

## MoBE 2017 Poster Abstracts

### (Poster #1)

**Title: US Army Engineers and the preservation of combat power through the prevention of microbial induced corrosion in the expeditionary warfare environment.**

**Author:** Will Pratt (Ft. Brag, NC)

War is a multidisciplinary endeavor that involves the economic application of combat power to defeat an enemy. Along the way to defeating enemies in austere places like Iraq, Afghanistan, Africa and worldwide, the US Department of Defense (DOD) and the US Army fight an ongoing war against corrosion. Annually, the DOD spends about \$22.5 billion corrosion prevention and remediation and of that amount, about \$4.5 billion on microbe corrosion prevention and remediation. This effort taken on by US Army Engineers and Preventative Medicine ensure the US Army can build, sustain, and decisively apply combat power worldwide.

The problems that the US Army faces when it comes to microbe (micro-algae, archaea, bacteria, and fungi) induced corrosion arise from the operational environment and from internal sustainment operations. An example of environmental influence are biologically active soils that, through erosion and wind, turn into dust and begin to corrode everything from tents to attack aircraft. The influence of internal sustainment operations are the use of black water collection ponds, burn pits, standing bodies of water from floods, burst pipes, collection of AC moisture amongst many things that ultimately result in the spread of mold, fungi, and bacteria that corrode and destroy tents, buildings, and equipment. Not to forget the health costs to the hundreds of thousands of Service Members that have microbe related ailments after 16 years of combat.

Like war, prevention and remediation of microbe induced corrosion requires a multi-disciplinary approach that involves Engineers and Preventative Medicine. Engineers deploy and construct base camps that reduce dust and prevent standing water. Preventative Medicine writes and enforces hygiene standards throughout the force. Together, with senior leaders and across all warfighting functions, they build, sustain, and apply combat power to achieve decisive results worldwide.

## **(Poster #2)**

### **Title: Viral aerosols in the built environment: Who's there and how do indoor conditions affect their viability?**

**Authors:** Aaron J. Prussin II, Kaisen Lin, Karen Kormuth, Seema Lakdawala, and Linsey C. Marr  
(Virginia Tech)

Although we have made major strides in understanding the bacterial microbiome, we still know very little about viruses in the built environment (BE).[1] Viral aerosols play an important role in microbial ecology and are responsible for many respiratory infectious diseases. We have identified various sources of viruses in air of the BE including humans, pets, plants, HVAC systems, dust resuspension, and the outdoor environment.[2] We have also shown the potential for wastewater systems to aerosolize pathogenic viruses, such as Ebola.[3] In the BE, the airborne concentration of viruses is similar to that of bacteria.[4]

Understanding the seasonal dynamics of the community structure of viral aerosols is important when considering new ways to engineer healthier BEs. We collected HVAC filter samples at a daycare center to study viral aerosol communities over the course of a year. Although our previous research showed that bacterial communities in the daycare did not vary by season,[5] we hypothesize that certain pathogenic viruses will exhibit seasonality; however, since many airborne viruses are bacteriophages, we do not expect the overall viral community to be shaped by season.

Conditions in the BE can modulate the viability of airborne viruses. We have shown that there is a "U-shaped" dependence of influenza virus viability vs. relative humidity (RH), with decreased viability in droplets at 40-75% RH in some media, although mucus appears to have a protective effect.[6] It is possible that by simply adjusting RH, we might be able create an environment that is less favorable for influenza survival and transmission. We are further exploring the viability of viruses in droplets and aerosols as a function of RH. Ultimately this work will inform strategies for creating a healthier BE.

#### References:

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**(Poster #3)**

**Title: Indoor plants are biodiversity and functional hotspots for the built environment**

**Authors:** Alexander Mahnert<sup>1</sup>, Rocel Amor Ortega<sup>1,2</sup>, Christian Berg<sup>3</sup>, Henry Müller<sup>1</sup>, Martin Grube<sup>3</sup>, and Gabriele Berg<sup>1</sup>

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Molds are unfavorable for buildings and its residents, since they degrade building structures and are suspected to cause “mold related illness” or the “sick building syndrome”. However, our study also shows the beneficial potential of fungi and bacteria from indoor plants to act as biodiversity and functional hotspots in the built environment as well as to counteract fungal pathogens.

