

Annual Microbial Communities Symposium

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Posters

Poster #	Presenter	Poster Title
1	Taylor Beck	Investigating the impact of a combined blueberry and galacto-oligosaccharide diet on the gut microbiome composition in a sickness-induced rodent model
2	Kyle J. Buffin	Patterns of symbiotic bacterial community composition and innate immune system complexity across insects
3	Kendall Byrd	Conservation Through Microbiome Analysis: Examining Salamander Disease Defense and Ecosystem Health
4	Jayun Kim	Characterizing factors for proliferation of cyanotoxin-producing cyanobacteria in eutrophic lake and river by data-driven models
5	Minseung Kim	Household environments and socioeconomic conditions affect breastmilk microbiome and infant gut microbiome
6	Jonathan Leopold	Water Microbiome, Resistome, and Sources of Fecal Contamination in a Guinea Worm Endemic Country
7	Zachary J. Lewis	Optimizing DNA extraction and host depletion for shotgun metagenomics of high-host low-microbial biomass urine samples.
8	Jia Liu	Metagenomic Analysis Reveals How a Low-carbohydrate/high-protein Diet Shapes Gut Microbiome in Individuals with Chronic Spinal Cord Injury
9	Yijing Liu	Impacts of specific UV wavelengths on antibiotic resistant bacteria and their genes during wastewater treatment
10	Kris Martens	Metagenomic sequencing detects injury-specific changes to the microbiome 30 days following traumatic brain injury in rodents
11	Alexander B. Michaud	The Life of Ice: Where, how, and why ice influences the ecology of microorganisms
12	Nora Jean Nealon	To Sample or Not to Sample: Capturing Feline Fecal Microbiome Changes With High-Frequency Sample Collection
13	Dawson Phan	Feedback Sought: Integrating multi-omics for capturing climate feedbacks
14	Nina Randolph	The long-term in vitro bacterial viability of lyophilized and frozen canine and feline fecal microbial transplantation products
15	Amanda Stickney	Influence of relative humidity on <i>Aureobasidium pullulans</i> degradation of polyurethane foam
16	Sophia Stokes	BefA protein stimulates increased growth and development in germ-free insects
17	Jaylen E. Taylor	Bile Inhibits Bacterial Toxin Activity by Instigating Structural Destabilization and Oxidation
18	Tessa H. C. Wilde	Monkeys and Microbes: Examining gut and oral microbiota in SIV-infected sooty mangabeys

----- poster no. 1 -----

Investigating the impact of a combined blueberry and galacto-oligosaccharide diet on the gut microbiome composition in a sickness-induced rodent model

Taylor Beck

The human diet aids in modulating the gut-brain axis, a two-way signaling system between the nervous system and microorganisms in the gut. Cognitive function is influenced by this relationship and has been shown to decline over time due to age-related structural and functional changes. Development of a functional food product for clinical studies to serve as an intervention for improved cognitive function via the gut-brain axis was achieved by creating a confection containing blueberry anthocyanins (ACNs, a polyphenol) and galacto-oligosaccharides (GOS, a prebiotic), which have both been previously studied to reduce systemic inflammation and promote healthy bacterial populations in the gut, respectively. It is hypothesized that consumption of blueberry ACNs and GOS will increase the diversity of the gut microbiome and mitigate effects of cognitive decline. A sickness induced rat model was used to evaluate behavioral and physiological parameters when fed a control and a treatment diet containing similar relative amounts of ACNs and GOS as the confection. Fecal samples were collected before and after inducing systemic inflammation via lipopolysaccharide (LPS, an endotoxin) injection at multiple time points to mimic cognitive decline in humans. Sickness scores were measured starting at 16 hours post LPS injection for five days and standard cognitive functions tests (Elevated Plus Maze, Forced Swim Test, and Morris Water Maze) were performed starting three days after LPS injection. The treatment diet given to rats pre-LPS injection showed a significant effect on the gut microbiome compared to the control diet. Further, effects of the diet post-LPS injection were maintained over fourteen days. These findings indicate that the intervention diet effectively modifies the gut microbiome and future studies will compare its composition as it relates to behavioral and physiological outcomes after induced sickness.

