Thursday September 6th – Tilly / Tupper
Genomic Applications 1 & 2
Moderator – Attila Karsi (Mississippi State University)

9:30 AM	Genomics 1	Polinski - The Consequences of IHNV on Resistant and Susceptible Sockeye Salmon – Assessing Transcriptomics to Physiological Performance
9:45 AM		**Xue - Functional Genomics Analyses of Molecular Mechanisms Involved in Atlantic Salmon Responses to the Bacterial Pathogen *Piscirickettsia salmonis***
10:00 AM		Umasuthan - Transcriptomic Response of Atlantic Salmon Fin to Sea Lice *Lepeophtheirus salmonis* Infestation
10:15 AM		Saleh - Ichthyophthiriosis: Insight Into Common Carp Immune Response by Quantitative Shotgun Proteomics
10:30 AM		**Refreshments**
10:45 AM	Genomics 2	**Ignatz - Immune and Stress Response of Growth Hormone Transgenic Female Triploid Atlantic Salmon (*Salmo salar*) Reared at Three Temperatures Following Intraperitoneal Polyriboinosinic Polyribocytidylic Acid Injection**
11:00 AM		**Walsh - Use of Molecular Techniques and Water Chemistry to Understand Fish Health in the South Branch, Potomac River, West Virginia**
11:15 AM		**Le - Genome Sequence and Phylogenetic Relationship of *Nocardia seriolae* Strains Isolated From Fish Farms in Vietnam**
11:30 AM		Lawrence - Comparative Genomics Reveals the Species-Based Diversity of *Edwardsiella* Genus Members
11:45 AM		Thune - You Can Never Have to Many Mutants

The Consequences of IHNV on Resistant and Susceptible Sockeye Salmon – Assessing Transcriptomics to Physiological Performance

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Aquatic Rhabdoviruses are globally significant pathogens associated with disease in both wild and cultured fish. Infectious hematopoietic necrosis virus (IHNV) is a Rhabdovirus that causes an internationally regulated disease (IHN) in most species of salmon. Sockeye salmon are a keystone species in the North Pacific and natural host for IHNV. Yet not all naïve salmon exposed to IHNV develop disease pathology, and the mechanisms by which some individuals are able to evade or rapidly clear infection following exposure are poorly understood. Through RNA-sequencing, we evaluated transcriptomic changes in Sockeye salmon following IHNV exposure and/or infection. Both waterborne exposure and acute infection had dramatic but discrete effects on the Sockeye salmon head kidney transcriptome and included the differential regulation of metabolic, acute phase and cell boundary processes. We then applied an integrated respiratory assessment paradigm (IRAP) to evaluate the respiratory capabilities and capacity of resistant and susceptible Sockeye following an intra-peritoneal injection challenge with IHNV. This demonstrated that primary resistance to IHNV does not compromise the physiological respiratory performance of the fish or ability to tolerate acute hypoxia. Taken together these findings suggests that primary resistance of naïve fish to IHNV may involve global responses that encourage a general state of reduced cellular signalling rather than promoting disseminated antiviral responses and that these primary resistance strategies (which encompass global transcriptomic changes) do not compromise the fish's general physiological performance or ability to tolerate physiological (i.e. hypoxic) stress.

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

Functional Genomics Analyses of Molecular Mechanisms Involved in Atlantic Salmon Responses to The Bacterial Pathogen *Piscirickettsia salmonis*

Xi Xue*[1], Jennifer R. Hall[2], Albert Caballero-Solares[1], Eva Jakob[3], Renate Kvingedal[4], Christopher Hawes[3], Jorge Pino[3], Juan Sepulveda[3], Richard G. Taylor[4], Javier Santander[1] and Matthew L. Rise[1]

