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

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

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

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

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

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

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

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

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

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

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

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

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

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

Emergence of Mucormycosis Among Marine Mammals in Pacific Northwest

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Refinement of *Moritella Viscosa* Challenge Model End Points

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

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

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

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

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