Thursday September 6th – Gray / Palmer / Pope
Virology / Emergent Disease
Moderator – Esteban Soto (Univ. of California at Davis)

3:15 PM	Virology / Emergent Disease	Harkness - Sav3 Challenge Model Optimization for Testing Dna Vaccine Duration of Immunity
3:30 PM		Soto - Isolation and Metagenomic Chracterization of a Novel Flavivirus from Chinook Salmon (*Onchorhynchus tshawytscha*)
3:45 PM		Clouthier - Nucleo-Cytoplasmic Large DNA Viruses of Wild Lake Sturgeon (*Acipenser fulvescens*) in Central Canada
4:00 PM		**de Kantzow - Ostreid Herpesvirus 1 (OSHV-1) In Vivo Growth Curve and Pathogenesis at a Semi-Permissive Water Temperature**
4:15 PM		**Lopez-Porras - Red Seabream Iridovirus Associated With Cultured Juveniles Florida Pompano *Trachinotus carolinus* Mortality in Central America**
4:30 pm		Lou - A Novel Viral Pathogen Causing Tongue Sole Spleen and Kidney Necrosis in China
4:45 PM		Mordecai - Evidence of a Divergent Arenavirus Infection in Farmed and Wild Salmon in British Columbia
5:00 PM		**Abdelrazek - Comparative Susceptibility of Cyprinidae, Cichlidae, Acipenseridae, and Salmonidae to *Veronaea botryosa***
4:45 PM		**OPEN**

SAV3 Challenge Model Optimization for Testing DNA Vaccine Duration of Immunity

Jennifer E Harkness*, Lisa M Phillips, Paul Tonita, Afton McMillan, Bryan Linton, Amy Garland

Elanco Canada Limited, Victoria, Prince Edward Island, Canada
harkness_jennifer_e@elanco.com phillips_lisa@elanco.com tonita_paul@elanco.com
mcmillan_afton@elanco.com linton_bryan@elanco.com garland_amy@elanco.com

Pancreas disease caused by salmonid alphavirus (SAV) has been observed in farmed Atlantic salmon in Norway since the 1980's and continues to pose a threat to the successful rearing of Atlantic salmon. It can cause acute mortality from heart and skeletal muscle damage, with mortality ranging from 1- 48%. In addition it causes chronic wasting in survivors due to loss of exocrine pancreas and impaired red and white muscle function which leads to fish "runting" and flesh quality down grades at the processing plant. Necropsy of infected fish may reveal petechial bleeding on the pyloric caeca and surrounding fat, ascites, yellowish liver and pale heart and yellow mucoid gut contents. Histopathological analysis of infected fish is characterized by necrosis of heart, red and white skeletal muscle, along with necrosis and/or complete destruction of the exocrine pancreas. The damage to the Norwegian industry continues, despite commercially available vaccines, with 176 cases reported in 2017.

In order to demonstrate clinically relevant duration of immunity for DNA vaccine development, a SAV3 cohabitation challenge was optimized and used to challenge Atlantic salmon in saltwater at 6, 9.5 and 12 months post-vaccination. The model, including behavioral observations, gross observations during necropsy and sampling, impact on average weight gain, mortality for all time points, and from 12 month immunized fish microscopic cardiac, pancreatic and skeletal muscle lesions at 19, 54 and 89 days post challenge will be presented for the saline control fish and the not vaccinated not challenged controls (NVNC).

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

Isolation and Metagenomic Chracterization of a Novel Flavivirus from Chinook Salmon (*Onchorhynchus tshawytscha*)

Esteban Soto[1,*], Alvin Camus[2], Susan Yun[1], John H. Leary[2] Jennifer A. Dill[2], Tomofumi Kurobe[3], Chelsea Lam[3], Akinyi C. Nyaoke[4], Mark Adkison[5], Allan Renger[6], Thomas Rosser[7], Terry Fei Fan Ng[2]

[1] Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA 95616, USA sotomartinez@ucdavis.edu, scyun@ucdavis.edu
[2] Department of Pathology, College of Veterinary Medicine, University of Georgia Athens, Athens, GA, USA camus@uga.edu, jhleary@uga.edu, jdilldvm@uga.edu, ylz9@cdc.gov;
[3] Department of Anatomy, Physiology and Cell Biology, School of Veterinary Medicine, University of California, Davis, CA 95616, USA tkurobe@ucdavis.edu, chylam@ucdavis.edu;
[4] California Animal Health and Food Safety Laboratory-San Bernardino, University of California at Davis, 105 W. Central Avenue, San Bernardino, CA 92408, USA canyaoke@ucdavis.edu;
[5] California Department of Fish and Wildlife, Rancho Cordova, CA 95670, USA Mark.Adkison@wildlife.ca.gov;
[6] California Department of Fish and Wildlife, Fortuna, CA 95540, USA Allan.Renger@wildlife.ca.gov
[7] Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS USA

