogs with an uncharged peptide backbone. PNA probes can effectively detect a target gene by amplification and a melting temperature signal. It was reported that PNA probes can effectively distinguish between mis-matched sequences by their different melting temperatures in amplified PCR products. The present study report a new RT-qPCR method using the 3F2R primer set towards VHSV N-gene [a validated primer set of the conventional RT-PCR (320 bp) for detection of all genotypes of VHSV, Aquaculture 492 (2018) 170-183] for simultaneous detection and genotyping of VHSV using PNA probes which was designed based on genotype specific sequences of VHSV.

In the sensitivity test, the new RT-qPCR was compared with virus titration on fish cell cultures, and included 6 VHSV isolates representing the VHSV genotypes Ia, Ib, II, III, IVa and IVb, respectively. For 5 isolates the cell culture and the RT-qPCR showed almost same detection levels except the IVa isolate. In case of this isolate, the virus titration revealed 10 fold higher sensitivity compared to the new RT-qPCR method. Thus, it was confirmed that the sensitivity of the new RT-qPCR for detection of VHSV showed almost same level as the cell culture method.

For the melting point analysis, the new RT-qPCR reactions were performed using extracted viral RNA from 81 VHSV isolates including all known genotypes. The melting temperatures of 40 genotype I VHSV isolates using FAM and ROX was 70.0° and 72.0°C, respectively. In the case of 4 genotype II isolates, FAM and HEX showed temperatures at 70.5° and 65.5°C while the melting temperatures of 11 genotype III isolates with FAM and HEX was 70.0° and 58.0°C . In the case of genotype IV, ROX showed a melting point of 72.0 °C in all 26 isolates, while FAM showed 58.0 °C in 20 subtype IVa, isolates) and 44.5 to 49.5 °C in 6 subtype IVb, isolates.

It was thereby showed this PNA based RT-qPCR can be a useful tool to distinguish between all VHSV genotypes by checking their melting temperatures.

Conference Sesson Designation: (Virology)
Presentation format: (Oral)

Molecular Identification of Viral Hemorrhagic Septicemia Virus (VHSV) in Rainbow Trout, Iran

Mehdi Raissy[1]*, Keyvan Sadeghian[2], Ardeshir Ganji[3], Hasan Momtaz[4] and Mahsa Ansari[5,6]

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Viral Hemorrhagic Septicemia (VHS) is one of the most important viral diseases of fish, especially rainbow trout. This research aimed to evaluate the rate of VHSV infection in rainbow trout in Chaharmahal va Bakhtiari, Lorestan, Esfahan and Kohkilooye va Boyerahmad Provinces which are the most important trout producing Provinces of Iran. For this purpose, between 2014 and 2016, suspected fish samples were collected from fish farms and studied by RT-PCR, after transportation to the laboratory in appropriate conditions. According to the results, it was found that 103 samples (10.6%) of a total of 964 studied samples were infected with VHSV. The rate of infection in 2014, 2015 and 2016 was 16.98, 8.92 and 7.82%, respectively. In three (3) years of study, the highest and lowest infection rates were observed in Chaharmahal va Bakhtiari and Kohkilooyeh va Boyerahmad Provinces, respectively, showing a significant difference ($p<0.05$). On the other hand, fishes with 1-50 g weight had the highest infection rate and the lowest infection rate was observed in fishes with more than 200 g weight ($p<0.05$). Considering the results, a decrease in disease incidence was recorded from 2014 to 2016, although full-scale actions are necessary for the eradication of VHS.

Conference Session Designation: (Virology)
Presentation Format: (Oral)

Hydrodynamic Dispersion Model of Ostreid Herpesvirus to Improve Surveillance and Emergency Responses

Shane D Roberts[1*] Elise K Spark[2], Evan J Rees[1], Charles E James[3] and John F Middleton[3]

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In 2014 a toxic algae bloom of *Karenia mikimotoi* caused a significant fish kill in South Australia, with concerned nearby fisheries and aquaculture sectors wanting to know its predicted spread over those coming days and weeks. Subsequently, the eSA-Marine system was developed to provide a hydrodynamic model of ocean conditions for future emergency responses (see www.pir.sa.gov.au/research/esa_marine).

The system maps past 'hind-cast', present 'now-cast' and future 'forecast' ocean conditions in South Australia. Conditions include sea surface height 'sea level', sea temperatures, sea salinity, ocean currents and wind speeds and directions. The system is directly applicable to aquatic animal health in South Australia providing predictions on the trajectories of harmful algal blooms, toxins, oil spill, and viral particles to allow for rapid emergency response actions.

Most recently the system was used to model the dispersal of ostreid herpesvirus (OsHV-1) particles from an outbreak in the Port Adelaide River estuary. This was the first incursion of OsHV-1 into South Australia, which put at risk the States $32 million / year oyster farming industry. The closest farming region is ~60km from Port Adelaide, while the closest oyster hatchery is ~25km away. Known biological parameters of the virus were used to run a series of hydrodynamic models to predict particle dispersal based on real time data. At the start of each run the model was seeded with 10,000 particles randomly arranged in a Gaussian distribution with a radius of approximately 1km of the mouth of the Port Adelaide River. The model demonstrated that live viral particles do not spread any greater than ~5km, while viral DNA may travel further at up to ~45km before deteriorating. Viral dispersal was dominated by tidal movements. The model informed the emergency response planning team of the likelihood of viral DNA reaching nearby regions and potentially interfering with PCR results of state-wide proof of freedom surveillance.

A current project funded by the Fisheries Research and Development Corporation is now utilising the system further by running a series of models in each of the oyster farming regions, and at other known feral oyster populations at ports and harbors. The hydrodynamic particle tracking outputs will assist with designing future surveillance activities by identifying epidemiologically relevant geographical areas (biosecurity zones) and thus defining sampling populations. Historically biosecurity zones have been defined by a conservative distance of 5 nautical miles (<10km) based on previous literature. Model outputs will also enhance future emergency responses to OsHV-1.

Conference Session Designation: **(** Epidemiology / Aquatic Animal Health Management)
Presentation Format: **(** Oral)

Refinement of *Moritella Viscosa* Challenge Model End Points

Amy N. Garland *, Bryan E. Linton and Thomas E. Wright

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In Norwegian salmonid aquaculture, *M. viscosa* infection and the resulting winter ulcer disease is a major animal welfare concern. The most prominent clinical sign of an infection with *M. viscosa*, is the deep sores which can penetrate into underlying musculature of fish. These sores, or ulcers, open the animal to secondary infections, interfere with osmotic regulation, and can result in death.

A clinical laboratory study was conducted to produce a number of salt-water challenge models using *M. viscosa* isolates, that could reliably produce at least 60% cumulative mortality, and clinical signs in the form of sores of winter ulcer disease in Atlantic salmon. Sores were categorized as follows: S0= no sores, S1= superficial sores, which do not penetrate the skin to underlying muscle, and S2= deep sore penetrating the underlying muscle. One model objective was to assess mortality kinetics of Atlantic salmon following a bath challenge with *M. viscosa* at two concentrations. An additional objective of this model was to assess if removal of fish with observable S2 sores would negatively affect or skew mortality data.

Challenge concentrations were ran in duplicate; one replicate allowed fish to reach a moribund state or die, and one replicate removed and humanely euthanized fish with obvious S2 ulcers. Any fish with S2 ulcers that were euthanized, were counted as mortalities. Once challenged, fish were monitored daily for mortalities, moribund fish, or development of S2 ulcers (in relevant tanks) for 15 (High Concentration) to 22 (Low Concentration) days. No difference was noted in cumulative mortality for fish challenged with the Low Concentration (78.8%), and a difference of 6.2% (93.8 – 100.0%) was noted between replicates for fish challenged with the High Concentration. All replicates produced acceptable mortality levels according to pass criteria required by the study objectives. All fish counted as mortalities produced clinical signs of an infection with *M. viscosa*, and all fish tested for specificity to *M. viscosa* via agglutiantion with *M. viscosa antibodies*, tested positive. Additionally, removing S2 ulcerated fish from one replicate of fish challenged with Low Concentration, reduced that tanks challenge duration by two days compared to the replicate tank where ulcerated fish were not removed immediately.

This study demonstrated that removing fish with S2 ulcers from a challenge tank does not negatively affect the challenge kinetics and, it is acceptable, recommended, and humane to remove fish challenged with *M. viscosa* once S2 ulcers develop.

Conference Session Designation: (Bacteriology / Mycology)
Presentation Format: (Oral)

Thursday September 6th – Gray / Palmer / Pope
Virology 6
Moderator – Jan Lovy (New Jersey Department of Environmental Protection)

1:15 PM	Virology 6	Spencer - Does Pilchard Orthomyxovirus Fill the Ecological Niche of ISAV in Tasmanian Salmonid Farming?
1:30 PM		**Hernandez - The Population Structure of Columbia River Basin Chinook Salmon *Oncorhynchus tshawytscha* and Linkages to the Landscape Ecology of Infectious Hematopoietic Necrosis Virus**
1:45 PM		Kurath - Biological Basis of Specialist and Generalist Infectious Hematopoietic Necrosis Virus in Pacific Salmon
2:00 PM		Padhi - Viruses As Biocontrol Agents for Invasive Common Carp in Minnesota
2:15 PM		Cuenca - Viral Haemorrhagic Septicaemia Virus (VHSV): Molecular Phylogenetics, Geography and Virulence
2:30 PM		Fusianto - Effective Disinfection Protocols for Megalocytiviruses
2:45 PM		Pham - VER-155008 Induced Hsp70 Proteins Expression in Fish Cell Cultures While Concurrently Impeding Replication of Two RNA Viruses

Does Pilchard Orthomyxovirus Fill the Ecological Niche of ISAV in Tasmanian Salmonid Farming ?

Emily E Spencer*, Richard Morrison, Scott Godwin, Martine Cornish, Graeme Knowles and Jeremy Carson

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Many salmonid pathogens in Australia and New Zealand appear to have evolved separately from those present in the rest of the world, presumably due to geographical isolation, limited importation of stocks and strict biosecurity measures. However, Tasmanian salmonid farms face production threats from unique pathogens that fill similar niches to the major salmonid pathogens seen in Europe and the Americas. One of these unique pathogens, pilchard orthomyxovirus (POMV), is related to infectious salmon anaemia virus (ISAV), but is distinct genetically and phenotypically. Like ISAV, POMV has a segmented, negative sense ssRNA genome and causes elevated mortalites in farmed salmon, but POMV shares less than 30% amino acid sequence with ISAV and exhibits different receptor-destroying and haemagglutination activities. POMV causes necrosis in the liver, haematopoietic and vascular tissues, and targets endothelial cells and hepatocytes. POMV has become the primary pathogen of concern for Tasmanian salmonid farmers in recent years, making the need for effective control strategies increasingly urgent. Ongoing research is focused on production of inactivated and subunit vaccines, with the aim of controlling POMV outbreaks on a long-term, sustainable basis.

Conference Session Designation: **(** Virology)
Presentation Format: **(** Oral)

The Population Structure of Columbia River Basin Chinook Salmon (*Oncorhynchus Tshawytscha*) and Linkages to the Landscape Ecology of Infectious Hematopoietic Necrosis Virus (IHNV)

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Chinook salmon populations of the Columbia River Basin (CRB) are genetically diverse with expressed phenotypic differences in behavioral patterns, life history, and geographical distributions. This investigation examined the epidemiological linkages between the dominant life history phenotypes observed across Chinook salmon populations of the CRB and the ecology of Infectious Hematopoietic Necrosis Virus (IHNV). Integrative data analysis of IHNV Virology, Genotyping and Surveillance records available for Chinook salmon, between the years 2000-2012, revealed intraspecific heterogeneity in the prevalence of IHNV infection in Chinook salmon of the CRB. Infection prevalence was higher in Chinook salmon of the Spring-run life history phenotype than in Chinook salmon of the Fall-run type. Observed differences in the prevalence of IHNV infection across the life history phenotypes does not appear to be driven by differences in abundance, as Fall-run Chinook salmon were more numerous than Spring-run Chinook salmon in the CRB between 2000-2012. Geostatistical analysis (ArcMap 10.4) revealed that IHNV positive cohorts of Spring-run Chinook salmon have a broader geospatial distribution within the CRB than virus positive cohorts of Fall-run Chinook salmon. Univariate analysis revealed that the majority of IHNV detections in Chinook salmon were of U genogroup virus in Spring-run fish. Controlled laboratory studies, examining the shedding kinetics of IHNV in juvenile Chinook salmon, showed that offspring of Spring-run Chinook salmon shed higher quantities of U genogroup virus than M genogroup virus. Taken together, the high prevalence of U genogroup IHNV infection in Spring-run Chinook salmon, the broad geospatial distribution of virus positive cohorts, and the high quantities of U genogroup virus shed, suggests that Spring-run Chinook salmon are closely linked to the ecology of U genogroup IHNV in the CRB. While our findings may be a result of ecologically driven differences in exposure to IHNV or inherent genetic differences in susceptibility of Spring-run fish to the virus, these data suggest biological and epidemiological relevance of the patterns observed. Additional laboratory studies are needed to better understand the epidemiological linkages between IHNV and genetically diverse populations of CRB Chinook salmon.

Conference Session Designation: (Aquatic Epidemiology)
Presentation Format: (Oral)
Student Presentation: (Yes)

Biological Basis of Specialist and Generalist Infectious Hematopoietic Necrosis Virus in Pacific Salmon

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The fish rhabdovirus infectious hematopoietic necrosis virus (IHNV) is a major target of long-term surveillance in salmonid populations of western North America. Genotyping of IHNV field isolates has demonstrated three IHNV genogroups (U, M, and L) in North America, and field prevalence data indicates both specialist and generalist virus patterns among various genetic subgroups. In general, viruses within the U, M, and L genogroups are specialists that occur mostly in single salmonid hosts: U in sockeye salmon (*Oncorhynchus nerka*), M in steelhead and rainbow trout (*O. mykiss*), and L in Chinook salmon (*O. tshawytscha*). However, in the Columbia River Basin a subgroup within the U genogroup, designated subgroup UC, occurs in all three hosts, and thus has an unusual generalist host specificity pattern. We are interested to understand how UC viruses evolved from ancestral specialist U virus to become generalists in the Columbia River Basin, and what biological features changed to allow them to successfully infect multiple hosts in the field. We are conducting a series of in vivo infection experiments using 12 IHNV strains including three each from the ancestral U (now referred to as the UP subgroup to distinguish it from UC), M, L, and UC subgroups. These viruses are being tested in sockeye salmon, steelhead trout, and Chinook salmon to quantify variations in virulence, infectivity, in-host replication, shedding kinetics, persistence, and stimulation of protective immunity. To date assays of virulence have confirmed varied host-specificity phenotypes that mirror the observed specialist and generalist field prevalence patterns. Although the generalist UC strains have moderate virulence in all three hosts, they do not have higher virulence than the ancestral UP strains in Chinook salmon or steelhead trout. Thus the high field prevalence of UC viruses in Chinook salmon and steelhead trout represents a major gain in fitness without increased virulence at the biological virus:host level. In contrast, UC viruses have reduced virulence relative to UP strains in the ancestral sockeye salmon host, as predicted by specialist-generalist theory. By quantifying the generalist phenotype for multiple biological traits we will define biological basis of generalism in IHNV. Results will be used to estimate R_0 for each virus:host combination, and to inform a landscape virus transmission model for IHNV in the Pacific Northwest.

Conference session designation: (Virology)
Presentation format: (Oral)

Viruses as Biocontrol Agents for Invasive Common Carp in Minnesota

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Common carp, *Cyprinus carpio*, are one of the most ecologically devastating aquatic invasive species in the world. Despite significant efforts to control this highly prolific and problematic species, managers have no options that are i) species specific, ii) highly lethal, iii) environmentally safe, and iv) cost effective. While fish health managers often aim to prevent the spread of viral pathogens, scenarios are conceivable where a virus could meet the aforementioned criteria for carp control. Based on this idea, we have conducted a survey in search of viruses that could be used as an alternative control agent for invasive common carp in Minnesota. Sampling of live healthy carp and dead carp collected from wild fish mortalities was conducted between June to October 2017 to obtain an overall viral population present in Minnesota through molecular methods such as PCR and next generation sequencing (NGS). Special emphasis was given for the detection of Spring Viremia of carp virus (SVCV), Cyprinid herpesvirus 3 (CyHV-3) and Carp Edema Virus (CEV) with targeted PCR's. During the survey period, species-specific mass mortalities of common carp were reported in a cluster of eight lakes in Le Sueur and Waseca Counties. Clinical signs of dead common carp included severe gill necrosis, dermal hemorrhaging, epithelial sloughing and discoloration, as well as sunken eyes and facial tissue. Ubiquitous presence of CyHV-3 in all eight lakes was confirmed through qPCR. Interestingly, out of eight CyHV-3 positive lakes two had confirmed co-infection with CEV. These two lakes had higher mortality rates as compared to the other six. Further, gel-based PCR and Sanger sequencing confirmed the presence of these viruses. The whole genome of CyHV-3 was determined with Illumina MiSeq from one lake. The genome was 295,016 bp in length and had higher identity to the European variant of CyHV-3 (KX544847). The results are suggestive of existence of CyHV-3 and CEV to wild common carp in the state of Minnesota that have the potential to cause future outbreaks. The use of viruses as biocontrol agents requires a vigorous in-depth scientific exploration since protecting native species and promoting pure ecosystem health is our ultimate priority.

Conference Session Designation: (Virology)
Presentation Format: (Oral)

Viral Haemorrhagic Septicaemia Virus (VHSV) : Molecular Phylogenetics, Geography and Virulence.

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Viral haemorrhagic septicaemia (VHS) is an important viral fish disease, widespread all over the northern hemisphere. The causative agent is VHS virus (VHSV), a rhabdovirus with a negative sense, single strand RNA genome of about 11 kb. One of the most intriguing characteristics of VHSV is its ability to cross species boundaries, not only to cause sporadic infection, but also to create stable intra-species transmission in novel fish species. Indeed, since its first isolation from cultured rainbow trout in 1963, VHSV has been found in more than 90 different fish species in freshwater and marine environments.

Phylogenetic analyses based on the sequence of the glycoprotein (G-gene) VHSV have clearly identified four main genotypes and eight subtypes. Different clades in the phylogeny show a strong geographic differentiation and, at a lesser extent, host specificity. In addition, virulence to certain fish species seems to be genotype specific. Phylogenetic analyses shown that fresh water farmed rainbow trout isolates have a marine ancestry, and that occasional jumps to fresh water from marine environments could occur.

Since discovery of the virus, our laboratory has constructed the largest repository worldwide of VHS viruses, including epidemiological data, genetic sequences and phenotypic characterization of virulence to rainbow trout. We use this platform for two inter-related studies:

First, we present phylogenetic reconstructions based on >100 full genome isolates of VHSV, including representatives of all major groups. The aim of this analysis is not only gain a better understanding to the evolution of VHSV, but also to gain insight in some of the genomic regions involved in virulence to rainbow trout. To do so, 70 isolates were tested for virulence to rainbow trout in vivo, and data mapped into the phylogeny.

Second, we constructed the most complete phylogenetic analysis of VHSV so far, including full-length G-gene sequences for more than 800 isolates. Based on this analysis we are proposing a new classification where sub-genotype IVd is included, as well as minor revisions within genotype Ia. Phylogenetic data combined with epidemiological data for the different isolates will help to better understand the evolutionary history of VHSV in marine and fresh water environments.

Conference session designation: **(** Virology)
Presentation format: **(** Oral)

Effective Disinfection Protocols for Megalocytiviruses

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The genus *Megalocytivirus* (MCV) includes *Infectious spleen and kidney necrosis virus* (ISKNV) and red seabream iridovirus (RSIV) which are listed by the World Organization for Animal Health (OIE). These viruses cause mass mortalities in both marine and freshwater aquaculture. MCV has a broad host range and can be present as a persistent subclinical infection in some fish. Australia is considered free from MCV and important aquaculture and wild fisheries are at risk. Consequently, biosecurity measures are implemented to ameliorate transmission pathways through imported ornamental fish which include certification of freedom from infection with MCV. Evaluation of practical and cost effective disinfection protocols suitable for recirculating aquaculture facilities is an important aspect of the biosecurity plan to facilitate eradication in the event of early detection of an outbreak.

An authentic sample matrix was prepared by *in-vivo* amplification of ISKNV in Murray cod (*Maccullochella peelii*) and the biological load of a clarified tissue homogenate was standardized by addition of 10% v/v foetal bovine serum. In the absence of a cell culture system for ISKNV, a bioassay was used to assess infectivity after disinfection. A cellulose membrane buffer exchange device was used to remove residual disinfectants before intraperitoneal injection of juvenile Murray cod. Fish were maintained in 100L aquaria at 23°C and observed for clinical signs over 14 days. Each bioassay was conducted in duplicate tanks with 18 fish per tank. The appropriate array of positive and negative control samples was also assayed. A positive assay was defined by an increase in ISKNV DNA quantified by qPCR in any fish from a subsample of challenged fish collected at 7d or those remaining at 14d. Negative biosassays were defined by the absence of ISKNV DNA in all fish at the both sampling times.

The bioassay provided a sensitive test for infectious ISKNV. Clinical disease and amplified viral DNA was detected after injection of a dilute positive control, indicating greater analytical sensitivity compared to qPCR. Further, the system was used to demonstrate that ISKNV can remain infectious in aquarium water without fish for at least 48 h at 25°C. Effective disinfection measures included: heating to 65°C for 20 min; pH 3; pH 11; 1% Virkon™; 1000 ppm sodium hypochlorite and benzalkonium chloride at the recommended concentration and contact time. These data can be interpreted to provide effective disinfection protocols for MCV in a wide variety of disease control scenarios.

Conference Session Designation: (Virology)
Presentation Format: (Oral)

VER-155008 Induced Hsp70 Proteins Expression in Fish Cell Cultures While Concurrently Impeding Replication of Two RNA Viruses

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The heat-shock protein 70 (Hsp70) inhibitor, VER-155008 (VER), was explored as a potential antiviral agent for two RNA viruses important to fish aquaculture, viral hemorrhagic septicemia virus (VHSV) and infectious pancreatic necrosis virus (IPNV). Studies were done at a temperature, 14 °C, and with cell lines commonly used to propagate these viruses. These were respectively EPC from fathead minnow for VHSV and CHSE-214 from Chinook salmon embryo for IPNV. Additionally, both viruses were studied with the Atlantic salmon heart endothelial cell line ASHe. For both VHSV and IPNV, 25 μM VER impeded replication. This was seen as delays in the development of cytopathic effect (CPE) and the expression of viral proteins, N for VHSV and VP2 for IPNV, and as less production of genome copy number and of viral titre. As VER inhibits the activity of Hsp70 family members, these results suggest that VHSV and IPNV utilize one or more Hsp70s in their life cycles. Yet neither virus induced Hsp70. Surprisingly VER alone induced Hsp70, but whether this induction modulated VER's antiviral effects is unknown. Exploring this apparent paradox in the future should improve the usefulness of VER as an antiviral agent.

