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Improved detection methods of Clostridium perfringens and Coliphage viruses in reclaimed water

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Background: With rapid population growth and economic development in North Carolina, increased quantities of water are demanded for industrial, agricultural, and domestic use. As such, reclaimed water has been suggested as a means to augment currently available water sources. The state of North Carolina has recently defined new performance requirements for indicator viruses and protozoan parasite surrogates allowable in Type II reclaimed water, a new category of higher quality water for agricultural irrigation of food crops that could be consumed raw and other beneficial uses. However, microbiological analysis methods for reclaimed water were not specified. Methods: This study investigated improved methods for analysis of coliphages and Clostridium perfringens as fecal indicators in reclaimed water to provide affordable, effective and practical means for other laboratories to test for them. For coliphage analysis bacterial hosts were tested for "total coliphages" against current standard requirements for separate analysis of both male-specific (F) and "somatic" coliphages on different E. coli hosts. Two standard coliphage methods were tested, two step enrichment-spot plate (EPA Method 1601) and Single Agar Layer (SAL) (EPA Method 1602). This study also compared different agar media to enumerate Clostridium perfringens in type II reclaimed water, as several candidate media with potentially different performance are available and none were specified by the regulation. Results: Total coliphage host E.coli CB390 gave the highest coliphage detectability for both somatic and F coliphages and provided similar results to the sum of coliphages separately detected by currently accepted somatic (E.coli CN13) and F (Famp) hosts, respectively. SAL and two step enrichment methods provided similar results for coliphage detection. However, the enrichment-spot plating method requires two steps, takes 48 hours and gives estimated MPN concentrations. In contrast, the SAL method requires only a single-step, takes 24-hours and provides direct enumeration. Clostridium perfringens media tested for membrane filtration included mCP, TsC, and Chromoselect (CS) agars. Samples were tested for unpasteurized (spores and vegetative clostridia) and pasteurized (spores only) samples. CS medium provided highest Cl. perfringens detectability on both pasteurized and unpasteurized samples, although some false positivity occurred. However, false positivity also occurred with the other media and these media had other deficiencies as well, such as ambiguous appearance of colonies. Conclusions: Based on the observed results, it appears that combined detection of total coliphages is an effective, efficient and less costly option to separate detection of somatic and F coliphages and that Clostridium perfringens detection by membrane filter enumeration on CS agar is also an effective analytical method. However, more data collection and statistical analyses will be needed to better assess the most effective assay techniques for the examination of these microbes in reclaimed water.

Release and runoff partitioning of manure-borne indicator bacteria during simulated rainfall

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Microorganisms, as well as other manure components, are released from land-applied manure during precipitation and irrigation events and, subsequently, transported in suspension with surface runoff or infiltrated water with the potential to contaminate surface water and groundwater resources used by humans. Understanding and modeling the release of manure-borne pathogen and indicator microbes as

well as other manure constituents is essential for making accurate predictions and risk assessment of microbial fate and transport in the environment. Current kinetic-based manure release models oversimplify bacteria release processes and need to be evaluated with new data on manure dissolution in order to better explain: 1) the relationship between the microbial concentrations found in manures and the microbial concentrations at different stages of release during rainfall and 2) the partitioning of manure constituents into runoff and infiltration. The objective of this work was to compare the contents of E. coli, enterococci, total coliforms, fecal coliforms, and organic carbon from two types of cattle manure (that from a dairy CAFO and that from grazing cattle) to the concentrations of these components released over time into runoff and infiltration water during rainfall simulations, using partitioning boxes. The partitioning boxes were designed to have manure applied on a mesh-covered frame (70cm x 70cm), and to have both runoff and infiltration collected from troughs on level with the mesh frame and below the base frame, respectively, during a simulated rainfall event. Concentrations of the indicator microbes in the initial release were, on average, 0.7 and 1.1 orders of magnitude less than their effective initial concentrations in the manure liquid phase (i.e., concentration in manure divided by the manure water content) for CAFO manure and grazing cattle manure, respectively. The microbial release kinetics appeared to follow a piece-wise, log-linear shape, beginning with a precipitous log-linear drop in concentration of released constituents during the first four to eight minutes of rainfall, followed by a much slower log-linear release. In contrast, the organic carbon displayed a consistent exponential release. The early release rates of E. coli, fecal coliforms, and total coliforms were significantly higher coming from CAFO manure than from grazing cattle manure. The release rates of microorganisms into synchronous runoff and infiltration did not differ significantly. In light of these preliminary results, manure release models should be re-evaluated to ensure that their formulations do not oversimplify bacteria release processes, which could lead to erroneous results. This research will be extended to advance parameterization and application of manure release kinetic models that are critical for risk assessment of microbial contamination of the environment.

