Monday September 3rd – Langevin / Cartier
Microbiome 1 & 2
Moderator - Matt Griffin (Mississippi State University)

9:30 AM	Microbiome 1	Marsh - Microbiome of The Sturgeon Egg
9:45 AM		Arias - The Channel Catfish (*Ictalurus Punctatus*) Microbiome
10:00 AM		**Ahasan - Fecal Microbiota of Wild Captured And Stranded Green Turtles on The Great Barrier Reef**
10:15 AM		**Pathirana - The Role Of Pacific Oyster *Crassostrea Gigas* Microbiome In The Pathogensis of Ostreid Herpesvirus-1 Infection**
10:30 AM		**Refreshments**
10:45 AM	Microbiome 2	Amthor - Efficacy Of A Bath Probiotic Application Before Seawater Exposure In Atlantic Salmon
11:00 AM		Fauske - Compatability of Application of A Probiotic Treatment In Anesthetic Bath Using Benzocaine or Tricaine Methanesulfonate (Tms)
11:15 AM		**Parker-Graham - Effect of Oxytetracycline Treatment on The Gastrointestinal Microbiome of Critically-Endangered White Abalone *Haliotis sorenseni* Treated For Withering Syndrome**
11:30 AM		Open
11:45 AM		Open

The Microbiome of the Sturgeon Egg

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Sturgeons are an imperiled group of fishes and conservation efforts have focused on all life stages but in particular during early development where losses are high. To better understand egg mortality caused by microbes, our work has investigated the assembly of the egg's microbiome. The egg, which is essentially sterile when extruded into river water during spawning, becomes coated within 15 minutes with a diverse collection of bacteria dominated by *Pseudomonas*, *Aeromonas*, *Geobacillus* and *Bacillariophyta.* At 135 minutes the community shifted modestly and was dominated by *Pseudomonas*, *Aeromonas*, *Geobacillus*, *Comamondacea* and *Burkholderia*. After 24 hours exposure, the community shifted significantly and was dominated by *Comamonadaceae*, *Rheinheimera*, *Undibacterium*, *Bacillariophyta*, *Rhodobacteraceae* and *Methylophilus*. Differences were also detected between different mating pairs of sturgeon, indicating that variation in the egg surfaces can select for different bacterial populations. Community analysis with 18S revealed ciliates, algae and *Saprolegnia* at low concentrations while analysis targeting the ITS region identified 30 genera in 20 sampled eggs. *Aureobasidium*, *Cryptococcus*, *Neobulgaria*, *Pythium* and two unidentified groups dominated.

Disinfection of eggs with formalin or peroxide to quench microbial pathogenesis is common practice in hatcheries. These treatments reduce the bacterial load but also alter the community. Both peroxide and formalin treated eggs had increases in *Flavobacterium* species and losses to *Sphaerotilus* and *Rheinheimera* populations. Community shifts are also detected when eggs are supplemented with putative probiotics or sugars that compete with glycan binding motifs on the surface of the egg during fertilization. The addition of an *Acidovorax* sp. isolated from the egg reduced mortality by 25% and the addition of glucose and galactose shifted the communities in statistically significant ways, diminishing the attachment of select aquatic populations. In addition, we have isolated and characterized over 400 bacterial strains from stream-captured sturgeon eggs. The isolates were dominated by *Pseudomonas* and *Aeromonas*, of which 65% were positive for extracellular cellulose and 52.3% positive for ß-hemolysin. Several isolates have antimicrobial and antifungal activities and have been considered for use as a probiotic.

These investigations point to possible interventions during the assembly of egg- associated microbial communities that will reduce mortality and the use of harmful chemicals in the hatchery. Controlling the community assembly early in the process appears critical to altering the phylogenetic structure of the community and improving mortality rates and larval robustness.

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

The Channel Catfish (*Ictalurus punctatus*) Microbiome

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Nowadays it is well accepted that the community of microbes occupying the gastrointestinal (GI) tract of vertebrates (gut microbiome) plays a critical role in host development, physiology, and health. One of the main reasons for studying the gut microbiome in fishes is the idea that those communities can be modified to improve host health. A prerequisite to this approach is the characterization of the gut microbiome of the species of interest. Many factors contribute to the composition of the gut microbiome in vertebrates including host genetics, environment, and nutrition among others. Discovering a core microbiome, i.e. members of the microbial community present in all individuals of a species, has been a primary goal for many researchers interested in understanding gut microbial communities. Our group has been studying the gut microbiome of channel catfish (*Ictalurus punctatus*) for several years in order not only to identify its core microbiome but also to characterize how the gut microbial community assemblies during ontogenesis. Channel catfish (*Ictalurus punctatus*) is the top farmed raised fish in United States with a production of more than 750 million pounds per year. The demand for farmed catfish is strong and growing worldwide. However, the US catfish industry faces losses during the entire commercial cycle due primarily to infectious diseases. In many cases, bacterial diseases affecting channel catfish are common opportunistic pathogens that fail to cause disease in healthy hosts. In order to elucidate if fish rearing under intensive conditions displayed an altered gut microbiome, we performed a series of studies that assessed changes in the gut microbiome of fish exposed to medicated feed, vaccination, and mechanical injury (some of those experiments were carried out in the animal model zebrafish *Danio rerio*). We utilized High throughput Illumina MiSeq of the 16S rRNA V4 region to characterize the gut microbiome.