Conference Session Designation: (Virology)
Presentation Format: (Oral)

Thursday September 6th – Gray / Palmer / Pope
Virology / Emergent Disease
Moderator – Esteban Soto (Univ. of California at Davis)

3:15 PM	Virology / Emergent Disease	Harkness - Sav3 Challenge Model Optimization for Testing Dna Vaccine Duration of Immunity
3:30 PM		Soto - Isolation and Metagenomic Chracterization of a Novel Flavivirus from Chinook Salmon (*Onchorhynchus tshawytscha*)
3:45 PM		Clouthier - Nucleo-Cytoplasmic Large DNA Viruses of Wild Lake Sturgeon (*Acipenser fulvescens*) in Central Canada
4:00 PM		**de Kantzow - Ostreid Herpesvirus 1 (OSHV-1) In Vivo Growth Curve and Pathogenesis at a Semi-Permissive Water Temperature**
4:15 PM		**Lopez-Porras - Red Seabream Iridovirus Associated With Cultured Juveniles Florida Pompano *Trachinotus carolinus* Mortality in Central America**
4:30 pm		Lou - A Novel Viral Pathogen Causing Tongue Sole Spleen and Kidney Necrosis in China
4:45 PM		Mordecai - Evidence of a Divergent Arenavirus Infection in Farmed and Wild Salmon in British Columbia
5:00 PM		**Abdelrazek - Comparative Susceptibility of Cyprinidae, Cichlidae, Acipenseridae, and Salmonidae to *Veronaea botryosa***
4:45 PM		**Open**

SAV3 Challenge Model Optimization for Testing DNA Vaccine Duration of Immunity

Jennifer E Harkness*, Lisa M Phillips, Paul Tonita, Afton McMillan, Bryan Linton, Amy Garland

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Pancreas disease caused by salmonid alphavirus (SAV) has been observed in farmed Atlantic salmon in Norway since the 1980's and continues to pose a threat to the successful rearing of Atlantic salmon. It can cause acute mortality from heart and skeletal muscle damage, with mortality ranging from 1- 48%. In addition it causes chronic wasting in survivors due to loss of exocrine pancreas and impaired red and white muscle function which leads to fish "runting" and flesh quality down grades at the processing plant. Necropsy of infected fish may reveal petechial bleeding on the pyloric caeca and surrounding fat, ascites, yellowish liver and pale heart and yellow mucoid gut contents. Histopathological analysis of infected fish is characterized by necrosis of heart, red and white skeletal muscle, along with necrosis and/or complete destruction of the exocrine pancreas. The damage to the Norwegian industry continues, despite commercially available vaccines, with 176 cases reported in 2017.

In order to demonstrate clinically relevant duration of immunity for DNA vaccine development, a SAV3 cohabitation challenge was optimized and used to challenge Atlantic salmon in saltwater at 6, 9.5 and 12 months post-vaccination. The model, including behavioral observations, gross observations during necropsy and sampling, impact on average weight gain, mortality for all time points, and from 12 month immunized fish microscopic cardiac, pancreatic and skeletal muscle lesions at 19, 54 and 89 days post challenge will be presented for the saline control fish and the not vaccinated not challenged controls (NVNC).

Conference Session Designation: (Virology)
Presentation Format: **(** Oral)

Isolation and Metagenomic Chracterization of a Novel Flavivirus from Chinook Salmon (*Onchorhynchus tshawytscha*)

Esteban Soto[1,*], Alvin Camus[2], Susan Yun[1], John H. Leary[2] Jennifer A. Dill[2], Tomofumi Kurobe[3], Chelsea Lam[3], Akinyi C. Nyaoke[4], Mark Adkison[5], Allan Renger[6], Thomas Rosser[7], Terry Fei Fan Ng[2]

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In November 2015 diseased Chinook salmon, *Oncorhynchus tshawytscha* were collected from the lower Eel River, Fernbridge, CA and submitted for diagnosis. Approximately, 10% of the fish presented with abnormal behavior (lethargy, decreased avoidance of humans, congregating at the banks of the river) and cloudiness and opacity in their eyes. Upon necropsy, the eye and brain were the only tissues exhibiting gross changes, specifically cataracts associated to metazoan parasites and petechial hemorrhages in the brain (optic lobes, cerebellum) and spinal cord. Sub-samples of brain, spleen, kidney, and gonad were pooled for virus isolation on multiple cell lines. Three weeks post-inoculation, only the striped snakehead (SSN-1) cell lines presented cytophatic effect. Total nucleic acid was extracted from cell culture supernatants and subjected to RNAseq. RNAseq identified two viral agents in the supernatants, the snakehead *retrovirus* previously identified in the SSN-1 cell line and a novel member of the genus *flavivirus*, family *Flaviviridae*. Additionally, phylogenetic analysis placed the salmon *flavivirus* as the base of flaviviruses described to date. The genome sequence was utilized to generate a reverse transcriptase real time PCR assay specific for the major capsid protein gene of the salmon *flavivirus*. Infectious challenges in rainbow trout (*Oncorhynchus mykiss*) and Chinook salmon fulfilled River's postulates and demonstrated virus replication in brain and kidney. This represents the first isolation and characterization of a *flavivirus* infecting fish.

Conference Session Designation: **(** Virology)
Presentation Format: **(** Oral)

Nucleo-Cytoplasmic Large DNA Viruses of Wild Lake Sturgeon (*Acipenser Fulvescens*) in Central Canada

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Namao virus (NV) is a sturgeon nucleo-cytoplasmic large DNA virus (sNCLDV) that can cause a lethal disease of the integumentary system in lake sturgeon *Acipenser fulvescens*. As a group, the sNCLDV are members of the *Mimiviridae* family with CroV as their closest extant virus relative. In this study, the spatial, temporal and genetic patterns of sNCLDV were evaluated for the first time for wild lake sturgeon from eleven rivers in central Canada. A total of 1329 pectoral fin biopsies were collected between 2010 and 2015. Quantitative PCR (qPCR) results with the Q2 test indicated that the virus was endemic in sturgeon of the Hudson Bay drainage basin with 23.7% (315/1329) of the fish testing positive. The sNCLDV-positive samples were from endangered populations in the Saskatchewan-Nelson River watersheds where virus was detected in 3 to 58% of the sturgeon tested in each population. The highest virus loads were observed in the Nelson River populations in northern Manitoba. Repeat testing of captured-recaptured individuals (n=26) revealed temporal heterogeneity with respect to their virus status in Landing River, a tributary of the Nelson River. Analyses of samples collected annually from the Landing River population over the six year study revealed that virus presence was inversely correlated with sturgeon age, cohort year, weight and the number of times sturgeon were handled (as part of the sturgeon monitoring program) prior to virus sample collection. These results suggest that NV infection may reduce lake sturgeon fitness and survival in the wild. Genetic typing of 114 virus isolates indicated that the NV genogroup of sNCLDV was dominant in the Hudson Bay drainage basin. The results of this study can be used to inform disease management strategies for lake sturgeon conservation, management and recovery programs.

Conference Session Designation: (Sturgeon Disease)
Presentation Format: (Oral)

Ostreid Herpesvirus 1 (Oshv-1) _In Vivo_ Growth Curve and Pathogenesis at a Semi-Permissive Water Temperature

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Microvariant genotypes of *Ostreid herpesvirus-1* (OsHV-1) are emerging pathogens that cause seasonally recurrent epizootics in Pacific oysters (*Crassostrea gigas*) across Europe, New Zealand and Australia. The incubation period ranges from 48 to 72 hours and is dependent on water temperature. Risk factors including water temperature impact mortality and are important to understand in order to develop strategies to reduce the impact of the disease. The aim of the current study was to define the growth curve of OsHV-1 using an *in-vivo* infection model at both 18°C and 22°C. Additionally, a change in water temperature was used to assess if a subclinical infection at 18°C might develop into disease at 22°C. The experiments were conducted in a physical containment level 2 aquatic animal facility. Samples were obtained to measure the concentration of OsHV-1 DNA in gill and mantle tissue at 2, 4, 6, 8, 10, 12, 18, 24, 48 and 72 hours after exposure to OsHV-1 by injection into the adductor muscle. Peak viral load occurred at 24 hours at 22°C and 36 hours at 18°C. This was 24 and 48 hours before onset of mortality was observed at 22°C and 18°C, respectively. In a separate cohort, the water temperature of surviving oysters was increased to 22°C 14 days after challenge at 18°C and monitored for a further 14 days. Control groups were challenged and maintained at 18°C or 22°C for 28 days. OsHV-1 prevalence at 18°C at 14 days was 33% (95% CI: 10% - 65%) after which mortality was 3% (95% CI: 1% - 8%) in oysters exposed at 18°C and raised to 22°C compared to 36% (95% CI: 26% - 47%) in those maintained at 22°C. The present results suggest that some oysters may recover and clear an infection at 18°C and indicate that recrudescence at a permissive water temperature may not result in mortality. These results also confirm that the pathogenesis of OsHV-1 is slowed at 18°C but not prevented. Raising oysters to a permissive water temperature has been recommended as a surveillance method to identify infected individuals including carriers. Further research is required to determine the disease and transmission risk from oysters which remain positive by qPCR, and surveillance methods for identifying persistent infection require validation.

Confernece Session Designation: **(** Virology or Invertebrate and Shellfish Disease)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Red Seabream Iridovirus Associated with Cultured Juveniles Florida Pompano *Trachinotus carolinus* Mortality in Central America

Adrian Lopez-Porras[1]; Juan A. Morales[1]; Gilbert Alvarado[2]; Samantha A. Koda[3]; Alvin Camus[4]; Kuttichantran Subramaniam[3]; Thomas Waltzek[3]; Esteban Soto[5]*

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Mariculture of Florida pompano *Trachinotus carolinus* in Central America has increased over the last few decades and is now a highly valued food fish. High feed costs and infectious diseases are important impediments to the expansion of mariculture. Members of the genus *Megalocytivirus* (MCV), subfamily *Alphairidovirinae*, family *Iridoviridae*, are emerging pathogens that negatively impact Asian mariculture. A significant mortality event in juvenile Florida pompano cultured in Central America occurred in October 2014. Affected fish presented with abdominal distension, darkening of the skin and periocular hemorrhages. Microscopic lesions included cytomegalic "inclusion body-bearing cells (IBCs)" characterized by basophilic granular cytoplasmic inclusions in multiple organs. Transmission electron microscopy revealed arrays of hexagonal-shaped virions (155-180 nm in diameter) with electron-dense cores within the cytoplasm of cytomegalic cells. Pathological findings were suggestive of an MCV infection and the diagnosis was later confirmed by partial PCR amplification and sequencing of the viral gene encoding the transmembrane amino acid transporter protein. The viral sequence revealed the juvenile Florida pompano were infected with an MCV strain, red seabream iridovirus (RSIV), previously reported only from epizootics in Asian mariculture. This case underscores the threat RSIV poses to global mariculture including the production of Florida pompano in Central America.

Conference Session Designation: **(Emergent Disease)**
Presentation Format: **(Oral)**
Student Presentation: **(Yes)**

A Novel Viral Pathogen Causing Tongue Sole Spleen and Kidney Necrosis in China

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Half-smooth tongue sole *Cynoglossus semilaevis* Gunther is a major cultured fish species in China, with high commercial value. From 2009 onwards, tongue sole culturing industry was almost destroyed by a previously unknown disease. The disease has been restricting the industry for near ten years, and farming of half-smooth tongue sole has been abandoned in some areas.
Generally, the spleen and kidney of diseased fish developed cysts, and their textures became uneven. White particles could be detected in the spleens of diseased fish, as well as in some kidneys. These signs were exhibited by both larvae and adults and were associated with mass death of breeding tongue sole. The disease was characterized by either acute or chronic visceral necrosis, both of which caused high mortalities, with cumulative mortalities as high as 96%. Bacterium couldn't be isolated from diseased organs using the streak method and there were no any parasites on the surface of the diseased fish. Results of TEM indicated the presence of virus particles in the cytoplasm of spleen cells. No bacterium and parasite pathogens were detected by TEM. The virus particles were about 30 nm in diameter and roughly circular. Viral inclusion bodies were also present in the cytoplasm.

Symptomatic spleens were collected and grounded with 0.85% NaCl. After centrifuged at 14,000g × 15 minutes, the supernatant was passed through 0.22μm filter. Infection of the filtration material induced spleen and kidney necrosis and death in tongue sole. We supposed the virus we observed under TEM is the causative agent of spleen and kidney necrosis disease.
We couldn't amplify any target segments by using primers for Nodavirus and known fish Picornaviurs whose size are the same with observed virus.
These results suggested that a novel viral pathogen was responsible for the development of splenic necrosis signs in tongue sole. Whole genome sequencing of the novel virus is in progress.

Conference Session Designation: (Virology)
Presentation Format: (Oral)
Student Presentation: (Yes)

Evidence of a Divergent Arenavirus Infection in Farmed and Wild Salmon in British Columbia

Gideon J. Mordecai[1], Emiliano Di Cicco[2], Angela D. Schulze[2], Karia H. Kaukinen[2], Tobi J. Ming[2], Shaorong Li[2], Jan Finke[1], Curtis A. Suttle[1,3,4,5,6], Kristi M. Miller[2]

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There is growing concern that infectious diseases may be contributing substantially to early marine losses of Pacific salmon populations, however, there is little data on diseases occurring in wild migratory salmon. In the current viral discovery study, we sought to determine if unknown viruses were associated with some of the pathologies of unknown etiology observed in farmed Chinook salmon. Initially, a divergent arenavirus was discovered in farmed chinook salmon from the Canadian Department of Fisheries and Oceans (DFO) regulatory audit program, in which farms are randomly sampled for daily mortalities, and subsequently, we identified a closely related virus in sockeye salmon. The novel viruses in Chinook and Sockeye salmon showed homology to arenaviruses and were named salmon pescarenavirus 1 and 2 (SPAV-1/2) respectively. SPAV, along with a recently sequenced arenavirus in frogfish likely represent a new genus of arenavirus. Histopathology observations and localization in both chinook and sockeye salmon confirm empirically that SPAV infects salmon cells and may be associated with pathology in a cultured setting. Furthermore, SPAV -1 RNA was detected in 24/235 farmed Chinook salmon, and 41/852 wild Chinook salmon, whilst SPAV-2 was detected in 145/1714 wild sockeye salmon. Furthermore, prevalence of SPAV-2 was over 20% in the Northern Johnstone strait and Discovery Islands, suggesting that in certain regions SPAV infection is common and may be an important driver of wild salmon population dynamics.

Conference Session Designation: (Virology)
Presentation Format: (Oral)

Comparative Susceptibility of Cyprinidae, Cichlidae, Acipenseridae, and Salmonidae to *Veronaea botryosa*

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In the Western United States, tilapia (*Oreochromis* spp.), koi carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*), and sturgeon (*Acipenser* spp.) farming is a multi-million dollar industry. *Veronaea botryosa* is a dematiaceous, saprobic fungus and cause of systemic fungal infections in cultured sturgeon. Mortality in adult female sturgeon caused by this emergent pathogen results in significant economic losses for the caviar industry. Known to producers as "fluid belly," the disease is now regarded as one of the most important diseases affecting sturgeon aquaculture in North America. Little is known regarding the epizotiology of the disease and host specificity of the fungus. This study aimed at investigating the susceptibility of white sturgeon, koi, Nile tilapia, blue tilapia, and rainbow trout to *V. botryosa* using laboratory-controlled challenge model. Our hypothesis was that *V. botryosa* is host specific and would only cause mortality in white sturgeon. In this study, fish were acclimatized for at least two weeks prior to challenge and maintained in flow-through fresh water at 18±1.2°C. Yearling trout, yearling sturgeon, sturgeon fingerlings, koi, and blue tilapia were exposed to 5.73 x 10^5 *V. botryosa* spores/fish via intramuscular (IM) injection. Trout fingerlings, and Nile tilapia fingerlings were exposed to the same dose of *V. botryosa* via intracoelomic (IC) injection. Daily mortality was recorded throughout a 30 d post-challenge period and persistence of the fungus in the spleens of moribund and surviving fish was investigated using culture and histopathological analysis. Results showed that, yearling trout, sturgeon fingerlings, and Nile tilapia had the highest rates of *V. botryosa*-related mortalities reaching 100% mortality within 30 d post-challenge. Affected fish exhibited abnormal orientation and/or failure to maintain neutral buoyancy, emaciation, coelomic distension, exophthalmos, cutaneous erythema, and ulcerated skin. Blue tilapia and trout fingerlings were also susceptible to the fungus presenting 26.7% and 10% mortality, respectively. Yearling white sturgeon were infected without exhibiting any clinical signs of diseases or mortality during the experimental challenge. Colonies of *V. botryosa* were recovered from all exposed fish except for one of the yearling sturgeon. No control fish died, nor presented positive isolation of *V. botryosa*. Multinucleated giant cell formation was a prominent feature of the inflammatory response to *V. botryosa*. In conclusion, our results suggest that *V. botryosa* is not a host specific pathogen as it can infect and cause mortality to different fish species. Additionally, age/size of fish appears to play a role in some of the tested fish species including white sturgeon and rainbow trout. This information should be taken into account by clinicians, biologists and farmers in the development of surveillance plans and diagnostic methods for this growing industry.

Conference Session Designation: (Emergent Diseases)
Presentation Format: (Oral)
Student Presentation **(** Yes)

Thursday September 6th – Tilly / Tupper
Genomic Applications 1 & 2
Moderator – Attila Karsi (Mississippi State University)

9:30 AM	Genomics 1	Polinski - The Consequences of IHNV on Resistant and Susceptible Sockeye Salmon – Assessing Transcriptomics to Physiological Performance
9:45 AM		**Xue - Functional Genomics Analyses of Molecular Mechanisms Involved in Atlantic Salmon Responses to the Bacterial Pathogen *Piscirickettsia salmonis***
10:00 AM		Umasuthan - Transcriptomic Response of Atlantic Salmon Fin to Sea Lice *Lepeophtheirus salmonis* Infestation
10:15 AM		Saleh - Ichthyophthiriosis: Insight Into Common Carp Immune Response by Quantitative Shotgun Proteomics
10:30 AM		**Refreshments**
10:45 AM	Genomics 2	**Ignatz - Immune and Stress Response of Growth Hormone Transgenic Female Triploid Atlantic Salmon (*Salmo salar*) Reared at Three Temperatures Following Intraperitoneal Polyriboinosinic Polyribocytidylic Acid Injection**
11:00 AM		**Walsh - Use of Molecular Techniques and Water Chemistry to Understand Fish Health in the South Branch, Potomac River, West Virginia**
11:15 AM		**Le - Genome Sequence and Phylogenetic Relationship of *Nocardia seriolae* Strains Isolated From Fish Farms in Vietnam**
11:30 AM		Lawrence - Comparative Genomics Reveals the Species-Based Diversity of *Edwardsiella* Genus Members
11:45 AM		Thune - You Can Never Have to Many Mutants

The Consequences of IHNV on Resistant and Susceptible Sockeye Salmon – Assessing Transcriptomics to Physiological Performance

Mark P Polinski[1]*, Matthew L Rise[2], Yangfan Zhang[3], Anthony P Farrell[3], Stewart C Johnson[1] and Kyle A Garver[1]

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Aquatic Rhabdoviruses are globally significant pathogens associated with disease in both wild and cultured fish. Infectious hematopoietic necrosis virus (IHNV) is a Rhabdovirus that causes an internationally regulated disease (IHN) in most species of salmon. Sockeye salmon are a keystone species in the North Pacific and natural host for IHNV. Yet not all naïve salmon exposed to IHNV develop disease pathology, and the mechanisms by which some individuals are able to evade or rapidly clear infection following exposure are poorly understood. Through RNA-sequencing, we evaluated transcriptomic changes in Sockeye salmon following IHNV exposure and/or infection. Both waterborne exposure and acute infection had dramatic but discrete effects on the Sockeye salmon head kidney transcriptome and included the differential regulation of metabolic, acute phase and cell boundary processes. We then applied an integrated respiratory assessment paradigm (IRAP) to evaluate the respiratory capabilities and capacity of resistant and susceptible Sockeye following an intra-peritoneal injection challenge with IHNV. This demonstrated that primary resistance to IHNV does not compromise the physiological respiratory performance of the fish or ability to tolerate acute hypoxia. Taken together these findings suggests that primary resistance of naïve fish to IHNV may involve global responses that encourage a general state of reduced cellular signalling rather than promoting disseminated antiviral responses and that these primary resistance strategies (which encompass global transcriptomic changes) do not compromise the fish's general physiological performance or ability to tolerate physiological (i.e. hypoxic) stress.

Conference Session Designation: (Virology)
Presentation Format: (Oral)

Functional Genomics Analyses of Molecular Mechanisms Involved in Atlantic Salmon Responses to The Bacterial Pathogen *Piscirickettsia salmonis*

Xi Xue*[1], Jennifer R. Hall[2], Albert Caballero-Solares[1], Eva Jakob[3], Renate Kvingedal[4], Christopher Hawes[3], Jorge Pino[3], Juan Sepulveda[3], Richard G. Taylor[4], Javier Santander[1] and Matthew L. Rise[1]

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Piscirickettsiosis, caused by the intracellular Gram-negative pathogen *Piscirickettsia salmonis*, is one of the most economically important diseases of salmonids. Atlantic salmon (*Salmon salar*) parr were infected with the EM-90-like *P. salmonis* isolate to investigate the genes and molecular pathways involved in piscirickettsiosis. All fish of the challenge group were intraperitoneally injected with 0.1 ml of bacterial inoculum (inoculum titer 10^0.83 $TCID_{50}$/ml), while fish in the control group were injected with 0.1 ml of a control medium that was used to prepare the bacteria inoculum (minimum essential medium, MEM). Mortalities began 20 days post-injection (DPI), and cumulative mortality reached ~30% by the end of the trial. Expression of four anti-bacterial biomarker transcripts (CAMPb, HAMPa, IL8a, sTLR5a), as well as pathogen level, was initially measured using qPCR on head kidney samples. The transcript expression of these genes except HAMPa, as well as pathogen level, peaked at 21 DPI. Multivariate statistical analyses (e.g. PCA) of qPCR data were conducted to classify the fish into low and high infection groups. Five fish from each group (mock control, low and high infections) at 21 DPI were selected for transcriptome profiling using Agilent 44K microarrays. The Significant Analyses of Microarray (SAM) approach identified a total of 3242 differentially expressed features when comparing both infected groups with the control group (FDR=0.01). Gene ontology (GO) enrichment analysis of *P. salmonis*-responsive biomarkers identified a large number of overrepresented terms, many of which related to immune system process, response to bacterium, iron ion hemostasis, redox hemostasis, leukocyte activation and antigen presentation. Key *P. salmonis*-responsive genes will be evaluated with RNA templates collected from multiple time-points by qPCR to understand the dynamic of salmon immune response during the infection process. The findings from this study will help provide insight into the molecular mechanisms involved in salmon response to *P. salmonis* infection, and may aid in the development of anti-piscirickettsial therapeutics.