Fungi and bacteria from 14 phylogenetically diverse plant species grown under different controlled greenhouse conditions in the Botanical Garden Graz (Austria) were investigated by 16S rRNA gene amplicon analysis, cultivation and the two-clamp volatile organic compounds assay (TCVA).

High fungal ( $H' = 2.8 - 6.5$ ) and bacterial ( $H' = 2.4 - 7.9$ ) diversity, richness (fungi: 449-1050; bacteria: 151 - 567 observed OTUs) and abundance (fungi: 103-106; bacteria: 102-106 CFU cm<sup>-2</sup> leaf surface) was associated with the studied plant species. The structure of fungal and bacterial communities was strongly plant species-dependent and only marginally driven by the ambient climatic variables. Additionally, the function of fungal and bacterial isolates to suppress the plant pathogen *Botrytis cinerea* by the production of antifungal volatile organic compounds (VOCs) showed that 1/3 of the tested fungi (mainly *Penicillium*, *Cladosporium*, and *Cryptococcus* spp.) was able to inhibit mycelial growth, and 2/3 of the fungal and up to 58% of the bacterial isolates (e.g. *Bacillus* and *Stenotrophomonas*) inhibit sporulation completely.

This study indicates that plants grown indoors enrich distinctive functional guilds, and harbor a stable phyllosphere fungal and bacterial diversity regardless of abiotic conditions of a room. An independent maintenance of the beneficial plant-surface mycobiome and microbiome from their surroundings can support attempts to counteract developments in the built environment to become a so-called microbial wasteland and increase diversity of our beneficial microbial co-residents.

#### **(Poster #4)**

#### **Title: The interaction of resistome and mobilome in confined built environments**

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Confined built environments are maintained by a defined microbial control. Such actions drive selection processes and alter structure and function of the residing microbiome. However, accompanying effects on the resistome and mobilome were not sufficiently investigated. Therefore, we examined several built environments, showing an increasing level of microbial control, (public buildings, public and private houses, intensive care units – ICUs and cleanroom facilities) to understand coherences of microbial confinement and resistances.

Shotgun metagenomics and 16S rRNA gene amplicon analyses from large surface samples were applied to analyze these built environments by genome centric as well as gene centric approaches. Binned genomes and extracted plasmids were further investigated for their resistance network to understand the transfer of mobile genetic resistance elements in microbial confined built environments.

Increasing microbial confinement was associated to a shift from gram-positive (Actinobacteria and Firmicutes) to gram-negative bacteria (Proteobacteria) encoding for many functions associated to virulence, disease, defense and resistance. Likewise, phenotypic traits shifted significantly for facultative anaerobic bacteria ( $P=4.17 \cdot 10^{-6}$ ), pathogens ( $P=0.0008$ ), stress tolerance ( $P=4.17 \cdot 10^{-7}$ ) and mobile genetic elements ( $P= 0.0004$ ), from uncontrolled to ICU environments. 377 different resistance features could be identified for 42 selected high quality binned genomes and 91 plasmids. While resistance to tetracycline and Pulvomycin were mainly exchanged on plasmids in uncontrolled built environments, confined built environments showed a completely different resistome and comprised many proteins associated to multidrug resistances (e.g. *mtrR* and *mexK*) as well as resistance to novobiocin in genomes assigned to *Acinetobacter*, *Agrobacterium* or *Pseudomonas*.

Deciphering the complex interaction network of microbes and their plasmids to exchange genetic resistant elements in confined compared to naturally uncontrolled built environments will help to identify and target microbial key players in these environments for an adapted microbial monitoring and biotechnological control to reduce resistance developments in the future.

**(Poster #5)**

**Title: Microbial dynamics in a sealed spacecraft mock-up simulating a full duration flight to planet Mars**

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A manned flight to planet Mars will confront its crew with an extremely isolated built environment. However, despite such rigid confinement the crew and their spacecraft still have to share their habitat with microorganisms. The MICHA (“Microbial ecology of Confined Habitats and humAn health”) experiment monitored microbial dynamics in the air and on diverse surfaces inside a confined sealed spacecraft mock-up for more than 500 days in the frame of the so-called Mars500 project. Over the full duration of this project 360 samples from the air and different surfaces were collected and analyzed by 16S rRNA gene amplicon sequencing, supported by PhyloChip G3 analysis and extensive cultivation.