Our results showed that the gut microbiome of hatchery-reared channel catfish was dominated by Firmicutes (~50% of the community) followed by Fuseobacteria (~25%). This differs from what we have reported previously in wild catfish in which >90% of their gut community was comprised of Fuseobacteria. As expected, the administration of medicated feed containing the antibiotic florfenicol elicited a drastic shift in the gut microbiome even after fish resumed normal feeding. The gut microbiome of treated fish experienced a significant decrease in diversity and an overall increase in Proteobacteria, which are typical traits of a dysbiotic gut. Most importantly, dysbiotic fish were more susceptible to infection by an opportunistic pathogen (*Aeromonas hydrophila*) than control groups. By contrast, vaccination practices did not have a significant effect on the gut microbiome (or on the skin and gill microbiomes). Finally, and this was an unexpected result, mechanical injury (adipose fin clipping) did produce a significant change in the gut community. The 'gut-brain axis' has been the subject of many studies in humans and mice and our results suggest that a similar pathway exists in channel catfish by which the gut microbiome can sense and react to external stimuli or assaults not directly infringed upon the gut microbiome.

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

Fecal Microbiota of Wild Captured and Stranded Green Turtles on the Great Barrier Reef

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Green turtle (*Chelonia mydas*) is an endangered marine herbivore that breaks down food particles, primarily sea grasses, through microbial fermentation. However, the microbial community and its role in health and disease are still largely unexplored. In this study, we investigated and compared the fecal bacterial communities of wild-captured green turtles to stranded turtles by PCR amplification of a hypervariable region (V1-V3) of the bacterial 16S rRNA gene**.** A total of 12 samples were sequenced using next generation high-throughput sequencing technology on an Illumina MiSeq platform. At a phylum level, Firmicutes predominated among wild-captured green turtles, followed by Bacteroidetes and Proteobacteria. In contrast, Proteobacteria (Gammaproteobacteria) was the most significantly dominant phylum among all stranded turtles, followed by Bacteroidetes and Firmicutes. In addition, Fusobacteria was also significantly abundant in stranded turtles. No significant differences were found between the wild-captured turtles from two different locations. At a family level, 25 of the 53 families were identified in both the wild-captured and stranded green turtles, while 14 families were found only in stranded turtles. At the OTU level, 256 (48.7%) of the total OTUs (>1% abundance) were shared between the wild-captured groups of turtles, while absent in stranded turtles. The predominance of *Bacteroides* in all groups indicates the importance of this bacteria in turtle gut health. In terms of microbial diversity and richness, wild-captured green turtles showed the highest microbial diversity and richness compared to stranded turtles. The marked differences in the bacterial communities between wild-captured and stranded turtles suggest the possible dysbiosis in stranded turtles in addition to potential causal agents.

Conference Session Designation: (Microbiomes)
Presentation format: (Oral)
Student presentation: (Yes)

The Role of Pacific Oyster (*Crassostrea Gigas*) Microbiome in the Pathogenesis of *Ostreid Herpesvirus-1* Infection

