No.	Name	Poster Title	Student
1	Landis, Eric	Aquaculture Drugs: What Are They And Why Does Fda Review Matter?	No
2	Crosby, Tina	Epidemiological Cutoff Value Analysis Of 229 Mics From Standard Testing Of Flavobacterium Columnare Isolates By Four Laboratories	No
3	Ulloa, Pilar	Effect Of B-Glucans On Intestinal Health In Zebrafish And Yellowtail Kingfish	No
4	**Soto-Davila, Manuel**	**Antibacterial Effects Of Cholecalciferol In Atlantic Salmon (Salmo Salar) Primary Macrophages Infected With Aeromonas Salmonicida.**	**Yes**
5	Kang, So Young	Efficacy Of Optimized Rhus Verniciflua Stokes Extracts Against Edwardsiellosis In Experimental And Field Trials In Olive Flounder Paralichthys Olivaceus	No
6	Soto, Esteban	Pharmacokinetic Parameters Following A Sinlge Intravenous Administration Of 10 Mg/Kg Of Natamycin To White Sturgeon (Acipenser Transmontanus)	No
7	Jung-Schroers, Verena	Development Of Antibiotic Resistances In Bacteria Isolated From Ornamental Fish And Fish For Food Production Between 2005 And 2017	No
8	Jung-Schroers, Verena	Diagnostic Methods For The Identification Of Different Aeromonas Spp. And Examination Of Their Pathogenicity Factors And Their Cytotoxicity And Adherence To Fish Mucus	No
9	Jung-Schroers, Verena	Identification And Pathogenicity Of Vibrio Spp. From Recirculating Aquaculture Systems For Pacific White Shrimps (Litopennaeus Vannamei)	No
10	Jung-Schroers, Verena	Methods For Identification And Differentiation Of Shewanella Spp. Isolates For Diagnostic Use	No
11	**Ghosh, Satyaki**	**Molecular Detection And Quantification Of The Fish Pathogen Saprolegnia Using Qpcr And Loop Mediated Isothermal Amplification (Lamp).**	**Yes**
12	Soto, Esteban	Investigating The Role Of The Type Vi Secretion System (T6ss) In The Emergent Fish Pathogen Francisella Noatunensis Subsp. Orientalis **(Changed to Oral)**	No
13	**Weatherhead, Rebekah**	**The Influence Of Water Temperature On Juvenile Nile Tilapia (Oreochromis Niloticus) Gene Expression Patterns When Challenged With Saprolegnia Parasitica**	**Yes**
14	Sebastião, Fernanda	Molecular Detection Of Francisella Noatunensis Subsp. Orientalis In Cultured Nile Tilapia (Oreochromis Niloticus) In Three Brazilian States	No
15	Olmos, Paola	Antimicrobial Susceptibility And Classification Of Piscirickettsia Salmonis Isolates From Southern Chile	No
16	Romero, Alex	Biofilm Formation By Piscirickettsia Salmonis Isolates Under Different Culture Conditions	No
17	**Hossain, Ahmed**	**Role Of Cold Shock Proteins In Aeromonas Salmonicida.**	**Yes**
18	Sanguinetti, Gina	Characterization Of A Pathogenic Yersinia Ruckeri Strain Isolated From A Fishfarm In Puno, Peru	No
19	Namba, Aki	Characterization And Pathogenicity Of Edwardsiella Piscicida From Peled, Coregonus Peled (Gmelin), In Japan	No

20	**Vasquez, Jose I.**	**Characterization And Draft Genome Of Vibrio Anguillarum J360 Marine Pathogen Isolated From An Outbreak In Lumpfish (Cyclopterus Lumpus)**	**Yes**
21	Phillips, Kenneth	Vagococcus Salmoninarum As The Causative Agent Of Mortality In Brook Trout Salvelinus Fontinalis	No
22	Huang, Tzu-Ming	Bacillus Cereus Infections In Soft-Shelled Turtles In Taiwan	No
23	Illanes, Oscar	Challenge Of Nile Tilapia Oreochromis Niloticus With With Salmonella Enterica Typhimurium In A Recirculating Aquaponics System.	No
24	**Powell, Ashley**	**Investigation Of Antimicrobial Resistance In Fecal Escherichia Coli In Seals From Canadian Atlantic And Arctic Waters**	**Yes**
25	**Choual, Khayr-Eddine**	**First Confirmed Report On Stranding Of A Pygmy Sperm Whale "Kogia Breviceps" (Blainville, 1838) At The Algerian Eastern Coast**	**Yes**
26	**Choual, Khayr-Eddine**	**Using The Macrobenthic Fauna As Bioindicator Of Microbiological Pollution Of The Lake Mellah (North-East Of Algeria)**	**Yes**
27	Kane, Andrew	Seafood Workers Are Aquatic Animals Too: Surveillance Of Health, Injuries And Fatalities Along The Us Gulf Coast **(Changed to Oral)**	No
28	Hsu, Hui-Min	Who's Driving The Bus? A Puzzling Case Of Tilapia Morbidity And Mortality In An Aquaponics System	No
29	**Ahasan, Shamim**	**Determining The Etiology Of An Emerging Ulcerative Disease In Invasive Lionfish**	**Yes**
30	Rashed, Rabar	Physiological Responses To High Carbohydrates In Common Carp (Cyprinus Carpio L.) Diet	No
31	Midtlyng, Paul	Improvement Of Environmental Parameters (Oxygen Saturation And Water Temperature) Using “Midt Norsk Ringen” In Salmon Cages …. **(Withdrawn)**	No
32	Loncarevic, Semir	Regulations For Equipment And Methods Of Desinfection For Water Used In Fish Slaughter And Processing Plant	No
33	**Falade, Abiodun**	**Effect Thymol and Cinnamaldehyde Essential Oils on Nutrition and Health Management of African Catfish (Clarias gariepinus)**	**Yes**
34	Powell, Mark	Fish And Shellfish Health Research At The Institute Of Marine Research, Bergen	No

35	Rhee, Jae-Sung	Comparative Analysis Of Distinctive Transcriptome Profiles With Biochemical Evidence In Bisphenol S- And Benzo[A]Pyrene-Exposed Liver Tissues Of The Olive Flounder Paralichthys Olivaceus	No
36	Quiroga, Isabel	Approaching The Puzzle Underneath Skeletal Anomalies In Senegalese Sole Solea Senegalensis	No
37	Rhee, Jae-Sung	Ichthyotoxic Dinoflagellates Induce Dna Damage, Lipid Peroxidation, And Antioxidant Response In The Gill Tissue Of Red Seabream Pagrus Major	No
38	**Petursson, Hrolfur**	**Source, Transmission And Development Of Ichthyophonus Hoferi Infection In The Icelandic Summer-Spawning Herring**	**Yes**
39	Mladineo, Ivona	Anisakis Pegreffii Extracellular Vesicles: Tem And Proteomic Characterisation **(Withdrawn)**	No
40	Cho, Jaebum	Amoebic Gill Infestation Of Juvenile Rockbream (Oplegnathus Fasciatus)	No
41	Mladineo, Ivona	Pathogenesis Of Anisakis Pegreffii Experimental Infection In The Accidental Host Model Inferred By Rna Seq: Clues To Elucidate Infection In Humans **(Withdrawn)**	No
42	Mladineo, Ivona	The Ultrastructure Of Anisakis Spp. L3 Larva **(Withdrawn)**	No
43	Skern-Mauritzen, Rasmus	A Copepodid Is Not A Copepodid	No
44	**Woodyard, Ethan**	**Investigations Into The Life Cycle And Pathology Of Odhneriotrema Incommodum In Wild-Caught Alligator Mississippiensis**	**Yes**
45	Gorgoglione, Bartolomeo	Modulating Vhsv And Ihnv Matrix Proteins To Regulate General Host Cell Transcription And Innate Antiviral Responses	No
46	Umasuthan, Navaneethaiyer	Impact Of Co-Stimulation With Sea Lice Lepeophtheirus Salmonis And Poly(I:C) On The Atlantic Salmon Dorsal Skin Transcript Expression	No
47	Godard, Danielle	Detection, Diagnosis, And Monitoring Of Acipenserid Herpesvirus 1 (Acihv1) In Lake Sturgeon In Wisconsin	No
48	Jung, Sung-Ju	Efficacy Of Plant Extracts, Kc-100 Against Viral Hemorrhagic Septicemia Virus (Vhsv)	No
49	Subramaniam, Kuttichantran	A Putative New Family Of “Minimal” Nucleo-Cytoplasmic Large Dna Viruses Distantly Related To Iridoviruses	No
50	Jung-Schroers, Verena	Infectious Spleen And Kidney Necrosis Virus (Isknv) In Ornamental Fish In Germany	No
51	Vieira, Diego	Morphological And Molecular Analysis Of Henneguya Sp., A New Gill Parasite Of Prochilodus Lineatus From Brazil	no
52	**Vieria, Diego**	**Phylogenetic Analysis Of Myxobolidae Family Parasites From Brazilian Fish**	**Yes**
53	Abdallah, Vanessa	Preliminary Phylogenetic Analysis Of Henneguya Sp. Found Parasitizing Stomach Of Serrassalmus Spilopleura From Brazil	No
54	**Capodifoglio, Kassia**	**Morphological And Molecular Characterization Of Two New Species Of Myxobolus And Of Myxobolus Colossomatis Infecting Colossoma Macropomum From The Amazon Basin**	**Yes**

55	Atkinson, Stephen	Novel Species And Cryptic Sources Of Ceratonova Parasites In The Upper Klamath Basin	No
56	Bailey, Christyn	Seq, Drugs And Pkd: An Rna – Sequencing Analysis Of A Biological And Chemical Stressor And Their Consequences For Fish	No
57	Bailey, Christyn	Do Fish Get Wasted? Assessing The Influence Of Effluents On Parasitic Infection Of Wild Fish	No
58	Kovacevic, Nikolina	Myxobolus Cerebralis Laboratory Testing In Alberta	No
59	Quiroga, Ma Isabel	Integrating Old And New Findings: Recent Advances In Turbot Enteromyxosis	No
60	**Murarolli Ribeiro, Amanda**	**Morphological Analysis And Molecular Of New Aurantiactinomyxon Type (Cnidaria: Myxosporea) In Oligochaetes From Fish Farms, Brazil**	**Yes**
61	Wang, Pei-Chi	Molecular Confirmation Of Kudoa Iwatai (Myxosporea: Multivalvulida) In Cultured Rock Bream Oplegnathus Fasciatus In Taiwan	No
62	Ali, Haythm	Hepatic Granuloma In A Wild-Caught King Soldier Bream (Argyrops Spinifer) From The Gulf Of Oman.	No
63	Nekouei, Omid	Spatiotemporal Distribution Of Infectious Agents In Juvenile Fraser River Sockeye Salmon	No
64	De Buron, Isaure	Pathology Induced By Blood Flukes Cardicola Spp. In Spotted Seatrout Cynoscion Nebulosus	No
65	Quintanilla, Juan	Infection And Horizontal Transmission Of Piscirickettsia Salmonis Between The Wild Fish Sub-Antartic Notothenioid Patagonian Blenny Eleginops Maclovinus And Rainbow Trout Oncorhynchus Mykiss By A Model Of Cohabitation Challenge	No
66	Silbernagel, Constance	Health Assessment Of Wild White Seabass Atractoscion Nobilis – A Species Cultured For Coastal Replenishment In California	No
67	Smith, Pedro	Testing For Piscine Orthoreovirus And Lesions Associated With Heart And Skeletal Muscle Inflammation In Wild Genypterus Chilensis From The Pacific Ocean In The North Of Chile	No
68	Kane, Andrew	Look Deep Into My Shell: Gross And Radiographic Observations Of Shell Damage By Boring Parasites In The Eastern Oyster Crassostrea Virginica **(Changed to Oral)**	No
69	**Lee, Do-hee**	**Monitoring Of Microplastic Dynamics And Anaysis Of Ecotoxicologycal And Physiological Effects In Marine Mysid**	**Yes**

70	**Le, Cuong**	**Genetic Relatedness Of White Spot Syndrome Virus Isolated From Imported Frozen Shrimp Products And An Outbreak In Shrimp Penaeus Monodon Farm In Australia**	**Yes**
71	**Perera, N. C. N.**	**Antiproliferative Responses Upon Viral Hemorrhagic Septicemia Virus Of Interferon Induced Gene Identified From Disk Abalone (Haliotis Discus Discus)**	**Yes**
72	**Sandamalika, Gayashani**	**A Cytosolic Glutathione S-Transferase, Gst-Theta (Gst-Θ) From Disk Abalone (Haliotis Discus Discus): Insights To Molecular And Biochemical Properties**	**Yes**
73	**Chakraborty, Setu**	**Immunization Against Vibrio Anguillarum In Cyclopterus Lumpus With Vibrogen 2 Commercial Vaccine**	**Yes**
74	**Chakraborty, Setu**	**Vaccination Of Atlantic Salmon (Salmo Salar) Against Aeromonas Salmonicida (Withdrawn)**	**Yes**
75	Carriero, Mateus	Molecular Cloning And Gene Expression Analysis Of The Cytokines Il1b And Il8 Of Pacu (Piaractus Mesopotamicus) Infected With Aeromonas Dhakensis	No
76	**Jung, Sumi**	**Insights Into Two Ferritin Subunits From Red-Lip Mullet (Liza Haematocheila): Potential Antibacterial Activity With Its Immune Response**	**Yes**
77	**Kankanamge, Omeka**	**Molecular And Transcriptional Insight Into Gstκ1 In Red-Lip Mullet Chelon Haematocheilus**	**Yes**
78	Chen, Shih-Chu	Differential Functional Gene Expression In Koi Carp (Cyprinus Carpio) After Challenge With Aeromonas Sobria	No
79	Gonzalez-Stegmaier, Roxana	Recombinant Flagellin Of Vibrio Anguillarum And Derived Peptides From D1 Domain Promote In Vivo An Acute Overexpresision Of Il-1β And Il-8 ProinflamatoryCytokines In Salmo Salar	No
80	**Liyanage, Dileepa**	**Molecular Characterization And Immune Profiling Of Cathepsin D From Redlip Mullet (Chelon Haematocheilus).**	**Yes**
81	**Perera, Neranjan**	**Antiviral Protein Viperin- Transcriptional Modulation Patterns Upon Immune Stimulants From Big Belly Seahorse Hippocampus Abdominalis.**	**Yes**
82	Hikima, Junichi	Interaction Of Soluble- And Membrane-Forms Of Toll-Like Receptor 5 Induce Expression Of Interleukin-1β Gene In Japanese Flounder, Paralichthys Olivaceus	No
83	Sakai, Masahiro	Il-17a/F1 Modulates Production Of Antimicrobial Peptides In The Intestine Of Japanese Medaka, Oryzias Latipes	No
84	**Trung, Cao Thanh**	**Vibrio Anguillarum Vaccine Candidates For Lumpfish (Cyclopturs Lumpus)**	**Yes**
85	Adamek, Mikolag	Immune Responses To Carp Edema Virus Infection In Common Carp – A Model For Immunosuppression?	No
86	Adamek, Mikolag	Resistance Vs Type I Interferon Responses In Viral Infection Models Of Common Carp	No
87	Adamek, Mikolag	Piscine Orthoreovirus 1 And 3 Detected As A Co-Infecting Agents During Furunculosis In Germany	No
88	Adamek, Mikolag	First Detections Of Carp Edema Virus In Hungary, Croatia And Serbia Confirm Very Wide Distribution Of The Virus In Europe	No
89	Adamek, Mikolag	Evaluation Of New Virus Infection Models For Zebrafish	No
90	**Thiel, Whitney**	**Antibody Development And Serologic Test Validation In Juvenile Northern Pike Esox Lucius Experimentally Infected With Viral Hemorrhagic Septicemia Virus**	**Yes**

91	**Rodrigues, Thais**	**Genomic Characterization Of A Novel Pegivirus Species In Wild Bottlenose Dolphins Tursiops Truncatus**	**Yes**
92	**Rodrigues, Thais**	**Phylogenomic Characterization Of A Novel Rhabdovirus Isolated From A Stranded Harbour Porpoise Phocoena Phocoena**	**Yes**
93	Björnsdóttir, Þórunn Sóley	Sequence Analysis Of The Hpr And Fusion Gene Of Hpr0 Isolates Of Isav In Iceland	No
94	Enriquez-Sais, Ricardo	Stress And Immune Gene Expression In Atlantic Salmon (Salmo Salar L) Broodstock Sub-Clinically Co-Infected With Chilean Prevalent Viruses	No

No. 1 Aquaculture Drugs: What are They and Why Does FDA Review Matter?

Eric D. Landis*

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What's a drug? A product's intended use determines whether it's a drug or not.
For example, when formalin is used to fix a biopsy sample from a fish, it isn't a drug because the intended use is to preserve the tissue for future study. But when formalin is used to kill external parasites on finfish, it's a drug because the intended use is to treat a disease (parasitism), which makes it a drug under federal law.

Why does FDA review animal drugs? Federal law requires that drug companies submit information to demonstrate that new animal drugs are safe and effective before they can be marketed. The FDA evaluates the information submitted by the drug company to make sure:

- The drug is safe and effective for its intended use;
- Food products from treated animals are safe for people to eat (if the drug is for a food-producing species);
- The drug is properly manufactured;
- The drug is properly labeled.

FDA's review also considers:

- The drug's impact on the environment; and
- The safety of the people who will come in contact with the drug.

FDA continues to monitor the drug's safety and effectiveness after it reaches the market. FDA also continues to monitor the drug's labeling, the drug's manufacturing process, and the company's marketing communications related to the drug.

Why should you care? As a fish producer, if you use an aquaculture drug with legal marketing status, you're assured that the drug is safe, effective, and manufactured to a high quality standard. The drug company has shown that the drug is safe and effective in a specific fish species when used according to the label. The label is written specifically for that species and includes all information necessary for you to use the drug safely and effectively, including the risks associated with the drug.

Currently, few legally marketed drugs are available for fish compared to other animals like cats, dogs, cattle, and chickens. Drug companies are often hesitant to spend a lot of resources to develop aquaculture drugs when there is so little return on their investment due to the small size of the U.S. aquaculture market. However, as a fish health researcher, you can help change this. You can collaborate with government agencies and other private and public fisheries professionals and use your facilities and expertise to help get more aquaculture drugs to legal marketing status.

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

No. 2 Epidemiological Cutoff Value Analysis of 229 Mics from Standard Testing of *Flavobacterium columnare* Isolates by Four Laboratories

Tina C Crosby[1]*, Annelies M Declercq[2], Patricia S Gaunt[3], John P Hawke[4], Nicholas R Hasbrouck[1], and Charles M Gieseker[1]

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Antimicrobial resistance is a major public health concern. Therefore, it is critical to have standard methods and criteria, called epidemiological cutoff values (ECVs, a.k.a. ECOFFs) to monitor for the development of resistance. To develop ECVs for the fish pathogen Flavobacterium columnare, we tested the susceptibility of 229 isolates using the standard broth dilution susceptibility testing method in the Clinical Laboratory and Standards Institute (CLSI) VET04-A2 guideline. Using the method, we determined the minimum inhibitory concentration (MIC) against 10 antibiotics: ampicillin, enrofloxacin, erythromycin, florfenicol, flumequine, gentamicin, ormetoprim/sulfadimethoxine, oxolinic acid, oxytetracycline, and trimethoprim/sulfamethoxazole. We analyzed frequency distributions for each antibiotic to estimate an ECV which separates the wild-type isolates without resistance from the non-wild-type isolates that have developed resistance using two statistical methods: ECOFFinder (Turnidge et al., 2006) and Normalized Resistance Interpretation (Kronvall, 2010). ECVs were estimated for ampicillin, enrofloxacin, erythromycin, florfenicol, flumequine, oxolinic acid, and oxytetracycline. The estimated ECVs showed that the isolates categorized similarly among the quinolones (enrofloxacin, flumequine, and oxolinic acid) as wild-type and non-wild-type. Therefore, laboratories could potentially use one of these compounds to monitor quinolone resistance. An ECV for the potentiated sulfonamides could not be estimated because the potentiators may be masking resistance to sulfonamide. Similarly, an ECV for gentamicin was not evaluated because there are currently no existing quality control ranges to verify the results. The CLSI Veterinary Antimicrobial Susceptibility Testing subcommittee is currently reviewing the data and the ECVs proposed from our analysis. If adopted, the ECVs will be included in the next revision of the VET04-supplement document.

Conference Session Designation: (General Session or Antibiotic Use/Pharmacology)
Presentation Format: (Poster)

No. 3 Effect of B-Glucans on Intestinal Health in Zebrafish and Yellowtail Kingfish

Pilar E. Ulloa[1,2], Mario Caruffo[2], Rodolfo Wilson[3], Patricio Dantagnan[4], Adrian Hernandez[4], Victor Martinez[5] and Carmen G. Feijóo[2]

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The limited availability of fishmeal in aquaculture has forced the industry to find alternative protein sources, being the soybean the most commonly used. Nevertheless, fish fed with soybean meal-based diet develop an intestinal inflammatory process which affects negatively growth. A possible solution to this problem is identify additives with "intestinal protective activity" that can be added to fish diet. Actually strategies for screening additives in cultured fish species are costly, lengthy and time consuming. We propose zebrafish as an excellent springboard in the selection of most beneficial additives to later be test in target fish species. We demonstrated that β-glucans (BG) exerted a protective effect on intestinal inflammation triggered by the intake of a soybean meal based diet. We took advantage of the *Tg(Bacmpx:GFP)* transgenic line, where neutrophils are fluorescently labeled allowing to make *in vivo* analysis and found a significant reduction in the number of neutrophils present in the intestine of larvae fed with the soybean meal diet supplemented with β-glucan. Beside, a significant decrease in the mRNA expression of pro-inflammatory cytokines; genes related to mucosal integrity and to extracellular matrix remodelation. To confirm these action of the addition of BG to the diet, we test the effects on yellowtail kingfish (*Seriola lalandi),* an important fish in aquaculture. The effects of BG were evaluated on growth parameters, molecular and intestinal level. To perform our research, a proinflammatory diet containing 34% of soybean meal (34SBM) was generated and one batch was supplemented with 1,5 g/kg of β-glucans (SBM+BG) and compared with the control diet (100FM). Growth parameters were significantly reduced in fish feed with SBM, thus altering negatively the productive parameters. Fish fed with SBM vs SBM+BG did not present significant differences, indicating that BG did not favor the productive parameters. mRNA expression of *tnf-α, il-1b, il-8, il-10 tjp2a, tjp2b, muc-2* and *fabp2* did not presented differences between diets. The histological analysis showed no signs of inflammation in any diet, however we detected a strong difference in the intestine plasticity. Fish fed with SBM+BG revealed greater turgidity/firmness in the intestinal folds, recovering the phenotype observed in fish fed with control diet. The lax condition in the intestine of fish fed SBM diet could negatively influence the nutrients absorption and contribute in part to the lower growth of these fish. These results in yellowtail show that despite the high level of soybean meal included in the diet, it did not cause damage to the intestine. On the other hand, β-glucans could improve morphology of the intestinal folds, thus playing a protective role from damage induced by high soybean meal inclusion in the diet. (Fonding: *Fondecyt 3130664 and 11170847 for PU and Fondecyt 1140297 for CGF*)

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

No. 4 Antibacterial Effects of Cholecalciferol in Atlantic salmon (*Salmo salar*) Primary Macrophages Infected with *Aeromonas salmonicida*.

Manuel Soto-Davila*[1], Matthew L Rise[2], Javier Santander[3]

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Vitamin D_3, also known as cholecalciferol, is a pro-hormone obtained from animal sources in fish through the diet (e.g. zooplankton or aqua-feeds supplemented with vitamins) and represent the major source of vitamin D in nature. In mammals, is involve in important processes including mineral metabolism, cell growth, tissue differentiation and the stimulation of antibacterial immune response. However, this mechanism has not been explored in fish cells. Here, we evaluate the effect of cholecalciferol on *A. salmonicida* subsp. *salmonicida* infection in *Salmo salar* primary macrophages. *S. salar* primary macrophages were isolated, incubated during 5 days and supplemented with cholecalciferol (100 ng/ml) for 24 h. *A. salmonicida* infection levels in *S. salar* primary macrophages, treated and non-treated with cholecalciferol, were evaluated by using the gentamicin exclusion assay at different time points. A multiplicity of infection (MOI) of 1:1 (bacteria : macrophages) was used. Expression of vitamin D receptor (VDR) and innate immune genes (IL-1β, IL-8, IL-10, TNFα, TLR5) at different time points pre- and post-infection was evaluated by qPCR. We found that cholecalciferol positively influenced the antibacterial activity of *S. salar* macrophages. In addition, *A. salmonicida* infection was significantly reduced in cholecalciferol treated cells versus non-treated cells. Collectively, our data suggest that the impact of cholecalciferol in *S. salar* macrophages innate immunity is similar to mammals. Collectively, our data suggest that the impact of vitamin D_3 on *A. salmonicida* infection of salmon macrophages may be similar to results described for bacterial infections of mammals.