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Piscirickettsiosis, caused by the intracellular Gram-negative pathogen *Piscirickettsia salmonis*, is one of the most economically important diseases of salmonids. Atlantic salmon (*Salmon salar*) parr were infected with the EM-90-like *P. salmonis* isolate to investigate the genes and molecular pathways involved in piscirickettsiosis. All fish of the challenge group were intraperitoneally injected with 0.1 ml of bacterial inoculum (inoculum titer 10^0.83 $TCID_{50}$/ml), while fish in the control group were injected with 0.1 ml of a control medium that was used to prepare the bacteria inoculum (minimum essential medium, MEM). Mortalities began 20 days post-injection (DPI), and cumulative mortality reached ~30% by the end of the trial. Expression of four anti-bacterial biomarker transcripts (CAMPb, HAMPa, IL8a, sTLR5a), as well as pathogen level, was initially measured using qPCR on head kidney samples. The transcript expression of these genes except HAMPa, as well as pathogen level, peaked at 21 DPI. Multivariate statistical analyses (e.g. PCA) of qPCR data were conducted to classify the fish into low and high infection groups. Five fish from each group (mock control, low and high infections) at 21 DPI were selected for transcriptome profiling using Agilent 44K microarrays. The Significant Analyses of Microarray (SAM) approach identified a total of 3242 differentially expressed features when comparing both infected groups with the control group (FDR=0.01). Gene ontology (GO) enrichment analysis of *P. salmonis*-responsive biomarkers identified a large number of overrepresented terms, many of which related to immune system process, response to bacterium, iron ion hemostasis, redox hemostasis, leukocyte activation and antigen presentation. Key *P. salmonis*-responsive genes will be evaluated with RNA templates collected from multiple time-points by qPCR to understand the dynamic of salmon immune response during the infection process. The findings from this study will help provide insight into the molecular mechanisms involved in salmon response to *P. salmonis* infection, and may aid in the development of anti-piscirickettsial therapeutics.

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

Transcriptomic Response of Atlantic Salmon Fin to Sea Lice *Lepeophtheirus salmonis* Infestation

Navaneethaiyer Umasuthan[1]*, Xi Xue[1], Albert Caballero-Solares[1], Jillian D. Westcott[2], Zhiyu Chen[2], Mark D. Fast[3], Barbara F. Nowak[4], Richard G. Taylor[2] and Matthew L. Rise[1]

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Sea lice (*Lepeophtheirus salmonis*) are ectoparasitic copepods that cause severe economic damage to the Atlantic salmon aquaculture industry globally. Since previous studies have proven that the attachment sites of early life stages of sea lice are mainly the fins, gene expression response of Atlantic salmon fin tissue may shed light on host immunity against sea lice infection. To better understand this, we sampled tissue from louse-attachment (Att) and non-attachment (NA; adjacent to Att) sites of the fin of salmon 8 days post-infestation (laboratory-reared louse at chalimus stage) for gene expression studies complemented with our ongoing histological analyses. Fin samples collected from fish prior to infestation served as the control group (C). A salmon 44K microarray experiment was used to investigate the sea louse-derived changes in the fin transcriptome and to screen potential fin-specific biomarkers of sea lice infestation based on which a multiplex qPCR is aimed to be developed. Significant Analyses of Microarray (SAM) approach identified a total of 2271 differentially expressed genes (DEGs) when three different groups (C, NA and Att) were compared [FDR (false discovery rate) 0.05]. Direct comparison of Att and NA groups revealed that 37 genes showed significant alteration in their transcription. Gene ontology (GO) term enrichment analysis of all DEGs identified six main functional groups related to extracellular matrix (e.g. matrix metalloproteinases, *mmp-2*, *-9*, *-13*; and *timp2*), stress (e.g. *gstA, gpx7, prx*), immunity (e.g. *il8, lect2, ccl20, rsad2*), wound healing (e.g. *mmp13, fn1, lgals1*), inflammation (e.g. *1l1b*), and Fe^{2+}/heme/oxygen transport (e.g. *hba, hbb*) that were affected. These preliminary data could improve our understanding of the molecular events underlying early stages of Atlantic salmon infestation by *L. salmonis.*

Conference Session Designation: (Parasitology Sea Lice – Ectoparasites)
Presentation Format: **(** Oral)

Ichthyophthiriosis: Insight into Common Carp Immune Response by Quantitative Shotgun Proteomics

Mona Saleh[1], Gokhlesh Kumar[1], Saleh Al-Quraishy[2] and Mansour El-Matbouli[1]

