Thursday September 6th – Gray / Palmer / Pope
Virology 6
Moderator – Jan Lovy (New Jersey Department of Environmental Protection)

1:15 PM	Virology 6	Spencer - Does Pilchard Orthomyxovirus Fill the Ecological Niche of ISAV in Tasmanian Salmonid Farming?
1:30 PM		**Hernandez - The Population Structure of Columbia River Basin Chinook Salmon *Oncorhynchus tshawytscha* and Linkages to the Landscape Ecology of Infectious Hematopoietic Necrosis Virus**
1:45 PM		Kurath - Biological Basis of Specialist and Generalist Infectious Hematopoietic Necrosis Virus in Pacific Salmon
2:00 PM		Padhi - Viruses As Biocontrol Agents for Invasive Common Carp in Minnesota
2:15 PM		Cuenca - Viral Haemorrhagic Septicaemia Virus (VHSV): Molecular Phylogenetics, Geography and Virulence
2:30 PM		Fusianto - Effective Disinfection Protocols for Megalocytiviruses
2:45 PM		Pham - VER-155008 Induced Hsp70 Proteins Expression in Fish Cell Cultures While Concurrently Impeding Replication of Two RNA Viruses

Does Pilchard Orthomyxovirus Fill the Ecological Niche of ISAV in Tasmanian Salmonid Farming ?

Emily E Spencer*, Richard Morrison, Scott Godwin, Martine Cornish, Graeme Knowles and Jeremy Carson

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Many salmonid pathogens in Australia and New Zealand appear to have evolved separately from those present in the rest of the world, presumably due to geographical isolation, limited importation of stocks and strict biosecurity measures. However, Tasmanian salmonid farms face production threats from unique pathogens that fill similar niches to the major salmonid pathogens seen in Europe and the Americas. One of these unique pathogens, pilchard orthomyxovirus (POMV), is related to infectious salmon anaemia virus (ISAV), but is distinct genetically and phenotypically. Like ISAV, POMV has a segmented, negative sense ssRNA genome and causes elevated mortalites in farmed salmon, but POMV shares less than 30% amino acid sequence with ISAV and exhibits different receptor-destroying and haemagglutination activities. POMV causes necrosis in the liver, haematopoietic and vascular tissues, and targets endothelial cells and hepatocytes. POMV has become the primary pathogen of concern for Tasmanian salmonid farmers in recent years, making the need for effective control strategies increasingly urgent. Ongoing research is focused on production of inactivated and subunit vaccines, with the aim of controlling POMV outbreaks on a long-term, sustainable basis.

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

The Population Structure of Columbia River Basin Chinook Salmon (*Oncorhynchus Tshawytscha*) and Linkages to the Landscape Ecology of Infectious Hematopoietic Necrosis Virus (IHNV)

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Chinook salmon populations of the Columbia River Basin (CRB) are genetically diverse with expressed phenotypic differences in behavioral patterns, life history, and geographical distributions. This investigation examined the epidemiological linkages between the dominant life history phenotypes observed across Chinook salmon populations of the CRB and the ecology of Infectious Hematopoietic Necrosis Virus (IHNV). Integrative data analysis of IHNV Virology, Genotyping and Surveillance records available for Chinook salmon, between the years 2000-2012, revealed intraspecific heterogeneity in the prevalence of IHNV infection in Chinook salmon of the CRB. Infection prevalence was higher in Chinook salmon of the Spring-run life history phenotype than in Chinook salmon of the Fall-run type. Observed differences in the prevalence of IHNV infection across the life history phenotypes does not appear to be driven by differences in abundance, as Fall-run Chinook salmon were more numerous than Spring-run Chinook salmon in the CRB between 2000-2012. Geostatistical analysis (ArcMap 10.4) revealed that IHNV positive cohorts of Spring-run Chinook salmon have a broader geospatial distribution within the CRB than virus positive cohorts of Fall-run Chinook salmon. Univariate analysis revealed that the majority of IHNV detections in Chinook salmon were of U genogroup virus in Spring-run fish. Controlled laboratory studies, examining the shedding kinetics of IHNV in juvenile Chinook salmon, showed that offspring of Spring-run Chinook salmon shed higher quantities of U genogroup virus than M genogroup virus. Taken together, the high prevalence of U genogroup IHNV infection in Spring-run Chinook salmon, the broad geospatial distribution of virus positive cohorts, and the high quantities of U genogroup virus shed, suggests that Spring-run Chinook salmon are closely linked to the ecology of U genogroup IHNV in the CRB. While our findings may be a result of ecologically driven differences in exposure to IHNV or inherent genetic differences in susceptibility of Spring-run fish to the virus, these data suggest biological and epidemiological relevance of the patterns observed. Additional laboratory studies are needed to better understand the epidemiological linkages between IHNV and genetically diverse populations of CRB Chinook salmon.