----- poster no. 2 -----

Patterns of symbiotic bacterial community composition and innate immune system complexity across insects

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All animals, including humans, evolved within a microbial world, resulting in metazoans maintaining a myriad of symbiotic relationships with microbes that range from antagonistic to mutualistic, single obligate endosymbionts to complex, species-rich microbiomes. Host-associated bacterial microbiomes have demonstrated multifaceted importance to animal health and development due to essential functions such as the ability to degrade dietary components into beneficial metabolites, remove toxic compounds, and provide protection from pathogens. Despite near universal essentiality, symbiotic microbiomes show very different compositions and functions across host diversity. We chose to examine insects, which among metazoans exhibit the greatest amount of diversity along the following axes: innate immune gene family repertoire, ecological niche occupancy, microbiome compositionality (i.e., diversity and abundance) and host-microbe relationships (i.e., mutualism, parasitism, commensalism). At the interface between insect host and symbiont is the innate immune system where these direct interactions influence microbiome assembly and maintenance. Innate immunity is present in all metazoans, with several features being conserved over deep evolutionary time, and, while its role in pathogen defense is acknowledged, its role in the maintenance of microbiome homeostasis is emergent. Previous studies have demonstrated that many animals maintain a microbiome with high specificity in composition, however the host genetics underpinning this maintenance is unclear. Therefore, we hypothesize that evolutionarily conserved interactions between host and microbiome are identifiable through the differences in microbiome diversity and innate immune system gene complexity between different species. Here we begin to address this hypothesis by examining patterns of both microbiome composition and immune gene evolution across 48 insect genera. We have taken a meta-analysis approach leveraging existing bacterial community and host genome sequencing efforts. We demonstrate the phylogenetic signal present across insect microbiome compositions, and how this intersects with patterns of innate immune gene evolution.

----- poster no. 3 -----

Conservation Through Microbiome Analysis: Examining Salamander Disease Defense and Ecosystem Health

Kendall Byrd

Emerging infectious diseases in wildlife, notably chytridiomycosis in amphibians caused by *Batrachochytrium dendrobatidis* (Bd), pose a significant threat to global biodiversity. This salamander microbiome study, using a one-

health approach, has the primary goal comparing the microbiome composition and function of captive bred and wild *Desmognathus ocoee* and *Eurycea wilderae* salamanders, aiming to support conservation efforts for these species. To achieve this goal, this study's objectives included assessing the presence of Bd-inhibiting microbes and characterizing functions of these in the cutaneous biofilms of both captive and wild salamanders. This research utilizes 16S rRNA gene sequencing to analyze the microbial community present on salamander skin. These findings reveal that captive conditions do not alter the salamander microbiome at the genus level, suggesting that the captivity itself may not negatively impact their immune responses or increase susceptibility to diseases through microbiome changes. This outcome is particularly encouraging for conservation practices such as rewilding. Maintaining a stable microbiome in captivity is crucial for ensuring that reintroduced species can adapt and thrive in wild environments, supporting ecosystem health and biodiversity. This work aids in understanding the complex interactions between captivity, microbiome alterations, and health in amphibians. It also explores potential implications for ecosystem health, as salamanders play a key role as environmental indicators. The study's findings advocate for a holistic approach to wildlife conservation, where the health of individual species is viewed as interconnected with the health of entire ecosystems and, by extension, human health.

----- poster no. 4 -----

Characterizing factors for proliferation of cyanotoxin-producing cyanobacteria in eutrophic lake and river by data-driven models

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Cyanobacterial harmful algal blooms (CHABs) have been increasing recently mostly due to climate change and nonpoint source pollution. Therefore, it is crucial to understand the conditions that give rise to cyanotoxins to minimize potential risks owing to CHABs. However, only a few studies have explored the influencing factors of cyanotoxins in freshwaters. This study aimed to examine the community composition of cyanobacteria genera across different regions; relate cyanobacteria to microcystin-LR (MC-LR); and assess the impact of environmental factors. In a eutrophic South Korean lake and river, differences in *Microcystis* prevalence were observed regardless of the total nitrogen to total phosphorous (TN/TP) levels. In warmer areas, including downstream of the Nakdong River (NR), *Microcystis* was prevalent during CHAB events. Multiple linear regression models revealed that 1300–1700 cells/mL of *Microcystis* produced 0.03 µg MC-LR/L (test accuracy up to 95%). Random forest models using environmental factors predicted *Microcystis* dominance with a performance of 52% at NR and Daecheong Lake. These models pointed to water temperature and nutrient levels (TN/TP or NH₃-N/PO₄-P) as the primary factors influencing *Microcystis*. Notably, higher TN/TP contributed to higher dominance of *Microcystis* only at Daecheong Lake when the water temperature was high. Overall, this study demonstrated the potential to predict toxicity levels using environmental variables through DDMs. It underscores the importance of implementing practices aimed at reducing nutrient pollution sources to mitigate cyanobacteria toxicity in surface waters.