Overall the microbiome was dominated by human associated bacteria assigned to *Corynebacterium*, *Ralstonia* and *Staphylococcus*. According to next generation sequencing microbial diversity decreased significantly over time. On the contrary, the proportion of opportunistic pathogens, stress-tolerant or potentially mobile element bearing microorganisms increased. Temporary fluctuations of microbial diversity and composition could be linked to procedures of microbial maintenance conducted by the crew. However, microbial communities did not only show a time-dependent, but also a location-dependent composition. Hence, microbial compositions in wet rooms, on table surfaces or a greenhouse, could be further linked to different surface materials and surface orientation, but only slightly to climatic variables.

This study clearly shows that despite confined conditions, human associated microbiota are still subject to fluctuations. A detailed monitoring of microbial abundance and diversity will be essential to counteract threatful developments, such as an increase of highly resistant microorganisms or labile and very low microbial diversity, to guarantee a safe and healthy journey to planet Mars.

## **(Poster #6)**

### **Title: Are Opportunistic Pathogens in Premise Plumbing the Source of Infection in Children with Cystic Fibrosis?**

**Authors:** Sarah-Jane Haig<sup>1,2</sup>, Nadine Kotlarz<sup>1</sup>, Lindsay Caverly<sup>2</sup>, Linda M. Kalikin<sup>2</sup>, John J. Lipuma<sup>2</sup> and Lutgarde Raskin<sup>1</sup>

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Opportunistic bacterial infection of the airways is an inevitable hallmark of cystic fibrosis (CF) and the major factor responsible for reduced life expectancy (median survival ~29 years of age). The source of opportunistic bacterial lung infections in patients with CF is often thought to be from the environment, although it is unclear which specific environmental exposures pose the greatest risk of infection. Previously, we have found that the top five CF opportunistic bacterial pathogens (*Achromobacter*, *Burkholderia*, non-tuberculous mycobacteria (NTM), *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*) are common in drinking water (DW) samples from homes of individuals whom do not have CF. In addition, we have identified several clinically relevant species and strains of NTM and *Burkholderia* in premise plumbing water and aerator biofilms. Therefore, it is likely that CF patients are exposed to opportunistic pathogens through use of their DW. However, few studies have compared genotypes of DW and CF patient-associated strains to make an epidemiologic link, primarily due to the lack of high-throughput and culture-independent methods capable of distinguishing bacterial strains.

In order to test whether CF patients acquire opportunistic bacterial infections through exposure to the microbial community in municipally treated drinking water, we sampled drinking water from 20 homes of pediatric CF patients who recently (within six months) became infected with a CF relevant opportunistic pathogen. Within each home, swabs of biomass from several aerators, filtered biomass from stagnated (> 6 h) cold premise-plumbing water and samples of hot water were collected. All homes were located within Michigan and were serviced by either chlorinated or chloraminated DW. To determine if a strain-level match exists between the child's clinical isolate and their DW, we will use our newly developed, accurate, and high-throughput Pacific Bioscience assays.

The findings from this study will provide insight into whether DW is a source of infection for individuals with CF. Overall, the tools developed by this study allow high throughput strain-level screening of opportunistic CF pathogens in environmental samples, without the need for prior cultivation. Furthermore, this novel approach will have far reaching applications for the identification of other sources of clinically relevant opportunistic pathogens that pose a risk for CF patients as well as other individuals susceptible to chronic bacterial infections.

**(Poster #7)**

**Title: Quantitative microbial risk assessment for opportunistic pathogens in roof-harvested rainwater**

**Authors:** Kerry Hamilton<sup>1</sup>, Warish Ahmed<sup>2</sup>, Simon Toze<sup>2</sup>, Charles N. Haas<sup>1</sup>

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A quantitative microbial risk assessment (QMRA) of opportunistic pathogens *Legionella pneumophila* (LP) and *Mycobacterium avium* complex (MAC) was undertaken for various uses of roof-harvested rainwater (RHRW) reported in Brisbane, Australia to identify appropriate usages and guide risk management practices. Risks from inhalation of aerosols due to showering, swimming in pools topped up with RHRW, use of a garden hose, car washing, and toilet flushing with RHRW were considered for LP while both ingestion (drinking, produce consumption, and accidental ingestion from various activities) and inhalation risks were considered for MAC. The drinking water route of exposure presented the greatest risks due to cervical lymphadenitis and disseminated infection health endpoints for children and immune-compromised populations, respectively. It is therefore not recommended that these populations consume untreated rainwater. LP risks were up to 5 orders of magnitude higher than MAC risks for the inhalation route of exposure for all scenarios. Both inhalation and ingestion QMRA simulations support that while drinking, showering, and garden hosing with RHRW may present the highest risks, car washing and clothes washing could constitute appropriate uses of RHRW for all populations, and toilet flushing and consumption of lettuce irrigation with RHRW would be appropriate for non-immune-compromised populations.