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

Biological Basis of Specialist and Generalist Infectious Hematopoietic Necrosis Virus in Pacific Salmon

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The fish rhabdovirus infectious hematopoietic necrosis virus (IHNV) is a major target of long-term surveillance in salmonid populations of western North America. Genotyping of IHNV field isolates has demonstrated three IHNV genogroups (U, M, and L) in North America, and field prevalence data indicates both specialist and generalist virus patterns among various genetic subgroups. In general, viruses within the U, M, and L genogroups are specialists that occur mostly in single salmonid hosts: U in sockeye salmon (*Oncorhynchus nerka*), M in steelhead and rainbow trout (*O. mykiss*), and L in Chinook salmon (*O. tshawytscha*). However, in the Columbia River Basin a subgroup within the U genogroup, designated subgroup UC, occurs in all three hosts, and thus has an unusual generalist host specificity pattern. We are interested to understand how UC viruses evolved from ancestral specialist U virus to become generalists in the Columbia River Basin, and what biological features changed to allow them to successfully infect multiple hosts in the field. We are conducting a series of in vivo infection experiments using 12 IHNV strains including three each from the ancestral U (now referred to as the UP subgroup to distinguish it from UC), M, L, and UC subgroups. These viruses are being tested in sockeye salmon, steelhead trout, and Chinook salmon to quantify variations in virulence, infectivity, in-host replication, shedding kinetics, persistence, and stimulation of protective immunity. To date assays of virulence have confirmed varied host-specificity phenotypes that mirror the observed specialist and generalist field prevalence patterns. Although the generalist UC strains have moderate virulence in all three hosts, they do not have higher virulence than the ancestral UP strains in Chinook salmon or steelhead trout. Thus the high field prevalence of UC viruses in Chinook salmon and steelhead trout represents a major gain in fitness without increased virulence at the biological virus:host level. In contrast, UC viruses have reduced virulence relative to UP strains in the ancestral sockeye salmon host, as predicted by specialist-generalist theory. By quantifying the generalist phenotype for multiple biological traits we will define biological basis of generalism in IHNV. Results will be used to estimate R_0 for each virus:host combination, and to inform a landscape virus transmission model for IHNV in the Pacific Northwest.

Conference session designation: (Virology)
Presentation format: (Oral)

Viruses as Biocontrol Agents for Invasive Common Carp in Minnesota

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Common carp, *Cyprinus carpio*, are one of the most ecologically devastating aquatic invasive species in the world. Despite significant efforts to control this highly prolific and problematic species, managers have no options that are i) species specific, ii) highly lethal, iii) environmentally safe, and iv) cost effective. While fish health managers often aim to prevent the spread of viral pathogens, scenarios are conceivable where a virus could meet the aforementioned criteria for carp control. Based on this idea, we have conducted a survey in search of viruses that could be used as an alternative control agent for invasive common carp in Minnesota. Sampling of live healthy carp and dead carp collected from wild fish mortalities was conducted between June to October 2017 to obtain an overall viral population present in Minnesota through molecular methods such as PCR and next generation sequencing (NGS). Special emphasis was given for the detection of Spring Viremia of carp virus (SVCV), Cyprinid herpesvirus 3 (CyHV-3) and Carp Edema Virus (CEV) with targeted PCR's. During the survey period, species-specific mass mortalities of common carp were reported in a cluster of eight lakes in Le Sueur and Waseca Counties. Clinical signs of dead common carp included severe gill necrosis, dermal hemorrhaging, epithelial sloughing and discoloration, as well as sunken eyes and facial tissue. Ubiquitous presence of CyHV-3 in all eight lakes was confirmed through qPCR. Interestingly, out of eight CyHV-3 positive lakes two had confirmed co-infection with CEV. These two lakes had higher mortality rates as compared to the other six. Further, gel-based PCR and Sanger sequencing confirmed the presence of these viruses. The whole genome of CyHV-3 was determined with Illumina MiSeq from one lake. The genome was 295,016 bp in length and had higher identity to the European variant of CyHV-3 (KX544847). The results are suggestive of existence of CyHV-3 and CEV to wild common carp in the state of Minnesota that have the potential to cause future outbreaks. The use of viruses as biocontrol agents requires a vigorous in-depth scientific exploration since protecting native species and promoting pure ecosystem health is our ultimate priority.

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

Viral Haemorrhagic Septicaemia Virus (VHSV) : Molecular Phylogenetics, Geography and Virulence.

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Viral haemorrhagic septicaemia (VHS) is an important viral fish disease, widespread all over the northern hemisphere. The causative agent is VHS virus (VHSV), a rhabdovirus with a negative sense, single strand RNA genome of about 11 kb. One of the most intriguing characteristics of VHSV is its ability to cross species boundaries, not only to cause sporadic infection, but also to create stable intra-species transmission in novel fish species. Indeed, since its first isolation from cultured rainbow trout in 1963, VHSV has been found in more than 90 different fish species in freshwater and marine environments.