Conference Session Designation: (Genomic Applications in Aquatic Animal Health)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Transcriptomic Response of Atlantic Salmon Fin to Sea Lice *Lepeophtheirus salmonis* Infestation

Navaneethaiyer Umasuthan[1]*, Xi Xue[1], Albert Caballero-Solares[1], Jillian D. Westcott[2], Zhiyu Chen[2], Mark D. Fast[3], Barbara F. Nowak[4], Richard G. Taylor[2] and Matthew L. Rise[1]

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Sea lice (*Lepeophtheirus salmonis*) are ectoparasitic copepods that cause severe economic damage to the Atlantic salmon aquaculture industry globally. Since previous studies have proven that the attachment sites of early life stages of sea lice are mainly the fins, gene expression response of Atlantic salmon fin tissue may shed light on host immunity against sea lice infection. To better understand this, we sampled tissue from louse-attachment (Att) and non-attachment (NA; adjacent to Att) sites of the fin of salmon 8 days post-infestation (laboratory-reared louse at chalimus stage) for gene expression studies complemented with our ongoing histological analyses. Fin samples collected from fish prior to infestation served as the control group (C). A salmon 44K microarray experiment was used to investigate the sea louse-derived changes in the fin transcriptome and to screen potential fin-specific biomarkers of sea lice infestation based on which a multiplex qPCR is aimed to be developed. Significant Analyses of Microarray (SAM) approach identified a total of 2271 differentially expressed genes (DEGs) when three different groups (C, NA and Att) were compared [FDR (false discovery rate) 0.05]. Direct comparison of Att and NA groups revealed that 37 genes showed significant alteration in their transcription. Gene ontology (GO) term enrichment analysis of all DEGs identified six main functional groups related to extracellular matrix (e.g. matrix metalloproteinases, *mmp-2*, *-9*, *-13*; and *timp2*), stress (e.g. *gstA, gpx7, prx*), immunity (e.g. *il8, lect2, ccl20, rsad2*), wound healing (e.g. *mmp13, fn1, lgals1*), inflammation (e.g. *1l1b*), and Fe^{2+}/heme/oxygen transport (e.g. *hba, hbb*) that were affected. These preliminary data could improve our understanding of the molecular events underlying early stages of Atlantic salmon infestation by *L. salmonis.*

Conference Session Designation: (Parasitology Sea Lice – Ectoparasites)
Presentation Format: **(** Oral)

Ichthyophthiriosis: Insight into Common Carp Immune Response by Quantitative Shotgun Proteomics

Mona Saleh[1], Gokhlesh Kumar[1], Saleh Al-Quraishy[2] and Mansour El-Matbouli[1]

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Ichthyophthirius multifiliis, a ciliated ectoparasitic protozoan, causes ichthyophthiriosis and leads to considerable economic losses to the aquaculture industry. Understanding the fish immune response and host-parasite interactions could support disease management and control. Fish skin mucus is the first line of defence against infections through the epidermis. Yet, the common carp, *Cyprinus carpio*, protein-based defence strategies against infection with *I. multifiliis* at this barrier is unknown. We investigated the skin mucus proteome of common carp at 1 day and 9 days post-exposure with *I. multifiliis*. Using nano liquid chromatography tandem mass spectrometry (nano-LC ESI MS/MS), the abundance of 44 proteins was found to be significantly different in the skin mucus samples between exposed and non-exposed carp. Proteins with increased abundance values were mainly involved in signal transduction, metabolism, immune response and stress, whereas proteins with decreased values were mainly structural. The extracellular matrix proteins such myosin, and keratin showed increased abundance values. The analysis revealed increased abundance values of epithelial chloride channel protein, galactose-specific lectin nattection, high choriolytic enzyme 1 (nephrosin), lysozyme C, granulin-3 and protein- glutamine gamma-glutamyltransferase proteins. Besides, we identified novel proteins with yet unknown function in common carp following penetrating injuries such as olfactomedin 4, lumican, dermatopontin and papilin. This analysis, therefore, represents a key for the search for potential biomarkers, which can help in a better understanding and monitoring of interactions between carp and *I. multifiliis* and gives insight into the important role that skin mucus plays in protecting fish against parasites.

Conference Session Designation: Parasitology General
Presentation Format: Oral Presentation

Immune and Stress Response of Growth Hormone Transgenic Female Triploid Atlantic Salmon (*Salmo Salar*) Reared at Three Temperatures Following Intraperitoneal Polyriboinosinic Polyribocytidylic Acid Injection

Eric Ignatz *[1,2], Laura Braden[1], Tiago Hori [3], Dawn Runighan [1], Tillmann Benfey [4], Mark Fast [5], Jillian Westcott [2] and Matthew Rise [6]

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AquAdvantage® salmon (growth hormone transgenic female triploid Atlantic salmon) offer aquaculture producers a faster growing alternative to conventional salmon. In order to determine optimal rearing conditions for their commercial production, a study was conducted to examine the effect of rearing temperature (10.5°C, 13.5°C, 16.5°C) on the immune and stress response of AquAdvantage® salmon. When each temperature treatment group reached an average weight of 800 g, a subset of fish was intraperitoneally injected with either polyriboinosinic polyribocytidylic acid (pIC), a known immunostimulant, or an equal volume of sterile phosphate-buffered saline (PBS). Blood and head kidney samples were collected before injection and 6, 24 and 48 hours post-injection (hpi). Transcript abundance of 7 immune-related genes (*IFN-γ, ISG15a, RSAD2, LGP2, STAT1b, TLR3, mxb*) was measured by reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) on all head kidney samples. Blood plasma cortisol levels from samples collected pre-injection and from pIC and PBS at 24 hpi were quantified by ELISA. Target gene activation was observed at 24 hpi, with transcript levels starting to return to baseline after 48 hours in pIC-injected fish. Overall, rearing temperature did not appear to have a significant effect on immune-related transcript expression in response to pIC. No significant differences were found between any comparisons of rearing temperature and treatment based on cortisol response. This information provides insight into the relationships between rearing temperature and response to an immunostimulant in AquAdvantage® salmon for use in commercial applications.

Conference Session Designation: (Genomic Applications in Aquatic Animal Health)
Presentation Format: (Oral)
Student Presentation: (Yes)

Use of Molecular Techniques and Water Chemistry to Understand Fish Health in the South Branch, Potomac River, West Virginia

Heather Walsh[1,2], Vicki Blazer[2], Ryan Braham[2], Kelsey Young[2], Brandon Keplinger[3], Patrick Phillips[4]

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Since 2005 when fish kills were first observed in the South Branch Potomac River in West Virginia, smallmouth bass have been routinely sampled for fish health assessments. Pathogens, parasites, largemouth bass virus, testicular oocytes, and skin lesions have all been documented in smallmouth bass in this area. However, no one factor has been associated with mortality. It is likely that these cumulative stressors have an immunomodulatory effect, particularly in the spring during spawning. In 2013-present, a more comprehensive sampling effort was initiated to include changes in transcript abundance, water and sediment chemistry, immune function, and various other endpoints. Bass were sampled for histopathology and RNA-Sequencing during the spring prior to spawning and in the fall during recrudescence. Liver and testes were sampled and partial transcriptomes were assembled in order to identify genes of interest that may be associated with pathological alterations or contaminant exposure. Nanostring nCounter® technology was used to identify changes in transcript abundance of genes involved in immunomodulation, oxidative stress, and chemical detoxification. Water was sampled monthly, bi-weekly, and during storm events for pesticides, hormones, phytoestrogens, and total estrogenicity. Sediment was sampled in the spring and fall for pesticides, hormones, and phytoestrogens. The integration of multiple data types may help explain the observed disease symptoms observed in smallmouth bass in this area.

Conference Session Designation: (Genomic Applications in Aquatic Animal Health)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Genome Sequence and Phylogenetic Relationship of *Nocardia Seriolae* Strains Isolated From Fish Farms in Vietnam

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Nocardiosis in fish is an infectious, systematic, granulomatous disease caused by infection with *Nocardia seriolae*, a Gram-positive, facultatively intracellular bacterium. This pathogen affects a wide range of marine and fresh water fish species at different age groups or sizes and can result in massive mortalities for infected farms. In Vietnam, nocardiosis is currently considered one of the leading threats to the sustainable aquaculture development of commercially important pompano fish (*Trachoditus blochii*) as outbreaks of the disease have caused significant economic losses for many farms throughout the country. To obtain an insight into the biology, origin, evolution and epidemiology of the pathogen, the genetic relatedness of strains were analysed using pulsed-field gel electrophoresis (PFGE) and Illumina NextSeq 500 whole genome sequencing (WGS). PFGE of 20 strains digested with *Xba*I were classified into one pulsotype while two pulsotypes with a similarity of > 80% were identified by *Ase*I digestions, suggesting close genetic relatedness of these strains. Consistent with PFGE, phylogenomic analysis of seven Vietnamese strains and all currently available *N. seriolae* genomes (*n*=7) using whole-genome-derived single-nucleotide polymorphisms (SNPs) indicated that the Vietnamese strains fall into two highly clonal genotypes that differed by just 1-2 SNPs and were irrespective of the geographic regions where they were isolated. The Vietnamese strains share a common ancestor with strains isolated from other countries in the same region, although they differed from the next closest known strain in Japan by 265 SNPs. These results suggest that the Vietnamese *N. seriolae* strains have been recently introduced to this country, although the precise origin is not yet known. The bacterium encodes a large genome of 7,785,433 bp, a G + C content of 68.2%, 7,420 predicted coding DNA sequences and 77 transfer RNA sequences. It was also found that the bacterium harboured genes coding for factors relating to virulence, pathogenicity and host defence mechanisms such as iron uptake systems, resistance to antibiotics and toxic compounds, and the biosynthesis of hemolysins, adhesins and proteases. These findings provide novel information about the Vietnamese *N. seriolae* population that is essential for development of new drugs and vaccines, and ultimately, the move toward nocardiosis-free aquaculture.

Conference Session Designation: **(** Genomic Applications in Aquatic Animal Health)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Comparative Genomics Reveals the Species-Based Diversity of *Edwardsiella* Genus Members

Hasan C. Tekedar[1], Mark L. Lawrence[1], Salih Kumru[1], Stephen R. Reichley[1,2], Geoffrey C. Waldbieser[3], Jochen Blom[4], Matt J. Griffin[1,2]

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The genus *Edwardsiella* is a member of the family *Enterobacteriaceae*, and it consists of five species isolated from fish, reptiles, and mammals (including humans). Some members of this genus cause disease in U.S. farm-raised catfish as well as other aquaculture industries in Asia and central America. To determine the genetic basis underlying the diversification of *Edwardsiella* genus members, we conducted genome sequencing of 22 *Edwardsiella* strains. In our comparative analysis, we included genome sequences from other available *Edwardsiella* genus members deposited in NCBI (National Center for Biotechnology Information). To analyze species diversity, we applied ANI (average nucleotide identity) and core genome comparison to construct phylogenetic trees. Functional analysis revealed that type 3 secretion system (T3SS) is not present in *Edwardsiella tarda* and *hoshinae*, whereas type 1 secretion system (T1SS) and type 5 secretion system (T5SS) are encoded by all the genus members. Interestingly, type 4 secretion system (T4SS) is encoded by most of the evaluated *E. ictaluri* and some of the other *Edwardsiella* genus members. *E. ictaluri* genomes tend to carry more types of insertion sequences and higher numbers compared to other species. Our findings reveal the utility of comparative genomics to elucidate genetic diversity and potentially enable improved diagnostics for *Edwardsiella* species. These findings also impact vaccine development for the species and reveal differences in potential pathogenic mechanisms.

Conference Session Designation: (Genomic Applications in Aquatic Animal Health)
Presentation Format: (Oral)

You Can Never Have to Many Mutants

Ronald L. Thune

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In 1961 Stanley Falkow showed that the ability to utilize lactose could be transferred from *Salmonella* strain ST-2 to many strains of lactose negative *Escherichia*, *Salmonella*, and *Shigella* via an episome. He further demonstrated that similar genetic elements, now called plasmids, carry and transfer antibiotic resistance genes. The potential for studying pathogenesis was realized when he used a plasmid to isolate a gene encoding a toxin from a diarrhea causing *E. coli*. That humble beginning revolutionized the study of bacterial pathogenesis and led to a variety of protocols to mutagenize bacterial pathogens and subsequently evaluate the mutant phenotype to assess the function of the gene in question. The mission here today is to discuss several procedures for making mutant strains of bacteria to assess for various aspects of pathogenesis, including deletion/insertion mutagenesis, random transposon mutagenesis, targeted transposon mutagenesis, targeted transfer mutagenesis, and site specific mutagenesis.

Conference Session Designation: (Genomic Applications in Aquatic Animal Health)
Presentation Format: **(** Oral)

Thursday September 6th – Tilly / Tupper
Co-infections in Fish
Moderator – Bartolomeo Gorgoglione (Wright State University)

1:15 PM	Co-infections in Fish	Gorgoglione & Jones - Co-infections in Fish
1:30 PM		Adamek - *Flavobacterium branchiophilum* as a Secondary Pathogen in Koi Sleepy Disease
1:45 PM		Getchell - *Pseudomonas mandelii* and Viral Hemorrhagic Septicemia Virus Co-Infection in Sodus Bay, NY
2:00 PM		**Jones & Wargo - Impacts of Vaccination and Genetic Disease Resistance on Transmission in Single and Co-Infections in Rainbow Trout**
2:15 PM		Long - Impact of Co-Infections on Gene Expression in Sockeye Salmon *Oncorhynchus nerka*
2:30 PM		Cabellero-Solares - Analyzing the Molecular Mechanisms Underlying *Lepeophtheirus salmonis* and Bacterial Co-Stimulation in Atlantic Salmon
2:45 PM		Karlsen - Complex Skin Disease, Co-Infection of Atlantic Salmon by *Moritella viscosa* and *Aliivibrio wodanis*

Special Session: Co-infections in Fish

Bartolomeo Gorgoglione [1,*] and Simon Jones [2]

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Infection with multiple pathogens is a more typical scenario than single pathogen infections, both in farmed and wild populations. Despite this, our understanding of host-pathogen interactions and disease outcomes is primarily based on knowledge gathered from single-pathogen studies and observations from ecological and disease surveillance programs. A body of knowledge is beginning to emerge that reveals complex and often poorly predicted host interactions occur during concomitant infections in fish. New studies are focusing on the impact and dynamics of heterogenous co-infections affecting teleost fish. Using several model pathogens in salmonid and non-salmonid species, current scientific advances target improvements in the assessment of diagnostic tools during multiple infections and characterise pathological, immunological and disease outcomes. Co-infections may enhance the impacts of disease in farming conditions or increase the ecological consequences of disease in wild fish. The goal of this Special Session is to raise awareness of ongoing research, and to foster new studies focusing on the interaction between infectious agents in fish hosts.

Conference Session Designation: (Co-infections in Fish)
Presentation Format: (Oral)

Flavobacterium Branchiophilum as a Secondary Pathogen in Koi Sleepy Disease

Mikolaj Adamek[1*], Felix Teitge[1], Verena Jung-Schroers[1], Max Heling[1], David Gela[2], Veronika Piackova[2], Martin Kocour[2], Dieter Steinhagen[1]

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Koi sleepy disease (KSD) is often fatal condition affecting common carp. Therefore, KSD is of increasing importance for global aquaculture. Despite the fact that the carp edema virus (CEV) is most likely the causative agent of KSD, the disease often seems to present itself as multifactorial. Several parasites and bacteria species are present on gills, skin or in internal organs of fish suffering from clinical KSD. In this study, we analysed a possible interaction of flavobacteria and CEV infections in the development of clinical KSD in carp suffering from proliferative gill disease. We examined selected field samples from Germany and Hungary and suggested the presence of CEV and flavobacteria co-infections in subsets of the samples. To confirm this, several infection experiments were performed to study the transfer and dynamics of both infections. We analysed which *Flavobacterium* species could be isolated/identified from KSD affected fish and concluded that *Flavobacterium branchiophilum* is a possible co-pathogen. Antibiotic treatment and studies involving differently KSD susceptible carp strains showed that CEV seems to be the primary pathogen causing an insult to the gills of carp and by this enabling other pathogens including *F. branchiophilum* to establish co-infections. Despite the fact that a *F. branchiophilum* co-infection is not required for the development of clinical KSD; it could contribute to the pathological changes recorded during the outbreaks of this disease.

Conference Session Designation: (Co-infections in Fish)
Presentation Format: (Oral)

Pseudomonas mandelii and Viral Hemorrhagic Septicemia Virus Co-infection in Sodus Bay, NY

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New York fishery biologists reported dead pumpkinseed (*Lepomis gibbosus*) and bluegill sunfish (*Lepomis macrochirus*) floating and laying on bottom all over Sodus Bay on 2 May 2018. Dead and moribund specimens were collected for necropsy. No significant external lesions were noted. Gill clips of five sunfish examined showed light to moderate presence of piriform ciliates, encysted digenes, and *Dactylogyrus sp.*, and skin scrapes showed few trichodinids. One pumpkinseed had an enlarged spleen and a darkened liver with small white cysts. Examination of fixed tissues revealed necrosis, congestion, and bacteremia in many organs. Kidney loop samples were inoculated onto TSA/5%SB and significant growth was observed after 2 days of incubation. Cornell's Animal Health Diagnostic Center Bacteriology Section identified the isolate with MALDI-TOF as *Pseudomonas mandelii*. EPCs were inoculated with tissue homogenates from sunfish and cytopathic effects were observed after 3 days incubation. VHSV was detected by RT-qPCR from pumpkinseeds brain and pooled organs, with the highest viral copy number near 5000 per 50 ng of total RNA. The diagnosis was complicated by the co-infection with both *P. mandelii* and VHSV. The severe bacteremia observed in histology slides suggests *P. mandelii* was the primary pathogen in this case. Both pathogens were observed in other fish kills documented in New York this spring, though not as co-infections. An outbreak of VHSV in late March 2018 involving thousands of dying gizzard shad (*Dorosoma cepedianum*) occurred in another part of Lake Ontario within Irondequoit Bay, approximately 60 kilometers west of Sodus Bay. Mortality events of sunfish also occurred in May 2018 in Conesus Lake and the Seneca River from which *P. mandelii* was cultured, but VHSV was not detected. In 2015, *P. mandelii* was also associated with a spring outbreak in pumpkinseed and bluegill sunfish from two different water bodies in New Jersey (Lovy et al. JFD 2017). Together, these reports indicate that *P. mandelii* is an emerging bacterial pathogen affecting freshwater fish during spring.

Conference Session Designation: (Co-infections in Fish)
Presentation Format: (Oral)

Impacts of Vaccination and Genetic Disease Resistance on Transmission in Single and Co-Infections in Rainbow Trout

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Globally, infectious diseases are responsible for major conservation and economic losses in wild and farmed fish populations. Prevention tools, including vaccination and breeding for genetic disease resistance, are used in many systems to prevent mortality by such diseases. Studies are often done to evaluate the efficacy of a preventative method at reducing disease, but the impact on transmission is rarely studied. Protection under diverse field conditions, such as variable pathogen exposure dosages, is also not fully understood. Furthermore, there is little information on how preventative methods alter host-pathogen relationships. For example, it is largely unknown how vaccination impacts non-target pathogens that co-infect the host. These knowledge gaps make it difficult to infer the epidemiological impacts of disease prevention tools. In an attempt to fill these gaps, we investigated the leading pathogens in rainbow trout (*Oncorhynchus mykiss*) aquaculture: infectious hematopoietic necrosis virus (IHNV) and *Flavobacterium psychrophilum*. We evaluated the impacts of vaccination and genetic disease resistance on mortality and transmission across a range of challenge dosages of IHNV and *F. psychrophilum* to accurately reflect field variability. There is evidence of a dose effect; as dose increases, shedding increases and vaccine efficacy decreases. We also evaluated how vaccination and genetic disease resistance impact transmission dynamics during simultaneous and sequential co-infection of IHNV and *F. psychrophilum*. Our results indicate co-infected fish shed more of both pathogens than they do in single infections, but the order that the pathogen infected the host may impact transmission. These studies are aimed at developing a more robust framework for inferring the efficacy of disease prevention strategies. Our results will also help to inform and improve disease management in one of the top aquaculture species in the United States.

Conference Session Designation: (Co-infections in Fish)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Impact of Co-Infections on Gene Expression in Sockeye Salmon

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Synergistic co-infections in fish increase disease severity and mortality but there is little information available on the impact of co-infections on host gene expression. Both infectious hematopoietic necrosis virus (IHNV) and the salmon louse *Lepeophtheirus salmonis* are enzootic in marine waters of Western Canada, and there is a high likelihood of co-infection. Transcriptomic studies have suggested infection with *L. salmonis* impairs the host ability to respond to a virus challenge. To gain a better understanding of the host transcriptomic response to co-infection, Sockeye Salmon *Oncorhynchus nerka* smolts were infected with *L. salmonis* (V-/SL+), IHNV (V+/SL-), both (V+/SL+), or neither (V-/SL-). Anterior kidney and skin samples were collected at 3 and 7 d post-lice infection (dpl) for gene expression analysis. Genes of interest (GOI) for this study were associated with acute phase response, cytokines, antigen display, interferon-induced, immunoglobulins, tissue repair, and iron transport and circulation. Expression of genes associated with the antiviral response (*interleukin-1β*, *interleukin-10*, *mx-1*, and *rsad2*) was significantly down-regulated in the V-/SL+ treatment, confirming earlier observations. Conversely, there was no significant difference in the up-regulated expression of these genes in the V+/SL- and V+/SL+ treatments. Up-regulation of tissue repair and iron transport genes in response to *L. salmonis* infection occurred in both the V-/SL+ and V+/SL+ treatments, suggesting little impact of the virus. Work is underway to determine whether infection with IHNV affected the previously reported T_H2 anti-inflammatory response to *L. salmonis*. In summary, despite the significant effect of co-infection on salmon survival, expression of the GOI examined here was not significantly impacted by co-infection. Transcriptomics was not a useful predictor of the host response to co-infection and likelihood of survive. Future studies will require a global assessment of host responses to adequately understand host-pathogen interactions and outcomes in the context of co-infection.