----- poster no. 5 -----

Household environments and socioeconomic conditions affect breastmilk microbiome and infant gut microbiome

Minseung Kim, Molly Mills, Vanessa Hale, Chris Hoffmann, Barbara Piperata, Jiyoung Lee

Objective: We investigated associations between household environment and socioeconomic factors and human breastmilk microbiota and its impact on infant gut microbiota in mother-infant pairs in a developing country.

Methods: We collected breastmilk and infant stool samples of mother-infant pairs from Belém, Brazil (n = 50) and DNA was extracted. We used high throughput sequencing platform targeting V4-5 region on 16S rRNA coding gene to analyze microbial community of breastmilk and infant stool samples. For household environment surveillance, questionnaire was conducted, and water and swab samples were collected, and DNA was extracted. Molecular assay was done to determine *Escherichia coli* and microbial source tracking was done to determine fecal contamination (universal fecal sourced Bacteroidales) in drinking water and surfaces. Finally, statistical analyses were done to determine relationships between household environment and socioeconomic factors, breastmilk microbiome, and infant gut microbiome.

Results: We observed 8 core genera from breastmilk microbiome (BM), *Streptococcus*, *Staphylococcus*, *Elizabethkingia*, Unclassified *Enterobacteriaceae*, *Acinetobacter*, *Pseudomonas*, *Cutibacterium*, and *Phyllobacterium*, in at least 95% of the samples. BM was associated with household socioeconomic status (generalized Unifrac, PERMANOVA, p < 0.01). We also observed a significant relationship between household drinking water *E. coli*

contamination and BM Shannon diversity (Wilcoxon test, $p < 0.05$). We identified 23 overlapping OTUs between breastmilk and infant stool sample pairs, seven of which were detectable in multiple breastmilk-stool dyads (Unclassified *Enterobacteriaceae*, *Streptococcus*, *Veillonella*). Notably, 6 of these overlapping OTUs showed different distribution among samples by socioeconomic status.

Conclusion: Our results indicate that household environments, including microbial contamination of drinking water, and socioeconomic status may affect breastmilk microbiota. Preliminary findings indicate that breastmilk microbiota might influence infant gut microbiota via various ways, implying that maternal factors can affect infant gut microbiome assembly.

----- poster no. 6 -----

Water Microbiome, Resistome, and Sources of Fecal Contamination in a Guinea Worm Endemic Country

Jonathan Leopold

Objective: Overarching objectives of this project use a One Health approach to promote human/animal health by correlating microbial water quality data with Guinea worm (GW) prevalence in waterbodies in Chad, Africa. Purpose of this pilot study was to test feasibility of microbial methods and characterize two waterbodies in our study region. We aimed to determine the abundance and sources of fecal contamination in these waterbodies and their relationship with the microbial community.

Methods: Surface water samples were collected from two bodies of water in Ali Garga, Chad. Water was filtered and DNA was extracted. Digital PCR was performed to test for various sources of fecal contamination (bird, canine, ruminant, and human) via microbial source tracking. Bacterial DNA samples underwent shotgun metagenomic sequencing to characterize the microbiome and resistome.

Results: Waterbodies had high levels of fecal contamination, with the largest contributor being avians and canines. Site 2 shows human-associated fecal contamination and a greater antibiotic resistance gene (ARG) abundance. Site 1 contained a higher abundance of ARGs that are potentially capable of conferring resistance to antibiotics that treat tuberculosis.