**(Poster #8)**

**Title: Identifying parasites in New York City sewage**

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Microbial eukaryotes (protists) are important components of terrestrial and aquatic environments, as well as animal and human microbiomes. Despite their ecological and economic importance little is known about protist diversity, incidence or emergence in urban environments. The 7,400-mile combined sewer system of New York City (NYC) collects human and animal waste, street runoff, and groundwater from ~8 million human inhabitants, providing an ideal system to study these microbes. As a part of our “Mapping the New York City Microbiome” project, we have used environmental metagenomics (shotgun metagenomic and amplicon sequencing of the 18S rRNA marker gene) to characterize the protist communities in raw sewage. Assisted by the NYC Department of Environmental Protection, sewage samples were collected over a 12 month period from 14 treatment plants representing all five NYC boroughs, and compared with samples from other NYC environments including: soil from parks and green spaces, storm water, and sediment. NYC sewage was found to contain a diverse protist community dominated by free-living clades, and communities were highly differentiated across the different environments. Protists typically associated with human and animal guts or feces, for example multiple species of *Entamoeba*, *Blastocystis* and trichomonads were detected. Abundance of these parasites, which in one case made up ~50% of the protist community, varied significantly both spatially and temporally suggesting that spikes could reflect trends in the source population and underscoring sewage as a valuable biomarker for monitoring urban microbes. We are currently expanding our study to investigate parasites in human pets (cats and dogs) and pests (rats, pigeons, cockroaches) in NYC, with the eventual goal of establishing a baseline protist microbiome of the city.



**(Poster #9)**

**Title: Graphitic carbon nitride nanomaterials for sustainable antimicrobial applications**

**Author:** Hongchen Shen and Danmeng Shuai (Civil & Environmental Engineering, The George Washington University)

Pathogenic biofilms developed on hospital surfaces, medical devices, and food processors can result in serious problems such as nosocomial infections and foodborne diseases. For example, more than 80% of pathogenic foodborne diseases are caused by biofilms in food industry every year, and great efforts have been spent for biofilm control in food. However, current biofilm control strategies suffer from low efficiency, continuous application of disinfectants, and high cost. Recently, graphitic carbon nitride (g-C<sub>3</sub>N<sub>4</sub>), a visible-light-responsive photocatalyst, attracts great attention in antimicrobial applications, and it has several advantages compared to many other antimicrobials. The material produces oxidative species, such as reactive oxygen species (ROS) and holes, to inactivate a broad spectrum of pathogens (including biofilms), by activating oxygen in the air under visible light irradiation. The material is non-consumable and stable in application, synthesized from earth-abundant precursors, reactive under ambient conditions, and its properties are highly tunable for improving photocatalytic performance. The objective of our study is to develop and evaluate the performance of g-C<sub>3</sub>N<sub>4</sub> for biofilm inactivation and eradication, and understand the key mechanism that determines the performance of g-C<sub>3</sub>N<sub>4</sub>. g-C<sub>3</sub>N<sub>4</sub> powder was first prepared via thermal polycondensation of a supramolecular complex of melamine, cyanuric acid, and barbituric acid, and next the powder was pressed into coupons. *Staphylococcus epidermidis* (*S. epidermidis*), a common pathogen resulting in nosocomial infections, was selected as a probe microorganism for developing biofilms on g-C<sub>3</sub>N<sub>4</sub> coupons. Confocal laser scanning microscopy and optical coherence tomography were utilized to determine biofilm morphology and viability under different treatment. Results revealed that a thick, live biofilm was developed in the dark (ca. 80-120 μm) for 3 days but no biofilm was formed under continuous irradiation of white LEDs for the same duration. Biofilms developed in the dark were eradicated under light treatment, leaving a thin layer of dead cells on coupon surface. A kinetic study of biofilm eradication under light revealed that the inactivation of bacterial cells was achieved from the biofilm boundary to the center, likely due to the combinational effect of diffusive ROS and surface holes. The effect of nutrient concentration was also investigated (0.02, 0.1, 0.5 X TSB broth), and biofilms were successfully eradicated at any concentration. Our study is the first to demonstrate the viability of g-C<sub>3</sub>N<sub>4</sub>-based photocatalysis for biofilm control, and it holds promise as a sustainable approach to prevent nosocomial infections and foodborne disease outbreaks by utilizing indoor visible light and inexhaustible air oxygen.