Conference Session Designation: (Co-infections in Fish)
Presentation Format: **(** Oral)

Analyzing the Molecular Mechanisms Underlying *Lepeophtheirus salmonis* and Bacterial Co-Stimulation in Atlantic Salmon

Albert Caballero-Solares[1] *, Navaneethaiyer Umasuthan[1], Xi Xue[1], Jillian D. Westcott[2], Zhiyu Chen[2], Mark D. Fast[3], Barbara F. Nowak[4], Richard G. Taylor[5] and Matthew L. Rise[1]

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Co-infection by sea lice and pathogenic bacteria occurs naturally at Atlantic salmon sea cages, and causes multi-million dollar losses to the aquaculture industry. Under laboratory conditions, two experimental feeds (Cargill Innovation) supplemented with two different functional ingredients (CpG and Boost) were tested on lice (*Lepeophtheirus salmonis*)-infested Atlantic salmon (initial weight 290 gr) in comparison with a commercial grower diet (Control). Four weeks after copepodid exposure, with the parasites having developed to the pre-adult stage, salmon were injected intraperitoneally with either phosphate buffered saline (PBS) or a suspension of formalin-killed *Aeromonas salmonicida* (ASAL). Twenty-four hours post-injection, fish were euthanized and dissected for dorsal skin samples. Lice were counted before injection and at the sampling time, which revealed a significant reduction of lice infestation by CpG and Boost diets. For each fish, an area of the skin with a louse attached and an adjacent area with no signs of previous lice attachment, were collected. Dorsal skin RNA was qPCR-analyzed for transcripts involved in inflammatory response (*il1b*, *saa5*), eicosanoid synthesis (*cox2*), antibacterial response (*stlr5a*), and wound-healing (*mmp13*). ASAL induced the transcription of *stlr5a*, *il1b*, and *saa5* significantly. Also, ASAL treatment showed effects on *il1b*, *saa5*, *cox2*, and *mmp13* mRNA levels that differed among dietary groups. Feeding Control and Boost diets resulted in a significantly stronger ASAL-induction of *il1b* and *mmp13* compared with CpG diet. Conversely, *saa5* was significantly ASAL-induced in the salmon fed the CpG diet and not in those fed Control and Boost. Curiously, *cox2* was not ASAL-induced among salmon fed Control and Boost diets, and was significantly repressed by ASAL in the CpG-fed fish, although only slightly (-1.6 ASAL/PBS fold-change). Interaction of louse attachment/ASAL was significant for *mmp13*, the transcription of which was more intensely louse-induced in the PBS-injected/Boost-fed fish than in the PBS/Control and the ASAL/Boost groups. Interestingly, CpG diet significantly increased *stlr5a* induction by louse attachment compared with Control diet. The present qPCR data will be used in the selection of representative samples from each treatment/dietary group for 44K microarray profiling. Relations between transcriptomic and phenotypic (i.e., lice counts, growth, histology) data will also be investigated. It is anticipated that the findings arising from this study will aid in the development of improved feeds to protect farmed Atlantic salmon against sea lice and bacterial co-infection.

Conference Session Designation: **(** Co-infections in Fish)
Presentation Format: **(** Oral)

Complex Skin Disease, Co-Infection of Atlantic Salmon by *Moritella Viscosa* and *Aliivibrio Wodanis*

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The Norwegian aquaculture of Atlantic salmon (*Salmo salar* L.) is hampered by ulcerative disorders associated with bacterial infections. However, the etiology of skin disorders is complex and mechanical injuries, environmental factors, and nutrition are central factors that could effect susceptibility to infections. *Moritella viscosa* is the causative agent of classical winter-ulcer disease. Other contributing bacteria to occurring field outbreaks may be *Aliivibrio wodanis* and *Tenacibaculum* spp. This presentation will be focused on *M. viscosa* and *A. wodanis* interactions. A co-infection model reproduced field observations confirming that both bacteria co-infect Atlantic salmon. It is further hypothesized that *A. wodanis* colonization might influence the progression of a *M. viscosa* infection. From *in-vitro* co-cultivation studies *A. wodanis* impedes the growth of *M. viscosa*. Using bacterial implants in the fish abdomen it is evident that the presence of *A. wodanis* is altering the global gene expression of *M. viscosa*, and that the inhibitory effect is not contact-dependent. Outbreaks of ulceration are associated with salmon reared in marine waters at temperatures below 8°C. Ongoing studies are focusing on RNAseq data to explore relationships between temperature reduction and the effect on the global transcription profile to help improve understanding of putative virulence mechanisms.

Conference Session Designation: (Co-infections in Fish)
Presentation Format: (Oral)

Thursday September 6th – Tilly / Tupper
Miscellaneous
Moderator – Matt Griffin (Mississippi State University)

3:15 PM	Miscellaneous	Dennis - Seeing Spots: What's Happening at the Microscopic Level in Caribbean Sea Fans With Purples Spots?
3:30 PM		Kane - Look Deep Into My Shell: Gross and Radiographic Observations of Shell Damage by Boring Parasites in the Eastern Oyster *Crassostrea virginica*
3:45 PM		Gonzalez - Surgical Resection of a Leiomyosarcoma in a Goldfish (*Carassius auratus*)
4:00 PM		Dennis - What's Getting Under the Skin of Caribbean Surgeon fishes? An Investigation of a Prevalent Pigmented Dermatopathy in St. Kitts' *Acanthurus* spp.
4:15 PM		Kane - Seafood Workers Are Aquatic Animals Too: Surveillance of Health, Injuries and Fatalities Along the US Gulf Coast

Seeing Spots: What's Happening at The Microscopic Level in Caribbean Sea Fans with Purple Spots ?

Michelle M. Dennis *, Anna Becker and Mark A. Freeman

Center for Conservation Medicine and Ecosystem Health, Ross University School of Veterinary Medicine MiDennis@rossvet.edu.kn

While coral reefs are catastrophically declining around the world, octocorals seem more resilient than scleractinian corals and may become increasingly important to future reefs. The sea fans *Gorgonia ventalina* and *G. flabellum* are valued in Caribbean reef communities, yet information about their diseases is limited. In the 1990's, epidemics of mass mortality of sea fans were attributed to the common terrestrial fungus, *Aspergillus sydowii*, and presently aspergillosis remains endemic and widespread. Aspergillosis is typically diagnosed based on the presence of round to annular purple areas surrounding a central region of tissue loss, yet other injuries can result in purpling of sea fan tissue, and *A. sydowii* has been isolated from healthy-appearing colonies. More recently, an emerging disease termed multifocal purple spots (MFPS) was described, characterized by the presence of 1-3mm circular purple nodules, and *Aplonchytrium* sp. labyrithulomycetes and *Sphaerippe* sp. copepods have been identified in these lesions. Our research aims to examine purple lesions in Caribbean sea fans and to establish histological case definitions for aspergillosis and MFPS. Microscopically, lesions grossly consistent with aspergillosis were composed of tissue loss with a margin of amoebocyte infiltrate and protein or melanin deposition. Atrophy, loss, or necrosis of polyps in the purple pigmented region were frequently observed. While fungal hyphae were present in the axis of all affected sea fans, they were typically not diffusely throughout the lesion, and in most cases they were associated with other etiologic agents, including algae and labyrithulomycetes. MFPS lesions microscopically consisted of peri-axial chambers of protein containing fungus, or metazoan parasites, and similarly bordered by amoebocyte infiltrate or melanin deposition. One metazoan morphotype was identified as a barnacle based on morphology and molecular sequencing. These findings emphasize the heterogenous nature of purple lesions in sea fans, and the need to move away from macroscopic diagnosis of sea fan diseases. Greater research is needed to identify the pathogens involved in sea fan disease, and to understand their causal role and coinfection dynamics.

Conference Session Designation: (Coral Diseases)
Presentation Format: (Oral)

Look Deep Into My Shell: Gross and Radiographic Observations of Shell Damage by Boring Parasites in the Eastern Oyster, *Crassostrea virginica*

Shelby C. Thomas[1,2], Ross M. Brooks[1], Hanzhi Gao[3] and Andrew S. Kane[1,2,4*]

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Shell-boring parasites on oyster reefs can reduce shell density, increase shell surface area and contribute to accelerated shell erosion. Prevalence and severity of shell damage associated with shell parasites as part of a basic health assessment in restoration monitoring is important. This project examined severity of parasite shell damage in Eastern oyster, *Crassostrea virginica*, associated with *Polydora websteri (*polychaete*), Diplothyra smithii* (clam) *and Cliona celata* (sponge) based on current technology, i.e., gross visual examination, versus diagnostic radiography. Oysters (n=347) representing four size classes from 20-120mm were sampled from Apalachicola Bay during 2016, and shells were evaluated by gross visual observation and x-ray (i.e., "gold standard") using an established 0-5 severity score based on percent area affected. Mean severity scores based on radiographic, visual internal and visual external shell observations for *Polydora* were 3.9, 1.6 and 1.0; for *Diplothyra* were 1.7, 0.7 and 1.2; and for *Cliona* were 2.3, 0.7 and 1.6, respectively. *Polydora* shell damage based on internal shell visual observations underestimated radiographic observations by 2.4 rank scores. *Diplothyra* shell damage based on internal and external visual observations underestimated radiographic observations by 1.0 and 0.4 rank scores, respectively. *Cliona* shell damage based on internal and external visual observations underestimated radiographic observations by 1.6 and 0.7 rank scores, respectively. While precision for visual and radiographic severity scoring is 0.5 ranks, and some cases of mean radiographic severity score minus mean visual severity score was <0.5 ranks, variability across severity scores indicated that visual severity data are not comparable with matched radiography data. Linear regression-derived correction factors for visual severity data are being validated and appear to provide statistically accurate shell damage estimates relative to gold standard radiographic data. Shell parasite presence and density in the environment is driven by temperature and salinity, and severity of parasite shell damage is associated with oyster height ($p<0.01$). Therefore, these studies in oysters also support an understanding of parasite-keystone host species under changing environmental and climate regimes. This study was supported by the National Fish and Wildlife Foundation, the Florida Fish and Wildlife Conservation Commission, the University of Florida Institute for Food and Agricultural Science (IFAS), and the Florida Sea Grant Program.

What's Getting Under the Skin of Caribbean Surgeonfishes? An Investigation of a Prevalent Pigmented Dermatopathy in St. Kitts' *Acanthurus* Spp.

Michelle M. Dennis *, Adrien Izquierdo, Kelsey Johnson, Paul Frye and Mark A. Freeman[1]

Center for Conservation Medicine and Ecosystem Health, Ross University School of Veterinary Medicine, St. Kitts, West Indies

Acanthurus spp. of St. Kitts and other Caribbean islands, including *A. bahianus* (ocean surgeonfish), *A. chirurgus* (doctorfish), and *A. coeruleus* (blue tang), are frequently observed to have multifocal, brown-black, circular cutaneous lesions. However, the pathology has not been described and the etiology is unknown. In free-living finfish, epidemics of multifocal pigmented dermatopathies are often ascribed to cutaneous parasite infections, and have also been observed with chromatophore neoplasia. This research aims to investigate the extent of the pigmented dermatopathy in *Acanthurus* spp. of St. Kitts and to describe its pathology. Surveys were undertaken which showed prevalence to be 33-52% across three locations. The pigmented dermatopathy was more common in *A. bahianus* and *A. coeruleus* relative to *A. chirurgus*. Thirty *Acanthurus* spp. showing the pigmented dermatopathy were collected by spearfishing. Affected regions of skin usually involved the dorsal, pelvic, and tail fins, and less frequently the body of the fish. The pigmented foci centrally contained a <1mm diameter cyst from which metazoan parasites were dissected. Histologically, pigmented areas had chronic perivascular dermatitis of variable severity, and the dermis contained an encysted metazoan parasite. In each species of fish, parasites dissected from pigmented skin lesions were morphologically and molecularly classified as a digenean of the family Heterophyidae. Future research is warranted to determine the ecology of the parasite and factors which may explain is apparently high prevalence in St. Kitts and potentially other Caribbean islands.

Conference Session Designation: (Caribbean Fish Health)
Presentation Format: (Oral)

Seafood Workers are Aquatic Animals Too: Surveillance of Health, Injuries and Fatalities Along the US Gulf Coast

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Surveillance studies with Gulf coast fishers, crabbers, shrimpers, and oyster and clam harvesters, supported by the National Institute for Occupational Safety and Health/CDC, are underway to identify risk factors associated with fatal and non-fatal injuries where the majority of workers are self-employed and uninsured. Community partnerships highlight the importance of engaging with seafood workers to implement an in-person questionnaire supplemented with workplace observations on harvesting and fishing vessels. Falls overboard and winch injuries are associated with many of the fatalities and severe injuries reported. Musculoskeletal injuries, cuts and lacerations, bites, spine punctures, and heat and sun exposure are common in these work sectors. Conditions associated with unstable work platforms in harsh settings, coupled with declining fisheries – related in part to climate and environmental change – appear to increase risk of onboard incidents, drug use and mental health issues. Surveillance data is being used to inform interventions and outreach tools to support Gulf coast seafood worker and aquaculture health and safety. Research investigators who engage in ship time for sample collections or observations, or who rely on commercial harvesters for samples, may also be subject to similar environmental conditions and hazards. Therefore, safety and health concerns related to working on the water, with equipment under strain, on a moving platform translates beyond commercial seafood workers.
This study is supported through the Southeastern Coastal Center for Agricultural Health and Safety, and the National Institute for Occupational Safety and Health (NIOSH) under CDC, grant # U54OH011230.

Surgical Resection of a Leiomyosarcoma in a Goldfish (*Carassius auratus*)

Jaime F. González[*1], Lisa M. Avila[1] and Paola A. Barato[2]

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Mesenchymal neoplasms are relatively uncommon in fish as compared to epithelial tumors. A specimen of goldfish (*Carassius auratus*) (51.7 g weight and 18 cm total length) was submitted by her owner for clinical evaluation to the laboratory of Aquatic Toxicology and Fish Medicine, School of Veterinary Medicine and Animal Science, Universidad Nacional de Colombia. The fish showed a unilateral mass invading the eyeball area. A surgical resection was scheduled considering possible risks for the fish and its tankmates. The fish was fasted 12 h prior to the surgery and induced to a deep surgical plane by immersion in an anesthetic solution of eugenol (100 ppm) (stock sol of 100 mg eugenol/ml ethanol from Eugenol®, 85 % EC). After reaching the loss of both its swimming axis and the opercular movements (3 min 36 sec), the specimen was maintained in a surgical plane during the procedure using a recirculating pump that allowed infusion of the branchial tissue with a 30ppm eugenol solution during approximately 15 minutes. The mass along with the eyeball was removed by enucleation. The fish was returned to a recovery tank and regained its swimming axis (7 min 25 sec) and recovered uneventfully after 20 min. The mass was fixed in 10% neutral buffered formalin and submitted for histopathological evaluation to Corpavet (Vet Path Corp). A leiomyosarcoma was diagnosed after hematoxylin & eosin, Masson trichromic (TM) staining and immunohistochemistry (IHC) to smooth muscle actin (SMA) and Ki-67 evaluation. Neoplasia involved superficial and deep dermis showing fusiform and oval shaped cells, highly infiltrative with moderate pleomorphic features. TM revealed red wine-coloured muscular cells compatible with leiomyosarcomas, leiomyomas, rhabdomyomas or rhabdomyosarcomas. SMA and Ki-67 markers determined the final leiomyosarcoma identification by showing a positive result in some pleomorphic cells. In short, resection of the mass using eugenol as the anesthetic protocol was successful in both the induction and maintaining of a surgical plane. Further histopathologic protocols allowed the identification of a leiomyosarcoma, a mesenchymal neoplasm uncommonly reported in fish.

Conference Session Designation: (Ornamental and Aquarium Medicine)
Presentation Format: (Oral)

Tuesday Sepember 6th – Langeve / Cartier
eDNA / Metagenomics
Moderator – Sascha Hallett (Oregon State University)

9:30 AM	eDNA / Metagenomics	**Bernhardt - Development of a Non-Invasive Method for Concentration and Detection of Salmonid Alphavirus From Seawater**
9:45 AM		**Barry - Rapid DNA Based Detection of Whirling Disease Causing Parasites From Environmental Samples**
10:00 AM		**Shea - Assessing Environmental Microparasites in Relation to Atlantic Salmon Farms in BC**
10:15 AM		Soto - Development of Multiplex Quantitative PCR Assays for the Detection of Invasive Species and Aquatic Animal Pathogens

Development of a Non-Invasive Method for Concentration and Detection of Salmonid Alphavirus from seawater

Lisa-Victoria Bernhardt*[1], Marit L. Kjellin[1], Lars Qviller[1], Mette Myrmel[2], Atle Lillehaug[1] and Simon C Weli[1]

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Commercial Atlantic salmon (*Salmo salar L.)* farming is a vital industry in the coastal areas of Norway. It is the second most important export product and provide high-value nutrients that represent a valuable part of a healthy diet to humans. Despite these contributions, significant losses of fish during seawater phase production persist. The causes of these high mortalities have yet to be fully unravelled. However, virus-related diseases are thought to constitute the most important causes. Virus transmission and disease control strategies are thus important issues in Atlantic salmon health management. Since Salmonid Alphavirus (SAV) has the ability to spread via horizontal transmission and seawater represent the natural environment of Atlantic salmon, the seawater from the fish environment could be used for evaluation of SAV transmission. Currently, SAV transmission in Atlantic salmon farms is largely surveyed using traditional manual methods. These methods are selective, invasive and are limited to *in vivo* sampling of live fish for identification of the virus. Development of a reproducible non-invasive method to confirm the presence of the virus in fish environment, will serve as an early warning system and may have significant impact on Atlantic salmon health management. The aim of this study is to establish a non-invasive method for detection of SAV in seawater by sampling from the fish environment for the purpose of virus detection without sacrificing live fish, thus satisfying the 3R requirement relating to experimental animals: replace, reduce and refine. The method is based on concentration of SAV in seawater through filtration and adsorption to charged membranes, before detection and quantification of the virus with reverse transcriptase quantitative PCR (RT-qPCR). First external clinical signs were observed on day 12 post cohabitation (lethargic fish and findings of faecal casts in high dosage tank). Pathological signs associated with SAV-PD were observed from day 16 post cohabitation in fish from high dosage tank (enlarged spleen, petechial bleeding on pylorus and ascites). Samples of seawater and Atlantic salmon tissues from both high- and low dosage tanks in the SAV-PD cohabitant challenge trial were analysed. Mid kidney and gill samples from high- and low dosage SAV-PD tanks were positive by PCR on day 20-25 post cohabitation. In addition, concentrated seawater from high and low dosage SAV-PD tanks were positive by PCR on day 20-25 post cohabitation. The correlations of the results from tissue and seawater samples and clinical signs provide evidence that suggest that filtration of seawater can be applied as an early warning system for the presence of virus in Atlantic salmon farms.

Conference Session Designation: **(** Virology or Aquatic Epidemiology)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Rapid DNA Based Detection of Whirling Disease Causing Parasites from Environmental Samples

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Whirling disease is a disease of fish caused by an invasive myxosporean parasite, *Myxobolus cerebralis*. It was first detected in Canada in Johnson Lake in Banff National Park, Alberta in August 2016, and little is known about the transmission of this parasite in Canada. It affects salmonid fish and has potential to impact the recreation sport fishing industry due to increased mortality in juvenile fish and waterbody closures to prevent spread. As well, many salmonid species that are currently threatened or endangered in North America are susceptible to whirling disease. Current testing focuses on detection of *M. cerebralis* in fish tissues, requiring lethal testing of both infected and non-infected fish. However, the parasite has an intermediate host, the oligochaete worm *Tubifex tubifex* and two environmental stages found in water and sediment that create other avenues for detection. We propose that using these environmental and *Tubifex* samples are a reasonable alternative to fish sampling and will be especially useful in large scale monitoring programs. In addition, *T. tubifex* susceptibility to *M. cerebralis* is not consistent across the species, with experiments showing some are refractory. Characterization of these worm populations will help target future monitoring and control programs based on the presence or absence of susceptible *T. tubifex*.

This project utilizes environmental samples collected from 45 stocked ponds and 300 wild sites from Central and Southern Alberta. These include sediment samples, invertebrate worm samples and water samples from stocked ponds. These samples are extracted for DNA using different methods tailored to the sample type and tested in a qPCR assay targeting the 18S gene of *M. cerebralis*. Additionally, worm samples are barcoded targeting the CO1 gene to determine species as identification by morphology is unreliable.

We will use these results to look at infection prevalence in worm samples within and across sample sites. Using the CO1 barcodes of Tubificid-like worms, we aim to create a phylogeny of worms in Alberta to look for cryptic species that may correlate to parasite susceptibility. Species composition of Tubificid-like worms will be analyzed and compared other sites, as well as to highlight connections to parasite presence. DNA extracted from sediment, worms and water will be tested for presence of *M. cerebralis* DNA and to be compared to results from fish samples.

We hope that with this work we can create a reasonable alternative to using fish samples for monitoring whirling disease presence in Canadian fresh water systems.

Conference Session Designation: (Myxozoa)
Presentation Format: (Oral)
Student Presentation: (Yes)

Assessing Environmental Microparasites in Relation to Atlantic Salmon Farms in BC

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British Columbia supports a large Atlantic salmon aquaculture industry in addition to a diversity of wild Pacific salmon stocks. Spatial overlap between farmed and wild populations is not uncommon, as many salmon farms operate along Pacific salmon migration routes. There is a growing concern regarding potentially harmful interactions between farmed and wild populations via their shared environment with an emphasis on the transmission of infectious disease. In fact, it has been found that salmon farms increase the risk of sea lice infection in wild salmon smolts; however, it remains unclear whether transmission of microparasites such as viruses and bacteria occurs between farmed and wild populations. We assessed environmental pathogen transmission by filtering water samples collected nearby and far from active salmon farms. We screened water samples for a diverse group of 37 viral, bacterial, and eukaryotic microparasites using a high throughput qPCR platform. I will present this methodology as well as preliminary results obtained via this sampling method.