Microbial community differences were found between sites, but the Pseudomonadota phyla dominated the microbiome for each site. Site 1 contained nematodes from the *Wuchereria* genus, while Site 2 had nematodes from genus *Brugia*.

Conclusions: Fecal contamination is highly prevalent in these Chadian waterbodies. Canines can facilitate the spread of GW, thus, canine-contaminated waterbodies could be associated with GW. The high prevalence of ARGs could pose public health issues for communities that use these sources of water.

----- poster no. 7 -----

Optimizing DNA extraction and host depletion for shotgun metagenomics of high-host low-microbial biomass urine samples.

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Recent studies highlight the role of microbiota in cancer development and therapeutic response. However, efforts to characterize the urobiota and their role in bladder cancer (BC) have been limited due to technical challenges including low microbial biomass and high host cell shedding in urine. While some studies have identified links between the urobiome and BC, additional mechanistic studies with standardized methods are needed. Few studies have attempted shotgun metagenomic sequencing in urine, and none have attempted to assemble microbial genomes from metagenomes (MAGs). Here we evaluate the effect of host cell removal on metagenomic urobiome profiles in healthy dogs. We collected urine from seven dogs and fractionated samples into 3.0 mL aliquots. Aliquots were spiked with a standard concentration of canine cells, modelling a biologically relevant host-cell burden, before DNA extraction and shotgun metagenomic sequencing. To evaluate effects of host cell removal we extracted aliquots

of each sample using six methods (Bacteremia (B, no host cell removal), DNA Microbiome (D), Molzym MolYsis (M), NEBNext, HostZero (Z), and Propidium Monoazide). Sequences were analyzed using MetaPhlan4 or standard MAG assembly pipelines, and statistics were performed in R. Methods D, M, and Z reduced the burden of host reads compared to samples extracted with Method B. Method D maximized the number of unique microbial species observed ($p=0.01$) and overall microbial diversity ($p=0.002$, Shannon entropy) assessed via MetaPhlan4. Inter-dog differences in microbial composition were detectable ($p=0.001$) and overwhelmed differences introduced by extraction; samples were not different in composition according to extraction method ($p=0.9$). Method D recovered the greatest number of assembled MAGs, though overall recovery of MAGs was low. Results demonstrate that host removal via Method D may be optimal when high host-cell burden is suspected, and that shotgun metagenomic profiling is feasible at both the gene- and genome-resolved level in urine samples.

----- poster no. 8 -----

Metagenomic Analysis Reveals How a Low-carbohydrate/high-protein Diet Shapes Gut Microbiome in Individuals with Chronic Spinal Cord Injury

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Objective: To evaluate the effect of a low-carbohydrate, high-protein (LC/HP, 40% energy from carbohydrates, 30% from protein, and 30% from fat) diet that includes healthy dietary components on the gut microbiome composition in individuals with chronic spinal cord injury (SCI) using next-generation sequencing techniques.

Participants: The study enrolled adult participants with chronic SCI ($n=24$, $43\pm 10y$, 17M/7F), at least 3 years post-injury, ranging from C2 to L2, on the American Spinal Injury Association Impairment Scale (AIS A-D).

Interventions: Participants were randomly assigned to either the LC/HP diet group or the control group for 8 weeks. The LC/HP group received weekly meal deliveries, while the control group maintained their usual diet. Stool samples were collected at baseline and at week 8. Metagenomic sequencing was performed to analyze gut microbiome diversity and species abundance utilizing the Biobakery package. Statistical analyses were performed using the NBZIMM R package to assess the effect of time, diet group, and their interaction on changes in species abundance. **Results:** No significant interaction effects were observed for alpha and beta diversity, except for a tendency for increased alpha diversity (gini Simpson, $p=0.09$) in the diet group. Changes in several species differed between groups, with increased abundance of *Fusicatenibacter saccharivorans*, *Eubacterium siraeum*, and *Ruminococcus torques*, and decreased abundance of *Hungatella hathewayi*, *Clostridium symbiosum*, and *Phocaeicola vulgatus* in the diet group compared to the control group ($p_{interaction}<0.05$).