Conference Session Designation: **(** Immunology or Bacteriology)
Presentation Format: (Poster)
Student Presentation: (Yes)

No. 5 Efficacy of Optimized *Rhus verniciflua* Stokes Extracts Against Edwardsiellosis in Experimental and Field Trials in Olive Flounder, *Paralichthys olivaceus*

Jae-Woong Lim, Su-Mi Shin, Sung-Ju Jung and So Young Kang*

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Previously, we developed an optimized extract with antibacterial and antiscuticociliate activities from lignum of *Rhus verniciflua* Stokes (RVS) using Box-Behnken design (Korean Patent Registration No. 10-1814175). The optimized extraction condition included 20% ethanol as solvent. For commercialization of plant extracts as feed additives for fisheries, the production cost is very important factor. In the present study, we attempted to mass-produce a hot water extract (HW)and 20% ethanolic extract (20E) of RVS using 1-ton extractor (RVSEs) and compare their efficacies in edwardsiellosis in olive flounder, *Paralichthys olivaceus*. Antibacterial activities of RVSEs against *Edwardsiella tarda* were evaluated by broth microdilution method. To investigate effects of RVSEs against *E. tarda* infection (immersion) in olive flounder, RVSEs were orally administered at doses of 10, 30, 100 and 300 mg/kg b.w./day for 2 and 4 weeks, respectively in laboratory and 100 mg/kg b.w./day for 3 weeks in field. The major components of RVSEs were quantified by a HPLC-UV and the method validation was performed. The extraction yields of RVSEs were 5% (HW) and 6.3% (20E), respectively. The contents of gallic acid and fustin in RVSEs were 5.5% and 17.8% in HW, and 2.9% and 20.9% in 20E, respectively. In the case of mass-produced extracts, the extraction yields were lower than those of experimental extracts, but there was little difference in the extraction of major components. RVSEs showed minimum inhibitory concentration (MIC) of 500 (HW) and 250 (20E) μg/ml against *E. tarda*. Fish administered with HW or 20E for each 2 and 4 weeks showed significant efficacies showing relative percent survival of 6.7 to 50.0% ($P < 0.001$). In field trial test, administered groups showed significant efficacies showing relative percent survival up to 90.0% ($P < 0.0001$). These results suggested that RVSEs may be used as safe and effective antibacterial alternatives in aquacultures.

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

No. 6 Pharmacokinetic Parameters Following a Sinlge Intravenous Administration of 10 mg/kg of Natamycin to White Sturgeon (*Acipenser transmontanus)*

Esteban Soto[1,*] Heather Knych[2], Susan Yun,[1] Kirsten Kenelty, [1] Valentina Tomasi,[3]

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Sturgeons (Osteichthyes: Acipenseridae) represent 25 species of anadromous and potamodromous fishes of North America and Eurasia, with an evolutionary history of over 100 million years. *Veronaea* is a small genus of saprobic fungi found in soil and on plant materials, belonging to the family Herpotrichiellaceae, order Chaetothyriales. "Fluid belly" is regarded as one of the most important emergent diseases in sturgeon aquaculture and is associated with disease in other aquatic animals and in humans. Despite it being considered one of the most important emergent pathogens for the caviar industry, there are no commercially available therapeutants against systemic fungal infection in cultured fish. Natamycin, also known as pimaricin is a macrolide polyene antifungal agent produced by the bacterium *Streptomyces natalensis*. Natamycin targets ergosterol in the cell wall of fungi and has been used for food preservation and treatment of fungal infections in more than 150 countries around the world at levels as high as 40mg/kg of finished product. In previous study it was found that at least 4 and 16 μg/ml of natamycin was needed to decrease 70% area under the curve of the fungus in Saboroaud dextrose or RPMI media growth curves. To gain an understanding of the tissue distribution of natamycin in fish, a pilot study was recently completed using white sturgeon yearlings as model. Briefly, the fish received a single 10 mg/kg intravenous injection of natamycin. Plasma samples were collected at 0, 5, 15, 30 and 45 minutes and 1, 2, 4, 6, 8 and 24 hours post drug administration (n=3 fish/time point). Natamycin was quantified in white sturgeon plasma by tandem liquid chromatography–mass spectrometry using a previously published method. Naïve pooling of datum points was used to combine data from different fish at each time point. Noncompartmental analysis for sparse data was performed on plasma natamacyin concentration at each time point using commercially available software for determination of total systemic clearance, volume of distribution, and the terminal phase half-life for natamycin in white sturgeon. Based on pharmacokinetic parameters determined in this pilot study and targeting a blood concentration of 16 ug/mL (MIC determined from previous studies), an intravenous dose of 40 mg/kg should allow us to effectively achieve the targeted MIC.

Conference Session Designation: (Antibiotic Use / Pharmacology)
Presentation Format: **(** Poster)

No. 7 Development of Antibiotic Resistances in Bacteria Isolated from Ornamental Fish and Fish for Food Production Between 2005 and 2017

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Antibiotic resistance is one of the biggest threats to human and animal health. Antibiotic resistances can occur naturally, but misuse of antibiotics is accelerating the process. As resistances against antibiotics occur in bacteria isolated from fish as well, it is recommendable to perform a sensitivity test for the detected bacteria before treatment of fish.

All bacteria isolated from diagnostic samples between 2005 and 2017 at the Fish Disease Research Unit were analysed for resistance against antibiotic substances. In total 19 substances were tested, whereas some substances were tested during the whole period of 13 years and others were tested only for two to 12 years. The antimicrobial susceptibilities of bacterial isolates were determined by the use of the disk diffusion method. Bacterial isolates were inoculated on blood agar plates. Antibiotic disks containing amoxicillin (10 µg), ampicillin (10 µg) chloramphenicol (30 µg), chlortetracycline (30 µg), colistin (50 µg), doxycyclin (30 µg), enrofloxacin (5 µg), erythromycin (15 µg), florfenicol (30 µg), flumequine (30 µg), furazolidone (100 µg), gentamicin (10 µg), kanamycin (30 µg), neomycin (10 µg), oxolinic acid (10 µg), oxytetracycline (30 µg), trimethoprim/sulfonamide (25 µg) tulathromycin (30 µg), or tylosin (30 µg) were used according to the manufacturer's instructions. Inhibition zone diameters were measured and evaluated inspired by CSLI if possible. According to the diameter of the inhibition zone, the results were given in resistant (R), intermediate (I) and sensitive (S).

The results show that the detected bacteria showed mainly resistances against amoxicillin, ampicillin, neomycin, oxolinic acid and tylosin. Yet, over the last 13 years, resistances of bacteria against antibiotic agents were decreasing in total and for most substances the resistance situation improved. Only for single substances, like trimethoprim/sulphonamide, the number of resistant bacteria increased. Differences were seen in bacteria isolated from fish from different keeping units. Especially in bacteria isolated from ornamental fish at wholesaler facilities more resistances were detected, whereas in bacteria isolated from fish for human consumption fewer resistances were found. Differences were also detected in the resistances of specific bacterial species. Especially Flavobacteria, some species of motile Aeromonads and Pseudomonads showed frequently resistances against a number of antibiotic substances.

Conference Session Designation: (Antibiotic Use / Pharmacology)
Presentation Format: (Poster)

No. 8 Diagnostic Methods for the Identification of Different *Aeromonas* spp. and Examination of their Pathogenicity Factors and their Cytotoxicity and Adherence to Fish Mucus

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The genus *Aeromonas*, belonging to the class *Gammaproteobacteria* and the family *Aeromonadaceae*, contains Gram-negative, non-spore forming, rod-shaped, facultative anaerobic bacteria. Aeromonads are ubiquitous in the environment, especially in aquatic habitats and act as obligatory or facultative pathogens of aquatic animals and man. *Aeromonas salmonicida*, the only non-motile species within the genus *Aeromonas* and the causative agent for furunculosis in salmonids, is an obligate fish pathogen whereas many motile Aeromonads are known as opportunistic pathogens of fish, amphibians and other aquatic animals and also in human disease outbreaks are described. In this study 44 already characterized isolates of *Aeromonas* spp. were analysed. For species identification biochemical techniques, 16S rRNA sequencing, sequencing of the *gyrB* gene that encodes the b-subunit of DNA gyrase, MALDI-TOF MS and the Sherlock Microbial Identification System (MIS) based on the composition of fatty acid ethyl esters were compared. The phylogenetic relationship, cytotoxicity in vitro, adherence to mucus in vitro and resistance against antibiotics were tested. The most reliable method for species identification was MALDI-TOF MS and *gyrB* sequencing. Most virulence factors were found in isolates of *A. dharkensis*, *A. hydrophila*, and *A. salmonicida* and especially isolates of *A. dharkensis* and *A. hydrophila* showed a high cytotoxic activity. Nevertheless, the virulence of Aeromonads is probably not only depending on the species but on the isolate itself. Many isolates of *Aeromonas* spp. were showing multi-resistances against antibiotic substances. This result has to be regarded as critical, because of the ubiquitous nature of *Aeromonas* sp. and the widely distributed virulence mechanisms. Testing the susceptibility of antibiotic substances before treating diseased fish should be therefore be a standard procedure, to reduce the development of resistant isolates and to ensure that an adequate substance is used for treatment.

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

No. 9 Identification and Pathogenicity of *Vibrio* spp. from Recirculating Aquaculture Systems for Pacific White Shrimps (*Litopennaeus vannamei*)

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Due to their pathogenic potential, the species identification of *Vibrio* spp. from recirculating aquaculture systems (RAS) for Pacific white shrimps (*Litopennaeus vannamei*) is of great importance especially during disease outbreaks and for performing food safety examinations. Commonly used methods for identifying *Vibrio* spp. include biochemical identification or sequencing of the 16S rRNA gene. The present study compares these standard methods, used in general diagnostic laboratories for identification in addition to sequencing of the uridylate kinase encoding gene *pyrH* and an identification using protein spectra assessed by MALDI-TOF MS. It is still unclear how to evaluate the potential pathogenicity of many *Vibrio* spp. and the regulatory systems are often unrevealed. Estimating the occurrence and virulence of certain *Vibrio* species in recirculating aquaculture systems (RAS) for the pacific white shrimp (Litopennaeus vannamei) may help to assess potential risks.

The results achieved by the different applied methods were highly divergent for most of the analysed isolates. For identifying *Vibrio* spp. from diagnostic samples it can be recommended to use 16 S rRNA sequencing containing the variable regions V1 – V8. The conducted study showed difficulties in reliably identifying several *Vibrio* spp. These difficulties mainly resulted from missing entries of sequences in digital databases, a low amount of comparable isolates analysed so far and high interspecific similarities of biochemical traits and nucleotide sequences.

Further investigation of the *Vibrio* spp. isolates on the expression of genes that contribute to the virulence (VPI, ToxR, ToxS, vhh, vfh, tdh, trh, flab, flaC, flaBvuln, flaCvuln, flaDvuln, T6SS1, T6SS2) give indications towards a potential risk of infection when these isolate are present in biofilm or water of a RAS.

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

No. 10 Methods for Identification and Differentiation of *Shewanella* spp. Isolates for Diagnostic Use

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Shewanella spp. are Gram-negative, rod-shaped, motile bacteria that are widely distributed in marine and freshwater environments. The bacteria are present in the physiological microflora of fish from temperate waters and are known as fish-spoilage species. From clinically healthy fish and from fish with skin ulcerations *Shewanella* spp. are regularly isolated, indicating a possible role as fish pathogen. In this study 74 isolates of *Shewanella* spp. were analysed. For species identification biochemical techniques, 16S rRNA sequencing, MALDI-TOF MS and the Sherlock Microbial Identification System (MIS) based on the composition of fatty acid ethyl esters were compared. The phylogenetic relationship, cytotoxicity in vitro and resistance against antibiotics were tested. The most reliable method for species identification was 16S rRNA sequencing. From diseased fish, clinically healthy fish and the aquatic environment different *Shewanella* species were isolated. This indicates that *Shewanella* spp. are widespread in the aquatic milieu and act as a secondary pathogen. The virulence of *Shewanella* spp. is probably not depending on the species but on the isolate itself. Many isolates of *Shewanella* spp. were showing multi-resistances against antibiotic substances, especially in samples derived from retailers and in routine diagnostics all *Shewanella* spp. should therefore be tested for resistances against antibiotic agents.

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

No. 11 Molecular Detection and Quantification of the Fish Pathogen *Saprolegnia* Using qPCR and Loop Mediated Isothermal Amplification (LAMP).

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Saprolegniasis is a serious emergent disease of fish in both natural and commercial systems, causing losses worth approximately $40 million annually in USA. It is caused by oomycete pathogens of the order Saprolegniales, specifically of the genera *Saprolegnia*. This disease affects all life cycle stages of fish. Zoospores are asexual stages of the *Saprolegnia* life cycle and are the predominant infective stage which can chemotactically locate injured or stressed fish. Fish become more prone to infection during the arrival of a cold front, where the sudden drop in temperature immunocompromises the fish, while facilitating the virulence of the *Saprolegnia* pathogens. The goal of this study is to establish rapid and sensitive molecular tools for the on-field detection and quantification of this pathogen from water samples, specifically focusing on the zoospores. This will facilitate making informed decisions about the timing and extent of chemical treatment. Conventional molecular markers such as ITS (Internal Transcribed Spacer of rDNA) and the mitochondrial COI &COII (cytochrome c oxidase) have been routinely used for molecular identification of oomycetes. In this study, we have developed a qPCR protocol, based on the ITS marker, that could detect *Saprolegnia* to concentrations as low as 2 picograms of mycelial DNA, Also, using this strategy, we could directly detect one *Saprolegnia* zoospore from water samples without a DNA isolation step. In comparison, Loop Mediated Isothermal Amplification (LAMP) was also developed as a tool for simple and rapid molecular detection and quantification of the *Saprolegnia* zoospores. LAMP reactions can be performed at a constant temperature without the need of a thermal cycler, and visual estimation of results can be obtained within 30 minutes, making it suitable for on field diagnosis. A combination of six primers were designed against the ITS region of rDNA, specific to the *Saprolegnia* genus. Visual detection of amplification was achieved by the addition of SYBR Green I. Using this technique, a minimum of 10 femtograms of mycelial DNA could be detected. Combining LAMP and qPCR, quantitative detection can be achieved, with enhanced sensitivity compared to qPCR alone. A variety of fluorescent dyes have been analyzed, to achieve highest sensitivity and least time to threshold for detection. Further, we intend to use this LAMP technique for direct on-field detection of the pathogen from water samples collected from Recirculating Aquaculture Systems (RAS).

Conference Session Designation: (Diagnostics and Quality Assurance)
Presentation Format: **(** Poster)
Student Presentation: (Yes)

No. 12 Investigating the Role of the Type VI Secretion System (T6SS) in the Emergent Fish Pathogen *Francisella noatunensis* subsp. *orientalis*

(CHANGED TO ORAL PRESENTATION)

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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: (Poster)

No. 13 The Influence of Water Temperature on Juvenile Nile Tilapia (*Oreochromis niloticus*) Gene Expression Patterns When Challenged with *Saprolegnia parasitica*

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The continued rise in aquaculture production, relative to the production plateau of capture fisheries, highlights the importance of aquaculture for food security. Ensuring aquaculture sustainability is therefore vital in supporting the growing global human population. This sustainability, however, remains threatened by multiple factors, particularly infectious aquatic diseases - a major economic and welfare concern. Strategies aiming to reduce the impact of infectious diseases have only been successfully implemented against a limited number of pathogens for a handful of fin-fish species. Improving understanding of the mechanisms underpinning host, pathogen and environmental interactions influencing disease risk, may assist with advancing aquatic disease control strategies.

The tropical cichlid Nile tilapia (*Oreochromis niloticus*) is a globally important aquaculture species. Production within temperate climates, however, remains limited, likely due to the requirement of tropical temperatures to achieve optimal production. Successfully lowering tilapia production temperatures would improve production cost-benefits and energy efficiency. However, being a poikilotherm, water temperature will influence tilapia's physiological processes. To investigate the influence of water temperature on juvenile Nile tilapia immune function, an infection-challenge was conducted at three different temperatures (19, 23 and 27°C) using freshwater oomycete *Saprolegnia parasitica*. Saprolegniasis prevalence was higher in tilapia housed at 19 and 23°C, relative to optimal production temperature of 27°C.

Transcriptomic analysis of infected and sham-control tilapia skin samples (19 and 27°C groups), revealed significantly differentially expressed immune-related genes. Through gene-network analysis, the patterns of gene expression are being explored. Increasing knowledge of the potential mechanisms contributing to the differences in disease susceptibility observed, will support the production of a tilapia stock more sustainable within temperate climates, as well as assisting with advancing disease management.

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

No, 14 Molecular Detection of *Francisella noatunensis* subsp. *orientalis* in Cultured Nile Tilapia (*Oreochromis niloticus*) in Three Brazilian states

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Francisella noatunensis subsp. *orientalis* (*Fno*), the etiologic agent of piscine francisellosis is an emergent Gram-negative, facultative intracellular coccobacilli, and considered one of the most important emerging pathogen in the global aquaculture industry. In Brazil, few reports since 2012 described the isolation and identification of the pathogen in the state of Minas Gerais and Santa Catarina. In this study we utilized a quantitative PCR assay (qPCR) specific for the *Fno* intracellular growth loci C gene (*iglC*) to diagnose the etiologic agent of Nile tilapia mortality during the winter of 2015 at three fish farms in the state of Paraná, São Paulo and Minas Gerais. Thirty-seven moribund fish were subjected to a complete necropsy and subsamples of anterior and posterior kidney, liver and spleen were subjected to histological and molecular analysis. Genomic DNA was extracted from 10 mg of tissue and used as template in PCR reactions. All fish were positive for piscine francisellosis, presenting a threshold cycle (Ct) < 40. Comparison of Ct obtained from subsamples of the anterior and posterior kidney, liver and spleen suggests the posterior kidney as the ideal tissue for molecular diagnosis. In conclusion, utilizing molecular methods, the presence of *Fno* as etiologic agent of diseases and mortality in cultured Nile tilapia in west Paraná, the largest region of tilapia culture in Brazil is reported for the first time. Further research is needed to adequately assess the impacts of *Fno* to the Brazilian aquaculture industry. Although the susceptibility of other fish species to *Fno* is largely unknown, epidemiological studies to determine the prevalence of this bacterium in native species and other culture fish is warranted.

Conference session designation: **(** Latin American Fish Health)
Presentation format: (Poster)
Student presentation: (Yes)

No. 15 Antimicrobial Susceptibility and classification of *Piscirickettsia salmonis* isolates from southern Chile

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Piscirickettsia salmonis is the aetiological agent of Rickettsial Salmon Septicemia (SRS) or Piscirickettsiosis, a bacterial disease that affects farmed salmonids, causing high mortalities and significant economic losses. In 2016, approximately 382.5 tons of antibiotics were used by the Chilean salmon industry and from these the 95% was administered in sea farms. Most of the antimicrobials used (92.5%) in sea farms were administered to treat outbreaks caused by *Piscirickettsia salmonis*. The aim of this study was evaluated the antimicrobial susceptibility and classified different *P. salmonis* isolates from diverse geographical and host origins, separating wild-type (WT) bacteria from those non-wild-type (NWT), according to the Minimum Inhibitory Concentration (MIC) values.

The isolates of *P. salmonis* were recovered from Piscirickettsiosis outbreaks occurred in various salmon farms as well as different periods of time and geographical locations along the South of Chile. Isolates were recovered from internal organs, including brain, kidney and liver of farmed salmonid species, Atlantic salmon (*Salmo salar*), Pacific salmon (*Oncorhynchus kisutch*), and rainbow trout (*Oncorhynchus mykiss*). The MIC value of Florfenicol (FFC) and oxytetracycline (OT) of all *P. salmonis* isolates were determined using a broth microdilution method following VET04-A2 and VET03/VET04-S2 guidelines of Clinical and Laboratory Standards Institute (CLSI) and classified according to the epidemiological cut-off (CO_{WT}) value (MIC $CO_{WT} \leq 0.25$ μg mL^{-1} for FFC and ≤ 0.5 μg mL^{-1} for OT).

A total of 35 isolates were analyzed from 13 different salmon farms between January 2017 and May 2018, from the Los Lagos Region and Aysen Region in the southern Chile.

Broth microdilution testing showed that 57.1% of the total isolates were WT to Florfenicol and 94.3% were WT for Oxytetracycline. From the Los Lagos Region isolates, the MIC values showed that 47.6% of isolates were WT to Florfenicol and 95.2% were WT for Oxytetracycline, for this Region it was observed that more than 50% of the isolates exhibited reduced susceptibility to this commonly used drug. In contrast, from the Aysén Region isolates, the MIC values showed that 71.4% of isolates were WT to Florfenicol and 92.9% were WT for Oxytetracycline.

This work shows the results of the MIC values for the first isolates obtained from the Monitoring and surveillance program for bacterial resistance to antimicrobials commonly used in Chilean salmon farming and contributes valuable data to the validation efforts to determine universal epidemiological cutoff values of this fish pathogen.

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

No. 16 Biofilm Formation by *Piscirickettsia salmonis* Isolates Under Different Culture Conditions

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Piscirickettsia salmonis is the etiological agent of Piscirickettsiosis, a severe disease that has caused major economic losses in Chilean salmon farm since its appearance in 1989. This pathogen is Gram negative, facultative intracellular, non-capsulated, non-motile, and pleomorphic. *P. salmonis* produce biofilm, related with persistence and survival strategies. However, characterization of culture conditions inducing the biofilm production in this pathogen has not yet been describe. The aim of this study is identify the culture conditions needed for induction of biofilm formation in *P. salmonis* isolates belonging to genogroups LF and EM. The bacterium was grown under different culture conditions of sodium chloride (0.1, 7.1, 24.6 and 32.1 g/L), ferric citrate (0.01, 0.08 and 0.16 mM) and pH (4.80, 7.02 and 8.40) for twelve days. Planktonic growth was measure by determining optical density at 620 nm at 2, 4, 8 and 12 days and biofilm formation was quantified using the crystal violet assay and measured at 595 nm. Additionally, at day 8 the bacteria were classified as weak, moderate or strong biofilm producer, using the Stepanovic scale. It was observed that LF-89 strain was a moderate biofilm producer in medium with 0.16 mM ferric citrate and was strong producer when grown at 24.6 g/L (estuary-like) and 32.1 g/L (seawater-like) NaCl. The IBM-004 (EM) isolate was classified strong for all the studied media, except at 0,1 g/L NaCl and pH 8.4, where behave as weak biofilm producer. AUS-111 (EM) isolate was moderate biofilm producer when grown in the medium containing 24.6 g/L NaCl. These findings showed that all the bacteria evaluated were able to produce biofilm *in vitro*, which was depending on the isolates and the medium conditions used to grow them. Supported by Fondecyt 1171357, Fondecyt Postdoc 3170356, Fondap 15110027.