Conference Session Designation: (eDNA/Metagenomics)
Presentation Format: (Oral)
Student Presentation: (Yes)

Development of Multiplex Quantitative PCR assays for the Detection of Invasive Species and Aquatic Animal Pathogens

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Batrachochytrium dendrobatidis, Ranavirus, *Ceratonova shasta*, *Myxobolus cerebralis*, *Tetracapsuloides bryosalmonae* and *Ichthyophthirius multifiliis* are important pathogens of cultured and wild fish and amphibians previously reported in the Pacific Northwest. Quagga Mussels, *Dreissena rostriformis bugensis*, Zebra Mussel, *D. polymorpha*, New Zealand Mudsnail, *Potamopyrgus antipodarum*, and the Asian clam, *Corbicula fluminea* are important invasive species currently monitored in the state of California, USA. Environmental DNA (eDNA), defined in this study as "genetic material obtained directly from substrate samples," has the potential to be a powerful tool for evaluating the presence of organisms, of which direct observation is impossible, and for assessing biodiversity in aquatic environments. In this study, the presence of 10 different organisms, including important amphibian and fish pathogens, as well as important invasive species to California, was investigated using eDNA analysis of river sediment samples collected in areas affected by recent fire activity in Plumas National Forest, California, USA. Extracted DNA from sediment samples collected in 2017 and 2018 from 38 different watersheds were used as template for recently developed and validated TaqMan probe quantitative polymerase chain reaction multiplex assays. The most consistent fish pathogens detected were *C. shasta* and *I. multifiliis*. None of the targeted invasive species DNA were detected. Future efforts to genotype the detected organisms is warranted to clarify the pathogen diversity detected in environmental samples.

Conference session designation: (eDNA / Metagenomics)
Presentation format: (Oral)

Thursday September 4th – Langeve / Cartier
Parasitology 1
Moderators – Ariadna Sitjà-Bobadilla (Inst. Aquacultura Torre de la Sal) **Sarah Poynton** (John's Hopkins Univ.)

10:45 AM	**Parasitology 1**	Poynton - A Global Review of Parasites in Finfish Aquaculture
11:00 AM		**Furtado - Antiparasitic Potential of the Nano-Emulsioned Oil of the Acicula and Resin of *Pinus taeda* Against the Larval Stages of *Lernaea cyprinacea***
11:15 AM		Sitjà-Bobadilla - Parafishcontrol, a European Funded Project to Mitigate Fish Parasitic Diseases in Aquaculture
11:30 AM		Bradley - An Outbreak Of *Bonamia exitiosa* in Victorian Native Oysters in 2015 and Examination of Risk Factors for Developing Clinical Disease.
11:45 AM		**Nguyen - Rapid and Specific PCR Assay for Diagnosis of Apicomplexan- "X" (APX) Associated With the Flat Oysters (*Ostrea chilensis*) in New Zealand**
12:00 PM		**Lunch**

A Global Review of Parasites in Finfish Aquaculture

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With the rise in global production of finfish in aquaculture, knowledge of the role and control of parasites on a regional basis becomes essential. Although literature is appropriately focused on taxa of parasites with economic importance, geographic coverage is very uneven. Most geographically focused reports are of host-parasite occurrence and ecology, rather than broader issues such as fish health infrastructure. Furthermore, representation in the literature is often disproportional to the global, regional, or local importance of finfish aquaculture, and the parasites therein. There are key gaps in the scientific literature in English, (the current international language of science), about parasite of finfish aquaculture for China, much of Asia, Russia, Africa, and some of Latin America. These gaps exist despite the key importance of some of these countries as significant producers, (whether determined by tonnage of fish produced, or monetary value).

To address these knowledge gaps, we are currently completing assembly of a multi-author book "Aquaculture Parasitology: Global Impacts and Management in Finfish" to be published by Wiley. The "Regional Review" focus of the book describes the global picture in seven chapters, largely following the organizational scheme adopted by the Food and Agriculture Organization (FAO (UN)): China, Asia (excluding China), Oceania, Europe (including Russia), Africa, North American, and Latin America and the Caribbean. For each region, the authors present an overview of aquaculture in the region, infrastructure for health and disease monitoring and management, current and emerging parasite diseases, current practices, and special topics.

When viewing parasites of farmed fish from a global perspective, many interesting contrasts emerge, for example: (ii) parasites of greatest economic importance range from myxozoan and digenean trematodes in North America (primarily salmonid and catfish production) to monogeneans in China (predominantly carp production), and crustaceans in Latin America (predominantly salmonid production); (ii) infrastructure ranges from substantial, as in parts of Europe and North America, and in China (where there has been significant government investment in research and surveillance), to rather limited, as is the case in Russia and in East Africa; and (iii) prevention and treatment practices are very diverse, including rare implementation in East Africa (where there is a history of subsistence aquaculture), to inclusion of traditional herbal medicines in China. Despite the great geographic diversity across regions, some common themes emerged including the importance of non-native fish species, environmental concerns, and the need for better disease control including availability of anti-parasitics suitable for use in foodfish.

Conference Session Designation: (Parasitology General)
Presentation Format: (Oral)

Antiparasitic Potential of the Nano-emulsioned Oil of the Acicula and Resin of *Pinus* c.f. *taeda* Against the Larval Stages of *Lernaea cyprinacea*

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Lernaea cyprinacea L. (Copepoda), popularly known as 'anchor-worm', is a crustacean ectoparasite with worldwide distribution and extremely common in the culture of commercial fish. It may be associated to great economic liabilities due to high mortality rates and its repugnance aspect to fish. Due to the high and frequently great potential damage in the use of therapeutic drugs in fish farms, the management of diseases should focus on less aggressive methods to sick animals, the environment and the professional involved. Phytotherapeutic drugs have proved to be potentially beneficent compounds in fish culture. The *Pinus* species should be underscored due to their bioactive compounds with associated anti-parasite properties. Current analysis established the Minimum Inhibitory Concentration (MIC) of nano-emulsioned essential oils extracted from fresh acicula and from the crude resin of *Pinus* c.f. *taeda* on parasite larvae (nauplii and copepods). Oil extraction was undertaken by hydrodistillation in a Clevenger apparatus in the laboratory of essential oils of the Agro-Livestock Research Firm and Rural Extension of Santa Catarina (Epagri) in Itajaí SC Brazil. Biological material was retrieved during an outbreak of lernosis from broodstocks of silver catfish (*Rhamdia quelen*) in a pond constructed during the summer of 2018 at the Unit for Genetic Improvement of Fish (Itajaí, Epagri). Thirteen animals parasited by *L. cyprinacea* (mean 192 ± 156 parasites per fish, 40 – 479) were captured, anesthetized with eugenol (75 mg/L) and euthanized by brain commotion. Parasites were removed from the host by hand and transferred to a petri plaque with distilled water. The egg-sacs were ruptured to release the eggs which hatched in up to 24 h after the process. Assessment of antimicrobial activity of essential nano-emulsioned oil from the resin, the acicula and of α-Pinene (major compound in the composition of essential oils: 69,96% of acicula and 45,56% of resin) was performed separately by the MIC methodology for the larvae of the parasite (nauplii and copepods). Further, 100 µL of the agent were added to the first well of the flat-bottom microplate of cell culture (96 wells) and 50 µL of distilled water were added as from the second well. A factor 2 series dilution was performed till the 19th well. Finally, 50µL of distilled water with 5 parasites were added to each well. Microplates were monitored during periods of 60 minutes and 24 h. MIC was determined as the lowest dilution of the agent in which total inhibition of the larvae of the crustacean occurred (total absence of body movements) in all triplicates. All tests included one control group with distilled water and the tensoactive used for the preparation of the oil nano-emulsions (Tween® 80). The nano-emulsioned essential oil of the acicula of *P. taeda* had the best result among the chemical agents tested. It inhibited nauplii and copepods of *L. cyprinacea* in concentrations ranging between 20 and 156 ppm, respectively, according to analysis time. Currently, the most efficacious treatment in Brazil against *L. cyprinacea* is based on different commercial solutions with trichlorphon, an insecticide with proved toxicity for fish and authorized for fish farms. Positive results showed the capacity of the Pinus extract as a prophylactic agent in fish.

Conference Session Designation: **(** Parasitology General)
Presentation Format: **(** Oral)
Student Presentation: **(** Yes)

Parafishcontrol, a European Funded Project to Mitigate Fish Parasitic Diseases in Aquaculture

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Horizon 2020 is the largest EU Research and Innovation funding programme ever, with nearly €80 billion of funding available from 2014 to 2020. It promises more breakthroughs, discoveries and world firsts by taking great ideas from the lab to the market. The goal is to ensure Europe produces excellent science, removes barriers to innovation and makes it easier for the public and private sectors to work together in delivering innovation. Here we introduce the H2020 project ParaFishControl (Advanced Tools and Research Strategies for Parasite Control in European Farmed Fish), granted with an EU contribution of € 7.8 million in the first call of this programme, which started on April 2015. The consortium, coordinated by CSIC, comprises 28 academic and public organisations, SMEs and research and industrial enterprises from 13 countries, which are experts in parasitology, immunology, epidemiology, pathology, genomics, nutrition and feeding, biotechnology, chemotherapy, food security, etc. FAO estimates that aquaculture contribution to human food will reach 62% by 2030. This could not be accomplished without reducing the impact of diseases, which can reach 20% production value. Some authors estimate that parasites can produce up to 10% of the annual weight harvest lost in the world. These economic losses in farmed fish can be due to poor growth performance, impaired welfare, and high mortality rates. Therefore, the goal of the ParaFishControl project is to increase the sustainability and competitiveness of the EU aquaculture industry by improving our understanding of fish-parasite interactions and developing innovative solutions and tools for the prevention, control and mitigation of the most harmful parasitic species affecting the main European farmed fish species (Atlantic salmon, rainbow trout, gilthead sea bream, European sea bass, turbot and common carp). The most threatening ecto- and endo-parasites are being studied, including crustaceans, monogeneans, myxozoans, microsporidians, ciliates, dinoflagellates, amoebas, oomycetes and zoonotic helminths. Activities are carried out over five years into nine cross-cutting work packages which integrate all fish host species and their relevant parasites, including: parasite genomics and transcriptomics, life cycles, transmission and host immune response (WP1); wild-farmed fish parasite transfer (WP2); vaccines and immunostimulatory feeds (WP3); diagnostic tools (WP4); innovative treatments (WP5); risk analysis and surveillance, creation of a biobank of parasites (WP6); fish product safety (WP7); dissemination, technology transfer and take-up (WP8); coordination and management (WP9). For more information visit the web site of the project: ***www.parafishcontrol.eu*.**

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Conference Session Designation: (Parasitology General)
Presentation Format: (Oral)

An Outbreak of Bonamia Exitiosa in Victorian Native Oysters in 2015 and Examination of Risk Factors for Developing Clinical Disease.

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Bonamia ostreae and *Bonamia exitiosa* are significant pathogens of oysters that cause high mortality rates and substantial economic losses to the oyster farming industry globally. Infection by an unidentified Bonamia sp. was responsible for the devastation of experimental aquaculture of the Native Oyster (*Ostrea angasi*) and adjacent wild beds in Victoria, Australia in the early 1990s.

Small scale aquaculture of the Native Oyster was re-commenced in 2010 in Victoria. Surveillance was undertaken by the government to assess the presence of *Bonamia spp* and the results of that testing and the subsequent outbreak of clinical *Bonamia exitiosa* will be described.

A project investigating the factors that cause the development of clinical disease from previously healthy but sub-clinically infected oysters was undertaken both in indoor tanks and in the field.

The tank trials were conducted to examine putative risk factors for clinical expression of Bonamia infection in Native Oysters held in tanks at the Government Queenscliff facility over 2 years. Risk factors examined include water temperature, starvation, agitation, size and provenance. Comparisons between risk factors were examined with measures including mortality rates, PCR and histopathology.

The 2 field trials were undertaken at the "infected" site where clinical *Bonamia exitiosa* has previously been confirmed and the "uninfected" site where the Bonamia parasite have not previously been detected. Different risk factors were examined over 2 years including stocking density, oyster size, depth held in the water column and cleanliness of cages. A full summary of the results for all these trials will be provided.

Conference Session Designation: (Parasitology General or Aquatic Epidemiology)
Presentation Format: (Oral)

Rapid and Specific PCR Assay for Diagnosis of Apicomplexan- "X" (APX) Associated with The Flat Oysters (*Ostrea chilensis*) in New Zealand

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A PCR assay designed based on *in situ* hybridisation probes was developed to specifically detect the parasite Apicomplexan-X in the flat oyster (*Ostrea chilensis*), endemic to New Zealand, targeted 723 bp DNA product. The specificity of the assay was proved as it didn't amplify any product of other apicomplexan species DNA including *Toxoplasma gondii*, *Neospora caninum*, *Selenidium* sp., *Cephaloidophorida* sp., *Lecudina* sp., *Platyprotepum* sp., and *Thiriotia* sp. The analytical sensitivity of the test was determined as 1pg of APX DNA using dilution series method. Following analytical validation, diagnostic performance was determined by testing samples from flat oysters infected with APX (n = 75) at different intensities estimated by histology. Of 73 flat oysters infected with APX identified by histology, 69 (95%) tested PCR-positive. Failure to amplify an internal control indicated the presence of PCR inhibitors in the 4 PCR-negative samples. This is the first PCR assay for specific detection of the parasite APX in the flat oyster *O. chilensis*. It should be useful for diagnostic testing and active surveillance programs for managing flat oyster health.

Conference Session Designation: **(** Invertebrate & Shellfish Disease / Parasitology)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Thursday September 4th – Langeve / Cartier
Parasitology 2
Moderators – Ariadna Sitjà-Bobadilla (Inst. Aquacultura Torre de la Sal) **Sarah Poynton** (John's Hopkins Univ.)

1:15 PM	**Parasitology 2**	**Nishiwaki - Histopathological and Ultrastructural Studies on Intracellular Parasites in the Ovary of Skipjack Tuna (*Katsuwonus pelamis*)**
1:30 PM		**Watanabe - Characterization of Proteases of Trophont, the Parasitic Stage of *Cryptocaryon irritans***
1:45 PM		**McAllister - Examination of Parasite-Induced Anemia and Erythropoietic Regulator Gene Expression in *Carassius* auratus During *Trypanosoma carassii* Infection**
2:00 PM		Warland - Nucleospora Cyclopteri (Microspora): Tissue Tropism, Shedding and Non-Lethal Detection
2:15 AM		**Omovwohwovie - Parasites of *Oreochromis niloticus* Observed in the Fisheries Unit of the Niger Delta University Teaching and Research Farm**
2:30 AM		Urawa - Impact of *Spironucleus salmonis* on the Growth and Mortality of Juvenile Masu Salmon *Oncorhynchus masou*
2:45 AM		**Wang - Trichodinid Ectoparasites (Ciliophora: Trichodinidae) From Freshwater Fishes in China, With Notes on Host–Parasite Relationship**

Histopathological and Ultrastructural Studies on Intracellular Parasites in the Ovary of Skipjack Tuna (*Katsuwonus pelamis*)

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Skipjack tuna (Katsuwonus pelamis) is known as a highly migratory fish and is distributed mainly in tropical and subtropical regions of the world. It is also one of the most important target fish species in fisheries. In a previous study, we reported the presence of intracellular parasitic protozoa on ovary tissue of skipjack tuna caught over a number of years in the western central Pacific Ocean (Ashida et al., 2007). Since the protozoa were stained using fluorochrome Uvitex-2B, which binds to chitin, it was presumed that they comprise microsporidian spores with a chitinous wall (Ashida et al., 2007). On the other hand, microsporidian DNA was not detected by general PCR. Therefore in this study we performed histopathological and ultrastructural observations to confirm the detailed morphological structure of the microsporidian-like organisms in relation to classification and localization in the ovaries.

The protozoa are round to ovoid, and 1-3 μm in diameter. No mitochondria were observed, although mitosome-like organelles were present. No cysts could be detected. The parasites were mainly localized in the cytoplasm of phagocytes in connective tissue of the ovary. Some were also found within oocytes. The morphological characteristics of this microsporidian-like organism shows that the protozoa are a new species which have not previously been reported for skipjack tuna.

Ashida H. et al. (2007) Nippon Suisan Gakkaishi, 73(5), 916-918.

Conference Session Designation: (Parasitology General)
Presentation Format: (Oral)
Student Presentation: (Yes)

Characterization of Proteases of Trophont, the Parasitic Stage of *Cryptocaryon irritans*

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Cryptocaryoniasis of marine teleosts is caused by *Cryptocaryon irritans*, an obligatorily parasitic ciliate. This parasite is a major threat to marine aquaculture in tropical and subtropical waters. Many studies have been carried out, aimed at development of control methods against the disease, such as therapeutic drugs and vaccines. However, treatments efficient enough to control the disease have not yet been developed. Recently, proteases have been suggested to play a crucial role in the infection and development of parasitic protozoa such as *Tritrichomonas foetus*, *Tetrahymena* spp., *Leishmania* spp. and *Miamiensis avidus*. It is also thought that proteases play a key role in the infection and development of trophont, the parasitic stage of *C. irritans*, and are a potential target for chemotherapy and vaccines against the parasite. However, proteases involved in the infection of *C. irritans* have not been understood or characterized. In this study, we conducted transcriptome analysis to identify various proteases of *C. irritans*, and examined the activity of trophont proteases by zymography. In addition, we examined the effect of protease inhibitors on the infection, survival and growth of the parasite in vitro. The results show that the activities of serine proteases and cysteine proteases were strong in trophonts. When inhibitors against the proteases were added into the medium for in vitro culture of the parasite, the survival rate declined and growth was delayed. These results suggest that serine and cysteine proteases are important for the parasitic stage of *C. irritans*. This knowledge will assist in the development of new chemotherapeutic drugs or vaccines for cryptocaryoniasis.

Conference Session Designation: (Parasitology General)
Presentation Format: (Oral)
Student Presentation: (Yes)

Examination Of Parasite-Induced Anemia And Erythropoietic Regulator Gene Expression In *Carassius Auratus* During *Trypanosoma Carassii* Infection

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Trypanosoma carassii is a flagellated bloodstream parasite of cyprinid fish. Pathogenesis of *T. carassii* manifests primarily as anemia in experimentally infected fish. This anemia is characterized by decreases in the number of circulating red blood cells (RBCs) during peak parasitemia. We examined changes in the key blood metrics and expression of genes known to be important in the regulation of erythropoiesis. Increasing parasitemia was strongly correlated with an overall decrease in the total number of circulating RBCs. Gene expression of critical erythropoiesis regulators was measured in contrast to fish made anemic through injections with phenylhydrazine; a chemical which causes RBC hemolysis leading to severe anemia. Significant upregulation of pro-erythropoietic genes was observed in chemically induced anemia, but not during peak parasitic infection. Mammalian trypanomastids have also been shown to alter erythropoiesis leading to increased morbidity and delayed recovery from infection by the hosts. To examine whether the modulation of key erythropoietic factors was responsible for the observed anemia, we generated recombinant goldfish EPO (rgEPO) and demonstrated that it promoted erythroid colony formation *in vitro*. The administration of rgEPO *in vivo* reduced anemia severity, but was unable to restore erythrocyte numbers in infected fish. The mechanism(s) by which *T. carassii* induce anemia during infection remain unclear. We know that proinflammatory cytokines (IFNγ, TNFα, IL-1β) are upregulated during *T. carassii* infection and that this upregulation has been shown to downregulate EPO levels and consequently, erythropoiesis. It is also possible that the parasites secrete molecule(s) that directly affect EPO production and we are currently testing the excretory/secretory products of *T. carassii* for this activity.

[Supported by NSERC, Canada]

Conference Session Designation: (Parasitology General)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Nucleospora cyclopteri (Microspora): Tissue Tropism, Shedding and Non-Lethal Detection

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Farmed lumpfish are now the most important cleaner-fish used in Norwegian aquaculture for salmon lice (*Lepeophtheirus salmonis*) control.

The loss of lumpsucker juveniles is high during a production cycle of Atlantic salmon, mainly due to bacterial diseases. A virus belonging to the Flaviviridae and certain parasites may also be important. *Nucleospora cyclopteri* is a microsporidian parasite mainly affecting lymphocyte-like leukocytes in lumpfish, and has previously been associated with disease and mortality in farmed populations. Renomegaly, sometimes extreme, has been associated with the infection.

A concern is that this parasite could be both vertically transmitted and immunosuppressive.

Among 85 wild caught lumpfish, renomegaly due to *N. cyclopteri* was not observed. Three fish exhibited pale patches on the kidney, particularly affecting the anterior part. A RT-qPCR study of 41 of these fish; included 10 tissues, 6 swab-sites, bile and urine samples. Whole blood and leucocyte fractions were also analysed.

All sample types were positive, but parasite densities were highest in anterior kidney, followed by mid-kidney, posterior kidney, spleen, heart and gills. Prevalence was 59%. Whole blood was positive in only 25% of the infected individuals, leucocyte fractions in 42%. Some bile and urine samples were positive for the parasite, and parasite load in urine correlated with density in the other tissues, suggesting parasite shedding via this route. The parasite could be detected in gill, vent and 4 skin swabs from infected fish, but these samples were also positive in uninfected fish from the same tanks. Control RNA from tank biofilm samples was also positive, so the presence of infected individuals likely contaminated the tank water. Urine and faeces (bile) from infected fish could be a source of this contamination.

Swabs and gill biopsies may be used to examine a lumpfish population, such as potential broodstock, for *N. cyclopteri* presence. However, due to contamination, individual carriers may best be revealed through RT-qPCR analyses on leukocyte fractions from blood samples. However, repeated sampling may be necessary to reveal all infected individuals.

Conference Session Designation: (Cleaner Fish Diseases / Parasitology)
Presentation Format: **(** Oral)

Parasites of *Oreochromis niloticus* observed in the Fisheries Unit of the Niger Delta University Teaching and Research Farm.

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Parasites of Orechromis niloticus was studied at the Teaching and Research Farm of the Niger Delta University. The prevalence, abundance and intensity of infection were determined. The prevalence of Dactylogyrus parasitae in Oreochromis niloticus showed the highest value of 65%, followed by Cestode, ligula 60%, Ergasilus 10% and Protozoa and Nematode 1.66% with the least prevalence. The intensity of Dactylogyrus obtained was 2.79,while Cestode,(ligula)had 1.10,, Ergasilus spp 1.33, Protozoa and Nematode 1.2 respectively.