Conclusions: The LC/HP diet led to changes in gut microbiome composition, characterized by increased levels of bacteria implicated in intestinal anti-inflammatory function and improved host metabolic health, and reduced levels of bacteria linked to inflammation and colon cancer. Our results suggest that the gut microbiome may partly mediate the beneficial effects of an LC/HP diet in individuals with SCI.

----- poster no. 9 -----

Impacts of specific UV wavelengths on antibiotic resistant bacteria and their genes during wastewater treatment

Yijing Liu, Dr. Natalie Hull

Antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARGs) have been detected in tap, bottled, and drinking water, throughout wastewater treatment plants and their disinfected effluent, and in environmental water sources. Ultraviolet (UV) irradiation is an alternative or complementary disinfection method that damages nucleic acids, such as ARG, and proteins. While UV irradiation has been observed to disinfect ARB effectively in different engineered water systems, the role of different UV wavelengths in shaping the microbial community, disinfecting ARB, and damaging ARG is still largely unknown. In this work, wastewater influents from three Ohio WWTP facilities were sampled as part of our participation in the Ohio Coronavirus Wastewater Monitoring Network and exposed to two UV wavelengths (254 nm emitted by a low pressure mercury lamp, and 222 nm emitted by a Krypton-chloride excimer lamp) at doses of 40 and 160 mJ/cm^2 . Whole genome sequencing was conducted by Nanopore sequencing to investigate microbiomes in untreated and UV treated samples using wf-metagenomics or What's In My Pot (WIMP) workflow and to investigate antibiotic resistomes using (The Fastq Antimicrobial Resistance (AMR)) workflow. Preliminary results indicated that 92% of reads belonged to bacteria, and the most dominant phyla across all samples included *Proteobacteria* which were most abundant, *Firmicutes*, *Bacteroidota*, and *Actinobacteria*. No impact on

richness by UV was observed, but abundance of dominant taxa increased after higher dose exposure at 254 nm and 222 nm in a preliminary analysis of a subset of samples. Further exploration of the full sample set will be visualized using phyloseq for microbiomes or only ARB datasets to gain a comprehensive understanding of impacts of UV on the microbial community and antibiotic resistance. This work will enhance the understanding of microbial structure in wastewater samples and provide some insights on how UV may shift microbial communities, including ARB, to promote the development of UV in engineered water systems.

----- poster no. 10 -----

Metagenomic sequencing detects injury-specific changes to the microbiome 30 days following traumatic brain injury in rodents

Martens, K. M., Gratzol, C., Bressler, N. M., Bailey, M. T., & Vonder Haar, C.

Traumatic brain injury (TBI) causes cognitive impairment, increases risk for psychiatric disease, and exacerbates related symptoms such as risky decision-making and impulsivity. Impaired monoamine neurotransmission is a likely contributor to such symptoms, with serotonin signaling contributing to anxiety and mood-related disorders, impulsive dysfunction, and impaired decision-making. Despite this knowledge, precisely why these systems are vulnerable to TBI is unknown. However, emerging data indicate a role for the gut microbiome. Gut dysbiosis occurs rapidly after TBI and may persist for years in patients.

In a previous study, our lab manipulated the microbiome of rodents using antibiotic dysbiosis. We then assessed function on the Rodent Gambling Task, a clinically relevant assessment of impulsivity and decision-making. The findings from the study showed a delay in the onset of TBI symptoms in the antibiotic cocktail group pointing to a potential causal role for the gut microbiome in psychiatric disease following TBI. 16S amplicon-based sequencing identified broad changes in the microbiome but could not identify species-level information and injury-specific differences resolved by 14 days post injury. To better understand the mechanisms at play, we performed metagenomic shotgun sequencing. From these data, we were able to construct bacterial metagenome-assembled genomes (MAGs) to determine changes occurring at key time points post injury and at the species level. The results of this study showed that TBI and antibiotics differentially affected the prevalence of multiple MAGs. These differences persisted 30 days following injury. Of particular interest to our lab, sequencing identified several MAGs associated with behavioral performance even after accounting for manipulations of TBI and antibiotics identifying potential therapeutic targets in the gut.