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

No. 17 Role of Cold Shock Proteins in *Aeromonas salmonicida*

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First described in the 19th century, *Aeromonas salmonicida* is one of the oldest known fish pathogen and the causative agent of furunculosis in salmonid fish. *A. salmonicida* is an important pathogen due to its nearly worldwide distribution, broad host range and potential devastating impacts on wild and farm fish. *A. salmonicida* chromosome and plasmids are susceptible to endogenous rearrangement and mutagenesis caused by thermal inducible insertion sequences (ISs). ISs appeared to maintain the bacterium in a psychrophilic lifestyle in order to conserve their genomic integrity. *A. salmonicida* endogenous mutagenesis is induced at temperatures over 26ºC, influencing physiology and virulence. Thermal inducible ISs truncates the *vapA* gene that codes for the A-layer. Regulation of *A. salmonicida* ISs is unknown. We determined that the IS*AS3* are the ISs that truncates the *vapA* gene. Genetic analysis of the IS*AS3* promoters showed conserved Crp and Csp binding boxes. Deletion of *crp* has a minor effect on endogenous chromosomal mutagenesis. In this study, we evaluated the role of *cspB* and *cspD* genes on endogenous chromosomal mutagenesis and virulence. *A. salmonicida* mutants of *cspB* and *cspD* were constructed using suicide vectors. The mutants were characterized by bacteriological techniques. The frequency of endogenous mutagenesis of *vapA* was determined based on A-layer synthesis on congo-red TSA. Virulence was evaluated in *Salmon salar* primary macrophages. *A. salmonicida* □*cspD* showed a low frequency (4.5%) of *vapA* truncation after heat shock and reduced growth at 28ºC. The wild type and □*cspB* strains showed 100% and 74% of *vap* truncation after heat shock, respectively. □*cspB* showed a faster growth at 28ºC in contrast to the wild type. Additonally we determined that CspD control biofilm formation. We found that *A. salmonicida* □*cspB* □*cspD* has a higher virulence in *S. salar* primary macrophage in contrast to the wild type. In summary, we found that Csp plays a major role in the *A. salmonicida* virulence and endogenous rearrangement mutagenesis caused by thermal inducible ISs.

Conference Session Designation: (Bacteriology)
Presentation Format: (Poster)
Student Presentation: (Yes)

No. 18 Characterization of a Pathogenic *Yersinia ruckeri* Strain Isolated from a Fishfarm in Puno, Peru

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Yersinia ruckeri, the causal agent of enteric redmouth (ERM) disease, is an endemic pathogen that affects Peruvian rainbow trout (*Oncorhynchus mykiss*) aquaculture. Puno is one of the most important producer regions with several farms located on Lake Titicaca where constantly several ERM outbreaks occur with high morbidity and mortality rates. The aim of this study was to analyze the virulence of a *Y. ruckeri* strain isolated from a recent disease outbreak in a rainbow trout farm located in Lake Titicaca in Puno, Peru. Affected fish evidenced disease symptomatology including the characteristic "red mouth" pattern and mortalities over 70%. The isolate was identified as *Y. ruckeri* through microbiological, biochemical and molecular tests. Pathogenicity assays of the isolated *Y. ruckeri* were done through evaluation of intraperitoneal (i.p.) injection of healthy juvenile rainbow trout with different doses of isolate (from 10^4 to 10^7 cfu per 0.1ml), 20 fish per dose group. Control group was challenged with sterile saline solution. Fish were maintained at 11°C and monitored for 20 days. Mortalities and clinic symptomatology were recorded daily. Death and moribund fish were analyzed microbiologically. Samples were aseptically collected from internal organs (spleen and anterior kidney) and cultured in trypticase soy agar (TSA) incubated at 25°C for 24-48 hours. Strains isolated were evaluated by biochemical tests. Presumptive colonies were confirmed by PCR technique. At the same time, all isolates were tested for antimicrobial susceptibility. Results evidenced that *Y. ruckeri* strain isolated from Puno was virulent for healthy juvenile rainbow trout inoculated by i.p. injection. LD_{50} value was stablished in $10^{7.7}$ cfu per ml. Affected fish showed clinical signs of hemorrhagic septicemia. Macroscopic lesions found were splenomegaly and several hemorrhages mainly at the base of fins, around the eyes, on the swim bladder and posterior intestine. *Y. ruckeri* isolates recovered on TSA evidenced high phenotypic homogeneity. Biochemically, isolates were Gram-negative fermentative rods, positive for catalase, citrate utilization and glucose fermentation. Strains showed negative reactions for oxidase, bile esculin, gelatine hydrolysis, indol and Voges-Proskauer tests. All isolates were non-motile. Results of antibiotics susceptibility testing showed moderate to high sensitivity of isolates to florfenicol, oxytetracycline, enrofloxacin and fosfomycin. *Y. ruckeri* isolated from Lake Titicaca was a virulent strain for rainbow trout challenged by i.p. injection and it was possible to re-isolate these challenge strains in pure culture from internal organs of inoculated fish. This isolates should be used for autologous vaccine development in order to evaluate protection against Peruvian *Y. ruckeri* strains of Lake Titicaca.

Conference Session Designation: (Latin American Fish Health or Bacteriology/Mycology)
Presentation Format: (Poster)

No. 19 Characterization and Pathogenicity of *Edwardsiella piscicida* from Peled, Coregonus Peled (Gmelin) in Japan

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The peled, *Coregonus peled* (Gmelin), is a species of freshwater whitefish in the family Salmonidae. Whitefish, *C peled* and *C. lavaretus maraena*, were introduced in Japan from Czechoslovakia in 1975, and have been maintained for the last several decades in some Prefectures of Japan. At present, the whitefish is a representative candidate in efforts to diversify fresh water aquaculture in Japan.

From August 2011 to 2016 a disease resulting in mass mortality occurred sporadically in cultured peled in a farm in the Tohoku region of Japan. Diseased fish exhibited clinical signs such as systemic hemorrhagic septicemia and skin lesions, and bacteria that differed from aeromonads and streptococcosis causing damage in Japanese salmonid aquacultures for the summer season were isolated from all examined fish. Utilizing biochemical tests and multilocus sequence analysis (MLSA), all isolates were classified into *Edwardsiella picicida*, which was recently described and separated from the species *E. tarda* (Abayneh et al., 2012). The present isolates also caused high mortality to salmonid fish species such as rainbow trout. These results indicate that the mortalities of peled found in the fish farm were caused by *E. picicida*.

This work was supported by JSPS KAKENHI Grant Number 17K07920.

Conference Session Designation: (Bacteriology)

Presentation Format: (Poster)

No. 20 Characterization and Draft Genome of *Vibrio anguillarum* J360 Marine Pathogen Isolated from an Outbreak in Lumpfish (*Cyclopterus lumpus*)

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Vibrio anguillarum is a Gram-negative marine pathogen that causes vibriosis, a fatal hemorrhagic septicemia of fish, crustaceans, and mollusks, in fresh and brackish water. This pathogen has a devastating economic impact on the aquaculture industry worldwide. Lumpfish (*Cyclopterus lumpus*), a native fish of the North Atlantic Ocean, is utilized as a cleaner fish to control sea lice (*Lepeophtheirus salmonis*) infestations on cultured Atlantic salmon. Lumpfish is an emerging aquaculture species in the North Atlantic. *V. anguillarum* is a recurrent bacterial pathogen in cultured lumpfish. Here, we described the phenotypic and genomic characteristics of *V. anguillarum* J360 isolated from lumpfish infected head-kidney. Koch postulates were utilized to determine the pathogenic nature of *V. anguillarum* J360.

Biochemical and enzymatic profiles were completed using API20NE and API ZYM. Motility, catalase activity, synthesis of type I fimbria, optimal growth temperature, siderophores synthesis, growth in different concentrations of NaCl, biofilm formation, and sensitivity to antibiotics were also determined. *V. anguillarum* J360 grows between 4° - 30°C, is halo dependent and incapable of growing on TCBS media. Siderophore and IROMPs synthesis were induced under iron limited conditions. Hemolytic activity was induced at 28ºC and inhibited at 15ºC. API 20NE profile suggested 99.7% *Vibrio fluvialis* (code 7777745).

The PacBio platform (Genome Quebec) was utilized for whole genome sequencing. Five contigs were obtained with 30x coverage, with an estimated total length of 5,500,000 bp, and 44.47% of G+C. The phylogenic analysis using the 16S rRNA showed that *V. anguillarum* J360 is closely related to *V. anguillarum* M3. However, *V. anguillarum* J360 genome is larger than the other *V. anguillarum* reported genomes, such as *V. anguillarum* NB10 (4,373,835 bp), *V. anguillarum* 775 (4,117,056 bp) and *V. anguillarum* M3 (4,117,885 bp). Replication mechanism, stress-associated, and housekeeping genes such as *ftsZ*, *gapA*, *gyrB*, *mreB*, *pyrH*, *recA*, *rpoA*, and *topA*, were analyzed. Also, diverse virulence mechanisms were studied *in silico*, including pathogenic islands, and iron acquisition mechanisms. Virulence was evaluated in lumpfish. Groups of 80 healthy lumpfish were i.p. injected with 10^7 or 10^6 CFU / dose. PBS mock infected lumpfish were used as control group. Vibriosis symptoms were detected in the infected group. *V. anguillarum* J360 killed 98% of the infected fish. The whole genome sequence of *V. anguillarum* J360 will allow us to understand the pathogenesis of *V. anguillarum* in lumpfish, and to design vaccines using reverse vaccinology.

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

No. 21 *Vagococcus Salmoninarum* as the Causative Agent of Mortality in Brook Trout (*Salvelinus fontinalis*)

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In December 2017, staff at the Iron River National Fish Hatchery observed significant post-spawn mortality in an anadromous form of brook trout (*Salvelinus fontinalis*) reared at the hatchery. Necropsy revealed necrosis of cardiac tissue, cloudy fluid around the heart, ascites fluid in the peritoneal cavity, and egg retention. Samples for bacterial analysis were collected from the brain, egg skein, heart, and kidney. A gram-positive, chain forming coccus bacteria was isolated from most samples; the isolate was identified as *Vagococcus salmoninarum* via biochemical and molecular methods. Antibiotic sensitivity testing of the two antibiotics available for use under the Investigative New Animal Drug (INAD) indicated florfenicol was marginal for the treatment of *V. salmoninarum*, and oxytetracycline was ineffective. A stacked treatment with florfenicol (15mg/kg for 10d) under the INAD program was unsuccessful. The evaluation of other antibiotics for possible treatment is ongoing. Observations made during this outbreak are consistent with outbreaks of *V. salmoninarum* reported in the literature. The outbreak is ongoing, with losses of adult brook trout at the hatchery approaching 40%. This is the first report of *V. salmoninarum* in the Midwest region of the United States, as well as the first known outbreak in a charr species.

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

No. 22 *Bacillus cereus* Infections in Soft-Shelled Turtles in Taiwan

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Several bacterial infections in soft-shelled turtles (SST) (Trionyx sinensis) have been noticed in southern Taiwan since 2014. Clinical signs of abnormal and slow swimming, anorexia, and sudden death were observed. Hemorrhage in laryngeal region, ascites, enlargement of liver and spleen were found. Completely hemolytic colonies were obtained on blood agar plates from ascites, heart, liver, spleen and kidney. Based on the Vitek 2 biochemical characterization, 16s rDNA sequencing, and no crystal toxin production, the etiology was identified as Bacillus cereus. The multilocus sequence type (MLST) analysis revealed that the SST isolate was closest to ST234 with one base mutation in glpF and pta, respectively. Virulence factors of hemolysin BL (hbl) and nonhemolytic enterotoxin (nhe) were detected by polymerase chain reaction in the SST isolates. The isolate was sensitive to tetracycline and erythromycin, but resistant to trimethoprim/sulfmethoxazole by minimum inhibitory concentration (MIC) test. The B. cereus isolate showed virulence in SST by intraperitoneal inoculation. To author's knowledge, this is the first case of B. cereus infection in SST in Taiwan.

Conference Session Designation: **(** Bacteriology)
Presentation Format: (Poster)

No. 23 Challenge of Nile Tilapia (*Oreochromis niloticus*) with *Salmonella enterica* Typhimurium in a Recirculating Aquaponics System

Oscar Illanes[1], Elizabeth Antaki-Zukoski[2], Rodolfo Nino Fong[1], Michele Jay-Russell[2], Geoffrey Mangalam[2], Peiman Aminabadi[2], Fernanda de Alexandre Sebastião[3], Beatriz Martínez López, Fred Conte[4], Sarah Taber[5], Esteban Soto[3]

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Aquaponics, which combines the production of aquatic organisms such as fish with hydroponics in a symbiotic environment, is a developing technology that has gained significant popularity during the last two decades. In order to investigate the fate and food safety significance of contamination, laboratory controlled challenges were performed to study the susceptibility and persistence of *Salmonella enterica* serovar Typhimurium (PTVS 337) strain in Nile tilapia (*Oreochromis niloticus*) fingerlings in an aquaponics system. Fingerlings were challenged with two non-lethal doses (10^5 and 10^9 colony forming units (CFU)) of the bacterium by intra-gastric gavage. No morbidity or mortalities were detected in any of the exposed or control treatments after a period of 42 days. At the end of the challenge fish were euthanized and samples of the spleen and gastrointestinal tracts were subjected to bacteriological and histopathological examination. Even though no lesions compatible with salmonellosis were detected in any of the fish, *S. enterica* were cultured from intestinal tract of two fish exposed with 10^9 colony CFU/fish. Immunohistochemistry (IHC) using rabbit polyclonal anti-*Salmonella* horseradish peroxidase was performed in sub-samples of spleen, stomach, intestine, liver and gills to investigate the localization of bacteria within those tissues. Positive staining was detected only in the spleen, in which the bacterium was harbor within melano-macrophage centers located around the splenic ellipsoids. No *Salmonella* sp. was cultured from the control fish or the fish challenged with 10^5 CFU/fish. Results of this study suggest that *S. enterica* may not only be transient within the gastrointestinal flora of exposed fish but may be carried by phagocytic cells to internal viscera and remain in there without producing detectable lesions or clinical disease. Research investigating the presence and prevalence of potential enteric pathogens in the water, biofilm, life support system and plants is warranted to improve food safety in aquaponics.

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

No. 24 Investigation of Antimicrobial Resistance in Fecal *Escherichia coli* in Seals from Canadian Atlantic and Arctic Waters

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Seals are a highly migratory group of animals that may temporarily reside in waters adjacent to densely populated areas. Therefore, there is a potential risk for the acquisition of antimicrobial resistance (AMR) genes by gastrointestinal *Escherichia coli* in seals. Subsequent exposure to AMR *E. coli* in seal feces could be a health risk to the northern aboriginal peoples who rely on seal hunts for sustenance. The objective of this study is to investigate the presence of AMR and pathotypes of fecal *E. coli* in two Canadian seal populations. Fecal samples were collected from young and adult seals as part of the Ringed Seal Health Project in the Magdalen Islands and Nunavut. The samples were cultured for isolation of *E. coli* in the Atlantic Veterinary College Diagnostic Services Bacteriology Laboratory (DSBL) and primary cultures were sent to the OIE Reference Laboratory for *E. coli* (EcL) at the Université de Montreal, for detection of virulence genes in selected *E. coli* isolates. These same isolates and their DNA lysates will be tested at the DSBL for phenotypic and molecular antimicrobial resistance, using disk diffusion, broth microdilution, and multiplex PCR assays as needed, with a focus on antimicrobials considered to have very high importance in human medicine. Preliminary results of isolates have shown the presence of extra-intestinal pathogenic *E. coli* (ExPEC) and enteropathogenic *E. coli* (EPEC) pathotype virulence genes. In the upcoming months, we will continue to collect and analyze samples for virulence genes and AMR, and conduct statistical analyses to compare isolates. With respect to the current literature, this will be the first study to describe the AMR of fecal *E. coli* in two Canadian seal populations.

Conference Session Designation: (Aquatic Mammals, Bacteriology)
Presentation Format: (Poster)
Student Presentation: (Yes)

No. 25 First Confirmed Report on Stranding of a Pygmy Sperm Whale "*Kogia breviceps*" (Blainville, 1838) at the Algerian Eastern Coast

Khayr-Eddine Choual[1, 2*], Farida Bouzebda-Afri[1, 2], Zoubir Bouzebda[1, 2], Haron Bouras, Alexis Ribas Salvador[3] And Zitouni Boutiba[4]

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A specimen of Pygmy sperm whale, *Kogia breviceps* (Blainville, 1838) failed at the North East of Algeria near El-Kala, Wilaya of El-Tarf, in June 2001. This animal (male, ≈ 180 cm long, ≈ 100 to 130 kg of weight) was found by local residents on the shore of the sea. Up to date, no Pygmy sperm whale was reported along the Algerian coast or in the entire Mediterranean Sea. This document confirms the first stranding of a pygmy sperm whale reported on the Algerian coast. However it is not an evidence provided to explain the possible distribution of a population of *Kogia breviceps*, but rather to demonstrate the existence of this species in the southern Mediterranean Basin.

Conference Session Designation: (Aquatic Mammals)
Presentation Format: (Poster)
Student Presentation: (Yes)

No. 26 Using the Macrobenthic Fauna as Bioindicator of Microbiological Pollution of the Lake Mellah (North-East of Algeria)

Khayr-Eddine Choual[1, 2*], Farida Bouzebda-Afri[1, 2], Zoubir Bouzebda[1, 2], Haron Bouras and Zitouni Boutiba[3]

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The deposits of Glaucous cockle in lake Mellah (North-East of Algeria) are not yet classified. Recently, an inventory was done using this bivalve mollusk as a bio-indicator of microbiological pollution. Various takings of Glaucous cockle distributed over several stations resulted in searching mainly coliforms and faecal streptococci. The study confirmed the classification of stations as unhealthy stations but it also showed that the northern part of the study area generally presented better bacteriological quality than the southern part. The possibility of developing aquaculture activities in the northern part of the study area, however, remains a subject to the confirmation of health improvement in this part.

Conference Session Designation: **(** Bacteriology/Mycology or Aquatic Epidemiology)
Presentation Format: **(** Poster)
Student Presentation: (Yes)

No. 27 Seafood Workers are Aquatic Animals Too: Surveillance of Health, Injuries and Fatalities Along the US Gulf Coast (CHANGED TO ORAL PRESENTATION)

Andrew S. Kane[1*], Ross M. Brooks[1], Shannon A. Hartsfield[1,2], Rose Cantwell[3], Sue Colson[3], Robert M. Durborow[4] and Melvin L. Myers[5]

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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.

Conference Session Designation: (Bridges between Aquaculture, Human and Environmental Health; or Aquatic Animal Health Management)

Presentation Format: (Poster)

No. 28 Who's Driving The Bus? A Puzzling Case of Tilapia Morbidity and Mortality in an Aquaponics System

Hui-Min Hsu[1*], Susan V Marcquenski[2], David M Vandever[3], Philip N Bochsler[1]

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Tilapia (*Oreochromis niloticus*) in an aquaponics facility experienced two periods of morbidity and mortality affecting fish of all ages over a period of 18 months. Fish culture conditions in an aquaponics facility are not ideal for either the fish or the plants, necessitating operational compromises. While Tilapia are thought to be a stress tolerant species of fish, chronic stressors may push even Tilapia to a "tipping point". We observed a consistent, but varying in severity, suite of histopathological changes in fish from these two events. The first event was triggered by a catastrophic drop in pH from 6.8-7.3 to 5.5. Weak and moribund fish were necropsied. Gross lesions included hyperplastic and inflamed gills, pale to brown friable liver, abundant visceral fat, congested and frayed fins, petechiae and abscesses on the skin and uveitis. Tissues and lesions were cultured on TSA and Hsu-Shotts media. Extreme numbers of motile bacteria in tank water were observed microscopically and water was also cultured. Kidney, liver, spleen, brain, heart and gills were fixed in 10% NBF for histology. Water quality compromises included temperature (72°F), fluctuating pH, nitrate and nitrite, extremely low alkalinity (20-30 ppm), mineral additions for plants and the irregular influx of fresh water. Fish feed contained 32-41% protein and 3-12% fat. Neem oil was used to treat aphid outbreaks. Recommendations were made to address nutrition, water quality, fish husbandry, sanitation and record keeping, and fish losses decreased. About nine months later, mortality rates increased following an aphid outbreak that required the use of Neem oil, concomitant with other water quality changes. Calculation showed all age groups were severely underfed (0.12-0.71 %body weight), indicating the fish biomass on hand was much greater than required for vegetable production. Significant mixed bacteria were cultured from each event, although not from every fish. Histopathology results were the most intriguing and puzzling. Marked diffuse lipidosis and hydropic changes with variable amounts of eosinophilic hyaline-like droplets were present in hepatocytes. Hyaline droplets accumulated within the epithelium of renal tubules. Marked variation in tubular epithelial morphology, including pyknotic nuclei and cytoplasmic vacuoles were present. Mild to severe chronic glomerulonephropathy with mild periglomerular fibrosis, reduced Bowman's space, loss of cellularity and accumulation of an amorphous substance comprised of fibrin and collagen were present within many glomeruli, especially in older fish. Mild to severe diffuse lamellar hyperplasia, with infiltrating leucocytes, eosinophilic granular cells and mucus cells occurred in the gills of all fish. We suspect some or all of these changes were induced by suboptimal rearing conditions, however, we could not link specific histological changes to specific operational compromises. We welcome your insight.

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

No. 29 Determining the Etiology of an Emerging Ulcerative Disease in Invasive Lionfish

Md. Shamim Ahasan[1*], William F. Patterson III[2], Roy P. Yanong[2], Salvatore Frasca Jr.[3], Kuttichantran Subramaniam[1], Theresa Cody[4], Thomas B. Waltzek[1]

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Invasive lionfish (*Pterois volitans*/*miles complex*) have become the planet's most successful marine fish invader since their introduction in the western Atlantic in the 1980s, now also being widespread in the Caribbean Sea and Gulf of Mexico (GOM). Removal efforts via spearfishing or trapping have been only marginally effective, and natural biological control of lionfish, whether via consumption by native predators, cannibalism, or disease, is considered to be orders of magnitude lower than the intrinsic rate of lionfish population increase. However, an emerging ulcerative disease has recently been observed in the GOM, U.S. Atlantic, The Bahamas, and the Cayman Islands, which may have much more substantial impacts on lionfish populations. In this study, we aimed to identify the causative agent(s) of the lionfish ulcerative disease. Lionfish displaying cutaneous ulcerations were collected throughout 2017-2018 in the GOM. Representative fish were subjected to microscopic wet mount evaluation, full necropsy, bacteriology, and histopathologic evaluation. Additionally, lionfish skin tissue/swab samples were processed for: 1) virus isolation using a suite of marine fish cell lines, 2) PCR assays targeting known fish DNA viruses, and 3) unbiased separate viral and bacterial metagenomics approaches via deep sequencing. Gross abnormalities noted in the ulcerated lionfish were limited to deep ulcerations through epidermis and into the skeletal muscle. Microscopic wet mount evaluation of fin, gills, and ulcerative skin lesions were negative for parasites. Results of bacterial cultures attempted from the leading edge of ulcers, kidney, liver, and brain were inconclusive, with no common bacteria cultured. Preliminary cell culture efforts and histopathological evaluations failed to identify an inciting pathogen. However, histopathologic evaluation of many ulcers demonstrated attempted tissue healing in some sections, suggesting that initial insult or infection may have occurred sometime (days or weeks) prior to collection of these fish. Nucleic acid extracts from ulcerated lionfish skin tissue/swab samples were used to build DNA and cDNA libraries for sequencing on an Illumina MiSeq sequencer and the data are currently being analyzed. The importance of identifying the cause(s) of this disease is critical for understanding the impact on invasive lionfish populations.

Conference Session Designation: (Emerging Disease)
Presentation format: (Poster)
Student presentation: (Yes)

No. 30 Physiological Responses to High Carbohydrates in Common Carp (*Cyprinus carpio* L.) Diet

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Feeding trial was carried out for 70 days to study nutritional effects of three levels of local carbohydrate sources (Standard ration SR 37 %, Medium carbohydrate ration MCR 42 % and high carbohydrate ration HCR 53%) in the diet of juvenile (30g) common carp. There is an ascending trend in blood glucose of fish with increasing the level of carbohydrate fed. It increased from an average value of 6.4 mmol/L in the control group to 7.5 mmol/L in MCR group and 10.3 mmol/L in HCR. The data of amylase activity in blood samples varied from 215U/L in the SR, 529U/L in MCR and 1132 U/L in HCR. Hepatosomatic Index varied from 1.50 to 1.58 with no significant differences between treatments. However, HSI was slightly higher for fish fed with high carbohydrate level diets. No harmful effect for feeding elevated rates of carbohydrates on liver condition. Reduction in body moisture from 72.21% for SR to 71.70 % and 72.14% in MCR and HCR was noted. Lipid contents decreased from 9.27% in SR to 9.22% in MCR and 9.18% in HCR. Protein contents increased slightly from 13.97 % to 14.56 %. The carbohydrate contents of the whole fish including skins, fins, bones and flesh varied slightly.