Conference Session Designation: (Parasitology)
Presentation Format: (Oral)
Student Presentation: (Yes)

mpact of *Spironucleus salmonis* on the Growth and Mortality of Juvenile Masu Salmon *Oncorhynchus Masou*

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The diplomonad flagellate *Spironucleus salmonis* was detected from the digestive tract of juvenile Masu *Oncorhynchus masou* and Chum Salmon *O. keta* reared at hatcheries in Japan. To elucidate the pathogenicity of *S. salmonis* for juvenile salmon, an infection experiment was conducted at laboratory. Two groups of juvenile Masu Salmon (mean weight 1.5 g; n = 250 fish each) were held separately in 23-l tanks. One group was cohabited with Masu Salmon (n = 50) infected with *S. salmonis* for 2 weeks. The other group served as uninfected controls. Each tank was supplied with running well water, and the water temperature was constant at 10.5ºC. The fish were fed with commercial dry pellet at 2% body weight per day for 10 weeks. Thirty fish were sampled from each group every two weeks, and measured and weighted individually. The contents of stomach, pyloric caeca and intestine removed from each fish were immersed in PBS, and parasite counts were made under the microscope. The intestine tissues were fixed in Bouin's solution or 10% neural buffered formalin, and processed by standard histological technique. Sections were stained with Giemsa's stain or Alcian blue (pH 2.5)/PAS. The parasite was dominantly distributed in the anterior intestine of juvenile Masu Salmon. The abundance of *S. salmonis* increased 2 weeks post infection, peaked at 4,800 parasites at week 4, and declined to less than 100 parasites at weeks 8 and 10. The mortality in the infection group accumulated to 17.9% for 10 weeks, compared with only 1.3% in the control group. The infected fish were significantly smaller than the controls at weeks 6 and 8, and the condition factor of infected fish was also significantly reduced between weeks 4 and 6. Light erosion was observed in the mucosal epithelium of heavily-infected intestine. The present experiment has confirmed that *S. salmonis* infection has a significant impact on the growth and mortality of juvenile salmon. Further laboratory and field studies are required to control the parasite infection at hatcheries.

Conference Session Designation: (Parasitology General)
Presentation Format: (Oral)

Trichodinid Ectoparasites (Ciliophora: Trichodinidae) from Freshwater Fishes in China, with Notes on Host–Parasite Relationship

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Trichodinids, which are morphologically characterized with the presence of a prominent denticular adhesive disc, are probably the most common ciliated protozoan parasites or symbionts of marine and freshwater organisms. Some of them can cause severe disease and mass mortality in their host, which results in considerable economic losses to fishery sector. During a parasitic ciliate survey in China from 2013 to 2018, nine *Trichodina* species and two *Paratrichodina* species were isolated from freshwater fishes. The small subunit ribosomal RNA gene (SSU rDNA) sequences of five *Trichodina* species, that are *T. paranigra*, *T. reticulata*, *T. acuta*, *T. hyperparasitis* and *T. hypsilepis*, were sequenced. Phylogenetic analyses revealed that the five *Trichodina* species investigated in the present study were nested within a clade including several freshwater *Trichodina* species, which indicates that the central granule is a useful taxonomic feature, but it may not be an important phylogenetic characteristic. Our study extended the host range of trichodinids and revealed that invasion of exotic fishe invasion may cause a potential threat to native fishes by carrying or spreading parasitic ciliates. Besides, histopathologic analyses revealed that trichodinids firmly colonized gills, which resulted in discrete hyperplasia and injuries of the gill filaments.

Conference Session Designation: (Parasitology General)
Presentation Format: (Oral)
Student Presentation: (Yes)

Thursday September 6th – Langeve / Cartier
Husbandry / Physiology
Moderator – Hamish Rodger (Fish Vet Group)

3:15 PM	Husbandry / Physiology	Timmerhaus - Effects of Low to Very High Water Velocities on Atlantic Salmon Post-Smolts: Part I: Growth, Muscle Development and Schooling
3:30 PM		Lazado - Effects of Low to Very High Water Velocities on Atlantic Salmon Post-Smolts: Part II: Welfare, Mucosal Health and Stress Responses
3:45 PM		Misk - Assessing the Effects of High Oxygen Freshwater Saturation on Atlantic Salmon (*Salmo salar*) Growth, Food Conversion Ratio and Overall Health Within a Simulated Commercial Hatchery Setting
4:00 PM		**Stockwell - Determining the Effects of Oxygen Supplementation on Cultured Salmon Behavior Using Acoustic Telemetry**
4:15 PM		Barker - Saprotect™ – A Plant Derived Product for the Maintenance of Optimal Health of Fish and Fish Eggs.

Effects of Low to Very High Water Velocities on Atlantic Salmon Post-Smolts: Part I: Growth, Muscle Development and Schooling

Gerrit Timmerhaus[1*], Carlo C. Lazado[1], Nikko Alvin Cabillon[1,2], Britt-Kristin Reiten[3], Lill-Heidi Johansen[4]

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Beneficial effects of induced water velocities on Atlantic salmon smolts have been described in several studies. These effects include elevated growth rates, feed conversion rates and disease resistance. However, the optimum water velocity for farmed salmon smolts remains unknown. Thus, in this study, we addressed the effects of different water velocities on growth and muscle development (histology and gene markers) to estimate optimum conditions for rearing of post smolts in a recirculating aquaculture system. In addition to individual parameters, we addressed the behavioral response in regards to schooling. We divided 2400 salmon smolts (average start weight 80g) into twelve tanks (200 fish per tank) and set the water velocities for four triplicate tanks to *low* – 0.5 body length per second (BL/s); *medium* – 1.0 BL/s; *high* – 1.8 BL/s; and *very high* – 2.5 (BL/s). The velocity for the *very high* group was the highest tested for salmon smolts to date. The trial lasted three month and organ samples were collected at three time points. Time-laps cameras were used to observe the schooling behavior in increasing water velocities and showed that fish in the *low* and *medium* group distributed mostly evenly in the tanks. In contrast, fish in *high* and *very high* displayed strong schooling behavior at specific spots in the tanks. We observed a close to linear relationship between water velocity and growth rate, which resulted in 5.4% higher average body weight in the *very high* group than the *low* group at the end of the trial. The condition factors of fish from the *low* group was lower than in the other groups and an analysis of the contour of the fish bodies showed that fish in higher velocities grew wider (distance between back and belly outlines). Histological analyses of the muscle fibers revealed increased somatic growth in *high* and *very high* groups, while the expression of some genes of myosomatic growth pathway s were increased in the same fish. In conclusion, the increased body weight of fish reared in high water velocities was likely due to enhanced somatic growth of muscle fibers. Thus, these findings provide further evidence that elevated water velocities have positive effects on the growth rate of smolts even at the highest levels tested to date.

Participants are highly advised to consult the talk entitled: **Effects of low to very high water velocities on Atlantic salmon post-smolts: Part II: Welfare, mucosal health and stress responses.** by Carlo C. Lazado et al., for additional results.

Conference Session Designation: (Aquatic Animal Health Management)
Presentation Format: (Oral)

Effects of Low to Very High Water Velocities on Atlantic Salmon Post-Smolts: Part II: Welfare, Mucosal Health and Stress Responses

Carlo C. Lazado[1*,] Gerrit Timmerhaus[1], Nikko Alvin Cabillon[1,2], Britt-Kristin Reiten[3], Lill- Heidi Johansen[4]

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There is ample evidence showing the beneficial effects of induced swimming, or exercise training in farmed fish. In Atlantic salmon, it has been shown that training through elevated water velocities has positive impacts on growth, feed conversion efficiency and robustness. However, there is little evidence demonstrating the effects on mucosal and stress responses, as well as on the external welfare of fish. More so, there is a question whether limits exist on the beneficial functions of elevated water velocities in salmon. In this study, we subjected salmon post-smolts (initial body weight circa 80 g) to four different training intensities by manipulating the water velocity in the tank: low – 0.5 body length per second (BL/s); medium – 1.0 BL/s; high – 1.8 BL/s; and very high – 2.5 (BL/s), for three months. The water velocity in the very high group has not been tested before in salmon. The external welfare status of fish was assessed following the FISHWELL handbook. Increased incidence of skin damage (i.e., scale loss, hemorrhaging) and pelvic fin damage (i.e., splitting) in the high and very high groups was documented. Nonetheless, the overall external welfare scores remained favourable in all groups. The skin and gills were subjected to quantitative histomorphometry and qPCR analysis of genes relevant to the mucosal defence. The expression of immune defence genes (e.g., cd8α, tcrα, mhc1, mhc2, mblc2) in the skin was negatively affected in the very high group, where significantly lower transcript levels compared with the other groups were observed. Interestingly, no significant differences between treatments were observed in the expression of selected marker genes in the gills. Histomorphological analyses of skin and gills are on-going. Plasma samples were collected and analysed for stress indicators. Plasma cortisol, glucose and lactate varied remarkably between groups at the beginning of the trial but such differences were not observed at the termination of the experiment. In conclusion, the welfare scores and the gene expression results in the skin revealed that the very high velocity may have some unfavourable consequences. Nonetheless, results from other response variables are suggesting that salmon subjected to a water velocity higher than the level previously thought to be the upper limit does not pose substantial negative consequences to health and welfare. The results of the study offer new frontiers in producing robust salmon through the benefits of induced swimming at higher water velocities.

Participants are highly advised to consult the talk entitled**: Effects of low to very high water velocities on Atlantic salmon post-smolts: Part I: Growth, muscle development and schooling.** by Gerrit Timmerhaus et al., for additional results.

Conference Session Designation: (Aquatic Animal Health Management)
Presentation Format: (Oral)

Assessing the Effects of High Oxygen Freshwater Saturation on Atlantic Salmon (*Salmo Salar*) Growth, Food Conversion Ratio and Overall Health Within a Simulated Commercial Hatchery Setting

Ehab Misk[1], Ellen D. Fanning[1], Anne F. McCarthy[1] Mike Battie[2] Chris Bridger[1]

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Atlantic salmon is a diadromous fish species and spends about 50% of its entire life cycle in culture conditions in freshwater land-based hatcheries. A critical aspect in commercial hatcheries is the provision of appropriate levels of oxygen within the freshwater environment to provide optimal growing conditions. Oxygen can represent a significant operational cost to the hatchery cash flow depending on its source, which can be further exasperated depending on the efficiency of the delivery system. Conversely, improper oxygen delivery and maintenance of elevated levels may be detrimental if this leads to physiological issues, such as gas bubble disease in the absence of appropriate stripping of other gases to control the total gas pressure. This project provide benchmarking between triplicate tanks treated with ambient freshwater dissolved oxygen concentration (90% ± 10%) with triplicate tanks receiving added dissolved oxygen concentrations of 150% ± 10% and 200% ± 10%, respectively. Study water was made up on demand using a proprietary gas infusion system to infuse oxygen to raise the measured dissolved oxygen concentrations while removing nitrogen from the water to maintain water total gas pressure at near 100%. This study design was maintained from the time when the tested Atlantic salmon were about 3g throughout the entire freshwater stage to provide a simulated freshwater hatchery environment. Specific growth rate and Fulton's condition factor were calculated using data collected during non-lethal sampling (n=10 ×3 /group) either monthly or during planned cutbacks that match the simulation production plan based on stocking density. Fish survival from each of the treatment replicate tanks was recorded through documentation of removed mortalities. Overall fish health was assessed by a lethal sub-sampling of each tank population (n=10 ×3 /group) during cutbacks, including hematocrits and general necropsy. After the first density-split, fish held in 150% and 200% dissolved oxygen saturation freshwater had higher survival compared with controls held within ambient conditions. The overall mean growth rate in 150% and 200% oxygen saturation was also higher than from ambient conditions by 47% and 44%, respectively. Interestingly, while fry held in 150% and 200% oxygen saturation had similar overall performance, only fish exposed to 200% oxygen saturation had significantly lower hematocrit values. This highlights the ability of exposed fry to perform better in increased oxygen saturation but yet limit their intake of dissolved oxygen as the increase in dissolved oxygen saturation was not directly correlated with increased performance.

Conference Session Designation: (Aquatic Animal Health Management)
Presentation Format: (Oral)

Determining the Effects of Oxygen Supplementation on Cultured Salmon Behavior Using Acoustic Telemetry

Caitlin L Stockwell[1,a,*], Meredith Burke[1,b], Ramon Filgueira[2], Jon Grant[1,c]

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The health and welfare of farmed fish is highly dependent on water quality, being dissolved oxygen (DO) one of the most critical factors. Some farms suffer from episodic low DO, which can be exacerbated with predicted rising sea level temperatures causing solubility of oxygen to decrease. The negative impacts of low DO have caused the farm managers to seek alternative solutions for sustaining the health of farmed fish by supplementing sea cages with oxygen. Low oxygen levels negatively affect fish behavior, which is a key component in determining fish welfare, and can therefore could be used as an early warning indicator of stress from low DO. In this study, the behavior of Atlantic salmon (*Salmo salar*), located in Southern Nova Scotia, was studied in response to the introduction of supplemental oxygen for 3 months (mid July-mid Oct) to test the suitability of using fish behavior as an early indicator of fish health with relation to changes in oxygen levels. Swimming depth and biomass density were recorded, before, during, and after oxygen supplementation trials, using CageEye, a sonar system used for tracking total biomass movement within aquaculture cages in real time. Additionally, health factors, such as mortality rate, swimming activity, and feed intake, were recorded to help understand the behavior during changing dissolved oxygen levels. Future work will combine this technology with VEMCO acoustic tags to test the applicability of using fish behavior as an indicator of other stress drivers such as storms, temperature changes, and diseases. Preliminary results suggest that real time data collection of fish behavior allows for an early warning indicator of fish health and can help to improve farm management.

Conference session designation: **(** Aquatic Animal Health Management **)**
Presentation Format: **(** Oral)
Student Presentation: (Yes)

SAPROTECT™ - A Plant Derived Product for The Maintenance of Optimal Health of Fish and Fish Eggs

Duane E. Barker[1], Subrata Chowdhury[2*] and Anne F. McCarthy[1]

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Infections by Oomycete "water mould fungi" (e.g., *Saprolegnia* spp.) are problematic at most freshwater fish hatcheries, including Canadian salmon hatcheries, with egg losses between 10-50%. The most commonly used approved therapeutant is formalin (Parasite-S™); however, there are concerns about its safety for the fish and the user. Other treatments (e.g., iodophores, salt, H_2O_2, etc.) exist but each has its limitations. Consequently, there is a need to develop an alternative product that can be safely applied to all stages of eggs and fish. RPS Biologiques, a PEI-based Canadian Biotechnology Company working in the field of aquatic, human and animal health, has developed a plant derived product called SAPROTECT™ to maintain health of fish and fish eggs. SAPROTECT™ is cost-competitive with existing anti-fungal therapeutants and the raw materials can be used for human consumption. As part of the ongoing product development and pre-regulatory testing process, a third party test facility (Huntsman) was contracted to evaluate target animal safety (TAS) studies. In pilot studies using an *in-vitro* infection model, low concentrations (LC_{50} = 5.28-26.18%, mean ($\pm$ SD) = 17.47 $\pm$ 8.88%) of SAPROTECT™ had similar efficacy as standard formalin concentrations. Using these baseline concentrations, an exploratory safety evaluation on Atlantic salmon, *Salmo salar*, embryos (E), alevins (A) and fry (F) was conducted, based on the standardized EAF-test methods of Environment Canada. For toxicity testing exposures, 50% SAPROTECT™ represented the highest dose, with subsequent dilutions 25, 12.5, 6.25, 3.13 and 1.56%. One reference control used hatchery water and a second reference control was formalin applied at typical treatment doses used in salmon culture settings (250 ppm for eggs, 167 ppm for fry). The study began with 160 fertilized, 'eyed' eggs per treatment (4 replicates of 40 eggs per treatment). For the E-stage, a series of one-hour, static bath treatments occurred every Monday, Wednesday and Friday (n=13 treatments). During the A-stage, there were daily observations but no treatments to facilitate yolk sac absorbance by the alevins without disturbance. For the F-stage, a series of one-hour, static bath treatments occurred every Monday, Wednesday and Friday (n=12 treatments). During all stages, there were no patterns of mortality associated with treatment. Because of low overall mortality and low percentage of any measured effects, values of LC_{50}, EC_{50} and EC_{25} could not be reliably predicted (by definition of the algorithm). Using mortality as an endpoint, the NOEC (no observable effect concentration) for fry was 50% SAPROTECT™ and the LOEC (lowest observed effect concentration) was > 50% SAPROTECT™. The results from the exploratory TAS study did not report any quantifiable toxicological effect associated with using SAPROTECT™ (1.56, 3.13, 6.25, 12.5, 25 and 50%) in repeated 1h static bath exposures on live embryos (within eggs) and fry of Atlantic salmon. RPS is now engaged in the regulatory approval process and has applied for product registration. It is anticipated that pilot scale production will soon begin.

Conference Session Designation: (Husbandy / Phsiology)
Presentation Format: (Oral)

Thursday September 6th – Archibald / Campbell
Aquatic Animal Health Management 1 & 2
Moderator – Kim Klotins (Canadian Food Inspection Agency)

9:30 AM	Health Management 1	Klotins - Compartment Recognition Program and Declaration as a Free Area
9:45 AM		Giffin - Country Freedom for a Foreign Animal Disease in Canada
10:00 AM		Klotins - Transboundary Freedom for Shared Waters
10:15 AM		Gautam - A Tool to Facilitate Risk-Based Surveillance Planning of Marine Aquaculture: An Example Using an ISAV Outbreak in Canada and the USA
10:30 AM		**Refreshments**
10:45 AM	Health Management 2	Larson - Response to Canada's First Finding of Whirling Disease in Alberta in 2016
11:00 AM		Jung-Schroers - Recommendations for Stunning and Killing of Common Carp (*Cyprinus carpio*) and Rainbow Trout (*Oncorhynchus mykiss*)
11:15 AM		Cunha - Tilapia and Other Tropical Fish Aquaculture Policy in Brazil: Diseases and Other Constraints
11:30 AM		Spark - Improving the Likelihood of a Definitive Diagnoses in Fish Kill Investigations
11:45 AM		Blackwell - Risk Assessment for Imported Finfish

Compartment Recognition Program and Declaration as a Free Area

Kim C. Klotins

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The Canadian Food Inspection Agency (CFIA) is the lead for the government of Canada for the development and implementation of the National Aquatic Animal Health Program (NAAHP), a program designed to prevent the introduction and spread of serious aquatic animal diseases. Fisheries and Oceans Canada (DFO) is our partner, delivering diagnostic services and research for the NAAHP under the National Aquatic Animal Health Laboratory System (NAAHLS). In order to prevent spread of enzootic diseases, the CFIA has zoned Canada for diseases that occur regionally. Permits are required to move declared susceptible species and things, such as used fish graders, from infected areas to free areas. As another option to issuance of a permit, the CFIA offers a compartment recognition program that allows a facility to achieve declaration as a free area. This program requires the development and implementation of a preventive control plan that addresses bioexclusion measures described in the national standards for prevention of introduction of disease. The CFIA inspects the plan against the national standards and its implementation by the facility, and conducts sampling for disease freedom and submits the samples for testing by NAAHLS. If the inspections and testing results are satisfactory, then the recognized compartment can apply for a declaration as a free area. Upon acceptance of the conditions for declaration by the facility, including ongoing inspections by the CFIA of at least annually, then the declaration is published on the CFIA's web site: www.inspection.gc.ca. The compartment recognition program also includes the ability to suspend and revoke the declaration.

Conference Session Designation: (Aquatic Animal Health Management)
Presentation Format: (Oral)

Country freedom for a Foreign Animal Disease in Canada

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Canada is in the process of documenting country level freedom for a foreign animal disease, specifically Salmonid Alpha Virus (SAV) in accordance with the World Organization for Animal Health (OIE) Aquatic Animal Health Code section 10.5.4. Several options for declaration of country freedom are available however the option for declaring freedom when the disease status is unknown prior to targeted surveillance (Article 10.5.4.s3) will be examined in more detail.

Declaration of country freedom requires that basic biosecurity conditions have been met and Article 10.5.4.s3 requires that targeted surveillance has been in place for a minimum time period which is outlined in the Aquatic Animal Health Code. This presentation will review Canada's development of a systematic process for evaluation of basic biosecurity conditions, historical and on-going provincial surveillance in order to determine requirements for development of targeted surveillance programs and declaration of freedom. Evaluation methods have included trade statistics review to determine import risk, internal audit of import controls and disease response policies. Both passive and historical surveillance need to be evaluated to determine the need for future targeted surveillance programs.

Conference Session Designation: (Aquatic Animal Health Programs)
Presentation Format: (Oral)

Transboundary Freedom for Shared Waters

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The World Organisation for Animal Health Collaborating Centre for Epidemiology and Risk Assessment of Aquatic Animal Diseases along with the Canadian Excellence Research Chair in Aquatic Epidemiology at the University of Prince Edward Island, and the Canadian Food Inspection Agency in close collaboration with the Veterinary Services section of the Animal and Plant Health Inspection Service in the US Department of Agriculture jointly convened a workshop in May 2017 in Ottawa on transboundary aquatic animal diseases and evaluation of freedom or managing spread of disease across borders. Potential spread via movements of animals or animal products which are regulated by government authorities were not considered during the workshop. For the purpose of evaluating country freedom or prevention of spread across boundaries, participants identified the following required components: a shared approach to evaluating disease-specific information, a shared approach to evaluation of generated aquatic animal health evidence, evaluation of each country's aquatic animal health system, and maintaining trade while managing risks. A more in-depth discussion of the requirements for each component, identified feasibility challenges and next steps will be presented. It was generally agreed that the magnitude of the impact of water and wild aquatic animal movement on aquatic pathogen transfer across borders and the effectiveness of certain surveillance strategies for early detection of cases and for declaration of disease freedom remain areas that can benefit from more research effort.

Conference Session Designation: (Aquatic Animal Health Management)
Presentation Format: (Oral)

A Tool to Facilitate Risk-Based Surveillance Planning of Marine Aquaculture: An Example Using an ISAV Outbreak in Canada and the USA

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In the event of disease incursion or re-occurrence in a farm, selecting susceptible farms to monitor for disease spread becomes a challenge because the extent of coverage for surveillance and control often has to be balanced against available resources. A tool to assess the risk of disease spread between farms can help make informed decision regarding monitoring and surveillance activities, and support the response strategy, given available resources. We developed three models using seaway distance, hydrodynamic information and the two combined to estimate and compare Infectious Salmon Anaemia virus (ISAv) transmission risks from an infected farm site to all other susceptible farm sites. The models were validated using 2002-2004 ISAv outbreak data for 30 farms (24 in New Brunswick, Canada and 6 in Maine, United States). The outbreak data included monthly infection status of the cages, which was used to determine time sequence of infection spread. An infected farm was considered to remain infected in subsequent time intervals during the outbreak until all fish had been harvested. The first infected farm was considered to be the index site, and was used to assess the risk of ISAv spread to all other active susceptible farms. In the second and subsequent outbreak time intervals infected farms were identified using the farm status in the given time period and all infected farms from the previous time periods to assess the risk of ISAv spread. The three models (hydrodynamic only, seaway-distance, and combined hydrodynamic-seaway-distance based models) were used to assess the risk at each outbreak time interval. At each time interval we ranked the susceptible farms by adding the transmission risks from surrounding infected farms and sorting them from highest to lowest. We converted the rankings to percentiles and assessed the models' predictive performance by comparing the farm sites identified as high risk at each level of the observed ranking with those farms that actually became infected during the next time interval. The overall predictive ability of the models was compared using area under the ROC curve (AUC). Farms becoming infected in the next period always remained within the top 50% of the rank predicted by our models. The overall predictive ability of the model that combined hydrodynamic and seaway-distance information (AUC = 0.833) was similar to the model using seaway distance alone (AUC = 0.827). Our results indicate that such models can aid in the efficient allocation of limited resources by suggesting appropriate levels of surveillance coverage (proportion of farms considered for surveillance) based on the desired level of confidence for correctly including farms that could become infected.