----- poster no. 11 -----

The Life of Ice: Where, how, and why ice influences the ecology of microorganisms

Alexander B. Michaud
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Ecosystems are complex webs of matter fluxes between organisms, trophic levels, and the abiotic environment. Polar ecosystems, especially those in the high Arctic and Antarctica, contain simple trophic structures and reduced ecological complexity due to the selection pressures and low energy budget. The reduced complexity of polar ecosystems provides a more tractable environment in which to test ecological hypotheses. A quantitative understanding of ecological interactions and the resulting biogeochemical fluxes within a polar ecosystem begins with understanding the microorganisms and their activity. Thus, forces which alter the microbial engines that drive biogeochemical cycles merit study. One such force is the change in the extent, duration, and timing of ice in polar and temperate regions. There is a growing need to quantify how aquatic ecosystems are responding to the decline and changing phenology of ice worldwide. It is the response of microorganisms and their ecology that is central to understanding these ecosystem changes in the polar regions. However, in many cases, we still do not know the extent of the biosphere in polar environments. Quantifying the effects of a changing global climate is a complex, multi-faceted problem that requires collaboration between physical, chemical, biological, and social scientists. The role of my team in helping to solve this complex problem is to use the tools and theory of microbial ecology and biogeochemistry to quantify the fluxes and transformations of compounds and elements relevant to aquatic ecosystem function. I will summarize and highlight the ongoing research projects in the Polar Geomicrobiology Group which help to address these outstanding questions.

----- poster no. 12 -----

To Sample or Not to Sample: Capturing Feline Fecal Microbiome Changes With High-Frequency Sample Collection

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Microbiota-based fecal evaluations are promising feline diagnostic tools. While once-weekly sampling is standard in research, daily fecal microbiota changes make the ideal sampling frequency unknown. The objective of this study is to evaluate how sampling frequency impacts the resolution of fecal microbiota data. Our hypothesis is that daily high frequency fecal sampling is more effective than less frequent sampling at capturing significant microbiota alterations in cats.

Six healthy, sterilized adult laboratory cats (3 male, 3 female) were assessed. To initiate an abrupt microbiota disturbance, a rapid diet change was performed. Feces was collected prior to diet transition and then daily for five weeks. 16s rRNA microbiota analysis (V4 region) was performed using the DADA2 pipeline. To examine impacts of sampling frequency, twelve sampling schemes, representing once-weekly to everyday sampling, were compared for their ability to identify microbial shifts over time. For each scheme, pairwise PERMANOVA and DESeq2 differential abundance testing were used to identify time-dependent microbiota compositional and taxonomic changes respectively. Significance was defined as $p < 0.05$ following posthoc corrections.

When comparing sampling schemes, high frequency daily sampling provided the best resolution for identifying microbiota alterations over time ($p < 0.01$) compared to low frequency once-weekly sampling ($p = 0.015$). Sampling frequency schemes differentially estimated fold changes in Fusobacteriota, a phylum important in protein metabolism, where daily sampling identified a 5.31-fold reduction in Fusobacteriota abundance between baseline and study week one ($p = 9.41E-20$) whereas the once-weekly sampling scheme identified a 3.13-fold decrease ($p = 0.0034$).

The results from this study support that high frequency sampling may be needed to accurately capture microbial community shifts occurring in response to an inciting agent, including diet and/or medical treatments. Ongoing analysis will integrate this data with daily feline dysbiosis index values, a widely used feline microbiota diagnostic tool, to further assess the impact of sampling frequency on biologically-relevant taxa.

----- poster no. 13 -----

Feedback Sought: Integrating multi-omics for capturing climate feedbacks

Dawson Phan

Department of Microbiology | Rich Lab & Sullivan Lab

Multi-omics datasets are critical for understanding complex interactions between microbes and their natural environment, and capturing climate feedbacks which induce short-term and long-term biological change. Our study aims to layer the multi-omics framework (environmental DNA, RNA, and protein), to capture the reciprocal interactions between feedbacks from climate change and microbiota in a globally-relevant thawing permafrost environment.

We collected sixteen paired thaw gradient soil samples initially described in Woodcroft & Singleton et al. (2018) including metagenomes (microbial and viral), metatranscriptomes, metaproteomes, and associated metadata. Preliminary results show that as soils become increasingly thawed, there is a positive association between key methanogenesis transcripts and proteins with methane flux. This indicates a possibility of increased methane emissions as more permafrost thaws and global temperatures increase.