In November 2015 diseased Chinook salmon, *Oncorhynchus tshawytscha* were collected from the lower Eel River, Fernbridge, CA and submitted for diagnosis. Approximately, 10% of the fish presented with abnormal behavior (lethargy, decreased avoidance of humans, congregating at the banks of the river) and cloudiness and opacity in their eyes. Upon necropsy, the eye and brain were the only tissues exhibiting gross changes, specifically cataracts associated to metazoan parasites and petechial hemorrhages in the brain (optic lobes, cerebellum) and spinal cord. Sub-samples of brain, spleen, kidney, and gonad were pooled for virus isolation on multiple cell lines. Three weeks post-inoculation, only the striped snakehead (SSN-1) cell lines presented cytophatic effect. Total nucleic acid was extracted from cell culture supernatants and subjected to RNAseq. RNAseq identified two viral agents in the supernatants, the snakehead *retrovirus* previously identified in the SSN-1 cell line and a novel member of the genus *flavivirus*, family *Flaviviridae*. Additionally, phylogenetic analysis placed the salmon *flavivirus* as the base of flaviviruses described to date. The genome sequence was utilized to generate a reverse transcriptase real time PCR assay specific for the major capsid protein gene of the salmon *flavivirus*. Infectious challenges in rainbow trout (*Oncorhynchus mykiss*) and Chinook salmon fulfilled River's postulates and demonstrated virus replication in brain and kidney. This represents the first isolation and characterization of a *flavivirus* infecting fish.

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

Nucleo-Cytoplasmic Large DNA Viruses of Wild Lake Sturgeon (*Acipenser Fulvescens*) in Central Canada

Sharon C. Clouthier[1]*, Elissa J.D. VanWalleghem[1], Tamara L. Schroeder[1], Don Macdonald[2], Carol A. McClure[3], Eric D. Anderson[4]

[1] Fisheries and Oceans Canada, 501 University Crescent, Winnipeg, Manitoba R3T 2N6, Canada Sharon.Clouthier@dfo-mpo.gc.ca ; Elissa.VanWalleghem@dfo-mpo.gc.ca ; Tamara.Schroeder@dfo-mpo.gc.ca

[2] Department of Sustainable Development, Box 28, 59 Elizabeth Drive, Thompson, Manitoba R8N 1X4, Canada Don.Macdonald@gov.mb.ca

[3] AquaEpi Research, 300 Heartz Road, Charlottetown, PE C1C 0H8, Canada cmcclure@upei.ca

[4] Box 28, Group 30, Ste Anne, Manitoba R5H 1R2, Canada mainebiotek@hotmail.com

Namao virus (NV) is a sturgeon nucleo-cytoplasmic large DNA virus (sNCLDV) that can cause a lethal disease of the integumentary system in lake sturgeon *Acipenser fulvescens*. As a group, the sNCLDV are members of the *Mimiviridae* family with CroV as their closest extant virus relative. In this study, the spatial, temporal and genetic patterns of sNCLDV were evaluated for the first time for wild lake sturgeon from eleven rivers in central Canada. A total of 1329 pectoral fin biopsies were collected between 2010 and 2015. Quantitative PCR (qPCR) results with the Q2 test indicated that the virus was endemic in sturgeon of the Hudson Bay drainage basin with 23.7% (315/1329) of the fish testing positive. The sNCLDV-positive samples were from endangered populations in the Saskatchewan-Nelson River watersheds where virus was detected in 3 to 58% of the sturgeon tested in each population. The highest virus loads were observed in the Nelson River populations in northern Manitoba. Repeat testing of captured-recaptured individuals (n=26) revealed temporal heterogeneity with respect to their virus status in Landing River, a tributary of the Nelson River. Analyses of samples collected annually from the Landing River population over the six year study revealed that virus presence was inversely correlated with sturgeon age, cohort year, weight and the number of times sturgeon were handled (as part of the sturgeon monitoring program) prior to virus sample collection. These results suggest that NV infection may reduce lake sturgeon fitness and survival in the wild. Genetic typing of 114 virus isolates indicated that the NV genogroup of sNCLDV was dominant in the Hudson Bay drainage basin. The results of this study can be used to inform disease management strategies for lake sturgeon conservation, management and recovery programs.