Phylogenetic analyses based on the sequence of the glycoprotein (G-gene) VHSV have clearly identified four main genotypes and eight subtypes. Different clades in the phylogeny show a strong geographic differentiation and, at a lesser extent, host specificity. In addition, virulence to certain fish species seems to be genotype specific. Phylogenetic analyses shown that fresh water farmed rainbow trout isolates have a marine ancestry, and that occasional jumps to fresh water from marine environments could occur.

Since discovery of the virus, our laboratory has constructed the largest repository worldwide of VHS viruses, including epidemiological data, genetic sequences and phenotypic characterization of virulence to rainbow trout. We use this platform for two inter-related studies:

First, we present phylogenetic reconstructions based on >100 full genome isolates of VHSV, including representatives of all major groups. The aim of this analysis is not only gain a better understanding to the evolution of VHSV, but also to gain insight in some of the genomic regions involved in virulence to rainbow trout. To do so, 70 isolates were tested for virulence to rainbow trout in vivo, and data mapped into the phylogeny.

Second, we constructed the most complete phylogenetic analysis of VHSV so far, including full-length G-gene sequences for more than 800 isolates. Based on this analysis we are proposing a new classification where sub-genotype IVd is included, as well as minor revisions within genotype Ia. Phylogenetic data combined with epidemiological data for the different isolates will help to better understand the evolutionary history of VHSV in marine and fresh water environments.

Conference session designation: **(** Virology)
Presentation format: **(** Oral)

Effective Disinfection Protocols for Megalocytiviruses

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The genus *Megalocytivirus* (MCV) includes *Infectious spleen and kidney necrosis virus* (ISKNV) and red seabream iridovirus (RSIV) which are listed by the World Organization for Animal Health (OIE). These viruses cause mass mortalities in both marine and freshwater aquaculture. MCV has a broad host range and can be present as a persistent subclinical infection in some fish. Australia is considered free from MCV and important aquaculture and wild fisheries are at risk. Consequently, biosecurity measures are implemented to ameliorate transmission pathways through imported ornamental fish which include certification of freedom from infection with MCV. Evaluation of practical and cost effective disinfection protocols suitable for recirculating aquaculture facilities is an important aspect of the biosecurity plan to facilitate eradication in the event of early detection of an outbreak.

An authentic sample matrix was prepared by *in-vivo* amplification of ISKNV in Murray cod (*Maccullochella peelii*) and the biological load of a clarified tissue homogenate was standardized by addition of 10% v/v foetal bovine serum. In the absence of a cell culture system for ISKNV, a bioassay was used to assess infectivity after disinfection. A cellulose membrane buffer exchange device was used to remove residual disinfectants before intraperitoneal injection of juvenile Murray cod. Fish were maintained in 100L aquaria at 23°C and observed for clinical signs over 14 days. Each bioassay was conducted in duplicate tanks with 18 fish per tank. The appropriate array of positive and negative control samples was also assayed. A positive assay was defined by an increase in ISKNV DNA quantified by qPCR in any fish from a subsample of challenged fish collected at 7d or those remaining at 14d. Negative biosassays were defined by the absence of ISKNV DNA in all fish at the both sampling times.

The bioassay provided a sensitive test for infectious ISKNV. Clinical disease and amplified viral DNA was detected after injection of a dilute positive control, indicating greater analytical sensitivity compared to qPCR. Further, the system was used to demonstrate that ISKNV can remain infectious in aquarium water without fish for at least 48 h at 25°C. Effective disinfection measures included: heating to 65°C for 20 min; pH 3; pH 11; 1% Virkon™; 1000 ppm sodium hypochlorite and benzalkonium chloride at the recommended concentration and contact time. These data can be interpreted to provide effective disinfection protocols for MCV in a wide variety of disease control scenarios.

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

VER-155008 Induced Hsp70 Proteins Expression in Fish Cell Cultures While Concurrently Impeding Replication of Two RNA Viruses

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The heat-shock protein 70 (Hsp70) inhibitor, VER-155008 (VER), was explored as a potential antiviral agent for two RNA viruses important to fish aquaculture, viral hemorrhagic septicemia virus (VHSV) and infectious pancreatic necrosis virus (IPNV). Studies were done at a temperature, 14 °C, and with cell lines commonly used to propagate these viruses. These were respectively EPC from fathead minnow for VHSV and CHSE-214 from Chinook salmon embryo for IPNV. Additionally, both viruses were studied with the Atlantic salmon heart endothelial cell line ASHe. For both VHSV and IPNV, 25 μM VER impeded replication. This was seen as delays in the development of cytopathic effect (CPE) and the expression of viral proteins, N for VHSV and VP2 for IPNV, and as less production of genome copy number and of viral titre. As VER inhibits the activity of Hsp70 family members, these results suggest that VHSV and IPNV utilize one or more Hsp70s in their life cycles. Yet neither virus induced Hsp70. Surprisingly VER alone induced Hsp70, but whether this induction modulated VER's antiviral effects is unknown. Exploring this apparent paradox in the future should improve the usefulness of VER as an antiviral agent.

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