Conference Session Designation: (Nutrition and Fish Health)

Presentation Format: (Poster)

No. 31 Improvement of Environmental Parameters (Oxygen Saturation and Water Temperature) Using " Midt Norsk Ringen " in Salmon Cages With and Without Skirts

(WITHDRAWN)

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In response to development of resistance against anti-sea louse medicines, use of impermeable sheets or tarpaulins ("skirts") around the upper 5-10 meters of marine net pens have become popular in order to shield farmed salmon from exposure to influx of copepodids. However, partial closure of the pens will inevitably reduce the natural water exchange and thereby potentially reduce oxygen availability and removal of CO_2 and other excretion products from the pen.

A hardware equipment named "Midt Norsk Ringen" aims at improving oxygen availability inside pens that are equipped with skirts. It consists of an aerator device for installation in the cage near the bottom of the net, a robust air supply tube, and a compressor to be installed at the feed barge. By blowing compressed air through the aerator, water is being lifted by bubbles from the bottom of the cage to the top, setting up a vertical water circulation inside the pen and at the same time increasing evacuation of water below skirt edge. Visual documentation showing the prevention of surface water influx and oxygen and water temperature data from a recently started project will be presented to document the real-life effect of Midt Norsk Ringen on essential environmental parameters inside semi-closed net pens.

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

No. 32 Regulations for Equipment and Methods of Disinfection for Water Used in Fish Slaughter and Processing Plant

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In Norway, methods for disinfection of intake water to and effluent water from aquaculture operations are regulated by Regulation No. 192 issued by Norwegian Ministry of Aquaculture 20th of February 1997. The Norwegian Veterinary Institute approves methods of disinfection, while the Norwegian Food Safety Authority (NFSA) manages legislation and supervision of compliance. The list of approved disinfection methods pertaining to the regulation is publish on NFSA home pages www.mattilsynet.no/regelverk.

The regulation incorporates procedures for the approval of technical disinfectants, the disinfection of aquaculture systems as well as procedure for the approval of technical disinfectants. Furthermore, Norwegian Medicines Agency has issued in 1997 "Guidance and application form for applications to the aquaculture disinfectant approval scheme" that is based on the Norwegian Regulation No. 821 from 17th Jun 2008 concerning the cleaning and disinfection of aquaculture sites etc. The regulations incorporate procedures for the approval of technical disinfectants. These requirements are based on a draft of the EU's Biocides and Pesticides Directive (91/414/EEC) with test conditions such as temperature and organic load, and specified and mandatory test organisms. Applications are submit on the forms issued for this purpose. Approval authority is delegate to the Norwegian Medicines Control Authority, which will ensure the uniform handling of these applications, in cooperation with the Norwegian Veterinary Institute.

The list of approved disinfectants is publish on NFSA home page at:
http://www.mattilsynet.no/mattilsynet/multimedia/archive/00073/Liste_over_preparate_73947a.pdf

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

No. 33 Toxicity Bioassay of Sodium Lauryl Sulfate on Nile Tilapia (*Oreochromis niloticus*) Juveniles.

Falade, Abiodun. E. and Olowolafe Tunde E.

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The toxic effects of sodium lauryl sulfate (SLS) on *Oreochromis niloticus* juveniles were evaluated in a 96h toxicity trial using Blood profile, Histopathological changes of tissues and Behavioral response as indexes of assessment. One hundred and eighty (180) healthy juveniles of

O. niloticus (12.05±0.25g) were acclimatized for a week, weighed and randomly distributed into eighteen bioassay tanks with stocking density of ten (10) fish per tank. A range finding test was conducted prior to the experiment which was followed by a 96h definitive test by exposing the test fish to six varying concentrations of sodium lauryl sulfate in a static bioassay procedure. The effect of SLS on haematological parameters of *O. niloticus* was determined after four weeks of exposure to sub-lethal doses. Erratic swimming, loss of equilibrium, respiratory disturbance, lethargies and mortality were observed in the exposed fish. Differences observed in the mortalities of *O. niloticus* at varying concentrations were significant ($P < 0.05$). Percentage mortality as shown by correlation analysis revealed an increasing trend with increasing concentration of the surfactant over the 96h exposure period. Hematological indices indicated a significant ($P < 0.05$) decreasing trend in the red blood cell, haemoglobin, leucocyte counts and the packed cell volume with increasing concentrations of sodium lauryl ether sulfate. The gills histological changes observed were lesion, vacoulations, severe erosion of the secondary lamella and erosion of the gill mucus membrane. Abnormalities observed in the liver tissue of the treated fish were severe cellular infiltration, hepatic necrosis, venous congestion with diffuse vacuolar degeneration of hepatocytes and vacuolar degeneration. The LC_{50} value for sodium lauryl sulfate by probit analyses was 56.92 ppm. Exposure of *O. niloticus* to SLS at concentrations above 57.0 ppm can be detrimental to the health and wellbeing of the fish. It can also have adverse effects on non-target species.

Conference Session Designation:	(Toxicology /Path)
Presentation Format:	(Poster)
Student Presentation:	(Yes)

No. 34 Fish and Shellfish Health Research at the Institute of Marine Research, Bergen

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Fish and shellfish health in the marine environment has a strong focus on aquaculture and associated production. At the Institute of Marine research, the focus on fish and shellfish health provides support to the aquaculture industry as well as assessing the risk posed by farmed-wild fish interactions. Besides general fish health assessments and an advisory role to the Norwegian fisheries department and food authority, research strategically focuses on:

- Infection and disease transmission – studies on the infection dynamics of viruses (e.g. piscine reovirus and salmonid alphavirus) in salmonids in both marine and freshwater.
- Pharmacology and ecotoxicology – studies on the effects of delousing chemical treatments on crustacea and other non-target fish species.
- Salmon lice – studies on farmed-wild fish interactions and ecology in migrating smolts and adult salmon.
- Shellfish – surveillance and studies on parasitic and viral infections in shellfish and the spread of invasive species (e.g. Pacific oyster)
- Closed system aquaculture – investigations into health management in Recirculating aquaculture systems and semi-closed sea cage production.
- Cleaner fish – investigation of pathogen transmission, disease surveillance and application as biological control of sea lice infestation of Atlantic salmon.
- Fish immunology, virology, bacteriology and parasitology – investigation of the biology and dynamics of infectious agents and their immunological
- interactions in fish.

Conference Session Designation: (General Fish Health)
Presentation Format: **(** Poster)

No. 35 Ichthyotoxic Dinoflagellates Induce DNA Damage, Lipid Peroxidation, and Antioxidant Response in the Gill Tissue of Red Seabream *Pagrus Major*

Yun Kyung Shin, Do-Hee Lee, and Jae-Sung Rhee*

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Ichthyotoxic dinoflagellates pose a significant threat to aquaculture and fisheries. In this study, we employed several molecular and biochemical response systems of the gill tissue of red seabream *Pagrus major* to understand potential mode of actions of two dinoflagellates, *Cochlodinium polykrikoides* and *Karenia* sp. after exposure to different cell concentrations for 24 h. Overall, both dinoflagellates dose-dependently increased DNA damage, lipid peroxidation (intracellular malondialdehyde; MDA), and glutathione (GSH) depletion/synthesis during both exposure (24 h) and depuration (3 h) phases. We also analyzed enzymatic activities of antioxidant defense systems such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR). Both dinoflagellates tested in this study significantly modulated enzymatic activity of antioxidant defense system with strong inductions of SOD and CAT, and the levels were maintained during depuration period. Principle component analysis (PCA) showed potential correlations between molecular markers (i.e. DNA damage, MDA, and GSH) and enzymatic responses by cell concentrations and time-courses. Taken together, our results indicate that representative dinoflagellates have potential hazardous effects on the gill of red seabream within relatively short time period, as the gill is the first organ exposed to water and diverse environmental factors including dinoflagellates. Our results also suggest that analyzing a series of molecular and biochemical parameters can be a way of understanding and uncovering the mode of action of ichthyotoxic dinoflagellates.

Conference Session Designation: (Toxicology / Tox Path)
Presentation Format: (Poster)

No. 36 Approaching the Puzzle Underneath Skeletal Anomalies in Senegalese Sole *Solea senegalensis*

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The high incidence of skeletal anomalies detected in Senegalese sole (*Solea senegalensis*) is one of the major bottlenecks for its aquaculture. This problem not only affects the welfare of this promising flatfish, but also impairs the quality of the product, causing severe economic losses to the industry. In Senegalese sole farms, anomalies are often diagnosed using macroscopic techniques when they already affect the fish morphology. Therefore, the sector demands diagnostic tools providing a detailed scan of the most common deformities in distinct stages of development. In the literature, factors such as rearing conditions, genetics, nutrition and others were associated with the development of skeletal anomalies in different fish species. However, little is known on the incidence and typologies of vertebral anomalies in sole farms, where these multilevel factors may interact on fish in unknown manners, difficult to mimic in experimental conditions. This work provides a comprehensive study of skeletal anomalies affecting the vertebral column of farmed Senegalese sole at different rearing stages and feeding regimes. Complementary diagnostic methodologies were integrated, from the macroscopic, stereoscopic, radiographic and histologic perspective. Results showed that a conventional monitoring technique in this species farms, as macroscopic evaluation, unnoticed the presence of skeletal defects in around 72-75% of the individuals considered as "normal". The stereoscopic and radiographic studies revealed a high frequency of skeletal anomalies (more than 75%) in reared larvae, early juvenile and juvenile Senegalese sole. This methodology, along the anomaly profile at different stages, constitutes a valuable tool towards the refinement of rearing protocols. The histopathological studies showed the presence of chondrocytes in the deformed endplates and within the intervertebral space, opening the way for further investigations using the recent genomic resources in Senegalese sole. This work contributed with an interdisciplinary approach on the skeletal anomaly problematic affecting the aquaculture sector. Research on different key points in the productive context is essential to tackle this multi-factorial issue to optimize the quality and the welfare of the fish.

The authors would like to thank Stolt Sea Farm (Spain) (especially A Riaza and I Ferreiro) for providing fish samples and technical support. This work was supported by "Consellería de Economía e Industria" of Xunta de Galicia (10MMA020E) and by "Programa de Consolidación e Estructuración de Unidades de Investigación Competitivas GPC2015/034", Spain. AM de Azevedo held a University Professorship Formation (FPU) grant from the Spanish Ministry of Education.

Conference Session Designation: (Diagnostics and Quality Assurance)
Presentation Format: (Poster)

No. 37 Ichthyotoxic Dinoflagellates Induce DNA Damage, Lipid Peroxidation, and Antioxidant Response in the Gill Tissue of Red Seabream *Pagrus major*

Yun Kyung Shin, Do-Hee Lee, and Jae-Sung Rhee*

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Ichthyotoxic dinoflagellates pose a significant threat to aquaculture and fisheries. In this study, we employed several molecular and biochemical response systems of the gill tissue of red seabream *Pagrus major* to understand potential mode of actions of two dinoflagellates, *Cochlodinium polykrikoides* and *Karenia* sp. after exposure to different cell concentrations for 24 h. Overall, both dinoflagellates dose-dependently increased DNA damage, lipid peroxidation (intracellular malondialdehyde; MDA), and glutathione (GSH) depletion/synthesis during both exposure (24 h) and depuration (3 h) phases. We also analyzed enzymatic activities of antioxidant defense systems such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR). Both dinoflagellates tested in this study significantly modulated enzymatic activity of antioxidant defense system with strong inductions of SOD and CAT, and the levels were maintained during depuration period. Principle component analysis (PCA) showed potential correlations between molecular markers (i.e. DNA damage, MDA, and GSH) and enzymatic responses by cell concentrations and time-courses. Taken together, our results indicate that representative dinoflagellates have potential hazardous effects on the gill of red seabream within relatively short time period, as the gill is the first organ exposed to water and diverse environmental factors including dinoflagellates. Our results also suggest that analyzing a series of molecular and biochemical parameters can be a way of understanding and uncovering the mode of action of ichthyotoxic dinoflagellates.

Conference Session Designation: (Toxicology / Tox Path)

Presentation Format: (Poster)

No. 38 Source, Transmission and Development of *Ichthyophonus hoferi* Infection in the Icelandic Summer-Spawning Herring

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Since the beginning of the 20th century the summer spawning herring (*Clupea harangus*) has been one of the most commercially important fish species in Icelandic waters. In the late 1960s the stock collapsed, probably due to overfishing, leading to an implementation of a total nationwide fishing ban in 1971. The following decade, the herring population recovered well and remained strong and stable until 2008-2009, when severe and highly prevalent (up to 70-80%) *Ichthyophonus hoferi* infections were experienced in the older cohorts of the stock, followed by a significant decline in the stock. The reason for this sudden increase in infections of this endemic pathogen is unclear and now 10 years later, the epidemic is still ongoing. *Ichthyophonus hoferi* is a unicellular eukaryotic parasite, formerly classified as a fungus but presently grouped within the Class Choanoflagellatea. Knowledge on the lifecycle of *I. hoferi* is scarce, however herring is generally thought to acquire infections via its diet, although direct transmission between fish could also occur. The aim of the project is to investigate the source, transmission and development of *Ichthyophonus* infections in order to shed light on this prolonged epidemic in the Icelandic population of summer spawning herring. To reach this goal, different age groups of herring as well as various species of pelagic crustaceans are examined for infections using PCR, conventional histology and *in situ* hybridization.

Preliminary results indicate that a number of pelagic crustacean species carry *Ichthyophonus* infections and therefore a reservoir for the parasite. Furthermore, infections do not seem to be restricted to any specific age-groups, as asymptomic juvenile herrings, previously thought to be free of infections, have subclinical infections suggesting that the apparent age-related presence of clinical signs cannot be explained by a difference in diet. Possibly the stressful process of maturation leads to intensified infections and a clinical disease to emerge. The *in situ* hybridization technique has successfully been optimized and applied on highly *I. hoferi* positive samples. Examinations on the route of transmission and the development of the parasite in fish- and crustacean hosts are in process.

Conference Session Designation: (Parasitology)
Presentation Format: (Poster)
Student Presentation: (Yes)

No. 39 *Anisakis pegreffii* Extracellular Vesicles: TEM and Proteomic Characterization

Jerko Hrabar, Anamarija Vrbatovic, Ivona Mladineo **(WITHDRAWN)**

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Anisakis sp. that parasitises crustaceans as the first intermediate hosts, fish and cephalopods as paratenic hosts, and cetaceans as main definitive hosts, can accidentally infect humans through the ingestion of its live third stage larvae, present in the raw or lightly processed seafood. Today anisakiasis has been considered an important emerging zoonosis, but still frequently miss-diagnosed and under-reported in the Europe. Our previous study evidenced that *Anisakis* secretory and excretory products, as well as the total larval crude extract, are able to elicit increased production of reactive oxygen species (ROS), activation of kinases that trigger inflammation and cell proliferation, inhibition of apoptosis marked by strong upregulation of Hsp70, and elevated induction of p53 in human fibroblast cell line HS-68. Therefore the purpose of this study was to evidence the secretion of extracellular vesicles (EVs) of the live larvae that are today considered as the main source of parasite's products involved in its infection, migration and survival within the host. In general, EVs represent a ubiquitous mechanism for communication between cells and organisms across wide kingdoms of life, and have been shown useful to study host-parasite interactions. After cultivation of live *Anisakis pegreffii* larvae in RPMI-1640 culture media, larvae were harvested and prepared for High-Pressure Freezing (HPF)/ Freeze Substitution (FS) to obtain superior ultrastructural preservation and structural contrast for TEM. For proteomic analysis of EVs, media without larvae was centrifuged multiple times and afterwards, the proteins from the pellet obtained by ultracentrifugation, were separated in 2-D gel, digested, separated by liquid chromatography and analysed by MALDI/TOF-TOF.

Analysis of the ultrastructure and protein profile of *A. pegreffii* infective larvae EVs reveals their versatile and multiple role crucial for nematode survival *in vitro* conditions, but suggests as well their involvement in parasite's *in vivo* infection.

The research has been funded by Croatian Science Foundation (HRZZ), project #5576 AnGEl (*Anisakis* spp: Genomic Epidemiology), HRZZ PhD grant program for JH, and Small Grant Scheme of the European Association of Fish Pathologists awarded to JH. TEM facility has been used by curtesy of the Czech Bioimaging infrastructure, Laboratory of Electron Microscopy, Biology Centre ASCR - Institute of Parasitology, Ceske Budejovice.

Conference Session Designation: (Parasitology General/ Bridges between Aquaculture, Human & Environmental Health

Presentation Format: (Poster)

No. 40 Amoebic Gill Infestation of Juvenile Rockbream (*Oplegnathus fasciatus*)

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Thirty Juvenile rock bream, *Oplegnathus fasciatus* with an average weight of 15g were obtained from hatchery of Republic of Korea. The fish were suffered severe mortality during acclimation at laboratory. Affected fish showed behavioral alterations including the increase of ventilation rates and eventually dead. In morphological and histopathological observation, gills of infected with amoeba, showed fusion and clubbing of the lamellae and inflammatory cell gathered in basement membrane. From these fish, Fan-shaped amoeba was isolated and we assigned this amoeba as genus *Vannella* based on recent studies in spite of their morphology were identical to genus *Platyamoeba*, because taxonomic key based on glycostyle on cell surface in ultrastructure were not reliable. We obtained sequence of this study was showed at BLAST highest similarities with *Vannella*, and phylogenetic analysis of SSU rDNA were closely related in both *Vannella* and *Platyamoeba*, because it does not form distinct clades in the tree. . In histopathological observation, gills of infected with this amoeba showed fusion and clubbing of the lamellae and inflammatory cell gathered in basement membrane.

Conference Session Designation: (Parasitology General or Gill Health)

Presentation format: (Poster)

No. 41 Pathogenesis of *Anisakis pegreffii* Experimental Infection in the Accidental Host Model Inferred by RNA Seq: Clues to Elucidate Infection in Humans **(WITHDRAWN)**

Ivana Buselic Garber[1], Zeljka Trumbic[2], Jerko Hrabar[1], Anamarija Vrbatovic[1], Ivona Mladineo[1*]

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Anisakis sp. are marine parasites that use crustaceans as the first intermediate hosts, fish and cephalopods as paratenic hosts, and cetaceans as main definitive hosts. Humans can become accidental hosts by ingesting live third stage *Anisakis* sp. larvae, present in raw or lightly processed seafood. The disease is termed anisakiasis, which in symptomatic form elicits major clinical manifestations, developing gastric, intestinal, ectopic (or extra-gastrointestinal) and allergic/gastroallergic character. It has been evidenced that in evolutionary long-term host-parasite interactions, parasites are able to modulate immune response of the host, facilitating own propagation while buffering clinical symptoms in the hosts, in contrast to the cases of recent host colonisation. Therefore, the aim of this study was to explore the early infection mechanisms of *Anisakis pegreffii* larvae and the response of its accidental host, Sprague-Dawley rat, representing a mammalian "novel-host" model that simulates human infection. High-throughput next generation RNA sequencing was used to evaluate patterns of the *in vivo* experimental rat infection in the stomach and muscle tissues. Sequencing using Illumina NextSeq 500 of rat samples resulted in approx. 31 million paired-end reads per sample, 75 bases long and of good average quality, subsequently mapped to *Rattus norvegicus* genome. Rat response differed between stomach and muscle tissues, but generally showed to be strongly directed towards inflammatory reaction, congruent with observed gross and histopathological findings, evidencing pathways of stimulated neutrophil chemotaxis, leukocyte migration, peptide secretion, as well as intense tissue remodelling marked by keratinocyte proliferation and apoptosis. The top five overrepresented KEGG metabolic and signalling pathways up-regulated in the rat stomach included 03010 Ribosome, 04610 Complement and coagulation cascades, 04060 Cytokine-cytokine receptor interaction, 04657 IL-17 signalling pathway and 04640 Hematopoietic cell lineage. Except for 03010 Ribosome, the rest of the top five KEGGs were found in the infected muscle, although not all of them appeared in the top five list. To our knowledge, this is the first report of the RNA-Seq transcriptomic analysis in the vertebrate host tissues affected by *Anisakis* larval migration.

The research has been performed within the project AnGEl (Anisakis spp: Genomic Epidemiology) funded by the Croatian Science Foundation.

Conference Session Designation: (Parasitology General / Bridges between Aquaculture, Human & Environmental Health)

Presentation Format: (Poster)

No. 42 The Ultrastructure of *Anisakis* spp. L3 Larva **(WITHDRAWN)**

Jerko Hrabar, Anamarija Vrbatović and Ivona Mladineo

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Genus *Anisakis* Dujardin, 1845 comprises nine species of marine parasitic nematodes, which utilize marine mammals as definitive hosts, and fish and cephalopods as paratenic hosts. Some of the members of this genus are the causative agent of the disease anisakiasis, contracted by consumption of raw or undercooked sea food contaminated with live third stage larvae (L3). Due to increasing trend of consuming raw or undercooked food, more cases of anisakiasis are reported each year, listing this zoonosis as an emerging public health concern. Despite their cosmopolitan distribution and high abundance, and extensive research being conducted, much remains unknown about their biology, especially their morphology at ultrastructural level. Our study presents the comprehensive ultrastructure of *Anisakis* spp. L3 larvae throughout the nematode body. Live L3 were cut into 1mm pieces representing different body regions and fixed by high pressure freezing/freeze substitution (HPF/FS). Fixed samples were then resin embedded, cut to ultrathin sections, double contrasted and inspected under 80 kV microscope.
Muscle layer, located below multi-layered cuticle, is composed of elongated cells with large pale areas in non-contractile part indicative of glycogen storage. Epithelial cells of alimentary tract, except for oesophagus, are lined with microvilli and contain many different vesicles and multivesicular bodies. Single-celled excretory gland is composed of tightly packed vesicles of different size, surrounding large nucleus, which open into one main and several minor collecting ducts. All nuclei appeared rich in euchromatin with a single nucleolus, except for excretory gland nucleus that contains many nucleoli. In the hind part of the worm, several exosomal vesicles were found, containing same amorphous matter lining the cuticle from the outside. Presence of countless different vesicles and their localisation in intestinal cells and excretory gland, rich rough endoplasmic reticulum and euchromatin suggests active synthesis of secreted products, which aid the infective larvae in invading host tissue.

The research has been funded by Croatian Science Foundation (HRZZ), project #5576 AnGEl (*Anisakis* spp: Genomic Epidemiology), HRZZ PhD grant program for JH, and Small Grant Scheme of the European Association of Fish Pathologists awarded to JH. We acknowledge Laboratory of electron microscopy, Institute of parasitology, Biology Centre of AS CR (České Budějovice) supported by the MEYS CR (LM2015062 Czech-BioImaging) and ERDF (No. CZ.02.1.01/0.0/0.0/16_013/0001775) for their support in obtaining scientific data presented in this poster.

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

No. 43 A Copepodid is not a Copepodid

Rasmus Skern-Mauritzen[1*] Tone Vågseth[2], Sussie Dalvin[3], Samantha Bui[4]

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It has been previously demonstrated that the infectivity of the copepodid salmon louse, *Lepheoptheirus salmonis*, varies with age. The use of copepodid distribution and density modelling is becoming increasingly important, as the most recent models have reached a level of accuracy that has lead to their use in aquaculture management in the Norwegian industry. However, no present models include the temporal infectivity dynamics, which potentially weakens the certainty of the results. We infected salmon with defined age cohorts of copepodids (0.5 – 13 days old) at three temperatures (5, 10 and 15 °C), and sampled the fish after parasite settlement. The results are congruent with earlier findings, showing large variation in infective capacity as the larvae progresses from being newly molded to being moribound. The largest difference in infectivity was observed at 15 °C, where copepodids were 23 times more successful at infecting a host when 0.5 days old, compared to 10 days old. The infection profile differeded among temperatures, with the cooler temperature exhibiting a plateau in the middle range of ages, whereas the warmest temperature displayed a skewed peak at 0.5 days age. The results expand our knowledge base by including more detailed information on the dynamics of infectivity. This increased resolution allows inclusion of this parameter in next-generation models and thus represents an opportunity to increase model realism by improving parameterization.