However, analyzing these data come with unique challenges associated with both biological and statistical constraints. This includes interrelating different levels of biological information under highly variable regulatory controls, impacting their quantitative relationships, albeit under some predictable structure. Additionally, while individual samples may be high-quality in nature, collective sample size remains a challenge under classical statistical hypothesis testing paradigms. Further investigations are warranted to generalize frameworks necessary to elucidate relationships between biomolecules and their environmental parameters in sample-limited, multi-dimensional space. This may include approaches borrowed from specialized quantitative fields such as bioinformatics and computational biology.

----- poster no. 14 -----

The long-term *in vitro* bacterial viability of lyophilized and frozen canine and feline fecal microbial transplantation products

NK Randolph^{1,2}, D Diaz-Campos¹, J van Balen¹, NJ Nealon^{1,2}, J Rowe^{1,2}, L Wetzel^{1,2}, JA Winston^{1,2}

Fecal microbial transplantation (FMT) is the transfer of feces from a healthy donor into the gastrointestinal tract of a diseased recipient to confer a health benefit. The mechanism in which FMT confers a health benefit is linked to the viability and engraftment of microbes and the correction of dysbiosis. Our study aims to quantitate the colony forming units (CFUs) of microbes within canine and feline FMT products using aerobic and anaerobic culture-based techniques. Three screened canine and feline fecal donors each provided three separate fresh fecal samples for processing. Fecal processing techniques include unprocessed and three double centrifuged fecal slurries with the following additives: 0.9% saline, 0.9% saline with 10% glycerol, and 0.9% saline with 25% maltodextrin and trehalose (M:D). FMT products were aliquoted for long-term storage at -20C, -80C, and lyophilized for storage at room temperature. Timepoints for CFU/gram quantitation include baseline, 1, 3, 6, and 12 months. Canine lyophilized products preserved with M:D yielded significantly greater total CFUs compared with other lyophilized FMT products ($p < 0.01$). For canine products frozen at -20C, FMT preserved with glycerol and M:D yielded significantly more CFUs than other products ($p < 0.01$). All canine FMT products, except 10% glycerol frozen at -80C, exhibited a significant decrease in total CFUs over the 12-month period ($p < 0.01$). Under all storage conditions, feline FMT products preserved with M:D exhibited the lowest mean log₁₀ drop over 12-months. All feline FMT products, except 10% glycerol and 25% M:D frozen at -80C, exhibited significant decreases in total CFUs over 12-months ($p < 0.01$). For all products, storage at -80C yielded significantly more total CFUs than storage at -20C ($p < 0.01$). This study will provide clinicians with evidence for producing and storing FMT in their own practice. Further research is needed to determine whether increased CFUs translates to improved microbe engraftment and ultimately improved clinical outcomes.

----- poster no. 15 -----

Influence of relative humidity on *Aureobasidium pullulans* degradation of polyurethane foam

Amanda Stickney

Polyurethane is a type of plastic used as insulation, sealants, cushions, and life jackets. This plastic can be broken down by fungi such as *Aureobasidium pullulans* (*A. pullulans*), a fungus that produces cutinase, an enzyme involved in plastic biodegradation. However, the impact of relative humidity on *A. pullulans* degradation of polyurethane foam and genetic degradation pathways are unknown. This project aims to determine the impact of relative humidity on *A. pullulans* degradation of polyurethane and the associated gene degradation pathways. Three strains of *A. pullulans* were selected for analysis of degradative capability and genetic degradation pathways. Degradation analyses show that high relative humidity is associated with weight loss in foam and visual pitting or cracking in SEM images. Nucleic acid sequencing and bioinformatics predict 10 cutinase genes among the 3 strains. Of these cutinase genes, 4 were up-regulated at 85% and/or 100% relative humidity compared to 50% relative humidity. These results indicate that relative humidity is an important factor that may increase fungal degradation of polyurethane foams and that cutinase enzymes may contribute to this degradation. Preventing biodegradation is one strategy to lower the cost to maintain environments reliant on plastics, allow for the reuse of old plastics, and reduce the need for new plastics.