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Ichthyophthirius multifiliis, a ciliated ectoparasitic protozoan, causes ichthyophthiriosis and leads to considerable economic losses to the aquaculture industry. Understanding the fish immune response and host-parasite interactions could support disease management and control. Fish skin mucus is the first line of defence against infections through the epidermis. Yet, the common carp, *Cyprinus carpio*, protein-based defence strategies against infection with *I. multifiliis* at this barrier is unknown. We investigated the skin mucus proteome of common carp at 1 day and 9 days post-exposure with *I. multifiliis*. Using nano liquid chromatography tandem mass spectrometry (nano-LC ESI MS/MS), the abundance of 44 proteins was found to be significantly different in the skin mucus samples between exposed and non-exposed carp. Proteins with increased abundance values were mainly involved in signal transduction, metabolism, immune response and stress, whereas proteins with decreased values were mainly structural. The extracellular matrix proteins such myosin, and keratin showed increased abundance values. The analysis revealed increased abundance values of epithelial chloride channel protein, galactose-specific lectin nattection, high choriolytic enzyme 1 (nephrosin), lysozyme C, granulin-3 and protein- glutamine gamma-glutamyltransferase proteins. Besides, we identified novel proteins with yet unknown function in common carp following penetrating injuries such as olfactomedin 4, lumican, dermatopontin and papilin. This analysis, therefore, represents a key for the search for potential biomarkers, which can help in a better understanding and monitoring of interactions between carp and *I. multifiliis* and gives insight into the important role that skin mucus plays in protecting fish against parasites.

Conference Session Designation: Parasitology General
Presentation Format: Oral Presentation

Immune and Stress Response of Growth Hormone Transgenic Female Triploid Atlantic Salmon (*Salmo Salar*) Reared at Three Temperatures Following Intraperitoneal Polyriboinosinic Polyribocytidylic Acid Injection

Eric Ignatz *[1,2], Laura Braden[1], Tiago Hori [3], Dawn Runighan [1], Tillmann Benfey [4], Mark Fast [5], Jillian Westcott [2] and Matthew Rise [6]

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AquAdvantage® salmon (growth hormone transgenic female triploid Atlantic salmon) offer aquaculture producers a faster growing alternative to conventional salmon. In order to determine optimal rearing conditions for their commercial production, a study was conducted to examine the effect of rearing temperature (10.5°C, 13.5°C, 16.5°C) on the immune and stress response of AquAdvantage® salmon. When each temperature treatment group reached an average weight of 800 g, a subset of fish was intraperitoneally injected with either polyriboinosinic polyribocytidylic acid (pIC), a known immunostimulant, or an equal volume of sterile phosphate-buffered saline (PBS). Blood and head kidney samples were collected before injection and 6, 24 and 48 hours post-injection (hpi). Transcript abundance of 7 immune-related genes (*IFN-γ, ISG15a, RSAD2, LGP2, STAT1b, TLR3, mxb*) was measured by reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) on all head kidney samples. Blood plasma cortisol levels from samples collected pre-injection and from pIC and PBS at 24 hpi were quantified by ELISA. Target gene activation was observed at 24 hpi, with transcript levels starting to return to baseline after 48 hours in pIC-injected fish. Overall, rearing temperature did not appear to have a significant effect on immune-related transcript expression in response to pIC. No significant differences were found between any comparisons of rearing temperature and treatment based on cortisol response. This information provides insight into the relationships between rearing temperature and response to an immunostimulant in AquAdvantage® salmon for use in commercial applications.

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

Use of Molecular Techniques and Water Chemistry to Understand Fish Health in the South Branch, Potomac River, West Virginia

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Since 2005 when fish kills were first observed in the South Branch Potomac River in West Virginia, smallmouth bass have been routinely sampled for fish health assessments. Pathogens, parasites, largemouth bass virus, testicular oocytes, and skin lesions have all been documented in smallmouth bass in this area. However, no one factor has been associated with mortality. It is likely that these cumulative stressors have an immunomodulatory effect, particularly in the spring during spawning. In 2013-present, a more comprehensive sampling effort was initiated to include changes in transcript abundance, water and sediment chemistry, immune function, and various other endpoints. Bass were sampled for histopathology and RNA-Sequencing during the spring prior to spawning and in the fall during recrudescence. Liver and testes were sampled and partial transcriptomes were assembled in order to identify genes of interest that may be associated with pathological alterations or contaminant exposure. Nanostring nCounter® technology was used to identify changes in transcript abundance of genes involved in immunomodulation, oxidative stress, and chemical detoxification. Water was sampled monthly, bi-weekly, and during storm events for pesticides, hormones, phytoestrogens, and total estrogenicity. Sediment was sampled in the spring and fall for pesticides, hormones, and phytoestrogens. The integration of multiple data types may help explain the observed disease symptoms observed in smallmouth bass in this area.