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

Ostreid Herpesvirus 1 (Oshv-1) _In Vivo_ Growth Curve and Pathogenesis at a Semi-Permissive Water Temperature

Maximilian C. de Kantzow [1]*, Richard J. Whittington [2], Paul M. Hick [3]

[1] Sydney School of Veterinary Science, The University of Sydney, 325 Werombi Rd Camden NSW, Australia. Maximilian.dekantzow@sydney.edu.au
[2] Sydney School of Veterinary Science, The University of Sydney, 325 Werombi Rd Camden NSW, Australia. Richard.whittington@sydney.edu.au
[3] Sydney School of Veterinary Science, The University of Sydney, 325 Werombi Rd Camden NSW, Australia. Paul.hick@sydney.edu.au

Microvariant genotypes of *Ostreid herpesvirus-1* (OsHV-1) are emerging pathogens that cause seasonally recurrent epizootics in Pacific oysters (*Crassostrea gigas*) across Europe, New Zealand and Australia. The incubation period ranges from 48 to 72 hours and is dependent on water temperature. Risk factors including water temperature impact mortality and are important to understand in order to develop strategies to reduce the impact of the disease. The aim of the current study was to define the growth curve of OsHV-1 using an *in-vivo* infection model at both 18°C and 22°C. Additionally, a change in water temperature was used to assess if a subclinical infection at 18°C might develop into disease at 22°C. The experiments were conducted in a physical containment level 2 aquatic animal facility. Samples were obtained to measure the concentration of OsHV-1 DNA in gill and mantle tissue at 2, 4, 6, 8, 10, 12, 18, 24, 48 and 72 hours after exposure to OsHV-1 by injection into the adductor muscle. Peak viral load occurred at 24 hours at 22°C and 36 hours at 18°C. This was 24 and 48 hours before onset of mortality was observed at 22°C and 18°C, respectively. In a separate cohort, the water temperature of surviving oysters was increased to 22°C 14 days after challenge at 18°C and monitored for a further 14 days. Control groups were challenged and maintained at 18°C or 22°C for 28 days. OsHV-1 prevalence at 18°C at 14 days was 33% (95% CI: 10% - 65%) after which mortality was 3% (95% CI: 1% - 8%) in oysters exposed at 18°C and raised to 22°C compared to 36% (95% CI: 26% - 47%) in those maintained at 22°C. The present results suggest that some oysters may recover and clear an infection at 18°C and indicate that recrudescence at a permissive water temperature may not result in mortality. These results also confirm that the pathogenesis of OsHV-1 is slowed at 18°C but not prevented. Raising oysters to a permissive water temperature has been recommended as a surveillance method to identify infected individuals including carriers. Further research is required to determine the disease and transmission risk from oysters which remain positive by qPCR, and surveillance methods for identifying persistent infection require validation.

Confernece Session Designation: **(** Virology or Invertebrate and Shellfish Disease)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Red Seabream Iridovirus Associated with Cultured Juveniles Florida Pompano *Trachinotus carolinus* Mortality in Central America

Adrian Lopez-Porras[1]; Juan A. Morales[1]; Gilbert Alvarado[2]; Samantha A. Koda[3]; Alvin Camus[4]; Kuttichantran Subramaniam[3]; Thomas Waltzek[3]; Esteban Soto[5]*

[1] Servicio de Patologia Veterinaria, Universidad Nacional de Costa Rica, Heredia, Costa Rica adriandelopez@gmail.com; juan.alberto.morales.emv@gmail.com
[2] Laboratory of Experimental and Comparative Pathology (LAPECOM), Biology School, University of Costa Rica, Costa Rica gilbert.alba@gmail.com
[3] Department of Infectious Diseases and Immunology, College of Veterinary Medicine, University of Florida, Florida, USA samanthakoda@ufl.edu; kuttichantran@ufl.edu; tbwaltzek@ufl.edu
[4] Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA camus@uga.edu
[5] Department of Epidemiology and Medicine, University of California, Davis-School of Veterinary Medicine, Davis, California, USA sotomartinez@ucdavis.edu