Conference session designation: **(** Sea Lice – Ectoparasites)
Presentation format: **(** Poster)

No. 44 Investigations into the Life Cycle and Pathology of *Odhneriotrema incommodum* in Wild-Caught *Alligator mississippiensis*

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The trematode *Odhneriotrema incommodum* is a parasite of the tongue of the American alligator *Alligator mississippiensis*. Adverse pathology associated with this parasite is characterized by the presence of fibrotic lesions at the site of attachment. While the associated pathology been characterized from experimentally-infected alligators in a laboratory setting, pathology in naturally infected hosts has not been previously documented. Using specimens and host tissue collected from a commercial alligator processor during Mississippi's 2017 alligator hunting season, adverse pathology associated with *O. incommodum* in the definitive host is characterized. Parasite species identity was confirmed by morphological and molecular comparison with previously published works. Host inflammatory responses to secondary bacterial infection associated with the presence of this trematode are noted. Additionally, experimental work to identify the first intermediate snail host of *O. incommodum* through experimental infection of the endemic Ramshorn snail *Planorbella trivolvis*, as well as the invasive ghost Ramshorn snail *Biomphalaria havanensis*, are discussed. Neither of these snail species served as viable experimental intermediate hosts for *Odhneriotrema incommodum*, thus the life cycle of this trematode remains incomplete.

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

No. 45 Modulating VHSV and IHNV Matrix Proteins to Regulate General Host Cell Transcription and Innate Antiviral Responses

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Novirhabdoviruses are enveloped (-)ssRNA viruses affecting a wide range of wild and farmed fish. Viral Haemorrhagic Septicaemia virus (VHSV) and Infectious Haematopoietic Necrosis virus (IHNV) can both be highly contagious and cause OIE notifiable diseases, albeit with different host reservoirs. VHSV M (Matrix) protein is a powerful suppressor of host cell transcription, mediating both virus-induced cell toxicity and viral replication. Targeted mutations in the VHSV-IVb M gene sequence, including either D62A or E181A single mutations, tempered its ability to suppress host transcription in EPC cells. Combining the single mutations in the same construct (D62A/E181A) dramatically reduced M's anti-transcriptional effects in EPC cells in comparison to either single mutations alone. Reverse engineered replicating viruses harbouring these mutations exhibited less cytotoxicity and reduced anti-transcriptional activities, when compared to the wild type strain.

An improved cell transfection protocol, including the use of new transfection reagents, allowed to both increase dramatically the transient transfection efficiency in EPC, and the successful transfection of BF-2, RTG-2 and RTgill-W1 cell lines. This enabled to explore the capacities of the M genes of VHSV-IVb and IHNV to modulate cellular processes in heterogeneous cells, as wild type or mutated proteins. Although the VHSV-IVb strain (MI03GL) is relatively non-pathogenic for rainbow trout, the -IVb M protein exerts a strong anti-transcriptional activity in either epithelial (RTgill-W1) or fibroblastic (RTG-2) cells derived from rainbow trout (*Oncorhynchus mykiss*). Comparable anti-transcriptional effect was exhibited upon IHNV M transfection in these cell lines. A much-attenuated capacity to interfere with the host cell transcription machinery was observed with the VHSV-IVb M double mutant in RTG-2 and RTgill-W1 cell lines These new results are in line with previous observations from EPC, and have been corroborated in BF-2 cells. Although single mutations to IHNV-M did not suppress its strong anti-transcriptional and immunosuppressant activities, we are currently extending these to include combinations of mutations (double and triple) to investigate whether the orthologous regions of IHNV M regulate activity as observed with VHSV.

Conference Session Designation: (Virology)
Presentation Format: (Poster)

No. 46 Impact of Co-Stimulation with Sea Lice *Lepeophtheirus salmonis* and Poly(I:C) on the Atlantic Salmon Dorsal Skin Transcript Expression

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Ectoparasitic marine copepod infections constitute a major economic loss to the worldwide Atlantic salmon industry. Sea lice (*Lepeophtheirus salmonis*) intensify the prevalence of infectious diseases by (1) physically damaging salmon skin and allowing passage of pathogens and (2) suppressing host immunity. The current study attempts to understand the host immune transcriptomics from a co-infection perspective by exposing salmon to sea lice followed by injection of a viral mimic. Two diets containing functional ingredients, namely, Boost (EWOS) and CpG, were also tested to compare their impact on immune-relevant transcript expression with respect to a commercial control diet called Dynamic. Three groups of fish fed with different diets were infested with copepods. After four weeks (when parasites had developed to the pre-adult stage), fish in each dietary group received an intraperitoneal injection of either phosphate buffered saline (PBS) or polyinosinic:polycytidylic acid [poly(I:C)] suspended in PBS. Dorsal skin samples were collected from louse-attachment (Att) and adjacent non-attachment (NA) regions from each fish 24 h post-injection. Total lice count indicated that both Boost and CpG fed salmon had significantly reduced lice-attachment. RNA extracted from dorsal skin samples was used in qPCR to determine the expression of transcripts associated with antiviral (*isg15b*, *tlr3* and *irf7b*), wound healing (*mmp13*), inflammatory (*saa5*) and antimicrobial (*cathb*) activities. Poly(I:C) induced all three antiviral genes regardless of other treatment conditions, where overall fold-change in *isg15b* and *irf7b* transcripts was always higher when louse was present (Att) compared with NA in all dietary groups. The impact of poly(I:C) on other genes varied and depended on diet and lice attachment. Poly(I:C)-induced *irf7b* transcript expression in Att samples was significantly lower ($p<0.05$) in Boost fed fish compared with control diet fed fish. In addition, lice-induced fold-change in *mmp13* mRNA expression appeared to be greater in fish fed with experimental diets compared fish fed with control diet. Interestingly, both Boost and CpG reduced the level of inflammatory transcript (non-significant; $p>0.05$), *saa5*, particularly when poly(I:C) was injected, indicating that they could have anti-inflammatory properties. Transcriptional expression data of these candidate genes will be used in choosing the samples for a 44K microarray experiment to further explore the interaction between lice-viral mimic co-stimulation and two functional diets. Our integrated approach will be useful for (1) the understanding of genes and molecular pathways involved in lice infestation and viral co-infection, as well as (2) developing novel dietary formulation(s) to protect salmon from infection or co-infections.

Conference Session Designation: (Co-infections in Fish)
Presentation Format: **(** Poster)

No. 47 Detection, Diagnosis, and Monitoring of Acipenserid Herpesvirus 1 (AciHV1) in Lake Sturgeon in Wisconsin

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Alloherpesviruses have been described in a wide range of fish species. In 1991, an alloherepsvirus was described in a population of white sturgeon (*Acipenser transmontanus*) from a commercial farm in California and was later described in farmed white sturgeon in Italy. This alloherpesvirus, referred to as Acipenserid herpesvirus 1, resulted in microscopic lesions within the epithelial cells of the skin and oropharynx. In the spring of 2017, during a transfer event on the Wolf River, cutaneous plaques were noted on two wild-caught lake sturgeon. Biopsies were collected from these lesions for histopathology and molecular diagnostics. The histopathology and PCR screening confirmed the lake sturgeon were infected with a strain of Acipenserid herpesvirus 1. Through the distribution of sampling kits to hatchery managers and field biologists in Wisconsin, efforts are underway to better understand the prevalence and any potential role of the virus in disease.

Conference Session Designation: (Emergent Disease)

Presentation Format: **(** Poster)

No. 48 Efficacy of Plant Extracts, KC-100 Against Viral Hemorrhagic Septicemia Virus (VHSV)

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Production of olive flounder (*Paralichthys olivaceus*) has been severely affected by infection of viral hemorrhagic septicemia virus (VHSV). Efficacy of plant extracts mixture from *Curcuma aromatica* and *Chinese Liquorice* (KC-100) for inhibiting VHSV in fathead minnow (FHM) cell line and in orally administered olive flounder was studied. In the CPE (cytopathic effect) reduction assay, the group treated with KC-100 showed delayed CPE compare to the untreated group. In *in vivo* experiment, KC-100 was mixed with 1%, 33% and 66% into commercial feed weight. It was fed with 3% of body weight for 2 weeks and 6 weeks then challenged with VHSV. Relative percent survival (RPS) was 50%, 28.6% and 42.9% in the 2 weeks administrated group and 42.9%, 21.4% and 42.9% in the 6 weeks administrated group, respectively. There were no differences of histology and serum AST (aspartate transaminase) and ALT (alanine transaminase) level among control and KC-100 fed groups after 6 weeks suggested no toxicity to olive flounder. We further conducted field trials by selecting two olive flounder farms. KC-100 was mixed with commercial pellet at the concentration of 1% of feed weight then fed 3-5% of body weights for 5 weeks. After 5 weeks of administration, fish were brought to laboratory for challenge test. Fish were divided into 2 groups then injected by $1 \times 10^{7.8}$ and $2 \times 10^{6.8}TCID_{50}$/fish, respectively. The RPS of fish from farm A was 60% and 25%. On the other hand, RPS of fish from farm B was 100% and 88.9%. These results suggest that KC-100 can be used as safe and effective antiviral agent to control VHSV.

Conference Session Designation: (Aquatic Animal Health Management)

Presentation Format: (Poster)

No. 49 A Putative New Family of "Minimal" Nucleo-Cytoplasmic Large DNA Viruses Distantly Related to Iridoviruses

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Panulirus argus virus 1 (PaV1) is the only known pathogenic virus to naturally infect the Caribbean spiny lobster (*Panulirus argus*) from the Caribbean Sea. Related viruses, Dikerogammarus haemobaphes virus 1 (DhV1) and Carcinus maenas virus 1 (CmV1), have recently been detected in hosts sampled from sites in the United Kingdom, namely, the non-native demon shrimp (*Dikerogammarus haemobaphes*) and the native European green crab (*Carcinus maenas*), respectively. The virion morphologies of these viruses share similarities to those of iridoviruses, but they so far remained unclassified due to the lack of genomic data. Using an Illumina MiSeq sequencer, we sequenced the complete genomes of PaV1, CmV1, and DhV1. Comparative genomic analyses show that PaV1, CmV1, and DhV1 each possess the 6 hallmark genes found in other nucleo-cytoplasmic large DNA viruses (NCLDV), namely, the major capsid protein, DNA polymerase, primase-helicase, two RNA polymerase subunits, and disulfide-thiol oxidoreductase. Maximum Likelihood phylogenetic analyses of the DNA polymerase show that PaV1, CmV1, and DhV1 form a well-supported clade branching between asco-iridoviruses and pithoviruses. Thus, the results of our phylogenomic analyses suggest that these crustacean viruses represent a distinct NCLDV family and demonstrate potential for infection by this group across diverse members of the Class Crustacea. With genomes of less than 70 kilobases, these viruses are by far the smallest known, "minimal" NCLDVs.

Conference Session Designation: (Virology)
Presentation Format: **(** Poster)

No. 50 Infectious Spleen and Kidney Necrosis Virus (ISKNV) in Ornamental Fish in Germany

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Megalocytiviruses, a group in the family *Iridoviridae*, can be divided into three major subgroups: Infectious Spleen and Kidney Necrosis virus (ISKNV), Red Sea Bream Iridovirus and Turbot Reddish Body Iridovirus. They cause systemic infections in a wide range of marine and freshwater fishes with heavy losses. Affected are aquaculture and ornamental fishes. In Germany the first reported case of an infection of ornamental fish, platy and angelfish, with ISKNV was described in 2014 by the authors of this study. The infected fish showed anorexia, lethargy, gill swelling and skin alterations and mortality was up to 100%. Histological examination of tissue of infected fish resulted in profound alterations in almost all internal organs. Especially necrosis in spleen, kidney and liver and a high number of hypertrophic, intensively pink stained cells which were distributed in liver, spleen and kidney, could be detected.

Since March 2016 fish retailers need to examine fish for ISKNV, if they want to export them to Australia. Therefore ISKNV examination was included in the routine diagnostic at the Fish Disease Research Unit at the Veterinary University of Hannover. Between 2016 and 2018 in total 455 examinations for ISKNV were evaluated by PCR. In total 31 different fish species from 9 different families from freshwater as well as seawater were examined. All examined seawater species originated from tropic areas. The freshwater species originated from North America, Middle America, South America, South Asia, South-East Asia and East Africa. In eight examined fish ISKNV genome fragments could be detected. One *Apistogramma nijesseni*, one *Symphysodon* sp., two *Betta splendens*, two *Colisa lalia*, one *Xiphophorus hellerii* and one *Xiphophorus maculatus* were tested positive for ISKNV. In none of the five examined marine fish species and in none of the 412 tested cichlids from East Africa ISKNV could be detected. Until today there are no reports on infections of East African cichlids with ISKNV and it remains unclear if these fish are susceptible for the virus.

Nevertheless, because of the severe progressive form of this infection and the high mortalities that can occur in infected fish, it should be considered to keep ornamental fish shipments separately in quarantine and to examine them directly after import before selling to home aquaria.

Conference Session Designation: (Virology)
Presentation Format: (Poster **)**

No. 51 Morphological and Molecular Analysis of *Henneguya* Sp., a New Gill Parasite of *Prochilodus lineatus* from Brazil

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Brazil is the fifth largest country in the world and has the highest species diversity among all megadiverse countries, accounting for around 14% of the world biota. With the growth of aquaculture in several regions of the world since 1990, the study of fish pathologies has become extremely important. Among the agents responsible for causing diseases in fish are the myxozoans. This study records and describes a new species of *Henneguya* parasitizing the gills of *Prochilodus lineatus*, an important commercial fish species from Brazil. Among the 30 *P. lineatus* specimens collected in the Batalha river, Brazil, 23 were parasitized by *Henneguya* sp. in the gills, obtaining a prevalence of 76%. Some plasmodia of *Henneguya* sp. were collected and assembled fresh on slides for morphometric analysis, while other plasmodia were collected and fixed in 100% ethanol and 5% formaldehyde for subsequent molecular and histological analysis, respectively. Mature myxospores presented an oval shape, measuring in average 46.89 μm in total lenght, 14.65 μm in body lenght, 5.27 μm in body width and 34.28 μm in tail lenght. Polar capsules were elongated and measured 6.7 μm in length and 1.28 μm in width. Polar filaments had 12-13 coils. A 1417 bp partial sequence of the SSU rDNA gene was generated. BLAST search using sequences already deposited for *Henneguya* on GenBank did not show any sequence with 100% similarity. The phylogenetic analysis presented *Henneguya* sp. grouped with other myxozoans that parasites fish of ther order Characiformes. Histopathological analysis showed that plasmodia developed in the main artery of the primary gill filaments, causing hyperplasia, but no inflammatory infiltrates were observed at the sites of infection. It is known that the presence of plasmodium in the gills, associated with hyperplasia, inflammation or increased mucus production, can cause adhesion between the primary or secondary lamellae and consequent death of the fish by asphyxiation. Given the spore morphology, the morphometric data and SSU rDNA gene sequence obtained in the present study, we reported a new specie of *Henneguya* parasitizing *Prochilodus lineatus*.

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

No. 52 Phylogenetic Analysis of Myxobolidae Family Parasites from Brazilian Fish

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The phylogenetic analysis of species of myxozoans parasites of fish makes it possible to observe the degree of relationship between these species and whether these parasites have a monophyletic or polyphyletic origin. Myxobolidae is a family of myxozoan that can infect freshwater fish, having a complex life cycle, involving an alternate stage in an invertebrate. *Henneguya* and *Myxobolus* are two of the genus that have the biggest diversity of species and belong to the family Myxobolidae, which shows the importance of the study of this family. This study analyzes phylogenetically the myxozoan species of the family Myxobolidae of Brazil, including sequences belonging to seven new species. All partial sequences of the SSU rDNA gene from species of the Myxobolidae family that parasites fishes from Brazil were collected at Genbank for analysis. In addition, seven new partial sequences of SSU rDNA gene from *Henneguya* sp. 1, *Henneguya* sp. 2, *Henneguya* sp. 3, *Henneguya* sp. 4, *Henneguya* sp. 5, *Myxobolus* sp. 1 and *Myxobolus* sp. 2 were added to analysis. These species were located and collected parasitizing the gills of the following hosts: *Rhamdia quelen*, *Astyanax altiparanae*, *Prochilodus lineatus*, *Apareiodon vladii*, *Rhamdia quelen*, *Proloricaria prolixa* and *Cyphocharax modestus* respectively. Partial sequences were edited and aligned and later Bayesian (BI) and Maximum Likelihood (ML) analyzes were performed. The BI and ML trees generated presented an identical topology. The species of the family Myxobolidae were grouped in two clades, one compound only of *Myxobolus* spp. and the other compound of species of *Myxobolus* and *Henneguya*. Among the partial sequences collected in GenBank and the new partial sequences analyzed in this work, it was possible to observe the clustering by host specificity. *Henneguya* sp. 1 and *Henneguya* sp. 4 were shown as sister species in phylogeny, while *Henneguya* sp. 2 appeared as sister species of *Henneguya* sp. 3. *Henneguya* sp. 5, *Myxobolus* sp. 1 and *Myxobolus* sp. 2 appeared as basal species of their subclades. The species were divided into two large groups, one composed only of myxozoans parasites of Siluriformes fish and the other composed only of myxozoan parasites of Characiformes fish. It was also possible to observe a grouping in relation to the parasitized organ. Mainly due to the similarity of morphometric characters, the myxozoans are known to include several paraphyletic and polyphyletic taxa. The clustering of myxozoans in clades by host order showed a tendency for parasites of the genus *Myxobolus* and *Henneguya* to have a paraphyletic origin, with host specificity influencing parasitism. This study contributes to the knowledge of the biodiversity of myxozoans in Brazil and the phylogenetic relationship between the species of the Myxobolidae family, as well as the relation with their hosts.

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

No. 53 Preliminary Phylogenetic Analysis of *Henneguya* Sp. Found Parasitizing Stomach of *Serrassalmus spilopleura* from Brazil

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Myxozoans are cnidarians parasites that present a complex life cycle, alternating between a vertebrate host, mainly fish, and an invertebrate host. Brazil presents a megadiversity of fish and aquaculture has been growing year by year in the national economic scenario. In this context *Serrassalmus spilopleura*, popularly known as piranha, is an economically important specie of fish due to its commercialization and use in gastronomy. This study makes a preliminary analysis of a specie of myxozoan, *Henneguya* sp., found parasitizing the stomach of *S. spilopleura*. Between September 2016 and May 2018 were collected 34 specimens of *S. spilopleura* in the Jacaré-Pepira river, Ibitinga, Brazil. The fish were euthanized and all organs were analyzed in order to find signs of parasitism by myxozoans. Fourteen fish were parasitized by *Henneguya* sp. in the stomach, presenting a prevalence of 41%. The plasmodia found in the stomach of *S. spilopleura* were collected and fixed in 100% ethanol for molecular analysis. The DNA was extracted with specific kit (DNeasy Blood & Tissue Kits, Qiagen) and a partial sequence of the SSU rDNA gene was amplified with the primer set MyxospecF-MyxospecR. The 774 bp partial sequence of the SSU rDNA gene was generated from plasmodia obtained from the stomach. BLAST search using sequences already deposited for *Henneguya* in GenBank did not show any sequence recordings with 100% similarity. For the phylogenetic analysis the partial sequence obtained was aligned with partial sequences of the SSU rDNA gene of species of myxozoans that parasitize fish of Brazil, obtained in GenBank. A Bayesian analysis was then performed with Mr. Bayes software. Phylogenetic analysis showed that *Henneguya* sp. is a sister species of *Myxobolus cuneus*. A clade was formed composed of species of the genus *Henneguya* and *Myxobolus*, where there was a division into two subclades, one being formed by species that parasitize gills and the other clade composed of species that parasitize the other organs of the fish. It was also possible to observe a clustering by host groups. *Henneguya* sp. appears in a subclade formed by species that parasitize hosts of the family Serrassalmidae. In conjunction with morphological and histological analysis to be made later, we hope soon describe *Henneguya* sp. as a new species or to add new molecular data to a species of myxozoan already described parasitizing *S. spilopleura*. This study contributes to the knowledge of the biodiversity of myxozoans in Brazil.

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

No. 54 Morphological and Molecular Characterization of Two New Species of *Myxobolus* and of *Myxobolus colossomatis* Infecting *Colossoma macropomum* from the Amazon Basin

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This study describes, through morphology and molecular analyzes, two news species of the genus *Myxobolus* and of *Myxobolus colossomatis* found infecting specimens of *C. macropomum* collected from the Amazon Basin, Brazil. A total of 51 *C. macropomum* specimens were examined between October of 2014 and January of 2016. Plasmodia of the myxosporeans were found infecting several organs of *C. macropomum*. *Myxobolus opercullun* sp. n. and *Myxobolus longcapsulae* sp. n. showed, respectively, 9.6±0.4 µm and 19.1±0.4 µm in length, 7.0±0.3 µm and 9.4±0.3 µm in width and 5.0±0.3 µm and 8.3±0.4 µm in thickness of the myxospore. The polar capsules, which were elongated, showed 4.3±0.4 µm in length and 1.9±0.1 µm in width for *M. opercullun* sp. n. and 10.5±0.2 µm in length and 2.5±0.1 µm in width for *M. longcapsulae* sp. n. *Myxobolus colossomatis* was identified based on the host and morphology of the myxospore that showed two body shape. The myxospores ellipsoidal shape measured 11.6±0.4 µm in length, 7.6±0.2 µm in width and 6.5±0.4 µm in thickness. The polar capsules were elongated, measuring 5.6±0.2 in length and 2.5±0.2 in width. The myxospores oval shape measured 10.4±0.5 in length and 7.7±0.3 in width. And the polar capsules presented 5.4±0.2 in length and 2.4±0.0 in width. The number of turns of the polar filament was 7- 8 coils. The molecular comparison of the 18S gene showed a genetic divergence of 22.4% between *M. opercullun* sp. n. and *M. longcapsulae* sp. n., 10.3% between *M. opercullun* sp. n. and *M. colossomatis* (this study) and 23.2% between *M. longcapsulae* sp. n. and *M. colossomatis* (this study). *M. colossomatis* and *M.* cf. *colossomatis* showed 11.1% of genetic divergence demonstrated to be distinct species. Phylogenetic analysis, based on sequences of the 18S rDNA, showed a grouping according to host order and family.

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

No. 55 Novel Species and Cryptic Sources of *Ceratonova* Parasites in the Upper Klamath River Basin

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Genus *Ceratonova* (Cnidaria: Myxozoa) includes freshwater, enteric fish parasites distinct from marine, gall bladder parasites of genus *Ceratomyxa*. The best-known species, *C. shasta*, exists as at least three strains ("genotypes"), strongly associated with host species of salmon and trout, i.e. genotype O (endemic rainbow/steelhead trout) , genotype I in Chinook salmon, genotype II in coho salmon and non-endemic rainbow trout. In this project, we wanted to discover the source of genotype II in the Upper Klamath River basin (Oregon), an area presently without any known fish hosts: coho salmon have been excluded by dams for almost 100 years; and although non-endemic rainbow trout were stocked 1925 - 2011 to supplement recreational fishing, these were assumed to die from the parasite. Our second goal was to identify the fish host of *Ceratonova* "genotype X", which has been detected in water samples but not linked to a particular fish. A better understanding of *Ceratonova* parasites present in the upper basin will inform risk assessments for re-introduction of native salmonids, including endangered coho salmon, after dam removals in the 2020s.

We mapped spatial distribution of *C. shasta* genotypes in the Williamson River, which is ~120 km long and forms the headwater of the Klamath River basin. We sampled 4 x 1-L water at 16 sites, in June and July 2015 - 2017, collected spores on a 5 µm mesh filter, and extracted total DNA. To determine reservoir fish hosts of parasite genotypes II and X, we electrofished up to 7 sites in the Williamson River in summer 2016 and 2017. Non-native salmonids and non-listed incidental fish species were sampled lethally (intestines collected), whereas non-lethal anal swabs were taken from native fish (trout and suckers). All samples were assayed for *C. shasta* by PCR or qPCR, then sequenced to determine the ITS-1 genotype or species ID.