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Mass mortality disease outbreaks have caused severe economic loss in the global Pacific oyster industry. *Ostreid herpesvirus-1* (OsHV-1) has emerged as an important cause of disease outbreaks, yet the outcome of this infection is impacted by many factors. A polymicrobial pathogenesis may be associated with mass mortality outbreaks associated with OsHV-1 including the influence of the commensal microbiota of oysters. As filter-feeders in variable estuarine environments, oysters host a microbiota that is influenced by the environment. Changes in the microbiome provide an indirect pathway by which the environment can alter susceptibility to disease and might explain some of the variability in mortality caused by OsHV-1. This study aimed to: (1) compare the microbiome of Pacific oysters from a common hatchery but grown in geographically distinct estuaries; and (2) evaluate changes in the microbiome with particular reference to *Vibrio* spp., during progression of an experimental OsHV-1 infection. Pacific oysters sourced from a single hatchery were grown by commercial farming methods in three geographically distinct estuaries. The oysters (10-16 months of age) were acclimated to the laboratory and challenged with a measured dose of OsHV-1. Samples were collected: A) before OsHV-1 challenge; B) soon after OsHV-1 challenge; C) from moribund oysters; D) from survivors 7 days after OsHV-1 challenge and; E) from unchallenged control oysters at the end of the experiment. Total bacterial, OsHV-1 and *Vibrio* genomic DNA were quantified from each sample, using real-time PCR assays. The bacterial community composition in oysters was identified by sequencing the bacterial 16S rRNA gene and the relative abundance, diversity and evenness of bacterial families were calculated. Non-metric multidimensional scaling was used to visualize the dissimilarity in bacterial community structures between samples, and was analysed using one-way permutational multivariate analysis of variance (PERMANOVA). The initial diversity and evenness of bacterial families was different for oysters grown in the three estuaries. Mortality after OsHV-1 challenge was also variable between the batches. A difference in relative abundance of bacterial families and an increase in diversity, after the viral challenge ($p<0.05$) occurred for oysters from two estuaries. In contrast, the same bacterial community structure was maintained by oysters challenged with a negative control and those that survived the viral challenge, for two sites of origin. Both OsHV-1 and *Vibrio* DNA concentration was higher in dead oysters compared to live oysters. A strong correlation was observed between the OsHV-1 DNA load and *Vibrio* in OsHV-1 infected oysters. In conclusion, the bacterial community composition in oysters was different for each geographic site at which they were grown. The bacterial community changed with the outcome of OsHV-1 challenge. A potential opportunistic role of *Vibrio* spp. in the disease associated with OsHV-1 was demonstrated. This study provided insights on the potential for different estuarine environments to shape the Pacific oyster microbiome and how different commensal bacterial populations are associated with different outcome of OsHV-1 infection.

Conference session designation: (Microbiomes)
Presentation Format: **(** Oral)
Student Presentation: (Yes)

Efficacy of a Bath Probiotic Application before Seawater Exposure in Atlantic Salmon

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This laboratory study was undertaken to evaluate and verify significant results from large scale field trials with probiotic bath treatment on weight gain, survival and wound development in Atlantic salmon with exposure to natural water borne challenge *Moritella viscosa* and *Aliivibrio wodanis*. Nine (9) weeks before seawater entry, duplicate tanks of PIT tagged fish (weight range 40-60 g, n=200 per group) were bathed in three dilutions of selected probiotic strains of *Aliivibrio* species or no bacteria as a control in brackish rearing water containing 25 ppt salt. The treatment was applied for 30 minutes as a static bath and was ended by a return to normal water flow.

During the acute phase of the outbreak with *M. viscosa* a significant reduction in the mortality of fish in the 1/100 treatment group was observed. Mortality observed 9-45 days after seawater introduction resulted 40% and 32% loss in the control groups, and 23.4% and 23.4 % in the treated groups. The relative percent survival was of 35% in the reduction of impact of acute infection. In contrast, there was no significant reduction in overall survival in the groups treated with 1/600 or 1/1000 dilution of the treatment seeding. To evaluate the possibility of an immunological effect, we analyzed blood plasma samples from treated and control fish. The result was that the anti-*M. viscosa* titers were inversely proportional to dose, i.e., the 1/100 dilution group had the lowest titer and the controls, followed by the 1/1000 and 1/600 group had the highest titer. This indicates that benefit afforded by the probiotic treatment was not related to a humoral antibody response to the microbial introduction. As a measure of overall effectiveness and tertiary growth factors, the incremental weight increase over the whole study period was analyzed against the start weight. The mean weight of fish in the treatment groups was not statistically significant from the control groups. However, due to the higher survival rate at the effective treatment concentration, the 1/100 treated group had a total biomass of 16.2% over that of the control group and an absolute weight gain difference from the start to end of study of 14.2%, over 229 days (7.6 months).

The results of the probiotic bacteria application clearly indicate that a good effect is afforded by seeding in terms of pathogen displacement and general overall health to combat a naturally occurring epidemic outbreak with ulcerative bacterial infection. The infection progressed after the acute phase period with more chronic losses including the involvement of *A. wodanis*, but a clear benefit to avoiding to a higher degree the infection in treated fish was evident. Selected strains of *Aliivibrio sp.* were protective at the effective concentration of 1 x10 ^{7} cfu-mL and supported significantly better growth in fish challenged with ulcerative disease.