Conference Session Designation: (Aquatic Animal Health Management)
Presentation Format: (Oral)

Response to Canada's First Finding of Whirling Disease in Alberta in 2016

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In August 2016, the first detection of *Myxobolus cerebralis (Mc)*, in Canada, was confirmed from fish sampled at Johnson Lake, Banff National Park (BNP) in Alberta. That first finding triggered a series of events and an emergency response involving numerous government partners (Alberta Environment and Parks (AEP), Parks Canada, Canadian Food Inspection Agency (CFIA), Alberta Agriculture and Forestry, and BC Ministry of Agriculture, Forestry and Fish) to determine and prioritize further fish sampling, tissues to be taken and testing facilities (i.e. where to find fish health laboratory capacity for *M. cerebralis* testing in a country where Mc was considered exotic). The aim was to determine the extent of the parasite range in both wild and cultured fish (and therefore traced stockings). BNP quickly implemented lake closure (Johnson Lake) and containment measures, as several important salmonid populations (including threatened west slope cutthroat trout) exist in the park. Interim biosecurity protocols were also quickly devised through AEP to prevent further potential spread of *M. cerebralis* spore stages through fish sampling operations, and an initial risk assessment of salmonid species susceptibility and Alberta salmonid range, based on temperature and gradient profiles, informed that active surveillance plan. A communication plan was developed to provide key messages to stakeholders, the science community and address media enquiries in a timely manner.

I'd like to provide a high level review of what happened in those frenzied few months of sampling/testing quarantined cultured fish before allowing any fall stocking, and selecting wild fish from key watersheds prior to fall freeze-up, so that samples were at least 'in the freezer' to be processed as we apportioned samples to various testing facilities and geared up labs and personnel under AEP to continue in the longer term surveillance. Since its discovery, whirling disease has become a prominent issue in Alberta, prompting the Canadian Food Inspection Agency to zone the province to prevent the spread of this disease to other parts of Canada, and Alberta Environment and Parks to initiate a Whirling Disease Program dedicated to managing the disease through a three-pillared approach built around delineation, education and mitigation. More detailed specifics of results of *Myxobolus cerebralis* testing will be provided by colleagues in other presentations.

Conference Session Designation: (Aquatic Animal Health Management)
Presentation Format: (Oral)

Recommondations for Stunning and Killing of Common Carp (*Cyprinus carpio*) and Rainbow Trout (*Oncorhynchus mykiss*)

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In Germany stunning of animals in general is regulated in a directive and special requirements for stunning of fish are given. For all fish species these regulations prescribe stunning by percussion or electric current. Only for salmonids, additionally stunning by CO_2 exposure in a water bath is authorised. Precise instructions on how these methods should be used are not available. It is known that stunning of special fish species, like common carp, is difficult by using the authorised methods.

In total 24 fish farms throughout Germany were visited and the whole process of stunning and slaughtering was evaluated. Some of these farms were slaughtering carp and trout; others were slaughtering only one of these species. Therefore the process of stunning and killing was documented 22 times in aquaculture farms for trout and 17 times in farms for carp. If possible catching of fish from the ponds was documented. In all farms, keeping fish in special tanks before slaughter and the transport of fish from these tanks to the stunning site was evaluated. Also in all farms stunning and killing of fish was documented.

An evaluation score with 93 points was established which includes all measured parameters and data about the process. Different evaluation factors were multiplied with scores assessing their importance. By combining the scores from different aspects of the harvesting process, an overall evaluation score was calculated. With the overall evaluation score a gradual classification and an assessment of different techniques and methods for stunning and killing of rainbow trout and carp was possible.

Most of the rainbow trout were stunned by electric current, followed by percussion. In two farms trout were stunned by a combination of both and in one farm CO_2 was used for stunning. In contrast, in most of the documented cases carp were stunned by a combination of electric current and percussion. Stunning by percussion was used in 3 cases and stunning by electric current was uses in four cases. Most of the rainbow trout were successfully stunned by all evaluated methods. Only around 60% of carp were successfully stunned by electric current and around 80% of carp were successfully stunned by percussion. Only a combination of both methods was leading to successfully stunned carp. With the collected data it could be shown, that for stunning by electric current, the conductibility of the water, the stunning time and the size and shape of the stunning tank can have an important influence of the success of stunning. Short stunning times of less than 2 minutes and water conductibility lower than 500µS/cm or higher than 1000µS/cm were leading to problems with stunning of especially carp. In conclusion, a combination of stunning by electric current with adequate conductibility and adequate stunning time and by percussion seems to be the best method for stunning of rainbow trout and carp.

Conference Session Designation: (Aquatic Animal Health Management)
Presentation Format: (Oral)

Tilapia and Other Tropical Fish Aquaculture Policy in Brazil: Diseases and Other Constraints

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Tilapia is the most important species in the Brazilian aquaculture whilst production of other tropical species has increased recently. Brazil was included in the group of the top 4 global tilapia producers and the national fish industry is advancing and maturing as a business with an average of 1.2 billion dollars annual revenues. In addition, native tropical fishes, such as Tambaqui (*Colossoma macropomum*), have emerged as a promise of profit due to their unique taste, vegetarian based diet, commercial appeal as Amazon Bay eco-friendly raised animals and also because these species are not considered commodities. Animal health challenges seem not to compromise the results of tropical fish aquaculture in the country. In fact, the industry has still not passed through any severe sanitary crisis. Currently, endemic bacteriological diseases are considered responsible for major losses in tilapia breeding, and parasitic infections are claimed to be the most important health problem for native Brazilian tropical fish husbandry. Biosecurity measures still need to be implemented in most of farms and inland reservoirs. Aquatic animal health awareness among stakeholders should be reinforced and risk perception remains primarily on parasitic diseases and on exotic diseases such as tilapia lake virus. Main constraints beyond diseases are still to be faced, such as political dispute among parties to rule the regulatory agenda of fisheries and aquaculture in Brazil, environmental issues for licensing in some regions of the country, formal organization of stakeholders, fully comprehension of the role of the private sector and public sector in policy making in aquaculture, technological packages to breed native species with competitive advantages, climate changes (harsh droughts, extended raining seasons) and economical approach to assess the implementation of health tools and sustainable husbandry practices. Despite all these matters, the scenario for aquaculture of tropical fish in Brazil is favorable and attracts robust investments of successful national companies in the terrestrial animal protein field and of international industries with large experience in aquaculture overseas.

Conference Session Designation: (Aquatic Animal Health Management)
Presentation Format: (Oral)

Improving the Likelihood of a Definitive Diagnoses in Fish Kill Investigations

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Despite the relatively high aquatic animal health status, in comparison to other Australian and international seafood sectors, South Australia (SA) remains susceptible to the increasing worldwide threat of both significant known and unknown aquatic animal diseases emerging and spreading. Protecting and maintaining this favorable status to secure production is a core role of PIRSA through investigating fish kill and aquatic mortalities, surveillance for high priority diseases, regulation of veterinary medicine use, regulation of livestock translocations and the development and implementation of emergency response plans.

While disease management is core work for PIRSA's aquatic animal health program and consequently the driver to investigate aquaculture mortalities and wild fish kills, broader government priorities, industry and community expectation requires other causes of fish kills to be determined, including: human health risks, chemical spills (including oil) and other anthropogenic causes that may have compromised the marine environment. For this reason, and in the face of limited resources, fish kill investigations are approached as a whole of government joint effort.

PIRSA investigate approximately 15-20 fish kill reports annually, with the large majority being attributed to environmental and / or anthropogenic causes. In recent years, a number of fish kills caused substantial media and public concern. As a direct result there was a need to develop and implement greater training for regional staff to improve the likelihood of definitive diagnoses. Although failure to reach a definitive diagnosis is not uncommon in fish kill investigations improvements in both site assessment and sample collection and submission were recognized as key focus areas. In South Australia mortality sites may be more than 1200km (or 750 miles) from both the laboratory and key aquatic animal health staff hence reliance on appropriately trained and equipped regional staff is essential.

A collaborative approach to the provision of investigation kits and the development and delivery of fish kill training was implemented between PIRSA Biosecurity SA and PIRSA Fisheries and Aquaculture Divisions. This training has begun and will be completed in 2018 with ongoing annual training proposed. The training involves a significant practical (fish dissection) component in order to preserve appropriate samples to avoid unnecessary delays in fixation to ensure both water and tissue samples are of diagnostic quality. This presentation will include a summary of the state fish kill database and key case studies as examples.

Conference Session Designation: **(** Aquatic Animal Health Management **)**
Presentation Format: **(** Oral **)**

Risk Assessment for Imported Finfish

Ron G Blackwell

Biosecurity New Zealand, Ministry for Primary Industries, Wellington, New Zealand

New Zealand is situated at Latitude 40º S and is remote, being over 1500 km from its nearest neighbour Australia and separated by deep ocean (depths greater than 1500 m). Our isolation and active biosecurity measures has meant New Zealand remains free of most OIE listed and other emerging and re-emerging finfish diseases such as Infectious haematopoietic necrosis virus (IHNV) and Infectious salmon anaemia virus (ISAV).

New Zealand's salmonid aquaculture is largely focused on chinook salmon (*Oncorhynchus tshawytscha*) while brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) support significant recreational trout fisheries with a substantial tourist component. These salmonids derive from broodstock introduced in 1911.

Fish and fish products are highly traded commodities, where 38% of the total global production was traded internationally in 2012. Total production in 2017 represented a 2.3% increase from 2016, when 83.6 million tonnes were produced from aquaculture and 90.4 million tonnes produced from capture fisheries. As captured fish are used for aquaculture feed, aquaculture actually represents 55% of total production for human consumption (FAO 2018).

Global trade can bring with it a significant risk of new disease introduction. To address the challenges around increasing risk of international trade, the Ministry for Primary Industries is currently reviewing the import health standards for all aquatic commodities. Risk assessments have now been completed by for non-viable crustacean imports and for eviscerated or trunked finfish, while a review of mollusc products is planned for 2018.

The 2018 risk assessment for non-viable eviscerated/trunked finfish identified 569 potential hazard organisms. Of these, 40 exotic pathogens were identified as risks (including 19 viruses, 12 bacteria, 4 fungi/microsporidia, and 5 metazoan pathogens) that required mitigation measures.

Simple evisceration was considered sufficient mitigation for most fish diseases prior to 2016, but the OIE has now recognised that additional processing and handling measures including freezing or cooking, may be necessary (Oidtmann *et al.* 2017). Possible risk mitigation options for finfish include further processing, temperature or freezing. As active monitoring is rarely undertaken for non-OIE listed diseases, country freedom was not considered a viable management option for non-OIE listed disease organisms.

The introduction of additional measures based on risk assessments as appropriate will strongly mitigate against the introduction of exotic aquatic diseases into New Zealand.

Conference Session Designation: (Aquatic Epidemiology)
Presentation Format: (Oral)

Thursday September 6th – Archibald / Campbell
Aquatic Animal Health Management 3
Moderator – Kim Klotins (Canadian Food Inspection Agency)

1:15 PM	Health Management 3	Phelps - The Potential Risks of the Baitfish Pathway and Implications for Fish Health Management
1:30 PM		Price - A Retrospective Assessment of the Effect of Fallowing Duration on Piscirickettsiosis in Salmon Farms in Chile
1:45 PM		Jung-Schroers - Influence of a Nanofiltration – Reactor on the Bacterial Microflora and on *Ichthyophthirius multifiliis* Theronts in Recirculating Aquaculture Systems
2:00 PM		Dhar - Current Status of Acute Hepatopancreatic Necrosis Disease in Shrimp: Biology, Diagnostics and Disease Management
2:15 PM		Cunha - The Brazilian Shrimp Strategy to Keep Growing With the Global Diseases
2:30 PM		Roberts - Emergency Response to Ostreid Herpesvirus Microvariant in Feral Pacific Oysters (*Crassostrea gigas*)
2:45 PM		Yoshinaga - Biosecurity for Abalones in the Distribution Process of Imported Live Abalones and Its Problems

The Potential Risks of the Baitfish Pathway and Implications for Fish Health Management

Nicholas B.D. Phelps *

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In an increasingly connected world, human activities introduce the risk of moving invasive species and pathogens to naïve populations, and drive disease spillovers between farmed and wild populations. This reality is playing out in the use of baitfish for recreational angling, as billions of farm-raised and wild-caught fish (and their accompanying hitchhikers!) are moved long distances overland and intentionally introduced into new environments. As a result, baitfish movement has been considered a high-risk activity for the movement of aquatic invasive species (AIS) and disease in Minnesota, with potentially major economic, ecological, and societal consequences. To obtain baseline data of risks posed by baitfish use, a survey of invasive species and pathogens was performed at baitshops across Minnesota. Golden shiners (n=30) were purchased at retail baitshops (n=34, 18 of which were sampled twice; 52 total) across the state during unannounced consumer visits. 33/52 cases included non-target species, such as fathead minnows, brook stickleback, and brown bullhead. Potentially significant pathogens were in many baitshops, inclulding *Aeromonas salmonicida* and *Ovipleistophora ovariae.* In addition, at least 9 novel viruses were identified from fish collected during the study. No regulated AIS or pathogens were found during the course of the survey; however, the presence of non-target species and important pathogens confirm that baitfish should remain a pathway of concern. Ongoing efforts to better define potential hazards, quantify the risk of introduction via bait movement, and risk mitigation strategies will also be discussed.

Conference Session Designation: (Aquatic Animal Health Management or Emergent Diseases)
Presentation Format: (Oral)

A Retrospective Assessment of the Effect of Fallowing Duration on Piscirickettsiosis in Salmon Farms in Chile

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Management of antimicrobial usage in salmon farming is a key issue in increasing the sustainability of the industry. In Chile, piscirickettsiosis, the disease caused by *Piscirickettsia salmonis*, has been the main reason to use antimicrobials during the seawater phase despite the efforts of industry and government to control this disease. Area-coordinated mandatory fallowing was introduced by authorities in recent years; however, the effectiveness of this measure to reduce the risk of piscirickettsiosis has not been evaluated. We assessed the effectiveness of fallowing using farm-level weekly production and mortality records provided by industry. We used a discrete-time survival model to estimate hazard of piscirickettsiosis and compared the hazards in farms with and without a history of piscirickettsiosis in the previous cycle while controlling for external sources of infection such as infected neighboring farms. In our data, the hazard of piscirickettsiosis was high regardless of species and fallow duration. No difference in hazard was observed when a farm was fallowed for three months or longer. Fallow periods shorter than three months were only assessed for rainbow trout. In this species, fallow periods shorter than three months lead to increased hazard at the beginning of the subsequent production period. These results suggest 3-month fallowing might be adequate to reduce exposure to *P. salmonis* from the previous production cycle.

Conference Session Designation: (Aquatic Epidemiology)
Presentation Format: (Oral)

Influence of a Nanofiltration - Reactor on the Bacterial Microflora and on *Ichthyophthirius multifiliis* theronts in Recirculating Aquaculture Systems

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Recirculating aquaculture systems offer the opportunity to keep high numbers of fish without the need of high amounts of fresh water due to recirculation and filtration of tank water. Problems can occur if the amount of nitrate, bacteria or parasites in the water increases.
To maintain a good water quality, nanofiltration of the water is described as one method to reduce the amount of bacteria in the water and to keep the chemical water parameters in an optimal range. We tested nanofiltration reactors with integrated denitrification membranes in four different recirculation aquaculture facilities. One system in each facility was run with the reactor and as control identical systems without reactor were used.

The aquaculture facilities were stocked either with carp, sturgeons, golden orfes or rainbow trout and the systems were run at a water temperature between 20 and 25°C. In three facilities the bacterial microflora was analysed in tank water, biofilms of tanks and partly also of the filters and on skin and gills of fish kept in the systems. In one of the systems cortisol measurements in the water and in the blood of fish were performed to determine the stress level of the animals in the system. In the fourth system fish were examined for infection with the parasite *Ichthyophthirius multifiliis* and the effectivity of nanofiltration against the theronts of this ciliate was determined.

Overall it could be shown that the reactor with a filtrating membrane could decrease the total amount of bacteria in the tank water of a recirculating aquaculture system. Also the amount of bacteria on the gills of fish was decreased in the systems with installed reactor. The diversity of bacteria was higher in the systems with installed reactor and the fish in this system seemed to have less stress. A reduction of stages of *Ichthyophthirius multifiliis* could also be detected in a system with installed reactor. One challenge was the increasing water temperature in systems with installed reactor and the operation of the reactor itself is time consuming. Yet, the usage of a reactor with filtrating-membrane can have a positive influence on fish health and welfare.

Conference Session Designation: (Aquatic Animal Health Management)
Presentation Format: (Oral)

Current Status of Acute Hepatopancreatic Necrosis Disease in Shrimp: Biology, Diagnostics and Disease Management

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Acute hepatopancreatic necrosis disease (AHPND) of shrimp, also known as an Early Mortality Syndrome (EMS), is an emerging disease that is threatening shrimp aquaculture worldwide. Since the emergence of the disease in China in 2009, the disease has spread to many countries in Asia and now it has spread to North America (Mexico and the US). The disease is caused by *Vibrio parahaemolyticus* carrying binary toxin genes, *pirA* and *pirB*. Recently, other *Vibrio* species carrying the binary toxin genes were shown to cause the disease. In experimental challenge, *V. parahaemolyticus* can cause 100% mortality within 48 hr. Typical AHPND acute phase presents multifocal necrosis and massive sloughing of epithelial cells from the medial region towards the distal region of the hepatopancreatic tubule. This is followed by massive bacterial infection in the HP lumen and hemocytic infiltration surrounding the affected tubule. OIE-recommended method for AHPND detection involves PCR amplification of *pirA* and *pirB* genes. Recently, we have sequenced the genome of a novel *V. parahaemolyticus* strain that carries both toxin genes, yet it does not cause AHPND. This shows the need to develop detection method alternative to DNA-PCR. Biosecurity and pond management remain the corner stone for the managing AHPND. Recently, functional feed has been developed as a therapeutic approach to contain AHPND. Efforts are now underway to develop AHPND-resistant lines of shrimp.

Conference Session Designation: (Aquatic Animal Health Management or Shellfish)
Presentation Format: (Oral)

The Brazilian Strategy to Keep Growing the Shrimp Industry with Global Diseases

Eduardo A P Cunha[1*], Marina K V C Delphino[2], Tatiana M P Azevedo[1] and Isabella Fontana[1]

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Transboundary diseases of whiteleg shrimp (*Litopenaeus vannamei*) are well documented as responsible for massive losses in the aquaculture industry worldwide. In Brazil, it has been registered the occurrence of infectious hypodermal and haematopoietic necrosis virus, infectious myonecrosis virus, white spot syndrome virus and infection with *Hepatobacter penaei* (necrotising hepatopancreatitis). Through molecular epidemiological methods, it has been observed that most of these infections were genetically similar to those occurring in other important shrimp producing countries. It is believed that the illegal introduction of risk material, mostly post larvae for genetic enhancement programs, has played a crucial role in the introduction and dissemination of highly contagious epidemic diseases in the country. National public policy for the legal import of risk material was considered adequate by recent assessment of OIE PVS Tool specifically applied for aquatic animal health service. Infectious diseases are considered the major constraint for the shrimp industry worldwide and the national stakeholders tend to defend a very conservative and protectionist approach when debating with the authorities legal imports of any crustacean materials, including species and products epidemiologically irrelevant for the introduction of any potential risk. Recent openings of important national shrimp companies for international investments are expected to rearrange the country's private sector, which has been continuously struggling with veterinarian authorities. Stakeholders support the application of precautionary measures whilst the Brazilian official veterinary service relies on international accepted and validated risk analysis tools. Compartmentalisation seems to be a proper plan to make possible the compliance with the World Trade Organization SPS Agreement for the import of post larvae for genetic enhancement programs in the country considering a globalized world full of economically important diseases not reported in Brazil. In the meanwhile, the Brazilian strategy to keep growing the shrimp industry with global diseases remains on the use of strains of specific pathogen resistant animals; post larvae coming from certified SPF broodstock; enhanced feed with vitamins, prebiotics, probiotics, enzymes, phytotherapics and other immunostimulants as well as the use of superintensive culture in biofloc technology system at different stocking densities in greenhouse-enclosed system, which is sustainable from an environmental perspective and more effective amongst a diverse number of biosecurity measures.

Conference Session Designation: (Aquatic Animal Health Management)
Presentation Format: (Oral)

Emergency Response to Ostreid Herpesvirus Microvariant in Feral Pacific Oysters (*Crassostrea Gigas*).

Shane D Roberts[1*] Elise K Spark[2], Evan J Rees[1], Marty Deveney[3], Roger D Paskin[2], Peter J Dietman[1] & Sean R Sloan[1]

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The South Australian (SA) oyster growing sector has substantial value ($32 million / year) and importance to regional communities across the State. The sector largely comprises Pacific oyster (*Crassostrea gigas*) culture. Pacific Oyster Mortality Syndrome (POMS) is a disease caused by Ostreid Herpesvirus type 1 (OsHV-1) microvariant, which causes rapid high mortalities (up to 100%) in Pacific oysters. The SA government have collaborated with industry since 2012 to undertake prevention, preparedness and response activities to mitigate the threat of POMS, including development of State and national disease response plans: www.agriculture.gov.au/animal/aquatic/aquavetplan/. To date POMS has not been detected in SA oyster farms. POMS occurs in Europe, New Zealand and Australia (NSW). On 1 February 2016 the Australian State of Tasmania reported their first detection of POMS in oyster farms experiencing high mortalities. In SA, government and industry immediately responded to the potential introduction of OsHV-1from frequent importation of spat from Tasmanian hatcheries.

In July 2016 a barge from Sydney, NSW, arrived in Port Adelaide with Pacific Oysters on its hull. The barge was immediately quarantined and removed from the water. One oyster tested positive to OsHV-1. Subsequent tracing and surveillance did not detect infection in feral or farmed oysters in SA. This case demonstrated that biofouling on vessels can translocate OsHV-1 across continental-scale distances.

