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

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

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

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

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

Review of Epizootic Ulcerative Syndrome in Louisiana

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

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

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

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

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

Going Viral Against Bacteria: Implications for Phage Therapy in Aquaculture

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

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

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

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

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

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

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

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

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

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

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

Conference Session Designation: (Bacteriology / Mycology)
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Characterization of *Streptococcus Iniae* Isolates from Diseased Wild and Farmed Fish Across the North American Continent

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

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