**(Poster #10)**

**Title: Quantification of opportunistic pathogens in shower water and aerosols formed during showering.**

**Authors:** Yun Shen<sup>1</sup>, Aaron Justin Prussin II<sup>2</sup>, Sarah-Jane Haig<sup>1</sup>, Linsey Marr<sup>2</sup>, and Lutgarde Raskin<sup>1</sup>  
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Respiratory tract opportunistic pathogens (RTOPs) are commonly found in treated drinking water and can transfer from water to indoor air by aerosolization during showering. In this study, we surveyed bacteria and selected RTOP abundances in shower water and aerosols generated during showering. The shower water and aerosols were collected from an apartment (with an in-unit water heater) and a university building (served by a campus wide hot water loop) in Ann Arbor, MI. The shower water samples were collected every 5 minutes during a 20-minute shower, while three 20-minute aerosol samples were collected, one before, one during, and one after showering using an SKC biosampler. The abundances of total bacteria, *Legionella* spp., and *Mycobacterium* spp. were determined by qPCR. In the apartment, the total bacterial concentrations in the shower water ranged from  $5.6 \times 10^6$  to  $1.2 \times 10^7$  16S rRNA gene copies/L. The total bacterial concentrations in aerosols collected during showering were  $(1.3-1.4) \times 10^5$  gene copies/m<sup>3</sup>, and these concentrations were statistically higher than those found in background air before showering (t-test,  $p < 0.05$ ). In the university building, the total bacterial concentrations in shower water decreased from  $4.6 \times 10^7$  to  $1.9 \times 10^3$  gene copies/L over the 20-minute shower, whereas bacterial concentrations in aerosols were  $(0.5-1.1) \times 10^5$  gene copies/m<sup>3</sup> during showering. At both sampling sites, *Legionella* spp. and *Mycobacterium* spp. were detected in shower water at concentrations up to  $7.9 \times 10^6$  copies/L and  $2.8 \times 10^4$  copies/L, respectively. *Legionella* spp. were detected in all aerosol samples from both sampling sites, but were below the qPCR quantification limit of 2,748 gene copies/m<sup>3</sup>. *Mycobacterium* spp. were only detected in the aerosol samples collected from the university building, but were below the qPCR quantification limit of 3,480 gene copies/m<sup>3</sup>. Future efforts will assess the abundance of the RTOPs at additional sites and under different showering conditions (temperature, flow rate, etc.). Ultimately, this work will allow application of quantitative microbial risk assessment to investigate the impact of different mitigation strategies.

**(Poster #11)****Title: Core microbiome shared across drinking water distribution systems****Authors:** [Joline El-Chakhtoura](#), Emmanuelle Prest, Pascal Saikaly, Mark van Loosdrecht, Hans Vrouwenvelder (TU Delft, King Abdullah University of Science and Technology, Saudi Arabia)