Mariculture of Florida pompano *Trachinotus carolinus* in Central America has increased over the last few decades and is now a highly valued food fish. High feed costs and infectious diseases are important impediments to the expansion of mariculture. Members of the genus *Megalocytivirus* (MCV), subfamily *Alphairidovirinae*, family *Iridoviridae*, are emerging pathogens that negatively impact Asian mariculture. A significant mortality event in juvenile Florida pompano cultured in Central America occurred in October 2014. Affected fish presented with abdominal distension, darkening of the skin and periocular hemorrhages. Microscopic lesions included cytomegalic "inclusion body-bearing cells (IBCs)" characterized by basophilic granular cytoplasmic inclusions in multiple organs. Transmission electron microscopy revealed arrays of hexagonal-shaped virions (155-180 nm in diameter) with electron-dense cores within the cytoplasm of cytomegalic cells. Pathological findings were suggestive of an MCV infection and the diagnosis was later confirmed by partial PCR amplification and sequencing of the viral gene encoding the transmembrane amino acid transporter protein. The viral sequence revealed the juvenile Florida pompano were infected with an MCV strain, red seabream iridovirus (RSIV), previously reported only from epizootics in Asian mariculture. This case underscores the threat RSIV poses to global mariculture including the production of Florida pompano in Central America.

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

A Novel Viral Pathogen Causing Tongue Sole Spleen and Kidney Necrosis in China

Yanan Lou[1,2,3*], Peng Xiao[1,2]

[1] CAS Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China YNLou2012@163.com
[2] Laboratory for Marine Biology and Biotechnology, Qingdao National Laboratory for Marine Science and Technology, Qingdao, China
[3] University of Chinese Academy of Sciences, Beijing, China

Half-smooth tongue sole *Cynoglossus semilaevis* Gunther is a major cultured fish species in China, with high commercial value. From 2009 onwards, tongue sole culturing industry was almost destroyed by a previously unknown disease. The disease has been restricting the industry for near ten years, and farming of half-smooth tongue sole has been abandoned in some areas.
Generally, the spleen and kidney of diseased fish developed cysts, and their textures became uneven. White particles could be detected in the spleens of diseased fish, as well as in some kidneys. These signs were exhibited by both larvae and adults and were associated with mass death of breeding tongue sole. The disease was characterized by either acute or chronic visceral necrosis, both of which caused high mortalities, with cumulative mortalities as high as 96%. Bacterium couldn't be isolated from diseased organs using the streak method and there were no any parasites on the surface of the diseased fish. Results of TEM indicated the presence of virus particles in the cytoplasm of spleen cells. No bacterium and parasite pathogens were detected by TEM. The virus particles were about 30 nm in diameter and roughly circular. Viral inclusion bodies were also present in the cytoplasm.

Symptomatic spleens were collected and grounded with 0.85% NaCl. After centrifuged at 14,000g × 15 minutes, the supernatant was passed through 0.22μm filter. Infection of the filtration material induced spleen and kidney necrosis and death in tongue sole. We supposed the virus we observed under TEM is the causative agent of spleen and kidney necrosis disease.
We couldn't amplify any target segments by using primers for Nodavirus and known fish Picornaviurs whose size are the same with observed virus.
These results suggested that a novel viral pathogen was responsible for the development of splenic necrosis signs in tongue sole. Whole genome sequencing of the novel virus is in progress.

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

Evidence of a Divergent Arenavirus Infection in Farmed and Wild Salmon in British Columbia

Gideon J. Mordecai[1], Emiliano Di Cicco[2], Angela D. Schulze[2], Karia H. Kaukinen[2], Tobi J. Ming[2], Shaorong Li[2], Jan Finke[1], Curtis A. Suttle[1,3,4,5,6], Kristi M. Miller[2]

[1] Department of Earth, Ocean and Atmospheric Sciences and Biodiversity Research Centre, The University of British Columbia, BC, Canada gmordecai@eoas.ubc.ca
[2] Pacific Biological Station, Fisheries and Oceans Canada, Nanaimo, British Columbia, Canada
[3] Institute for the Oceans and Fisheries, The University of British Columbia, Vancouver, BC V6T 1Z4, Canada
[4] Department of Microbiology and Immunology, The University of British Columbia, Vancouver, BC V6T 1Z3, Canada
[5] Department of Botany, The University of British Columbia, Vancouver, BC V6T 1Z4, Canada
[6] Integrated Program in Microbial Biodiversity, Canadian Institute for Advanced Research, Toronto, ON M5G 1Z8, Canada