Passive surveillance over recent years has increased, with farmed oyster mortalities immediately investigated to rule out OsHV-1. Mortality events are generally localised (i.e. one farm) and likely causes have been attributed to a combination of environmental and/or husbandry stressors, and opportunistic *Vibrio* spp.

On 28 February 2018 SA confirmed its first detection of POMS, in Port Adelaide River feral Pacific oysters. Plankton samples (e-DNA) and Pacific oysters from the Port produced qPCR CTS <20, indicating high viral load. The closest oyster farming region is ~60km from Port Adelaide, while the closest oyster hatchery is ~25km away. The emergency response involved statutory restrictions on fishing vessel movements, livestock movements, feral oyster destruction using flame guns and other methods, and biofouling management. A communication and awareness campaign was developed and hydrodynamic modelling of viral particle dispersal and epidemiological analysis were used to inform surveillance. Extensive surveillance across SA has detected the virus only in Port Adelaide feral oysters.

Conference Session Designation: **(** Aquatic Animal Health Management)
Presentation Format: **(** Oral)

Biosecurity for Abalones in The Distribution Process of Imported Live Abalones in Japan and Its Problems

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Based on risk assessments, Japan revised rules for biosecurity of aquatic animals and expanded the list of aquatic animals and their epidemics subjected to import and domestic biosecurity measures in July 2016, which newly includes bacterial blister disease of Haliotis discus hannai, Haliotis discus discus, H. gigantean and H. madaka, and infection with abalone herpes virus of H. diversicolor diversicolor and H. diversicolor aquarilis. According to the new rule, when importers import and stock live abalones of those species in tanks and facilities draining directly to public water without disinfection of pathogens, they should obtain import permission in advance from the Ministry of Agriculture, Forestry and Fisheries by submitting import application attached with an inspection certificate issued by the competent authority of the exporting countries. Knowing the distribution process of imported live abalones is essential to make the biosecurity measures efficient. We examined the import statistics, and visited and interviewed importers and wholesalers to overview the distribution process of imported live abalones. We will introduce the distribution process and problems found in it from the view point of biosecurity.

Conference Session Designation: (Aquatic Animal Health Management)
Presentation Format: (Oral)

Thursday, September 6th – MacDonald
QASH 1 & 2
Moderators – Karin Pittman, Mark Powell, Robin Shields & Linda Andersen

9:30 AM	**Welcome to QASH – The QASH Core Team**
Biomarkers Session - Karin Pittman (Moderator)	
9:35 AM	Powell - A Healthy Fish Can Handle What Nature Throws At It: Allostasis In Fish Health
9:50 AM	Braceland - Challenges In The Biomarker Pipeline
10:05 AM	Gutiérrez - A Risk Assesment Matrix For Smolt Welfare In Atlantic Salmon: Insights From Chile
10:20 AM	Auchterlonie - Declining Marine Ingredient Inclusion Levels And A Hypothesized Link With Fish Health In Farmed Atlantic Salmon
Barriers and stressors session - Mark Powell (Moderator)	
10:35 AM	Pittman - Barrier Status In Skin, Gills And Guts: Mapping The Dynamics Of The Innate Immune System Throughout The Production Cycle With Statistically Robust Results
10:50 AM	Chikwati - Gut Health Monitoring During The Seawater Phase Of Farmed Atlantic Salmon In Different Produciton Regions Of Norway - The GutMatters Project
11:05 AM	Mella - Practical Applications of Quantitative Image-Based Assesment Of Digital Pathology Slides In Chilean Salmon Industry
11:20 AM	Sveen - Wound Healing And the Effect Of Chronic Stress In Post-Smolt Atlantic Salmon (*Salmo salar*)
11:35 AM	**Workshop 1 - Biomarkers and barriers - criteria, cutoff levels, long-term effects, remedial actions? - Mark Powell and Karin Pittman**

A Healthy Fish Can Handle What Nature Throws at it: Allostasis in Fish Health

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Determining the criteria for what is a healthy fish poses many challenges and alternate definitions. Given that health is not merely the absence of disease, that an infected fish is not a diseased fish, it is important to look at the physiological capacity of a fish to respond to a wide range of biological and environmental challenges. The concept of allostatic load is one in which an organism can respond to a range of challenges within an allostatic range – a range of adaptation and tolerance. However, once a threshold is reached, the response goes from being one of adaptive to maladaptive crossing over the patho-physiological limit. In response to infectious and non-infectious challenges, determination of the pathophysiological threshold is difficult. The application of current studies using clinical chemistry and histopathological responses to infectious and non-infectious disease, environmental and management challenges in salmon highlights the plasticity of fish patho-physiological processes. Using current evaluation techniques for gill responses, gill pathophysiology and histopathology we examine whether the cure can sometimes be worse than the disease and how compounding effects of treatments can compromise a fish that is in a state beyond the pathophysiological threshold.

Conference Session Designation: (QASH)
Presentation Format: **(** Oral)

Challenges in the Biomarker Pipeline

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Defining, and our abilities to do so, healthy populations is one of the greatest challenges faced to livestock industries generally. As such a multitude of scientific literature aims to establish biomarkers of infection, infestation, sub-clinical, clinical, and projected disease outcomes. However, few of these candidate biomarkers establish in a clinical setting. The pipeline of development includes several stages from discovery to implementation. However, due to the failures and issues with each of these few pass from discovery to implementation. Furthermore, the ability of a marker to pass through this pipeline is often blocked due to pre-conceived notions and the entrenchment of established practices which may not be appropriate and/ or better than that in development. This presentation aims to exemplify the process of a biomarker's discovery, validation, translation, evaluation, and implementation with the pitfalls of these steps being explained.

Conference Session Designation: (Quantitative Atlantic Salmon Health)
Presentation Format: (Oral)

A Risk Assessment Matrix for Smolt Welfare in Atlantic Salmon: Insights from Chile

Xavier A. Gutiérrez*, Tomás S. Mosquera and Felipe A. Briceño

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Salmon farming productivity strongly relies on smolt adaptability success on seawater (SW) environment after transference from the freshwater (FW) phase, especially during the first weeks after the arrival. According to current understanding, sub-optimal water quality conditions at tank level are able to alter key smolt physiological traits (e.g osmoregulation) which can be critical for fish growth and survival. Even though important advances on smolt welfare from land-based farm exist, there is a lack of quantitative tools able to better link fish physiological traits with SW smolt performance (e.g smolt index). A risk assessment on key water quality parameters, as well as smolt physiological indicators has been proposed as a first step towards a physiological smolt welfare index in Chile.

The current study is based on data from an ongoing smolt physiological monitoring program undertaken by NIVA Chile from both RAS-based and flow-through (FT) fish farms since 2015. The risk assessment is based on three components: key water quality, blood parameters and metals accumulated in target organs (gills and liver). A total of 20 consecutive batches of smolts were examined under these components only days before the smolts are transferred to the sea farms. Batch sampling was based on 3 tanks in which water quality parameters from effluents were analyzed. For each tank, 6 individuals were sampled to measure blood parameters, from which 3 individuals were randomly chosen to collect gills and liver samples.

Using the database of previous projects conducted in fresh water salmon farms, in conjunction with revising scientific literature, limits and recommended levels were established for all components considered. This enabled the categorization of each parameter by providing ranges in which they represent low, medium, or high implications on fish welfare. The frequency (or probability of occurrence) at which each variable presented low, medium or high implications was also determined. The combination of implication/severity level and frequency level results in a qualitative matrix for risk assessment considering the most critical variables.

This matrix appears as a suitable tool for visualizing the main risks for smolt welfare depending on production system (RAS or FT). Outcomes from this matrix can serve as a guideline for decision making process to correct and minimize the risk of these variables. The producer, for example, is able to prioritize and improve aspects of water quality conditions which in turn, result in better fish welfare.

Conference Session Designation: **(** Quantitative Atlantic Salmon Health)
Presentation Format: **(** Oral)

Declining Marine Ingredient Inclusion Levels and an Hypothesised Link with Fish Health in Farmed Atlantic Salmon

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The decline in inclusion rate s for fishmeal and fish oil is well understood, occurring since the late 1990s for salmon feed in particular, but across all the major farmed fish species groups and their formulations. The current situation is a consequence of both supply volume, and price, and the feed companies need to develop aquafeed volume supply to meet growing demand over time. Annual fishmeal and fish oil production is finite and additional feed volume has come from other ingredients, notably vegetable-based materials such as soya and wheat, of necessity.

The story is one of supplementation as the marine ingredients continue to be the foundation for aqafeeds but in much decreased concentrations in comparison to the feeds that were used in the early years of the modern aquaculture industry. In order to achieve effective substitution of marine ingredients in diets feed companies invested heavily in research in order to ensure that growth performance has not been impacted by changing raw materials use. One aspect of the reduction of fishmeal in particular is the change in supply across the micronutrients that are known to be found in rich concentrations in fishmeal, and which are not found in other protein sources to the same extent. In that respect, the minerals such as Fe, Ca, Zn, Se are important as well as the B-group vitamins and vitamin D, all possibly playing a role in immunocompetence and the ability to cope with pathogen challenge. In some respects the possible impact on fish health of the reduction in supply of these materials is unknown with traditional deficiency studies focused on meeting minimum requirements rather than optimal levels. There also exists the question of how feed composition may influence the gut microbiome, and the link that may have with fish health. Improving farmed salmon's ability to cope with pathogen challenge has the potential to improve production efficiencies.

The situation will vary for different species and production systems, and will certainly be very complex, but it is important to know the full impact that substitution and supplementation has had, and its impact on fish health. The Atlantic salmon as a species is an excellent model to look at these impacts in the first instance.

Conference Session Designation: (Quantitative Atlantic Salmon Health)
Presentation Format: (Oral)

Barrier Status in Skin, Gills and Guts: Mapping the Dynamics of the Innate Immune System Throughout the Production Cycle with Statistically Robust Results

Karin Pittman[1, 2], Mearge Okubamichael[2], Grigory Merkin[2], Natalie Brennan[1], Mark Powell[3], Linda Andersen[4] , Nini Sissener[3], Arthur Lyngøy[5] and Ole Jacob Myre[2]

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Mucosal barriers of skin, gills and guts are the primary tissues protecting the fish from outside challenges like pathogens and parasites and the internal challenges of suboptimal feeds. The mean size and volumetric density of mucous cells producing this protective slime were first quantifiable in 2010 (Pittman et al. 2011, 2013) and the technique has since been applied in over 50 large and small scale trials in 6 countries, 6 species and 3 tissues with subdivisions. Now trademarked as Veribarr, the verification of the living barriers, results show that there is a reproducible "behaviour" from these tissues in response to a variety of inputs and as such may indicate "herd health" in addition to reflecting individual status. Mean cell size, volume of mucous in the skin epithelium and the combination of these factors shows how each tissue responds both in concert with the others and independently in response to eg. diet. The growing database allows the ascertainment of normal ranges for each tissue and species, while the objective measures allow comparison across species, time, treatment and tissue. Currently two projects are exploring the link between microarray data, RNA analyses and Veribarr results to look for reliable markers of healthy homeostasis in skin or guts. Results further show that a sufficiently sized non-lethal gill biopsy will give rise to reproducible results and may be used to possibly indicate general health. The method is complementary to all other existing methods investigating fish health.

Conference Session Designation: (QASH)
Presentation Format: (Oral)

Gut Health Monitoring During the Seawater Phase of Farmed Atlantic Salmon in Different Production Regions of Norway – The Gutmatters Project

Elvis M. Chikwati[1,2], Paul J. Midtlyng[1,3*], Åshild Krogdahl[2]

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Optimization of feed resource utilization, production costs, fish growth performance, and environmental impact of Atlantic salmon industrial production is highly dependent on a healthy, optimally functioning gut. Overt clinical signs of gut dysfunction are rare in field conditions, yet subclinical gut health disorders can significantly diminish fish performance and health. Regardless, gut health of farmed fish populations at sea is not routinely monitored for impacts of feed, noxious, infectious or parasitic agents. As part of the ongoing GutMatters project funded by the Norwegian Seafood Research Fund, a national survey to establish the prevalence of gut health disorders and their incidence during a production cycle in sea farmed Atlantic salmon was therefore initiated in the autumn of 2017. Six sea farming sites along the Norwegian coast were monitored starting at about 5 weeks after sea-transfer until about 12 months of the fish at sea. Standardized procedures were developed for comprehensive sampling of up to 20 fish per site, including external and abdominal gross pathology, fish weight, length and blood plasma, content and tissue from intestine, liver, head kidney, spleen, and heart for histology and/or gene expression, microbiota, metabolomics, and digestive enzyme activity analyses. At each of 3 sampling events per farm, site physico-chemical data, fish stock feeding, growth, and health history were collected. Histology was used as the initial screening tool for gut health status of the sampled fish from which subsequent analyses will be based on. This presentation reports results from a semi-quantitative histology scoring for selected inflammatory and degenerative morphological changes in the mucosa of the pyloric caeca, mid-, and distal-intestine and the liver. Main findings from the histological evaluation of the fish sampled after 5 weeks post sea-transfer were mild to moderate inflammatory changes in the distal intestine of most of the fish sampled from one of the participating farms, as well as mild to marked enterocyte steatosis in the pyloric caeca in most of the groups evaluated. The inflammation resembled the well-documented soybean meal induced distal intestinal enteritis observed in salmonids fed diets containing soybean, or other legumes, as a protein source. The steatosis is thought to represent a lipid transport or metabolism disorder in enterocytes that in severe cases manifest as lipid malabsorption, steatorrhea and 'floating faeces'. Both disorders of inflammation and steatosis may markedly diminish gut function, fish growth and health and are candidate indicators of reduced feed utilization in Atlantic salmon. Details from the histological assessment of the gut and liver from fish sampled during the first and second round of monitoring the participating sea farms will be presented.

Conference Session Designation: **(** Quantitative Atlantic Salmon Health or Nutrition)
Presentation Format: (Oral)

Practical Applications of Quantitative Image-Based Assessment of Digital Pathology Slides in Chilean Salmon Industry

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Pathology diagnosis has been performed by pathologists observing the stained specimen on the slide glass using a microscope. In recent years, attempts have been made to capture the entire slide with a scanner and save it as a digital image (Whole slide image, WSI). Researchers both in the image analysis and pathology fields have recognized the importance of quantitative analysis of pathology images. Since most current pathology diagnosis is based on the subjective (but educated) opinion of pathologists, there is clearly a need for quantitative image-based assessment of digital pathology slides. In VeHiCe we adopt these analyses to be applied in farmed salmon industry in Chile. Currently this method is applying to assess pathologies evolution, health status, organs responses to drugs and diets among others. Allowing farmers take objectives measures regarding health and productions issues.

This study reports the assessment of the effects of anti-inflammatory drug in the evolution of an inflammatory process in the heart fibers caused by PRV. Two groups were tested; control group and T1. In total, the heart of 50 fish with were histologically processed, transverse sections of the ventricle were made and then the whole histopathological slide was capture with scanner and save as a digital image (WSI). The 85%-95% of total surface of the ventricle was analyzed in each slide. The morphometric analyze of the images was realized using ImageJ v1.49 (National Institut of Health, EE.UU.). In total 550 images were analyzed. In control group, there was 6,18% of the total heart surface presenting an inflammatory process, and in T1 group only a 0,47% of the total heart surface presented inflammation.

Using this method, we were able to measure precisely the % of inflammation in the different groups analyzed and consequently the evolution of the inflammatory process and the response to the tested drug.

Conference Session Designation: (Quantitative Atlantic Salmon Health)
Presentation Format: (Oral)

Wound Healing and the Effect of Chronic Stress in Post-Smolt Atlantic Salmon (*Salmo Salar*) **(WITHDRAWN)**

Lene Rydal Sveen [a,b,*], Gerrit Timmerhaus[b], Aleksei Krasnov, Harald Takle[d], Sigurd Handeland[c], Sigurd Stefansson[a], Elisabeth Ytteborg[b].

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The skin of Atlantic salmon (Salmo salar), is a coherent and dense barrier that protects the interior of the fish against the outer environment. The skin covers the entire outer surface, including the head, fins and eyes. Lesions in the skin are a major welfare issue for the fish.

Atlantic salmon post-smolts (mean weight 120g) were divided into two identical tanks (500L), and two treatments were established. High production intensity, HPI (mean fish density 126 kg/m^3) and normal production intensity, NPI (mean fish density 16 kg/m^3) . Three cylinders of tissue were excised with a 5 mm biopsy punch. Samples (n=12 per treatment) for gene expression analyses (microarray), histology, immunohistochemistry and scanning electron microscopy were collected 1, 3, 7, 14, 36, 42 and 57 days post wounding (dpw).

In general, the wounds from both HPI and NPI followed the normal progression of wound healing, with hemostasis, re-epithelialization, inflammation, tissue formation and tissue remodeling. The first 14 days of the healing process was dominated by acute inflammation and epidermal repair as shown through imaging, histological evaluation and transcriptomics. In the early inflammatory phase a more adherent mucus layer was observed, which further correlated with altered transcription of glycosyl transferases and mucin genes. This may indicate different properties and functions of the mucus during the acute inflammatory phase. Formation of scales and granulation tissue started approximately at 14 days post wounding. This was followed by wound contraction and formation of dermal structures.

At the transcriptomic level the greatest differences between NPI and HPI were found at 2-14 dpw, with more than > 500 DEG at each sampling point. In general, inflammation was enhanced in the HPI wounds, while cell proliferation and tissue regeneration was repressed. Histological examinations showed transient delays in the formation of epidermis, mucus response, scale mineralization, wound pigmentation and formation of dense connective tissue in HPI wounds. The overall wound morphology was also altered in fish reared at HPI. Wounds from NPI fish contracted in an elongated manner, while the wounds from HPI fish were more circular. The fish reared at HPI had significant higher cortisol levels compared to NPI fish, thus we suggest that cortisol are one of the main factors contributing to the delayed wound healing responses in fish reared at HPI .

The presented description of the wound healing processes in Atlantic salmon and the effect of HPI, gives insight into comparative ulcerative biology in fish and provides both novel and updated knowledge that can be applied for improved best operational practices for fish welfare in aquaculture.

Conference Session Designation: (QASH)
Presentation Format: (Oral)

Thursday Afternoon, September 6th – MacDonald
QASH 3
Moderators – Karin Pittman, Mark Powell, Robin Shields & Linda Andersen

1:10 PM	**Welcome Back !**
Available Tool Boxes Session - Robin Shields & Linda Andersen (Moderators)	
1:15 PM	Nylund - The Do's And Don'ts Of Real Time RT-PCR As A Tool In Fish Diagnostics: Evaluating Important Parameters, Pitfalls and Results Bias
1:30 PM	Berg - HealthPortal: Putting Production, Health And Environmental Data To Use For The Aquaculture Industry
1:45 PM	**Workshop 2 - Tool boxes available - Ease of use, global applicability, time usage? Integrated pest management and threshold values to elicit a response?**
2:15 PM	Overview Of Results, Comments, Volunteers For Core International QASH Proposal Work
2:45 PM	The Knowledge Gaps And Missing Tools In The Toolbox; Core QASH Objectives For 2019
3:15 PM	Concluding Remarks

The Do´S and Don`Ts of Realtime RT-PCR as a Tool in Fish Diagnostics: Evaluating Important Parameters, Pitfalls and Result Bias

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Molecular methods for use in fish diagnostics have been around for quite some time, both as tools for detection of pathogens and for use in quantitative analyses of gene expression. It has to a large degree replaced tried and true diagnostic methods in veterinary medicine, both in universities and veterinary colleges, and is supplied as commercially available products by several private laboratories linked to the aquaculture industry.

In Norway, realtime RT-PCR has become the staple method for monitoring and detecting pathogens during production of Atlantic salmon, due to its advantages in high sensitivity of detection, high specificity and the possibility to perform high- throughput analyses on a population scale. As a result, many different laboratories strive to design the highest performing assay for detection, identifying the best method for fish tissue processing, and how to interpret the outcoming results for use in setting a diagnosis. This increase in method development and optimization of performance, while positive, also have some negative impacts. Since different laboratories can employ different methodology, results are often not directly comparable between laboratories, and the interpretation of the results and their relevance in setting the correct disease diagnosis, will not always be straightforward.

The topic of this talk is to highlight some of the key parameters that will affect results of a realtime RT-PCR run. This includes choice of tissue for sampling, method for nucleic acid extraction and relevant factors that have to be taken into account when designing an assay for use in fish diagnostics. How to interpret the results, and how they are used in decision making, alongside complementing methodology will also be discussed.

Conference Session Designation: (QASH)
Presentation Format: **(** Oral)

Healthportal: Putting Production, Health And Environmental Data To Use For The Aquaculture Industry

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Benchmark Holdings have developed "HealthPortal" with input from leading Norwegian health care providers, including Marin Helse. HealthPortal is a tailor-made cloud-based data portal enabling aquaculture producers to keep track of, share and make use of production, health and environmental data. It is designed for producers and health providers to seamlessly collaborate, analyse and view critical information to improve the management of fish health and welfare and the environment. HealthPortal allows for the collection and integration of key health and welfare indicators such as mortality and sea lice burden within a user-friendly dashboard. These data are represented graphically and used to observe trends over time, allowing producers to monitor the health and welfare of their fish and communicate easily with health care providers.

Health care providers can use HealthPortal for everyday report writing, documentation storage, prescription provision and ordering analyses from laboratories. Producers can use HealthPortal to collate and streamline inputs from several health care providers, and to examine in-depth data such as causes of mortality e.g. disturbances, transport and sea lice by individual life-cycle stages. Through alert functions, producers can be warned if key indicators change – such as oxygen levels, feeding levels and mortality - enabling fast corrective action. Furthermore, health care providers can use HealthPortal to schedule visits with producers and generate post-visit veterinary reports which appear directly in producers' portals. From the dashboard, producers can order prescriptions and send samples for analyses such as qPCR; the results of which are uploaded by the laboratory and easily accessed by producers. Producers can share data with scientists, enabling scientists to make use of previously unavailable production, health and environmental data

HealthPortal is currently being used by health care providers, producers and scientists in Norway and Scotland. This new technology is highly valuable for both small-scale producers and for companies operating sites in multiple locations, as it allows for worldwide management and benchmarking of all sites. By integrating numerous indicators of health, welfare and the environment, HealthPortal represents a powerful tool to monitor and ensure long-term health and high productivity in farmed fish. HealthPortal has the potential to help the salmon industry lead the worldwide trend towards more sustainable aquaculture, incorporating high animal welfare standards, improved production and a low environmental footprint.

Conference session designation: (QASH)
Presentation format: **(** Oral)