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

Genome Sequence and Phylogenetic Relationship of *Nocardia Seriolae* Strains Isolated From Fish Farms in Vietnam

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Nocardiosis in fish is an infectious, systematic, granulomatous disease caused by infection with *Nocardia seriolae*, a Gram-positive, facultatively intracellular bacterium. This pathogen affects a wide range of marine and fresh water fish species at different age groups or sizes and can result in massive mortalities for infected farms. In Vietnam, nocardiosis is currently considered one of the leading threats to the sustainable aquaculture development of commercially important pompano fish (*Trachoditus blochii*) as outbreaks of the disease have caused significant economic losses for many farms throughout the country. To obtain an insight into the biology, origin, evolution and epidemiology of the pathogen, the genetic relatedness of strains were analysed using pulsed-field gel electrophoresis (PFGE) and Illumina NextSeq 500 whole genome sequencing (WGS). PFGE of 20 strains digested with *Xba*I were classified into one pulsotype while two pulsotypes with a similarity of > 80% were identified by *Ase*I digestions, suggesting close genetic relatedness of these strains. Consistent with PFGE, phylogenomic analysis of seven Vietnamese strains and all currently available *N. seriolae* genomes (*n*=7) using whole-genome-derived single-nucleotide polymorphisms (SNPs) indicated that the Vietnamese strains fall into two highly clonal genotypes that differed by just 1-2 SNPs and were irrespective of the geographic regions where they were isolated. The Vietnamese strains share a common ancestor with strains isolated from other countries in the same region, although they differed from the next closest known strain in Japan by 265 SNPs. These results suggest that the Vietnamese *N. seriolae* strains have been recently introduced to this country, although the precise origin is not yet known. The bacterium encodes a large genome of 7,785,433 bp, a G + C content of 68.2%, 7,420 predicted coding DNA sequences and 77 transfer RNA sequences. It was also found that the bacterium harboured genes coding for factors relating to virulence, pathogenicity and host defence mechanisms such as iron uptake systems, resistance to antibiotics and toxic compounds, and the biosynthesis of hemolysins, adhesins and proteases. These findings provide novel information about the Vietnamese *N. seriolae* population that is essential for development of new drugs and vaccines, and ultimately, the move toward nocardiosis-free aquaculture.

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

Comparative Genomics Reveals the Species-Based Diversity of *Edwardsiella* Genus Members

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The genus *Edwardsiella* is a member of the family *Enterobacteriaceae*, and it consists of five species isolated from fish, reptiles, and mammals (including humans). Some members of this genus cause disease in U.S. farm-raised catfish as well as other aquaculture industries in Asia and central America. To determine the genetic basis underlying the diversification of *Edwardsiella* genus members, we conducted genome sequencing of 22 *Edwardsiella* strains. In our comparative analysis, we included genome sequences from other available *Edwardsiella* genus members deposited in NCBI (National Center for Biotechnology Information). To analyze species diversity, we applied ANI (average nucleotide identity) and core genome comparison to construct phylogenetic trees. Functional analysis revealed that type 3 secretion system (T3SS) is not present in *Edwardsiella tarda* and *hoshinae*, whereas type 1 secretion system (T1SS) and type 5 secretion system (T5SS) are encoded by all the genus members. Interestingly, type 4 secretion system (T4SS) is encoded by most of the evaluated *E. ictaluri* and some of the other *Edwardsiella* genus members. *E. ictaluri* genomes tend to carry more types of insertion sequences and higher numbers compared to other species. Our findings reveal the utility of comparative genomics to elucidate genetic diversity and potentially enable improved diagnostics for *Edwardsiella* species. These findings also impact vaccine development for the species and reveal differences in potential pathogenic mechanisms.

Conference Session Designation: (Genomic Applications in Aquatic Animal Health)
Presentation Format: (Oral)

You Can Never Have to Many Mutants

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In 1961 Stanley Falkow showed that the ability to utilize lactose could be transferred from *Salmonella* strain ST-2 to many strains of lactose negative *Escherichia*, *Salmonella*, and *Shigella* via an episome. He further demonstrated that similar genetic elements, now called plasmids, carry and transfer antibiotic resistance genes. The potential for studying pathogenesis was realized when he used a plasmid to isolate a gene encoding a toxin from a diarrhea causing *E. coli*. That humble beginning revolutionized the study of bacterial pathogenesis and led to a variety of protocols to mutagenize bacterial pathogens and subsequently evaluate the mutant phenotype to assess the function of the gene in question. The mission here today is to discuss several procedures for making mutant strains of bacteria to assess for various aspects of pathogenesis, including deletion/insertion mutagenesis, random transposon mutagenesis, targeted transposon mutagenesis, targeted transfer mutagenesis, and site specific mutagenesis.

Conference Session Designation: (Genomic Applications in Aquatic Animal Health)
Presentation Format: **(** Oral)