There is growing concern that infectious diseases may be contributing substantially to early marine losses of Pacific salmon populations, however, there is little data on diseases occurring in wild migratory salmon. In the current viral discovery study, we sought to determine if unknown viruses were associated with some of the pathologies of unknown etiology observed in farmed Chinook salmon. Initially, a divergent arenavirus was discovered in farmed chinook salmon from the Canadian Department of Fisheries and Oceans (DFO) regulatory audit program, in which farms are randomly sampled for daily mortalities, and subsequently, we identified a closely related virus in sockeye salmon. The novel viruses in Chinook and Sockeye salmon showed homology to arenaviruses and were named salmon pescarenavirus 1 and 2 (SPAV-1/2) respectively. SPAV, along with a recently sequenced arenavirus in frogfish likely represent a new genus of arenavirus. Histopathology observations and localization in both chinook and sockeye salmon confirm empirically that SPAV infects salmon cells and may be associated with pathology in a cultured setting. Furthermore, SPAV -1 RNA was detected in 24/235 farmed Chinook salmon, and 41/852 wild Chinook salmon, whilst SPAV-2 was detected in 145/1714 wild sockeye salmon. Furthermore, prevalence of SPAV-2 was over 20% in the Northern Johnstone strait and Discovery Islands, suggesting that in certain regions SPAV infection is common and may be an important driver of wild salmon population dynamics.

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

Comparative Susceptibility of Cyprinidae, Cichlidae, Acipenseridae, and Salmonidae to *Veronaea botryosa*

Samah M.R. Abdelrazek,[1*] Denver J. Coleman,[1] Alvin Camus,[2] and Esteban Soto[1]

[1] Department of Medicine and Epidemiology, School of Veterinary Medicine, Davis, California, 95616, USA smrazek@ucdavis.edu; denverjcoleman@gmail.com; sotomartinez@ucdavis.edu
[2] Department of Pathology, College of Veterinary Medicine, University of Georgia Athens, Athens, GA 30602 USA camus@uga.edu

In the Western United States, tilapia (*Oreochromis* spp.), koi carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*), and sturgeon (*Acipenser* spp.) farming is a multi-million dollar industry. *Veronaea botryosa* is a dematiaceous, saprobic fungus and cause of systemic fungal infections in cultured sturgeon. Mortality in adult female sturgeon caused by this emergent pathogen results in significant economic losses for the caviar industry. Known to producers as "fluid belly," the disease is now regarded as one of the most important diseases affecting sturgeon aquaculture in North America. Little is known regarding the epizotiology of the disease and host specificity of the fungus. This study aimed at investigating the susceptibility of white sturgeon, koi, Nile tilapia, blue tilapia, and rainbow trout to *V. botryosa* using laboratory-controlled challenge model. Our hypothesis was that *V. botryosa* is host specific and would only cause mortality in white sturgeon. In this study, fish were acclimatized for at least two weeks prior to challenge and maintained in flow-through fresh water at 18±1.2°C. Yearling trout, yearling sturgeon, sturgeon fingerlings, koi, and blue tilapia were exposed to 5.73 x 10^5 *V. botryosa* spores/fish via intramuscular (IM) injection. Trout fingerlings, and Nile tilapia fingerlings were exposed to the same dose of *V. botryosa* via intracoelomic (IC) injection. Daily mortality was recorded throughout a 30 d post-challenge period and persistence of the fungus in the spleens of moribund and surviving fish was investigated using culture and histopathological analysis. Results showed that, yearling trout, sturgeon fingerlings, and Nile tilapia had the highest rates of *V. botryosa*-related mortalities reaching 100% mortality within 30 d post-challenge. Affected fish exhibited abnormal orientation and/or failure to maintain neutral buoyancy, emaciation, coelomic distension, exophthalmos, cutaneous erythema, and ulcerated skin. Blue tilapia and trout fingerlings were also susceptible to the fungus presenting 26.7% and 10% mortality, respectively. Yearling white sturgeon were infected without exhibiting any clinical signs of diseases or mortality during the experimental challenge. Colonies of *V. botryosa* were recovered from all exposed fish except for one of the yearling sturgeon. No control fish died, nor presented positive isolation of *V. botryosa*. Multinucleated giant cell formation was a prominent feature of the inflammatory response to *V. botryosa*. In conclusion, our results suggest that *V. botryosa* is not a host specific pathogen as it can infect and cause mortality to different fish species. Additionally, age/size of fish appears to play a role in some of the tested fish species including white sturgeon and rainbow trout. This information should be taken into account by clinicians, biologists and farmers in the development of surveillance plans and diagnostic methods for this growing industry.

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