We detected *C. shasta* in all water samples in all years, with genotypes O, II and X present together at some sites. No new genotypes were discovered. Electrofishing yielded 93 fish in 2016 (3 salmonid species and 8 other species) and 104 fish in 2017 (2 salmonid species and 6 other species). Non-lethal sampling was an effective means of sampling the parasite (redband trout had genotype O, as expected); *C. shasta* was not detected in any other salmonid. The only other fish positive for *Ceratonova* were 4/58 dace, which sequenced as genotype X. But no mature spores were seen in the intestines, which meant we could not be certain that dace are the fish host of genotype X. Genotype II was not detected in any fish, thus its host remains an outstanding question. Future work will focus on sampling greater numbers of all salmonid species (entailing a modification to the electrofishing protocol), and additional dace collection to determine if this species is a true or incidental fish host of genotype X.

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

No. 56 Seq, Drugs and PKD: an RNA – Sequencing Analysis of a Biological and Chemical Stressor and Their Consequences for Fish

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In nature, organisms are exposed to multiple stressors including biological, chemical and physical entities. Anthropogenic activities have far-reaching effects and have undoubtedly compounded the multiplicity of stressors affecting the planets ecosystems. The cumulative impact of such multiple stressors may result in nonlinear effects and ecological surprises potentially affecting numerous biological targets across varying organizational levels. Examining the roles of multiple stressors is essential if we are to understand how organisms acclimate to environmental change. The aim of the present study was to examine the molecular and organism reaction of rainbow trout, *Oncorhynchus mykiss*, to the combined impact of two environmental stressors: (1) the myxozoan parasite, *Tetracapsuloides bryosalmonae*, which is the causative agent of proliferative kidney disease (PKD) and naturally affects salmonid populations and (2) an estrogenic active compound, ethinylestradiol (EE2), which is ubiquitously present in the aquatic environment. Both stressors co-exist in Swiss rivers and are potential factors contributing to the decline of Swiss brown trout populations over the last decades. Experimentally, young-of-the-year rainbow trout, were (i) infected with the parasite, *T. bryosalmonae,* (ii) exposed to the environmental contaminant, EE2, or (iii) treated with a combination of the parasite and EE2. In this study, RNA sequencing (RNA-seq) was employed to investigate differentially expressed genes (DEGs) in the kidney of the rainbow trout. Transcriptome sequencing in the abovementioned experimental groups, as well as those without treatment was performed using the Illumina HiSeq 2500 platform. Gene pathway analysis of the DEGs indicated key pathways involved in aspects of adaptive and innate immunity, metabolic processes, extra-cellular matrix components and cellular adhesion and signalling molecules. We report on these pathways and the individual stressors impact and how this changes under exposure to the stressor combinations. Our results provide unprecedented insight into the combined effects of a biological and chemical stressor and their potential physiological and immunological modulations of wildlife.

Conference Session Designation: (Toxicology / Tox Path)
Presentation Format: **(** Poster)

No. 57 Do Fish Get Wasted? Assessing the Influence Of Effluents on Parasitic Infection of Wild Fish

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Many ecosystems are influenced simultaneously by multiple stressors. One important environmental stressor is aquatic pollution via WWTP (wastewater treatment plant) effluents. WWTP effluents may contribute to eutrophication or contain anthropogenic contaminants which directly and/or indirectly influence aquatic wildlife. Both eutrophication and exposure to anthropogenic contaminants may impact the dynamics of fish-parasite systems. With this in mind we studied the impact of WWTP effluents on infection by the parasite *Tetracapsuloides bryosalmonae*, the causative agent of proliferative kidney disease (PKD). PKD is associated with the long-term decline of wild brown trout (*Salmo trutta*) populations in Switzerland. We investigated PKD infection of brown trout at two adjacent sites (≈400m apart) of a Swiss river. The sites are identical in terms of ecology except that one site receives WWTP effluents. We evaluated the hypothesis that fish inhabiting the effluent site will show greater susceptibility to PKD in terms of prevalence and disease outcome. We assessed susceptibility by (i) infection prevalence, (ii) parasite burden, (iii) host health in terms of pathology and (iv) estimated apparent survival rate. At different time points during the study, significant differences between sites with regards to all measured parameters were found, thus providing evidence of the influence of effluents on parasitic infection of fish in our study system. However, from these findings we cannot determine if the effluent has a direct influence on the fish host via altering its ability to manage the parasite, or indirectly on the parasite or the invertebrate host via increasing bryozoa (the invertebrate host) reproduction. On a final note the WWTP adhered to all national guidelines and the effluent only resulted in a minor water quality reduction assessed via standardised methods in this study. Thus, we provide evidence that even a subtle decrease in water quality can have consequences for wildlife.

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

No. 58 *Myxobolus cerebralis* Laboratory Testing in Alberta

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Myxobolus cerebralis, the etiological agent of Whirling Disease, was detected in Alberta in August 2016, making it the first confirmed detection in Canada. To manage this parasite, Alberta Environment and Parks created the Whirling Disease Program which has established a Whirling Disease laboratory – Canada's first laboratory exclusively dedicated to testing for *M. cerebralis*. The laboratory supports diagnostic and surveillance programs assessing both wild and cultured fish populations in the province. Two different sample methods are utilized: (1) pepsin-trypsin digest (PTD) which targets collection and concentration of mature myxospores, and (2) homogenization (HG) of samples which allows for detection of presporogenic and spore stages of *M. cerebralis.* These samples are then assessed using quantitative PCR (qPCR). Using both methods allows for detection of *M. cerebralis* DNA at all developmental stages.

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

No. 59 Integrating Old and New Findings: Recent Advances in Turbot Enteromyxosis

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Enteromyxum sp. are intestinal myxozoan parasites which affect several cultured teleost species. *E. scophthalmi* is the causal agent of enteromyxosis in the flatfish turbot *Scophthalmus maximus*, and represents a major threat for the aquaculture of this species. The disease is directly transmitted from fish to fish and presents very elevated morbidity and mortality rates. These characteristics along with the lack of effective therapeutic options make culling the infected units and subsequent disinfection the only control measures to minimize losses. In order to devise successful disease prevention strategies, a proper understanding of host-pathogen interaction is crucial. In this work a review of the pathogenesis studies of turbot enteromyxosis is presented, focused on the advances obtained by an integrated approach based on genomic and morphological techniques. The morphopathological picture of turbot enteromyxosis has been comprehensively described since the first outbreaks registered in the 90s. The traditional histological stains and immunohistochemistry have been later extensively employed to characterize the lesions associated to the parasitosis, starting to elucidate host-parasite interactions. With the recent revolution in transcriptomic analyses represented by the RNA sequencing (RNA-Seq) technology, a formidable tool for a better understanding of the underlying pathways controlling the disease progression in a host, a multidisciplinary approach has been undertaken. RNA-Seq technology was applied in control and diseased turbot in the early and advanced stages of enteromyxosis. The results obtained, analyzed in relation to the morphopathological picture observed through the disease course, provided novel information on the infection mechanisms and evasion strategies of *E. scophthalmi*, as well as on the molecular basis of the morphological changes and physiopathology of the disease. The knowledge of the tissue scenario along with the employment of a powerful transcriptomic tool demonstrated to be a reliable approach for advancing in the knowledge of host-parasite interaction and towards the identification of suitable targets for early diagnosis and disease management strategies.

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

No. 60 Morphological Analysis and Molecular of New Aurantiactinomyxon Type (Cnidaria: Myxosporea) in Oligochaetes from Fish Farms, Brazil

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Myxosporea are cnidarians and some species act as fish pathogens in various parts of the world, are spore-forming obligate parasites of vertebrates (where they form myxospores) and invertebrates (where they form actinospores). In this work sediment samples containing oligochaetes were collected of shallow parts in a fish farm of breeding of pintado hybrid (*Pseudoplatystoma fasciatum* Linnaeus, 1766, X *Pseudoplatystoma reticulatum* Eigenmann & Eigenmann, 1889) in the state of Mato Grosso do Sul, Brazil. The oligochaetes were input individually in 96 well plates containing water, and checked this water daily by microscopy until individual infected oligochaetes were found. Among the 96 oligochaete specimens examined, one of them (1.04%) was observed releasing actinospores and was identified morphologically as a *Pristina synclites* species. The images of the actinospores were obtained through a Nikon microscope (80i) equipped with phase contrast and coupled with a camera by using the 60x objective. The morphometric data from spores were obtained according to Lom et al. (1997). The body of the spores showed 8 μm (6.79-9.01) of diameter. Caudal processes of equal size, measuring 14.43 μm (12.42-15.98) in length, 5.63 μm (4.61-6.18) in bottom width. Each caudal process showed a moving core inside. Three spherical polar capsules measuring 1.56 μm (0.91-1.53) of diameter were positioned, in apical view, in the periphery of the spore body. The actinospores released into of well were concentrated, their DNA extracted, and the ssrDNA went amplified by Polimerase Chain Reaction (PCR) (Milanin et al., 2018). A 1970 nt sequence was obtained after sequencing the gene, which together with the morphological analysis indicated to be an Aurantiactinomyxon type. The BLAST research showed a greater proximity with *Myxobolus kisutchi* (AB469988), *Myxobolus articus* (HQ113227) and *Myxobolus cordeiroi* (KF296353) with 79%, 78% and 80% of similarity, respectively. The result indicates if it is a specie not yet described**.** This is the second report of actinospore belonging to the collective group Aurantiactinomyxon in fish farms of the state of Mato Grosso do Sul and the second report in the literature of *Pristina synclites* involved in the transmission of Myxosporea.

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

No. 61 Molecular Confirmation of *Kudoa iwatai* (Myxosporea: Multivalvulida) in Cultured Rock bream *Oplegnathus fasciatus* in Taiwan

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Rock bream is a very important economic fish species in Asia. Myxosporean parasites have been reported in several fish species. Myxosporean infection can cause economic loss due to the pseudocysts lodged in several organs of the fish. In this study, three cases of the economic fish species Rock bream *Oplegnathus fasciatus* were identified as *Kudoa iwatai*. The diseased fish exhibited numerous creamy-white pseudocysts, in the eye, muscle and other internal organ. The number of pseudocysts per infected fish was different with some aggregation seen in the brain, muscle and eye. Mature spores were round to subquadrate in apical view. The junction of shell valves appeared as overlapping, straight lines. The polar filament formed 2 to 3 coils. A PCR (polymerase chain reaction) primer for Kudoa amplified the small subunit (SSU) rDNA sequences, and the amplified gene was sequenced. It was evident from the phylogenetic tree that the 3 strains tested were identical and belonged to the *K. iwatai*. The evolutionary tree showed that these strains form a unique clade, at a distance from other *Kudoa* species and myxosporeans. This is the first report in fish in Taiwan.

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

No. 62 Hepatic Granuloma in a Wild-Caught King Soldier Bream *(Argyrops spinifer)* from the Gulf of Oman.

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Potential causes of granulomas in fish include bacteria (particularly *Mycobacterium spp.* and *Francisella spp*.), fungi, metazoan infestations, heavy metals, feed binders, and vaccinal oil adjuvants. In this study, we report a case of multiple focal hepatic granulomas in a wild- caught king soldier bream (*Argyrops spinifer).* The king soldier bream is a significant commercially species of wild marine fish in Oman, locally known as "*Kofar"*. The fish was bought from As Seeb fish market, Muscat, Oman in 2017. External examination of the fish did not reveal any significant lesions. Necropsy showed yellow to whitish nodular lesions in the liver with sizes varying from 1 millimeter and up to 1 centimeter in diameter. Some nodules coalesced to form larger lesions with hemorrhage at their periphery. The granulomas were confined to the liver. Histologically, the granulomas consisted of either necrotic centers or aggregates of macrophages and epithelioid cells surrounded by fibroblasts. All the sections were negative with Ziehl-Neelsen, Periodic Acid Schiff and Gram stain. Although bacteriological culture was not performed, detection of the etiological agents using the 16s rRNA sequence assay was attempted. Despite the successful amplification of the 16s rDNA, sequencing of the amplified product did not reveal any known bacteria following the use of the BLAST tool. Based on the histopathological findings of the granulomas, mycobacteriosis was the presumptive diagnosis, although this assumption could not be confirmed the testes employed. Further studies are required to evaluate the importance and prevalence of hepatic granulomas in king soldier bream.

Conference Session Designation: (Diseases of Wild Fin-Fish)
Presentation Format: (Poster)

No. 63 Spatiotemporal Distribution of Infectious Agents in Juvenile Fraser River Sockeye Salmon Omid Nekouei[1]*, Raphael Vanderstichel[1], Tobi Ming[2], Karia H. Kaukinen[2], Krishna Thakur[1], Amy Tabata[2], Emilie Laurin[1], Strahan Tucker[2], Kristina M. Miller[2,3]

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Infectious diseases are likely contributors to declines in Fraser River Sockeye salmon (*Oncorhynchus nerka*) stocks, but a clear knowledge gap exists around which infectious agents and diseases are important. This study was undertaken to: 1) determine the presence, prevalence, and burden of 46 infectious agents in juvenile Fraser River Sockeye salmon, and 2) evaluate spatial patterns in prevalence and burden over initial seaward migration, contrasting patterns between two years of average and poor productivity. Overall, 2,006 out-migrating Sockeye salmon were collected from four regions along their migration trajectory in British Columbia, Canada, in 2012 and 2013. High-throughput microfluidics quantitative PCR was employed for simultaneous quantitation of 46 infectious agents. Twenty-six agents were detected at least once, including nine with prevalence > 5%. *Candidatus Brachiomonas cysticola*, *Myxobolus arcticus*, and Pacific salmon parvovirus were the most prevalent agents. Infectious agent diversity and burden increased consistently upon smolts entry into the ocean. Notably, both freshwater- and saltwater-transmitted agents were more prevalent in 2013 than in 2012, leading to an overall higher infection burden in the first two sampling regions. A reduction in the prevalence of two agents, erythrocytic necrosis virus and *Paranucleospora theridion*, was observed between regions 2 and 3, which was speculated to be associated with mortality during the first month at sea. The most prevalent infectious agents were all naturally occurring. However, in a small number of samples, seven agents were only detected post exposure to salmon farms, including four established farm pathogens: piscine orthoreovirus, *Piscirickettsia salmonis*, *Tenacibaculum maritimum*, and *Moritella viscosa*. Our findings provide the first comprehensive list of infectious agents detected in juvenile Sockeye salmon and can be considered in future prioritization for research and control of important infectious agents that may contribute to Fraser River Sockeye population declines.

Conference Session Designation: (Diseases of Wild Fin-Fish / Aquatic Epidemiology)
Presentation Format: (Poster or Oral)

No. 64 Pathology Induced by Blood Flukes, *Cardicola* Spp., in Spotted Seatrout (*Cynoscion nebulosus*)

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In estuaries of South Carolina USA, sciaenid seatrout (*Cynoscion nebulosus*) are commonly infected by the blood flukes *Cardicola laruei* and more rarely by *C. parvus*. (Aporocotylidae). A two year survey showed infection by worms was seasonal and peaked in the summer with ~70% fish being parasitized. Pathology induced by granulomatous reactions against the worms' eggs can be severe and remains even after adult worms are eliminated. Granulomas were studied with histology, immmunohistochemistry, and transmission electron microscopy. Eggs were shown to be encapsulated by neutrophils and large epitheloid cells that flattened and formed desmosomes. Large granulomas detached from neighboring tissues, which were damaged and had become fibrotic. Numerous inflammatory cells appeared mobilized around granulomas located near the epicardium and at times formed 'pus-like pockets' scattered in the damaged epicardium. At the gross level, some granulomas possessing eggs with live miracidia, as well as blister-like formations, were observed at the surface of the epicardium. This suggests that granulomas carrying both dead and live eggs may clear the myocardium by being transported into the pericardium.

Conference Session Designation: (Parasitology General or Disease of Wild Fish)

Presentation Format: (Poster)

No. 65 Infection and Horizontal Transmission of *Piscirickettsia salmonis* Between the Wild Fish Sub-Antarctic Notothenioid Patagonian Blenny (*Eleginops maclovinus)* and Rainbow Trout (*Oncorhynchus mykiss*) by a Model of Cohabitation Challenge.

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Piscirickettsia salmonis is the aetiological agent of Rickettsial Salmon Septicemia (SRS) or Piscirickettsiosis, a bacterial disease that affects farmed salmonids, causing high mortalities and significant economic losses. Although there is evidence of pathogens transmission from farmed to wild fish, the frequency and consequences of transmission are still unknown. Moreover, it is possible that wild fish could act as reservoirs of bacterial or viral agents, facilitating transmission and pathogens dissemination. The aim of this study was to determine the bidirectional transmission of *P. salmonis* between Patagonian blenny (PB) and Rainbow trout (RT), through a model of cohabitation challenge. A total of 400 individuals were used, which included trojan fish ($_T$) which were intraperitoneal inoculated with 0,2 ml of *P. salmonis* isolate, and cohabitant fish ($_C$). The experimental groups were: PB_T/RT_C (group 1), RT_T/PB_C (group 2), and a control group (RT_T/RT_C). Fishes were randomly assigned in 1 m^3 tanks at a 20 kg m^{-3} of density. Water temperature, dissolved oxygen and water salinity were recorder. The mortality was removed daily during the course of the challenge (59 days), and morphometric analysis and necropsy were performed. Organ samples (kidney-spleen) were taken, and then analyzed by qPCR. In the group 2, the RT_T mortality started at 2 dpi, reaching 100% at 51 dpi, and no mortality of PB_C individuals occurred during the challenge. For the group 1, no PB_T mortality was recorder throughout the challenge, however, mortality induced by *P. salmonis* in RT_C individuals started at 13 dpi, reaching 46% by the end of the challenge. For the control group, mortality was recorded both trojan and cohabitant individuals, reaching 98% and 50%, at 21 dpi and 54 dpi; respectively. The mortality of trojan and cohabitant RT individuals, from all groups, showed clinical signs attributed to *P. salmonis*. Additionally, the qPCR analysis confirmed the *P. salmonis* presence in RT_T and RT_C individuals, recording Ct values of 20.2 and 29.3, respectively. The multiple comparison of the survival curves, showed no significant differences between group 2 and control group RT_T individuals ($p = 0.584$), and also between group 1 and control group RT_C individuals ($p = 0.342$). This is the first study that demonstrate the horizontal transmission of *P. salmonis* from a non-salmonid native species to a salmonid species, inducing infection and mortality in rainbow trout by using a cohabitation challenge under controlled conditions. Apparently, *P. salmonis* infections have no observable effects on the native fish species, Patagonian blenny, since no mortalities were recorded for the inoculated individuals, suggesting that this species can carry the pathogen without expressing the clinical disease, as well as in salmonids. Additional studies are required to establish the epidemiological role of wild species in the transmission and dissemination of this disease.

Conference Session Designation: (Diseases of Wild Fin-Fish and Shellfish)
Presentation Format: (Poster)

No. 66 Health Assessment of Wild White Seabass (*Atractoscion nobilis*) – A Species Cultured for Coastal Replenishment in California

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Normal histology and presence and prevalence of infectious and non-infectious diseases of many species of marine fish important to existing and emerging mariculture opportunities are not well described. Documenting disease prevalence in wild populations is essential to understanding endemic diseases and to ensuring novel pathogens are not exchanged between wild and cultured populations, particularly for fish species involved in stock enhancement programs. Here we report histopathology and viral survey results in wild *Atractoscion nobilis* (white seabass), a popular sportfish in Southern California that has been the subject of a public-private replenishment program since 1982. Juvenile and adult fish (23 total) were wild-caught by a combination of hook-and-line and gillnet sampling in partnership with the local fishing community. White seabass range from Juneau, Alaska to Magdalena Bay, Baja California, Mexico, and our catch locations ranged from Ventura Cove to Imperial Beach, California. An estimated age range of 7 months to 17 years was determined based on total body length measurements. Age class and sex ratios were as follows: 11 adults:12 juveniles at an 9 male:13 female:1 unknown ratio. Gross post-mortem exams were performed on each fish, and formalin-preserved tissues were submitted to the Washington Animal Disease Diagnostic Laboratory for histologic evaluation. Significant pathologic findings included filarial nematodes in the intestine and coelomic cavity, intestinal cestodes, gill hyperplasia and hypertrophy, microsporidia in the renal tissue and nephrocalcinosis. All cardiac specimens had mixed lymphocytes, macrophages and eosinophilic granular cells in varying numbers in the epicardial adipose tissue that surrounds the bulbus arteriosus; these leukocytes are not associated with an identified pathogen within the heart tissue. This consistent finding among wild white seabass may reflect an immune reservoir (as previously described in sturgeon [*Acipenser naccarii*]) rather than an actual inflammatory process. Fourteen frozen brain samples tested negative via RT-PCR for all clades of Viral Nervous Necrosis Virus, historically documented to affect white seabass.

Conference Session: (Aquatic Epidemiology/Aquatic Animal Health Management)
Presentation Format: (Poster)

No. 67 Testing for Piscine Orthoreovirus and Lesions Associated With Heart and Skeletal Muscle Inflammation in Wild *Genypterus chilensis* from the Pacific Ocean in the North of Chile

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Piscine orthoreovirus (PRV) infections have been found to be highly prevalent among Atlantic salmon (*Salmo salar*) reared in sea cages in Chile since the year 2011. The virus was first found only in healthy salmon, but more recently some infected animals have shown lesions consistent with "Heart and Skeletal Muscle Inflammation" (HSMI), the disease associated with PRV infection. Recently, PRV has been detected in coho salmon (*Oncorhynchus kisutch*) cultured in Chilean sea sites with some fish showing a condition resembling HSMI. In order to search for the presence of PRV and investigate its association with HSMI in native wild marine fish in Chile (Salmonines are introduced species in the Southern Hemisphere), fifteen red cusk-eels (*Genypterus chilensis)*, captured in the Pacific Ocean near the "Punta Lengua de Vaca" peninsula in the Coquimbo Region of Chile, were studied. Necropsy, heart histopathology and PRV testing of head kidney samples using a real-time reverse transcription polymerase chain reaction were performed for each fish. Although several lesions associated with HSMI or HSMI-like diseases, such as ascites, liver paleness, hydropericardium, epicarditis and, particularly, mononuclear cell infiltration in heart, were found, all fish tested negative for PRV and therefore the presence of HSMI, as described so far, was questioned. Thyroid follicles close to the atrium in one fish and the lack of stratum compactum of the ventricle in most of them were interesting non-pathological findings. Results suggest that PRV is not present in red cusk-eels in the sampled area; however, it would be useful to expand this research to establish if Chilean native fish species are really PRV free.

Conference Session Designation: (General Sessions or Virology or Diseases of Wild Fish)
Presentation Format: **(** Poster)

No. 68 Look Deep Into My Shell: Gross and Radiographic Observations of Shell Damage by Boring Parasites in the Eastern Oyster, *Crassostrea virginica*

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(CHANGED TO ORAL PRESENTATION)

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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.

Conference Session Designation: (Invert & Shellfish Disease; or Climate Change)
Presentation Format: (Poster)

No. 69 Monitoring of Microplastic Dynamics and Analysis of Ecotoxicologycal and Physiological Effects in Marine Mysid

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Mysids are one of the most important food items for numerous aquariums, fisheries, and even for human. Mysids are relatively small-sized crustaceans that are commonly found in most aquatic environments, such as brackish, estuarine, coastal, and oceanic environments. For more than 20 years, they have served as an ecotoxicology model taxonomic group because of its ease of culturing and handling in the laboratory, wide geographical distribution, short life-span, and physiological sensitivity to various environmental factors. In this study, juvenile and adult marine mysids (*Neomysis awatschensis*; Crustacea; Mysidae) were exposed to different sizes of microplastics, and the bioconcentration dynamics and responses of ecotoxicological and physiological responses were measured during the exposure and additional depuration periods. Microplastics bioconcentrated by age- and size-specifically and the levels reduced gradually during the depuration phase. We measured morphological growth parameters and quantified the hormone ecdysterone (20-hydroxyecdysone: 20E), which controls molting in mysids. The lengths of the whole body, antennal scale, exopod, endopod, and telson were significantly smaller microplastics-exposed juvenile mysids than control group. However, no significant modulation in the levels of 20E was observed by microplatics exposure in juvenile. After exposure to different sizes of microplastics, a series of parameters of antioxidant defenses system were significantly modulated during exposure and early depuration periods in juvenile mysids, while adult mysids showed no significant change. Our results suggest that microplastics could affect mysid growth and the significances are strongly associated with microplastics' sizes in *N. awathchesis*.