----- poster no. 16 -----

BefA protein stimulates increased growth and development in germ-free insects

Sophia Stokes

The symbiotic relationship between organisms and their associated microbiota has been shown to play a vital role in host health and fitness. American cockroaches (*Periplaneta americana*) have diverse gut microbiomes that resemble the levels of complexity seen in human microbiomes. When reared without their normal gut microflora, these organisms exhibit notable growth and developmental issues. A previously uncharacterized protein, named BefA (Beta Cell Expansion Factor A), has been shown to restore some wild-type phenotypes in germ-free hosts. This experiment investigates whether the BefA protein can rescue several growth defects seen in germ-free *P. americana*. By inoculating germ-free cockroach nymphs with *Escherichia coli* genetically modified to either produce or not produce BefA, we found that the presence of the protein is correlated with increased growth and development. The nymphs inoculated with BefA-producing bacteria expressed larger body sizes, more consistent sizes between individuals, and faster molting than the group without BefA. These data suggest that the BefA protein plays a functional role in the symbiotic relationship between the host and its microflora.

----- poster no. 17 -----

Bile Inhibits Bacterial Toxin Activity by Instigating Structural Destabilization and Oxidation

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Bile salts are a diverse class of antimicrobial, amphipathic, cholesterol derivatives that are synthesized in the liver and secreted to the small intestine. They primarily function to emulsify fats and aid in metabolism but have also been implicated in modulation of virulence factors (e.g., toxins) expressed by pathogenic bacteria. While the vulnerability of bacterial membranes to bile components is recognized, the effects of bile on bacterial toxins have not been systemically studied. We speculate that due to their recognized high conformational plasticity, virulence factors are easy targets for the denaturing activities of bile salts. In this work, we explored the effects of reconstituted bovine bile on the stability and activity on the effector domains of multifunctional auto-processing repeats-in-toxin (MARTX) from *V. cholerae* and *A. hydrophila*. Structural stability experiments in the presence of whole bile reflected local destabilization of virulence effector secondary structure and exposure of hydrophobic regions leading to increased precipitation. Upon entry to the host cell, MARTX domains are separated by activation of the cysteine protease domain (CPD). Whole bile treatment of CPD-containing constructs inhibited cleavage in a DTT- (but not TCEP-) dependent manner and labeling by TMR-5-maleimide, suggesting formation of a covalent Cys adduct. Pre-incubation of LPS depleted *A.h.* media with whole bile at 37°C inhibited toxicity of T1SS toxins against IEC-18 cells in a time-dependent manner. Overall, bile compromises the structural integrity of the tested bacterial toxins, instigates their precipitation, and induces inactivation via cysteine oxidation – preventing toxicity and justifying the need for bacteria to tune toxin expression levels in the presence of bile.

----- poster no. 18 -----

Monkeys and Microbes: Examining gut and oral microbiota in SIV-infected sooty mangabeys

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The microbiome plays a critical role in primate health and is shaped by many environmental factors. In Human Immunodeficiency Virus (HIV) and Simian Immunodeficiency Virus (SIV), gut epithelial damage, and microbial translocation from the gut to systemic circulation are key determinants of disease progression, and are also associated with increased viral loads and CD4+ T-cell depletion. However, in natural SIV host species such as sooty mangabeys (*Cercocebus atys*), the gut barrier remains intact, and microbial translocation does not occur despite high levels of viral replication. Moreover, disease progression to AIDS is not commonly observed. This phenomenon has promoted interest in the microbial communities that play a role in maintaining gut homeostasis in SIV. Here we examine gut and oral microbiota of sooty mangabeys from Emory National Primate Research Center (NPRC) and their free-ranging counterparts living in Ivory Coast's Tai Forest – completing the first characterization of SIV in the saliva of wild sooty mangabeys. Our results indicate that saliva sampling is more accurate than fecal sampling for noninvasively detecting SIV in sooty mangabeys. SIV viral load in saliva was found to be significantly higher in mangabeys housed at Emory NPRC than those living in the Tai Forest. We observed differences in alpha and beta diversity metrics of the salivary microbiota based on SIV status. We establish correlations between the gut and oral microbiota, viral load, immune markers, and several social and ecological variables. These results highlight the importance of a holistic approach in examining microbiome-disease interactions in wild primate populations.

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