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

Compatibility of Application of a Probiotic Treatment in Anesthetic Bath Using Benzocaine or Tricaine Methanesulfonate (TMS)

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This laboratory study was undertaken to determine the safety and efficacy of application of live probiotic bacteria in combination with anesthesia commonly used for sedation prior to vaccination. The parameters assessed were survival by cultivation of the probiotic bacteria in the presence of the anesthetics with prolonged exposure (3,5 h), blood uptake of the bacteria in treated fish within 5 minutes of exposure, survival and safety of the treatment for 21 days after treatment, and time to stage II anaesthesia with and without the concurrent application of probiotic bacteria. The probiotic, Stembiont™ is a bath treatment of active cultures of specified strains of bacteria for microbial enhancement of the microbiome and has been shown to reduce the disease, and increase growth and survivability after exposure to sea water in treated smolt (Amthor *et al*., this session).

For the survivability determination *in vitro*, fresh cultures of the probiotic were exposed with Benzocaine (0,89 ml/L) or TMS (80 mg/L) in 1 % (10 ppt) salinized tap water and samples plated on blood agar plates at 30 min intervals. An initial decrease in cfu to time 60 minutes of 0.5 $x10^1$ in both the anesthetic groups and the controls was observed. Following 60 min, all groups maintained stabile end-point viability >1 x 10^8 cfu/mL to time 3.5 h.

Groups of fish (n=20) were introduced to anesthetic bath containing the same concentrations of anesthetic as above. Individual fish were observed to determine behavior and the time to loss of ability to right Stage II anesthesia). The mean time to reach anesthesia varied from t=36 sec to t=41 seconds and was not significantly different between the treatments.

Blood uptake by positive cultivability was sampled from 5 fish per group 5 min of introduction to the baths containing probiotic with or without anesthetic. Bacterial counts of 2000-10,000 cfu/mL blood were recorded and no biologically significant differences between anesthetic and without anesthetic were observed. In addition, no adverse behavioral characteristics or mortality were observed over 21 days in any of the treated groups.

The results of the combined application of specified probiotic strains and either Benzocaine or TMS did neither effect the effective concentration of anesthesia or probiotic and can be considered compatible for co-administration. Blood uptake of probiotic bacteria is rapid from bath application and easily applied at the time of anesthesia before vaccination where it is useful to augment the microbiome in the face of handling stress and vaccination.

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

Effect of Oxytetracycline Treatment on the Gastrointestinal Microbiome of Critically-Endangered White Abalone (*Haliotis sorenseni*) Treated for Withering Syndrome

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The white abalone (*Haliotis sorenseni*) is a critically-endangered marine gastropod native to the northeastern Pacific and is at risk of extinction in the wild due to overfishing. Despite closure of the white abalone fishery in 1996 there has been no significant recruitment in remaining wild populations. Captive-rearing programs in California have been successful in culturing white abalone with the intent to re-establish wild populations throughout the species' native range. Withering syndrome (WS) is a fatal disease caused by colonization of the gastrointestinal tract by an intracellular prokaryotic Rickettsiales-like parasite (WS-RLP), identified as *Candidatus xenohaliotis californiensis.* In other abalone species, induction of disease following infection with WS-RLP is significantly accelerated with increased water temperatures, making the disease of special interest with regards to climate change and ocean warming. Studies have shown that white abalone have the highest susceptibility and the lowest intrinsic resistance to WS of all Pacific abalone species. WS-RLP has been identified in wild white abalone populations and poses a considerable threat to captive culturing operations. Oxytetracycline (OTC) is effective in eliminating WS-RLP infections from the gastrointestinal tract of affected abalone. OTC concentrates in the digestive gland of exposed abalone and provides protection against reinfection with WS-RLP for up to six months after the completion treatment. OTC baths (500 mg/L) are used to treat and protect captive populations from WS. Clinically, OTC treatment is well-tolerated by abalone and there is no significant difference in growth rates between treated and untreated abalone. While the genetic composition of the normal white abalone gastrointestinal biome has not been fully characterized, as kelp-eaters they are also suspected to rely on a balanced microbiome for optimal feed utilization; dysbiosis could result in reduced fitness of white abalone in the wild. Because many white abalone that are destined for release into the wild undergo OTC treatment during their captive-culture phase it is important to investigate the long-term impacts that this antimicrobial may have on the fitness of these animals. This study is a metagenomic comparison between the gastrointestinal microbiomes of OTC-treated and untreated control captive-cultured white abalone to evaluate the impact of OTC treatment on the gut microbiome. Gastrointestinal tracts from five treated individuals and five untreated controls were sampled at each time point: time 0, at the end of the 21-day OTC treatment, and 200-days post-treatment to coincide with the withdrawal period of the OTC. Gastrointestinal tracts were analyzed via 16S metagenomics and compared to evaluate for any statistical differences between bacterial populations between the groups. This study achieves two goals: to characterize the gastrointestinal microbiome of normal white abalone and to evaluate the potential for long-term impact of OTC treatment for WS-RLP on individuals that are part of a captive-breeding program with the intent to release into the wild.

Conference session designation: (Invertebrate and Shellfish Disease)
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