Conference Session Designation: (Bridges Between Aquaculture, Human and Environmental Health)
Presentation Format: (Poster)
Student Presentation: (Yes)

No. 70 Genetic Relatedness of White Spot Syndrome Virus Isolated From Imported Frozen Shrimp Products and an Outbreak in Shrimp *Penaeus monodon* Farm in Australia

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Of more than the twenty deadly shrimp viruses, White Spot Syndrome Virus (WSSV) is the most lethal pathogen and has been the main threat to the shrimp farming industry worldwide for more than two decades. The Australian shrimp industry was considered free of WSSV until a serious outbreak of the disease occurred in commercial *Penaeus monodon* shrimp farms in South East Queensland in late 2016. The disease showed rapid spread and high mortalities with a significant loss of all shrimp production. To determine the possible origin of the pathogen, farmed shrimps, shrimp feeds from the infected farm and imported shrimp products purchased from retail shops in Australia were screened for WSSV using the WSSV detection method recommended by World Organisation for Animal Health. DNA of WSSV infected shrimps collected from the north and south of Vietnam were also included for comparison. No PCR positives were detected from the feeds used at the time of the outbreak in Australia. However, all Australian farmed shrimp samples and 90 % of retail shrimp products tested positive for WSSV. The Australian WSSV DNA sequences amplified using the 146F2R2 primers spanning relatively conserved regions, were identical and had close similarity to overseas WSSV strains whose sequences were obtained in this study and from Genbank. This result suggests that the outbreak was caused by an invasive strain from overseas. To assess more specifically the possible regional origin of the WSSV infection, we developed novel primers to amplify the hypervariable regions of the WSSV genomes to be used as molecular markers for epidemiological studies of WSSV disease. Phylogenetic analyses of concatenated variable sequences generated from the new primers showed that Australian WSSV sequences were most similar to published sequences from China, and less closely related to sequences from other regions, for example sequences derived from retail products from Vietnam. Our data indicate that there may be a more recent introduction rather than a longstanding endemic of WSSV disease in Australian crustaceans. Moreover, the presence of WSSV in the imported shrimp products and the DNA sequences tested could be indicative of a route of invasion of the virus.

Conference Session Designation: Aquatic Epidemiology
Presentation Format: Poster
Student Presentation: Yes

No. 71 Antiproliferative Responses upon Viral Hemorrhagic Septicemia Virus of Interferon Induced Gene Identified from Disk Abalone (*Haliotis discus discus*)

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Increasing pressure with the invasive pathogens, environmental stress conditions and contaminant pollutants may considerably affect for the occurrence of the diseases and the production losses of the abalone in marine culture. Interferons (IFNs) are cytokines which released by the host as the response for the presence of pathogen, that mainly characterized by antiviral and immunomodulatory activities. Though, IFN has not been publicized in lower order invertebrate species few studies have conducted on interferon induced proteins in abalone. Putative *AbIFI44* coding sequence (CDS; including stop codon) encoded 711 bp which could be coded 236 amino acids polypeptide with a 26 kDa molecular mass and 8.3 pI. Polyadenylation signals (^{1440}AATAAA^{1445}, ^{1457}AATAAA^{1462}) and poly-A tail were identified at the end of 3' UTR. Molecular domain architecture of AbIFI44 showed that presence of GTP/Mg^{2+} binding sites which might be collectively involved in transcription and signal transduction in abalone. Since, this is the first study of molluscan IFI44 molecule; pairwise IFI44 sequence comparison revealed that AbIFI44 shared quit low highest identity (23.5%) and similarity (40.3%) with teleostean IFI44 counterpart (*Takifugu rubripes*). Phylogenetic analysis also depicted AbIFI44 belongs to the invertebrate IFI44 clade. Transcriptional analysis exhibited that *AbIFI44* mRNA markedly expressed in hepatopancreas, digestive tract and gill. Kinetic expression of *AbIFI44* transcripts in gill tissue was examined upon viral hemorrhagic septicemia virus (VHSV) challenge and significant mRNA upregulation was noted at 12 h and 72 h post infection. Taken together, abalone interferon induced 44 like (*AbIFI44*) molecule could be a novel outcome to the molluscan immune studies.

Conference Session Designation: (Genomic Applications in Aquatic Animal Health)
Presentation Format: (Poster)
Student Presentation: (Yes)

No. 72 A Cytosolic Glutathione S-Transferase, GST-Theta (GST-Θ) from Disk Abalone (*Haliotis discus discus*): Insights to Molecular and Biochemical Properties

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Glutathione S-Transferase isoenzymes consists with a complex group of proteins, involving in phase II detoxification. Out of different classes of GSTs, GST -θ is considered to be as an ancient group of the family. In this study, Glutathione S-Transferase theta (GST -θ) in disk abalone (*Haliotis discus discus;* AbGST θ*)* was characterized in molecular transcriptional and functional perspectives to determine its potential capacity to perform as detoxification agent in immune stress. The deduced AbGST θ protein consists with 230 amino acids, with a predicted molecular weight of 26.7 kDa and theoretical iso-electric point (pI) of 8.9. In silico analysis revealed that AbGST θ possesses a thioredoxin like domain, G-sites and H-sites with catalytic residue in it while having no signal peptides in it. Highest sequence identity was observed with Japanese scallop (*Mizuhipecten yessoensis*) (62.0%). Highest mRNA expression was observed in the digestive tract indicting its highest detoxification ability. After challenging with lipopolysaccharide (LPS), Poly (I:C) & *Vibrio parahaemolyticus*, significant upregulations were observed, showing the involvement to protect the host from various pathogens. The enzyme kinetics towards specific substrates were determined and it showed relatively low conjugation activities over CDNB. The optimum temperature and pH of AbGST θ were determined as 35℃ & pH 8, respectively.

Conference Session Designation: (Genomic Applications in Aquatic Animal Health)
Presentation Format: (Poster)
Student Presentation: (Yes)

No. 73 Immunization against *Vibrio anguillarum* in *Cyclopterus lumpus* with Vibrogen 2 Commercial Vaccine

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Infectious diseases cause the significant losses in aquaculture industries. The close proximity between large individuals in a hostile environment facilitates the opportunistic pathogens to contribute to economic disruption. The Gram-negative bacterium, *Vibrio anguillarum*, is the etiological agent of vibriosis, a deadly haemorrhagic septicaemic disease affecting various marine and fresh/brackish water fish, bivalves and crustaceans. The syndrome ranges from mild cases to death. Modest performance of vaccines is due to the marginal application of the knowledge about *V. anguillarum* pathogenesis and vaccine design. Vibrogen 2 is a commercial bacterin from Elanco Canada Ltd. that contains formalin-inactivated cultures of *V. anguillarum* serotypes I and II and *V. ordalii*. Here, we evaluated Vibrogen 2 effectivity in lumpfish. Groups of 200 fish (mean weight ~2 g) were bath immunized, bath boost 4 weeks post primary-immunization, and intraperitoneally (i.p.) boost immunized 8 weeks post primary-immunization. Samples of blood, head-kidney, spleen, and liver were collected at different time points for histology, ELISA, and gene expression analysis. Twenty-seven weeks post primary-immunization the fish were i.p. challenged with 10 or 100 times the *V. anguillarum* J360 LD_{50} dose (2.3×10^5 cells). Monck PBS injected control fish died within 11 days post-challenge whereas immunized fish took 40 days to die. To conclude, commercial vaccine Vibrogen 2 delay mortality in about 3 weeks, but did not confer immune protection to *C. lumpus* against a local *V. anguillarum* isolated.

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

No. 74 Vaccination of Atlantic Salmon (*Salmo salar*) Against *Aeromonas salmonicida*

(WITHDRAWN)

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Infectious diseases cause the significant losses in aquaculture industries. The close proximity between large individuals in a hostile environment facilitates the opportunistic pathogens to contribute to economic disruption. The Gram-negative bacterium, *Aeromonas salmonicida* subsp. *salmonicida* is the etiological agent of furunculosis in salmon and salmonids. The syndrome ranges from asymptomatic and mild cases to death. Modest performance of vaccines is due to the marginal application of the knowledge about *Aeromonas salmonicida* pathogenesis and vaccine design. Iron acquisition mechanisms, specifically iron uptake outer membrane proteins (IROMPs), are the essential virulence factor for pathogenic bacteria. Based on this idea we use *A. salmonicida* purified outer membrane proteins (OMPs) and IROMPs as a vaccine candidates. OMPs and IROMPs were purified from *A. salmonicida* grown under iron-rich and iron-limited conditions, respectively. Antigens were characterized by Western-blot analysis. Vaccine doses were formulated with a commercial adjuvant Freund's. Groups of 25 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 for histopathology, ELISA, and gene expression analysis. Twenty-one weeks post prime-immunization the fish were i.p. challenged with a high dose of the *A. salmonicida* (8×10^6 cells per fish) to evaluate vaccine efficacy.

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

No. 75 Molecular Cloning and Gene Expression Analysis of the Cytokines IL1β and IL8 of Pacu (*Piaractus mesopotamicus*) Infected with *Aeromonas dhakensis*

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The cytokines IL1β and IL8 play crucial regulatory roles in the inflammatory response against pathogens in fish. In this study, we characterized a partial sequence of the mRNA of IL1β and the full-length mRNA of the IL8, as well as the expression profile of these genes in pacu (*Piaractus mesopotamicus*) following the experimental infection with a pathogenic strain of *Aeromonas dhakensis*. The partial sequencing of IL1β mRNA produced a 612 nt sequence of the open reading frame (ORF) constituting a putative partial protein with 204 amino acids. The SMART domain search found a conserved IL1 domain with 120 amino acid residues, and a search with the BLAST tool showed high correspondence with other fish IL1β. The complete IL8 mRNA consisted of 1.019 nt with an ORF of 285 nt, a 5' untranslated region (UTR) of 115 nt and a 3' UTR of 619 nt. The deduced protein sequence showed 95 amino acid residues with a theoretical molecular weight of 10.43 kDa and an isoelectric point of 8.35. The domain analysis revealed that this protein contains the Chemokine CXC domain at position 32 and other two conserved cysteines at positions 58 and 74. These four conserved cysteine residues are involved in two disulfide bonds that are key to the formation of the IL8 3-dimensional shape. The pacu IL8 lacks the ELR motif found in other chemokines, which indicates that this protein likely interacts with T and B cells during inflammatory processes. A 20 amino acid signal peptide was identified at its N-terminal region. The pacu IL1β and IL8 expression profile was analyzed in the spleen, head kidney and liver in the periods of 12, 24 and 48 h post-infection with *A. dhakensis* by qPCR. The IL1β showed significant upregulation in the spleen and in the head kidney at 12 h post-infection, with no significant variation when compared to the control group at any other time period. No significant regulation was observed for this gene in any of the analyzed periods in the liver. Similarly, IL8 showed significant upregulation in the spleen and head kidney 12 h post-infection. This gene also showed a significant downregulation at 24 h post-infection in the spleen, as well as in the liver. Given that these two cytokines are known to have pro-inflammatory activities, the observed increase in transcripts of these genes in the spleen and head kidney at 12 hours post-infection could indicate that the *A. dhakensis* infection elicited an early inflammation in pacu, probably beginning even earlier than 12 h and, after this period, anti-inflammatory pathways may be activated inhibiting the expression of these genes, which could explain the expression decrease to similar levels of the control group, and even lower, observed for IL8 at 24 h in the spleen and in the liver. Furthermore, the spleen was the most immune active organ, showing the highest regulation, followed by the head kidney, with the liver showing little influence on cytokine production, after the bacterial infection. These results suggest that IL1β and IL8 are involved in the early response to bacterial pathogens in pacu, and that this response occurs as soon as 12 h post-infection, mainly in the spleen and head kidney.

Conference Session Designation: **(** Immunology General)
Presentation Format: **(** Poster)

No. 76 Insights into Two Ferritin Subunits from Red-Lip Mullet (*Liza haematocheila*): Potential Antibacterial Activity with its Immune Response

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Iron is considered to be one of the essential micronutrients due to the diverse functions in organism. Ferritins are iron binding proteins that play an essential role in the regulation of free-iron levels *in vivo* by sequestrating to excess iron. Ferritins are basically composed of H subunit with ferroxidase center and L subunit with iron nucleation site. Interestingly, ferritin M subunit contains characteristic features of both H and L subunits and it could be identified in lower vertebrate organisms, including fish. In this study, two ferritin subunits (H, M) with ferroxidase center were characterized in the red-lip mullet (*Liza Haematocheila*) and their putative involvement in innate immune responses were determined. The open reading frames of red-lip mullet ferritin H (*MuFerH*) and ferritin M (*MuFerM*) subunits consist of 534 and 531 bp, which encode putative polypeptides of 177 and 176 amino acids. The overexpressed recombinant MuFerH and MuFerM proteins exhibited remarkable Fe (ll) ion depriving activity and potential antibacterial activity. Quantitative real time PCR results showed that highest expression of *MuFerH* and *MuFerM* in blood among the tissues analyzed. Interestingly, expression of two ferritin genes was found to be regulated against immune stimuli mounted by lipopolysaccharides (LPS), poly I:C, *Lactococcus garvieae*. Both ferritin genes were highly expressed in the blood, but in the presence of immune stimuli, *MuFerH* expression was upregulated in other immune organs (head kidney, spleen). In contrast, expression of *MuFerM* was increased in blood during the poly I:C and *L. garvieae* immune challenge compared to the control. Collectively, our results suggest that two MuFer subunits are actively involved in free iron (ll) homeostasis and antibacterial defense mechanism in red-lip mullet.

Conference Session Designation: (Genomic Applications in Aquatic Animal Health)
Presentation Format: **(** Poster)
Student Presentation: (Yes)

No. 77 Molecular and Transcriptional Insight into Gstκ1 in Red-Lip Mullet (*Chelon haematocheilus*)

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Glutathione S-transferases (GSTs) are ubiquitously distributed in all the aerobic organisms including fungus and plants. They are involved in phase II enzymatic detoxification thus regulate the xenobiotic metabolism. In aquatic environment, xenobiotics and toxins from biotic and abiotic sources cause massive impact on marine organisms. Red-lip mullet is economically valuable fish in South Korea and culture along the west coast of Korea. High mortality rate during cultivation is the major problem for mullet farming but reasons remain unknown due to insufficient research. Therefore, aim of this study is to investigate the influence of GSTκ1 on pathogenic stress. Accordingly, GSTκ1 (MuGSTκ1) was identified from the Red-lip mullet transcriptomic database and studied for their molecular and transcriptional properties. According to the *in-silico* analysis, coding sequence of MuGSTκ1 consisted with 687 bp open reading frame that encodes 227 amino acids. Phylogenetic reconstruction and multiple sequence analysis showed that MuGSTκ1 sequence clustered with respective orthologs from fish distinctly. Further, MuGSTκ1 share the highest identity and similarity *with Labrus bergylta*. Tissue-specific expression profile demonstrated that MuGSTκ1 highly expressed in gill followed by kidney. According to the temporal expression in gills, MuGSTκ1 exhibited significant transcript modulation against polyinosinic:polycytidylic acid and lipopolysaccharides. Hence, these results indicate that MuGSTκ1 can be induced by PAMP stimuli with different expression profiles. Altogether, results in this study suggest that MuGSTκ1 involving immune responses.

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

No. 78 Differential Functional Gene Expression in Koi carp (*Cyprinus carpio*) after Challenge with *Aeromonas sobria*

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Koi carp (*Cyprinus carpio*), are common hosts of an epizootic bacterial infection by *Aeromonas sobria*.. In the present study, we conducted transcriptome profiling of *C. carpio* to understand the host immune response to *A. sobria* infection, using the Illumina ultra-high-throughput sequencing platform. *De novo* assembly of paired-end reads yielded 69,480 unigenes, the total length, average length, N50, and GC content of which were 70,120,028 bp, 1,037 bp, 1,793 bp, and 45.77%, respectively. Annotation was performed by comparison against various databases, yielding 42,229 (non-redundant protein sequence [NR]: 60.78 %), 59,255 (non-redundant nucleotide [NT]: 85.28%), 35,900 (Swiss-Prot: 51.67 %), 11,772 (clusters of orthologous groups [COG]: 16.94 %), 33,057 (Kyoto Encyclopaedia of Genes and Genomes [KEGG]: 47.58%), 18,764 (Gene Ontology [GO]: 27.01%), and 32,085 (Interpro: 46.18%) unigenes. These DEGs were further categorised with KEGG. Enrichment analysis of the DEGs and unigenes revealed major immune-related functions, including Toll-like receptor signalling, and antigen processing and presentation.

Conference Session Designation: (Immunology General)

Presentation Format: (Poster)

No. 79 Recombinant Flagellin of *Vibrio anguillarum* and Derived Peptides from D1 Domain Promote *In Vivo* an Acute Overexpresision Of Il-1β and Il-8 Proinflamatory Cytokines in *Salmo salar*

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Flagellin is the major component of the flagellum, ligand for Toll-like receptor 5, and it can promote proinflammatory cytokines expression and chemokines in vertebrates. As reported, recombinant flagellin (rFLA) from *Vibrio anguillarum* and two derived peptides (rND1, 518) promoted *in vitro* upregulation of pro-inflammatory genes in gilthead seabream and rainbow trout macrophages. In this work, we verified those as immunomodulators in *Salmo salar*. Each molecule was validated *in vitro* using SHK-1 cells and isolated head kidney leucocytes (HKL), and finally assessed the effective dose for each molecule by *in vivo* assay. IL-1β and IL-8 levels were measured by qPCR. Overall, results for the in vitro assays showed IL-8 and IL-1β transcripts increased 6-8-fold using rFla and 1.2-1.3-fold using the derived peptides. For the in vivo assays, IL-1β and IL-8 induction was measured in head kidney 4, 24, and 72 hours after intraperitoneal injection with 5 μg rFLA or 15 μg of each derived peptide. Results showed that rFla and both D1-domain derived peptides induced an acute pro-inflammatory response time-dependent. IL-1β upregulation reached 25-fold above the PBS-control after 4 hours and it decreased progressively until 3 to 6-fold over the baseline. IL-8 showed an acute response. At 4 hours, it reached 13-fold above its basal levels using rFla or rND1, and 3-fold using 518-peptide, and after 24 hours IL-8 was almost undetectable. Altogether, our results suggest that the whole rFla and D1-domain derived peptides are valid candidates to be used as an immuno-stimulant or adjuvants in farmed salmon.

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Conference Session Designation: (Immunology General or Immunology Vaccine)
Presentation Format: (Poster)

No. 80 Molecular Characterization and Immune Profiling of Cathepsin D from Redlip Mullet (*Chelon haematocheilus*)

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The redlip mullet has a worldwide distribution in the sea and significantly used as a food source in different places of the world. According to the statistics published by Korean ministry of maritime affairs and fisheries, 2012 the mullets has accounted for the 8% of the total consumption and cultivation in Korea. Also, red lip mullet is one of the most treasured species in aquaculture sector and they are mostly cultivated in the west coastal area of South Korea. They are susceptible to emerge diseases by affecting from different kind of microorganisms like *Lactococcus garvieae* during the cultivation. Additionally, Amyloodinium and Myxobolus infections reported previously. In this study Cathepsin D (CTSD) from mullet were characterized. This gene encodes a lysosomal aspartyl protease composed of a protein dimer of disulfide-linked heavy and light chains, both produced from a single protein precursor. CTSD is an aspartic endo-protease that is ubiquitously distributed in lysosomes. The main function of CTSD is to degrade proteins and activate precursors of bioactive proteins in pre-lysosomal compartments. According to the in-silico analysis performed, evolutionary relationship identified CTSD belongs to the Cathepsin_D2, pepsin family of proteinases superfamily and cleaved into CTSD light chain and CTSD heavy chain. CTSD consisted with 396 amino acids with molecular weight 42.97 kDa. Pairwise alignment of CTSD with other orthologs showed that highest sequence similarity 96.0% and identity 91.7% with *Sparus aurata*. The catalytic sites of CTSD include two critical aspartic residues located on the light and heavy chains. These two chains are linked by hydrophobic bonds. Quantitative real time PCR (qPCR) results revealed the highest tissue specific immune expression in the blood, kidney and liver, others have very small level of expression among twelve different tissues from healthy mullet fish. As these blood and kidney tissues actively participating in the microbial invading process intensive expression can be observed. Mullets subjected to immune stimulation with lipopolysaccharides (LPS), polyinosinic:polycytidylic acid (Poly I:C), *Lactococcus garvieae* to observe the transcriptional pattern in the CTSD. Blood tissue specific immune expression showed, upregulation happened 48 hours after postinfection. In response to the immune stimulation, CTSD was found to be activated within phagolysosomes and may exert its pro-apoptotic effects either on substrates in the phagolysosome or in the cytosol after translocation from phagolysosomes. A fall in cytosolic pH has been identified as a consequence of bacterial phagocytosis and killing in phagocytes. CTSD is an aspartic protease that depends critically on protonation of its active site Asp residue. Along with Asp-protonation, lower pH also leads to conformational switch in CTSD: The N-terminal segment of the protease moves out of the active site as pH drops. Similar to other aspartic proteinases, CTSD accommodates up to 8 amino acid residues in the binding cleft of the active site. The metabolic degradation in CTSD play a major role in defend against harmful microorganisms. Altogether the CTSD gene can be identified as an immunologically important gene correspond to the innate immune system.

Conference Session Designation: (Genomic Applications in Aquatic Animal Health)
Presentation Format: (Poster)
Student Presentation: (Yes)

No. 81 Antiviral Protein Viperin - Transcriptional Modulation Patterns upon Immune Stimulants from Big Belly Seahorse *Hippocampus abdominalis*

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Viperin is an antiviral protein that has rapid ability to induce many types of interferon-regulated genes (ISGs). Viperin could be induced in a numerous of cell types by different cellular elements such as DNA and RNA viral proteins, type I, II and III IFNs, poly (I: C) and polysaccharide. Moreover Viperin has been induced by either IFN- dependent or IFN-independent pathways. In this study, Viperin of big belly seahorse ((*Hippocampus abdominalis; HaVip*) was recognized and characterized at sequence and transcriptional levels under the immune stimulants as well as healthy animals. Viperin contains coding sequence of 1047 bp which encodes 348 amino acids in length. The 3D structure of HaVip was constructed similar to that of the human viperin. Phylogenetic tree clearly appeared the close evolutionary relationship for vertebrate viperin countrerparts, with clustering to the fish homologs. Overexpressed HaVip showed antiviral activity against VHSV. The highest tissue expression of HaVip was the heart then in skin tissue, while the lowest expressions were detected in spleen and liver tissues. In blood and intestine, under LPS and poly (I: C) challenges, HaVip expressions were significantly up regulated in all time points, but not with *Streptococcus iniae* challenge. In kidney, HaVip transcripts has been increased significantly upon LPS, poly (I: C) and *S. iniae* challenges respectively. According to results obtained, viperin may involve in immune defense mechanism in seahorse.

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

No. 82 Interaction of Soluble- and Membrane-Forms of Toll-Like Receptor 5 Induce Expression of Interleukin-1β Gene in Japanese Flounder, *Paralichthys olivaceus*

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Bacterial flagellum is essential for their motility and consists of several different proteins whose flagella fiber possesses D1, D2 and D3 domains. In mammals, the D1 domain of flagellin is recognized by toll-like receptor 5 (TLR5) to activate innate immune responses. In fish, there are two forms of TLR5, membrane (-M) and soluble (S) forms. The TLR5S is observed only in fish and still unknown the mechanism of recognition pathway with flagellin. In this study, we attempted to understand the function of TLR5S and -M with flagellin to induce immune response in Japanese flounder (*Paralichthys olivaceus*). Over-expressing vector DNAs encoding *Edwardsiella tarda* flagellin, TLR5S and TLR5M genes (*i.e.*, pEtFliC, pTLR5S, pTLR5M) were transfected into the flounder embryonic (HINAE) cells. The expression of interleukin (IL)-1β gene was more strongly induced in the cell transfected with pEtFliC + pTLR5S or pEtFliC + pTLR5M compared to transfected with the empty vector or pEtFliC only. The expression levels of IL-1β gene in the cells transfected with both of pTLR5S and -M were much higher than those of the cells transfected with one of them. Furthermore, mutation of two cysteine residues, C593 or/and C620 in TLR5S indicated that C620 could be involved in dimerization of homo- or hetero-TLR5s to induce the expression of IL-1β gene in HINAE cells. These results suggest that TLR5S interacts with TLR5M to activate immune response in Japanese flounder. [This work was supported by JSPS KAKENHI Grant Number 17H03863.]