Drinking water distribution systems (DWDSs) are complex engineered systems designed to transport clean and safe water. Our objective was to determine (i) the impact of water distribution/age on the water bacterial communities, (ii) the effect of network flushing and (iii) whether there is a baseline, indigenous bacterial community– using 16S rRNA gene sequencing and flow cytometry. Seven full-scale DWDSs in The Netherlands were studied differing in water source, treatment strategy and network characteristics. Water samples were collected from (i) the treatment plant outlet and from the network (ii) before and (iii) during flushing. The network samples (ii and iii) were taken from different locations, representing short, middle and long distance from the treatment plant. A change in water quality parameters was detected during distribution and flushing. Tap water samples showed a notable increase in biomass. The values were significantly higher in the flushed water, revealing that planktonic bacteria represent a small fraction of the microbial load established in a DWDS, whereby bacterial activity can be heavily influenced by pipe biofilms and loose deposits. While a core microbiome dominated by Gammaproteobacteria was found within the treated, distributed and flushed water, an increase in the relative abundance of Betaproteobacteria was detected in the network. All TAP and FLUSH samples had a similar community structure, indicating that the bacterial community converged during distribution, despite different plant origins. Distance or residence time did not impact the water microbiology. This study shows the presence of a core microbiome shared between the treated, distributed and flushed water, with stable communities observed during distribution– in the same network and within different networks. The “network quality” seems to be governing the water quality, with biofilm and sediment communities driving the major microbial processes in the network, causing the key change in bacterial ecology and resultant tap water quality.

**(Poster #12)**

**Title: Fungi in the Wind: A Decade-Long Study of the Diversity of Fungal Spores Captured at the Mauna Loa Observatory in Hawai'i**

**Authors:** Laura Tipton, Geoffrey Zahn, Erin M. Datlof, Patrick Sheridan, Anthony S. Amend, Nicole A. Hynson (University of Hawaii at Manoa)

Many fungi produce spores small enough to enter the upper atmosphere and disperse long distances via global air currents. An active air sampler system was placed above the cloud layer at the Mauna Loa Observatory (MLO) on the island of Hawai'i, 3397 meters above sea level, and captured all particles smaller than 10  $\mu\text{m}$  on borosilicate glass fiber filters for 13 years. We expected these filters to capture a variety of the small spores floating around in the upper atmosphere. Using Illumina targeted amplicon sequencing of the fungal ribosomal internal transcribed spacer (ITS) region, we identified 160 operational taxonomic units (OTUs), representing 153 unique taxonomies, from 172 filter samples. Each air filter represents a snapshot of the aerobiota present at MLO during the time of collection. The community richness of the filters was low, averaging 35 OTUs (SD = 7.36) on each filter, and showed no significant trends over seasons or the decade-long sampling effort. Similarly, the beta diversity between communities was low, with an average Bray-Curtis dissimilarity of 0.41 (SD = 0.08). Surprisingly, beta diversity showed no trend over the decade-long sampling effort, but we did find significantly increased dissimilarity between filters collected in the first quarter of the year (January, February, and March). Characteristics based on taxonomy, including spore characteristics and ecological guild or function showed no trend over the sampling effort or seasonality. Local weather and atmospheric variables were poorly correlated with community composition, however, climate variables from long distance source locations may better explain the community compositions of these potentially long distance dispersing fungi.

**(Poster #13)**

**Title: Role of water treatment, nutrients, and environmental factors in regulating viral and microbial composition, diversity, and function in aquarium built environments using metagenomic approaches**

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Viruses have been found to be the most abundant biological life forms on earth with high abundance, particularly in the ocean where they have been associated with marine biomass destruction. Although native aquatic environments have been the focus of recent viral metagenomics (virome) studies, there is a need to understand the role of viruses in maintaining optimum living conditions of the built and controlled aquarium environment. The goals of this study were to apply metagenomic approaches for (i) characterizing viral and bacterial communities of tropical, marine mammal, and temperate human-contact aquaria habitats and (ii) examining the role of water treatment systems, nutrients, and environmental factors in shaping viral and bacterial populations of these habitats. Water samples were collected from three aquaria: Oceanarium, Wild Reef, and Sturgeon Touch, and their water treatment systems: sand filter, ozone, and UV water disinfection at the Shedd Aquarium in Chicago (25 sampling sites distributed across 3 aquaria habitats). Twenty-five viral water samples (n = 25) were concentrated, enriched, purified and DNA/RNA extracted, reverse transcribed, randomly amplified, and sequenced by Illumina HiSeq 4000. Bacterial water samples (n = 25) were collected by Sterivex filters, DNA extracted, and amplicon-sequenced (*16S rRNA*, V3-V4) by Illumina MiSeq. Viral and bacterial metagenomics sequences will be analyzed for characterizing composition, diversity, function, and variation in aquaria habitats as a function of water filtration system, nutrients, and environmental factors. Interactions between virus and host, particularly virus and bacteria will also be explored using Clustered Regularly Interspaced Palindromic Repeat (CRISPR).