Conference Session Designation: **(** Immunology General)
Presentation Format: **(** Poster)

No. 83 IL-17A/F1 Modulates Production of Antimicrobial Peptides in the Intestine of Japanese Medaka, *Oryzias latipes*

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In mammals, interleukin (IL)-17A and IL-17F are hallmark inflammatory cytokines, which are expressed by Th17 cells and play key roles in protection against infection and intestinal mucosal immunity. Although, fish IL-17A and -F homologs named as IL-17A/F have been identified (*i.e.*, actually 3 isotypes including IL-17A/F1, -2 and -3), their functional aspects, especially mucosal immunity in intestine are still unknown. In this study, IL17A/F1-mutated (IL17AF1-KO-) Japanese medaka (*Oryzias latipes*) was established using CRISPR/Cas9 system, and a 7-bp deletion (-7bp) and a 11-bp addition (+11bp) were confirmed in the two lines of IL-17A/F1-KO-medakas. The results of RNA-Seq analysis of the IL17AF1-KO- medaka (-7bp) intestine using a MiSeq next generation sequencer showed that expression levels of immune related genes [*e.g.*, c-type lysozyme, g-type lysozyme, transferrin A, interleukin (IL)-1β, *etc*.] and protein/lipid-digestive enzyme genes (*e.g.*, chymotrypsin-like protease, carboxypeptidase A, carboxyl ester lipase, *etc*.) were significantly decreased in the intestine of IL17AF1-KO-medaka compared to those of the wild-type (WT). Expression of the two lysozymes, transferrin A and IL-1β was confirmed by the quantitative real-time PCR. Furthermore, decreases of g-type lysozyme and IL-1β genes was also confirmed in the other line, +11bp-KO-medaka. The results suggest that IL-17A/F1 could play an important role in production of antimicrobial peptides and digestive enzymes in the intestine of Japanese medaka. This work was supported by JSPS KAKENHI Grant Number 17H01486.

Conference Session Designation: **(** Immunology General)
Presentation Format: **(** Poster)

No. 84 *Vibrio anguillarum* Vaccine Candidates for Lumpfish (*Cyclopturs lumpus*)

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Lumpfish (*Cyclopterus lumpus*), a native fish of the North Atlantic Ocean, is utilized as cleaner fish to control sea-lice in salmon aquaculture. *Vibrio anguillarum* is a Gram-negative pathogen that infects a wide variety of freshwater and seawater fish species, including lumpfish. The ability of bacterial pathogens to uptake iron from the host during infection is necessary for their multiplication within the host. However, host high-affinity iron-binding proteins limit the levels of free iron in fluids and tissues. To overcome this deficiency of iron during infection, bacterial pathogens have developed iron uptake systems that are up-regulated in the absence of iron. *V. anguillarum* iron-regulated outer membrane proteins (IROMPs) are essential virulence factors that could be utilized as immune protective antigens.

Effective vaccine programs against bacterial pathogens, like *V. anguillarum*, have been identified as a high priority area for lumpfish production. Therefore, the objective of this study was evaluated several vaccine formulations against *V. anguillarum*. *V. anguillarum* J360 bacterin expressing the iron up-take outer membrane proteins (IROMPs) and purified IROMPs were utilized as immune protective antigens. Purified outer membrane proteins (OMPs) and a commercial vaccine (AlphaJet V) were employed as comparative references. Naïve fish mock immunized with phosphate buffer saline (PBS) or PBS-Adjuvant were utilized as a control group. *V. anguillarum* J360, isolated from a vibriosis health event in lumpfish at a research facility in Newfoundland and Labrador, was utilized for bacterin preparation and protein isolation. Outer membrane protein profiles were utilized to confirm the presence of IROMPs in the bacterin preparations. Microscopy was used to evaluate bacterin integrity. No adjuvant was utilized for the bacterin-base vaccine preparation. Purified IROMPs or OMPs mixed with adjuvant (Freund's, Sigma) were utilized as protein-base vaccine preparations. Duplicated groups of 300 lumpfish (20g) were intraperitoneally immunized. Independent groups of fish were boost immunized at 4 weeks post-primary immunization. The immunized fish were challenged with 100 LD_{50} (10^7 CFU / dose) of *V. anguillarum* J360 at 8 weeks post-primary immunization. The vaccine preparations did not influence fish growth. Adhesion and vaccine residues were observed in all the adjuvant-based vaccine preparations. Preliminary results showed that adjuvants delay fish mortality. The vaccine preparation triggered different levels of immune protection. Our results suggest that some vaccine formulations protect the fish against *V. anguillarum* challenge. These results could be used for the development of local vaccine programs for the emergent lumpfish fish aquaculture industry.

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

No. 85 Immune Responses to Carp Edema Virus Infection in Common Carp – A Model for Immunosuppression?

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Viruses with larger genomes tend to encode multiple immunomodulatory proteins which evolved to ensure successful replication and transmission of viruses. Poxviruses are one of the families most known for evading immune responses. They are able to modulate onset of apoptosis, production of interferons, inflammatory cytokines, and the activity of cytotoxic T-lymphocytes, B-lymphocytes, and natural killer cells. Fish are host for only a limited amount of poxviruses, with the carp edema virus (CEV) and the salmon gill pox virus (SGPV) being studied to any noticeable extent. The establishment of an infection model with CEV resistant and susceptible strains of common carp allowed for the first time to study immune responses of fish towards fish-infecting-poxviruses. The mortality of koi can reach 100% within 2 weeks post infection while in Amur wild carp (AS) no clinical signs of the disease can be noticed. During the peak of clinical KSD (days 6 and 9 post infection), the development of severe leukopenia and granulocytosis could be noticed in koi. In KSD resistant AS differential blood cells counts remained stable. This led to the question how main immune responses are regulated during CEV infection in these two groups of fish. We measured mRNA expression of selected immunological marker genes in gills (the target tissue for the virus) and kidney (one of main immunological tissues of fish, but harbouring a very limited infection). Generally, the upregulation of only pro-inflammatory and a downregulation of several other responses could be noticed. The magnitude of changes was higher in gills than in kidney. The mRNA levels of *il1b* were extremely increased (over 200 fold) while the expression levels of the genes *cd4*, *tcr a2*, *casp8-9*, *igm* were 2-14 fold downregulated. For other genes: *mpo* and *cd8* the expression remained largely unchanged. Interestingly the KSD resistant fish did not respond to the infection in any noticeable manner and only *inos* upregulation was noticed. These initial results could indicate that the development clinical of KSD is accompanied by a suppression of T- and B-cell responses which could be a cause for development of secondary infections.

Conference Session Designation: (Immunology General)
Presentation Format: (Poster **)**

No. 86 Resistance vs Type I Interferon Responses in Viral Infection Models of Common Carp

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Carp with a different genetic background are presenting diverse susceptibility to viral pathogens. This was most clearly shown in the resistance to koi herpesvirus disease (KHVD) caused by *cyprinid herpesvirus 3* (CyHV-3) infection. Carp strains of Asian origin, especially Amur wild carp (AS) and Ropsha scale carp (Rop) were proven to be far less susceptible to KHVD than strains originating from Europe like the Prerov scale carp (PS) or koi from the Czech Republic. We hypothesised that it could be associated with a higher magnitude of type I interferon responses as a first line of cellular defences in the case of viral infections. Three rhabdo-, pox- and alloherpesviral infection models (*spring viremia of carp virus* SVCV, carp edema virus CEV and CyHV-3) were used to evaluate this hypothesis. The development of clinical signs was linked with an evaluation of virus spreading through selected tissues at several time points in four strains of common carp (AS, Rop, PS and koi). An infection experiment confirmed significant differences in mortality and virus load during CyHV-3 infection where Rop and AS (mortality of 22% and 47%) were performing better than PS and koi (mortality of 65% and 90%). When the susceptibility to a CEV infection and the development clinical of KSD were investigated, Amur wild carp were more resistant to the infection and did not develop clinical signs for KSD while in koi mortality reached 100%. An infection with SVCV induced low (22%) mortality in PS, while Rop (with 0% mortality) was the most resistant. The evaluation of the virus loads and replication showed significant differences which correlated with mortality. The evaluation of type I IFN responses showed that there are fundamental differences between the virus infection models. The alloherpesvirus CyHV-3 and poxvirus CEV were generally inducing low responses while IFN responses to the rhabdovirus SVCV were much higher. However the results proved the initial hypothesis wrong. The magnitude of the type I IFN response was not a good marker of resistance because in infected fish it was positively correlated with virus load.

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

No. 87 Piscine Orthoreovirus 1 and 3 Detected as a Co-Infecting Agents During Furunculosis in Germany

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Piscine orthoreoviruses (PRVs) are emerging viral pathogens causing circulatory disorders in salmonids. PRV-1 is the etiological cause of heart and skeletal muscle inflammation (HSMI) in farmed Atlantic salmon (*Salmo salar*), characterized by epicarditis, inflammation and necrosis of the myocardium, myositis and necrosis of red skeletal muscle. The recently discovered PRV-3 (also called PRV-*Om*) is inducing a HSMI-like disease in rainbow trout (*Oncorhynchus mykiss*). In 2017, two German farms breeding Atlantic salmon or rainbow trout, respectively, experienced disease outbreaks with accumulated mortalities of 10% and 20%. The main clinical signs were exhaustion and lethargic behaviour. Virological examination indicated PRV-1 infection in the Atlantic salmon and the PRV-3 infection in rainbow trout. Further analyses indicated also the presence of *Aeromonas salmonicida* in internal tissues of both species. While PRV-1 was most likely the causative agent of the disease in Atlantic salmon, most of the rainbow trout suffered also from a systemic infection with *A. salmonicida*. Interestingly, the German PRV-3 isolate resembled a closer genetic relationship to a Chilean PRV-3 isolate from coho salmon (*Oncorhynchus kisutch*) than to PRV-3 form the first detection in rainbow trout in Norway. The results from Germany confirm a wide geographical distribution of both viruses and could suggest their dispersal by global trade with live salmonids. Our findings indicate that diseases induced by PRVs should be considered when investigating mortalities in salmonid fish.

Conference Session Designation: (Emergent Disease)
Presentation Format: (Poster)

No. 88 First Detections of Carp Edema Virus in Hungary, Croatia and Serbia Confirm Very Wide Distribution of the Virus in Europe

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Koi sleepy disease (KSD) caused by carp edema virus (CEV) infection is considered by some as an emerging viral disease in certain regions of the world. Especially in Europe the virus was not detected for long time and only in recent years after its detection in UK it came more into the focus of diagnostic laboratories. However, we hypothesise that both the virus and the disease existed in the European common carp aquaculture for longer time and only due to a particular set of reasons like a less dramatic clinical presentation, the season of occurrence, some similarity to koi herpesvirus disease (KHVD) or intoxication with ammonia, no ability to detect virus by cell culturing, it was not detected. One result which could support this hypothesis would be a wide geographical distribution of the virus in both the main carp producing countries (e.g. Hungary) as well as in countries with a limited carp production (Serbia and Croatia). Therefore, samples were collected in these countries in 2015-2018 and screened for the presence of CEV. Prevalence of CEV in Hungary was the highest with 76% (13 CEV positive out of 17 locations screened), while 14% prevalence was recoded in Croatia were 6 out of 44 locations were CEV positive. In Serbia fish with clear signs of clinical KSD from only two farms were sampled and both locations were confirmed to be CEV positive. The differences in prevalence most likely were related to the season of sampling and purpose the samples were collected – in Hungary and Serbia the sampling was focused on clinically affected fish in autumn-winter-spring, when KSD is more likely to occur, while in Croatia samples were collected during spring-summer. Phylogenetic analyses indicated that some virus isolates are distributed in geographically related locations e.g the same virus could be found in neighbouring regions of Hungary and Serbia. Our findings together with results of epidemiological studies in Germany indicate that CEV (especially from genogroup I) is widely spread in the European carp population. Therefore, KSD should be considered in whole Europe when investigating disease outbreaks in common carp at temperatures below the optimum for KHVD.

Conference Session Designation: (Emergent Disease)
Presentation Format: (Poster)

No. 89 Evaluation of New Virus Infection Models for Zebrafish

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Zebrafish (*Danio rerio*) belong to the *Cyprinidae*, the same family as the three top farmed fish in global aquaculture: silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idella*) and common carp (*Cyprinus carpio*). The production of these fish reached 15.3 M tonnes in 2015, which covers 20% of global aquaculture production. With the significant biotechnological progress in genetics, zebrafish is becoming an interesting model for studying immune responses to infections causing diseases in other cyprinid fish. In a search for new viral models which could be explored in zebrafish, we tested ZF4 and SJD1 cells for their susceptibility to several viruses including *cyprinid herpesvirus 1* and *3* (CyHV-1 and CyHV-3), *chum salmon reovirus* (CSV), common carp iridovirus (CCIV) and common carp paramyxovirus (CCPV). As positive controls *viral haemorrhagic septicaemia virus* (VHSV), *infectious pancreatic necrosis virus* (IPNV) and *spring viremia of carp virus* (SVCV) were used. The CSV, CCIV but not CCP were able to replicate in ZF4 and SJD1 cells. Interestingly also CyHV-1 and CyHV-3 were able to replicate, however the virus mRNA was at a very low level and no cytopathic effect could be noticed which suggests only a partial compatibility of these viruses to the cells. Type I interferon responses were measured for selected virus models. ZF4 cells of embryonic origin showed very weak type I IFN responses to all virus infections while SJD1 cells (fibroblasts from adults) showed increased type I IFN response towards CSV but not to CyHV-1 and CyHV-3. This could suggest that ZF4 cells are of limited usability for monitoring antiviral responses. Furthermore it seems that CyHV-1 and CyHV-3 infections remain in SJD1 cells at a very low level which is difficult to detect in the cells. Based on the *in vitro* results, we selected CyHV-3, CCIV and CSV for testing *in vivo* and initial results from infection experiments of adult zebrafish will be presented.

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

No. 90 Antibody Development and Serologic Test Validation in Juvenile Northern Pike (*Esox Lucius*) Experimentally Infected with Viral Hemorrhagic Septicemia Virus

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Viral hemorrhagic septicemia virus (VHSv) is an ongoing cause of disease and mortality in freshwater fishes across the Midwestern United States. The virus was introduced into the Great Lakes and has caused recurring large-scale multi-species mortality events. VHS is also a notable disease requiring extensive testing and regulation of fish movement.. The current validated diagnostic assay for VHSv is cell culture with confirmation by polymerase chain reaction. This testing method requires lethal sampling to obtain internal organs and takes a minimum of four weeks to obtain results. The main goal of this study is to validate a non-lethal serological testing method for VHSv that has a significantly shorter turn-around-time while maintaining high sensitivity and specificity by experimentally infecting a susceptible species (northern pike, *Esox lucius*) and tracking the development of a detectable antibody response over time. In the fall of 2017, hatchery-reared juvenile pike were placed in a recirculating system and allowed to acclimate for 6 months. Pike were then experimentally infected with VHSv either by intraperitoneal injection or static immersion at a dose of $5x10^5$ PFU/mL. Infected fish were then monitored over a 12 week (84 day) period for signs of disease, and weekly serum samples were obtained. At the end of the experiment, all fish were euthanized using MS-222 and necropsied. Analysis of survival data shows no difference between injection and immersion groups (median survival times of 50 days post infection and 69 days post infection, respectively; survival percentages of 48% and 75% respectively; p-value of 0.17 when survival distributions of these two groups are compared by log-rank test). Fish infected by either method did, however, die significantly more quickly than control fish (Median survival time of 84 days post mock infection, 100% survival, p-values of 0.0382 and 0.21 respectively when compared to injection and immersion groups by log-rank test). All serum samples collected from the pike during the infection trial were tested by a previously published enzyme linked immunosorbent assay (ELISA) at the Wisconsin Veterinary Diagnostic Laboratory. ELISA testing is still in progress with results pending (will have results prior to conference). Results of this study will lend insights into the disease progression of VHSv while validating the published ELISA in northern pike, with the hope that ELISA may eventually replace lethal testing for VHSv in this economically important sport fish.

Conference Session Designation: (Virology)
Presentation Format: **(** Poster)
Student Presentation: (Yes)

No. 91 Genomic Characterization of a Novel Pegivirus Species in Wild Bottlenose Dolphins *Tursiops Truncatus*

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Several notable human and animal pathogens belong to the family *Flaviviridae* including: dengue fever, yellow fever, West Nile, Zika, hepatitis C, and classical swine fever viruses. The flavivirus genus *Pegivirus* contains viruses that infect humans, nonhuman primates, bats, horses, rodents, and pigs. Herein, we report the discovery of a novel species of pegivirus detected in a population of bottlenose dolphins (BD) *Tursiops truncatus*. We screened 50 serum samples from 32 adult wild (n=20) (U.S. NMFS permit no. 14352) and managed (n=12) BD for viral discovery. Ten five-sample pools were created and RNA was extracted using a commercial kit. We prepared cDNA libraries that were sequenced on an Illumina MiSeq sequencer and assembled *de novo* using CLC Genomics Workbench. BLASTX searches of the assembled contigs against a proprietary viral database recovered the near complete genome (9,649 bp) of a virus most closely related to porcine pegivirus and Theiler's disease associated virus. A Maximum Likelihood phylogenetic tree using the amino acid sequences of the NS3 protease domain of this novel virus and 28 other pegiviruses classified within the 11 accepted species retrieved from the NCBI GenBank database supported the BD virus as a unique branch between porcine and equine pegiviruses. Genetic analysis performed within the Sequence Demarcation Toolv.1.2 revealed the NS3 amino acid sequence identity of this novel BD virus to other pegiviruses ranged from 40.6-54.6%, lower than the 69% threshold proposed for species demarcation. Virus species in the genus *Pegivirus* are named alphabetically according to their chronological discovery. Thus, we propose this dolphin pegivirus (DPgV), to be formally named Pegivirus L, pending acceptance by the International Committee on Taxonomy of Viruses. To the best of our knowledge, DPgV is the first member of the family *Flaviviridae* sequenced from a cetacean species. Further research is needed to evaluate the DpgV host range (including zoonotic risk), route of transmission, prevalence, and potential role in disease within wild and managed dolphin populations.

Conference Session Designation: (Aquatic Mammals)
Presentation Format: **(** Poster)
Student Presentation: (Yes)

No. 92 Phylogenomic Characterization of a Novel Rhabdovirus Isolated from a Stranded Harbour Porpoise *Phocoena Phocoena*

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An adult male harbour porpoise (*Phocoena phocoena*) stranded off the coast of Alaska displaying poor body condition, scattered mild ulcerative dermatitis, and necrotizing balanoposthitis. Necropsy findings included severe verminous panniculitis, pneumonia, hepatitis, and enteritis. Histopathological examination of skin lesions revealed a pustular dermatitis, with ballooning degeneration of keratinocytes and occasional eosinophilic cytoplasmic inclusion bodies. Swabs taken from the ulcerative penile lesions were processed for virus isolation and resulted in cytopathic effects observed in primary beluga whale kidney (BWK) and Vero.DogSLAMtag cells. Transmission electron microscopy revealed bullet-shaped virions with 73nm mean width and 111nm mean length budding from the cell surface of infected BWK cells, consistent with a rhabdovirus. A cDNA library was prepared using RNA extracted from the supernatant of infected BWK cultures and sequenced on an Illumina MiSeq sequencer. The near complete genome (11,192 nucleotides) of a novel rhabdovirus was recovered. Genetic and phylogenetic analyses based on the amino acid sequence of the RNA-dependent RNA polymerase (L gene) supported the virus as a new species to be referred to as the harbour porpoise rhabdovirus (PRV). The PRV was supported as the sister species to the previously sequenced dolphin rhabdovirus and this cetacean rhabdovirus clade was found to be the sister group to genus Perhabdovirus, which includes rhabdoviruses that infect fish. Our results are consistent with a previous hypothesis that cetacean rhabdoviruses may have arisen following a host jump from fish. Further research is needed to evaluate the DRV/PRV host range (including zoonotic risk), route of transmission, prevalence, and potential role in disease within managed and wild cetacean populations.

Conference Session Designation: (Aquatic Mammals)
Presentation Format: **(** Poster)
Student Presentation: (Yes)

No. 93 Sequence Analysis of the HPR and Fusion Gene of HPR0 Isolates of ISAV in Iceland

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Infectious salmon anemia (ISA) is a serious viral disease of Atlantic salmon *(Salmo salar L.)* caused by the ISA virus (ISAV) and is notifiable to the World Organization for Animal Health (OIE). Virulent strains ISAV-HPRdel have deletions in a highly polymorphic region (HPR) of the hemagglutinin-esterase (HE) gene on segment 6, whereas avirulent strains ISAV-HPR0 have none. In addition, a Q266L substitution or insertion adjacent to the putative proteolytic cleavage site of the fusion protein (F) encoded by segment 5 has been suggested as a virulence marker. Outbreaks of ISAV-HPRdel have occurred in farmed Atlantic salmon in countries geographically close to Iceland, but only ISAV-HPR0 has been detected in Iceland. Only 0.63% of samples from Icelandic farmed salmon in 2011-2015 were positive for ISAV-HPR0.

Amplification of the HPR on segment 6 from 112 ISAV-HPR0 isolates was done by RT-PCR. Sequencing of the amplicons showed great homogeneity within the Icelandic isolates. The Icelandic HPR0 isolates were most similar to Norwegian and Faroese strains. Preliminary sequencing results from a partial sequence of the fusion gene on segment 5 RT-PCR amplicons suggest little variation there as well. Knowledge of the genotypes of the Icelandic ISAV-HPR0 isolates will be useful for further research, analyses and risk assessment for ISAV in Iceland.

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

No. 94 Stress and Immune Gene Expression in Atlantic Salmon (*Salmo salar L*) Broodstock Sub- Clinically Co-Infected with Chilean Prevalent Viruses

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Viral co-infections are being increasingly recorded in salmon production. Much of this information proceed from passive surveillance programs where apparently healthy animals are examined. The consequences of these sub-clinical co-infections in long term production performance are largely unknown. This study focused on stress and immune responses in broodstock co-infected with viral agents ubiquitously found in Chilean salmon. A total of 266 Atlantic salmon from 14 breeding centers belonging to 4 different geographical areas were sampled and infection status for Piscine Orthoreovirus (PRV), Atlantic Salmon Calicivirus (ASCV) and Infectious Pancreatic Necrosis Virus (IPNV) was assessed as single or co-infection. Transcripts of stress and immune genes including *gr-1, gr-2, nf-□b, i□b□, ifn-□/□, ifn-□, tnf-□, mx, cd4, cd8, mhc-II, igm* and *igt* were determined from head kidney samples. From the total, 15 individuals were co-infected with two or three viral agents. The most frequent co-infection was PRV-ASCV (14 of 15 co-infected animals). Gene expression analyses showed that stress response pattern in non-infected fish was indistinguishable from infected (single or co-infected). Accordingly, the innate immune response in infected fish (*ifn-□/□ □*and *mx* transcripts) was not upregulated. And innate gene expression in single- infected or co-infected fish did not differ between them either ($p > 0.05$). Interestingly, the adaptive immune response was upregulated in single-infected (PRV or ASCV) as well as in PRV-ASCV co-infected fish when *igm* was evaluated ($p < 0.05$). Moreover, other adaptive immune genes were differentially expressed in PRV-ASCV co-infected in comparison to single infected fish. Co-infected fish showed upregulation of *ifn-□, cd4, cd8* genes in comparison to single infected or non-infected animals ($p < 0.05$). These results suggest the involvement of Th1 cytotoxic antiviral activation in this particular group of animals but not an effective response as viral pathogens were still infecting the fish. Further analysis should be conducted in order to corroborate the increasing in the cellular antiviral response for instance, cytokine measurement.

Conference Session Designation: (Virology or Co-infections in Fish)
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