Fate of human noroviruses in shellfish water catchments in England and Wales

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The routes of environmental transmission of human noroviruses (NoV) remain largely undescribed in the scientific literature which constrains the development of measures to reduce the incidence of shellfish-related gastroenteritis. This study investigated these routes via a two-stage approach involving desk and field studies. The relationships between levels of NoV in oysters and a selection of risk factors were investigated using correlation analyses. The variables correlated were NoV quantities (genome copies/g digestive gland for genogroups I and II) and most probable number of E. coli quantified in 31 oyster sampling sites around the coast of England and Wales from May 2009 to April 2011, and descriptive information on risk factors (total resident population, population density, catchment area, treatment level and volume of impacting wastewater treatment works (WwTW), fluvial distance from oyster beds to the sewage outfall, river flows, rainfall and tidal range). In addition, a source apportionment study was carried out to evaluate the contribution of sewage sources and riverine inputs to NoV contamination and compare the decay of NoV (GI and GII) with that of E. coli in the receiving shellfish water. This element of the study used levels of NoV and E. coli quantified in sewage (UV-disinfected and settled storm) and in oysters contained in bags placed at 6 sites at various distances from the WwTW outfall from October 2012 to March 2013. The results indicated significant positive

correlations between levels of NoV in the oysters with river flows and positive correlations between E. coli levels and both river flows and rainfall. On a site average basis, levels of NoV were positively correlated with total resident human population in the catchment, size of catchment area and volume of sewage discharged to the shellfish waters. In contrast, urbanised area and population density did not significantly influence NoV contamination. This demonstrates that the transmission of the virus in the environment is not directly linked to the level of catchment urbanisation. The source apportionment study showed that storm tank discharges represented 97.6%-99.9% of the total (GI + GII) NoV load from the WwTW during the study period. The NoV loadings from the storm tank varied widely between sampling occasions. In the shellfish water, mean levels of total NoV were of the same order of magnitude over a fluvial distance of 7km from the WwTW outfall, decreasing only 0.6log10 at the station positioned 10km away from the sewage source. Rainfall events (>0.2mm) in combination with stormwater sewage spills resulted in total NoV detection in oysters exceeding 1,000 copies/g at all stations in the receiving water. Fluvial distance accounted for 37% of the variation in log10 of total NoV levels and 30% of the variation in log10 of E. coli in oysters in the study site. These results indicate that storm overflows are the dominant sources of NoV impacting shellfish beds in catchments with combined sewerage infrastructure. Furthermore, the geographical extent of NoV contamination could be very significant considering the potentially long residence times of the estuarine waters and relatively small areas associated with commercial shellfisheries. Fundamentally, these results provide insight into the understanding of the processes driving the persistence and distribution of NoV in shellfish water catchments. Improved knowledge on these processes will be critical for the development of measures to prevent transmission pathways and/or control by introduction of NoV food safety standards.

Cross-laboratory Comparison of a Duplex Digital PCR Assay for Simultaneous Quantification of Enterococcus spp. and Human Fecal-associated HF183 Marker

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Quantitative PCR (qPCR) provides indirect quantification by comparing unknown samples to known standards. While its speed and flexibility led to its wide use in water monitoring, the lack of reliable and consistent quantitative standards remains the biggest obstacle for qPCR applications, particularly in scenarios where comparison and integration of results across laboratories and studies are essential. Digital PCR provides direct, standards-free quantification based on limiting dilution and Poisson statistics and therefore eliminates quantification biases associated with inconsistent standards. Nevertheless, little work has been done to assess inter-laboratory consistency of digital PCR results. Here, we compared the performance of a duplex droplet digital PCR (ddPCR) assay targeting Enterococcus spp. and the HF183 marker across two laboratories. Each laboratory analyzed, in duplicate, DNA aliquots of standards, sewage and septage samples from 4 states, composite animal fecal samples for each of the 9 sources (cow, elk, deer, dog, horse, chicken, duck, goose, and raccoon) collected in 7 states, and ambient waters from southern California. Basic assay performance metrics such as linearity and detection limits of the ddPCR assay were similar between the laboratories regardless of quantification targets. For the human fecal-associated marker, both laboratories could quantify HF183 down to approximately 0.005ng sewage DNA and 0.05ng septage DNA. Neither laboratory showed cross creativity with any animal fecal DNA when tested at 2.5 ng per reaction except for two cow composite samples. The interlaboratory difference and intralaboratory variability were similarly low. The mean interlaboratory differences for Enterococcus spp. and HF183, respectively, were 0.06-0.15 and 0.004-0.17 log10 unit for various samples tested, much lower than those reported for qPCR (0.5-2 log10 unit)

due to inconsistency in standards. Overall, the duplex ddPCR assay demonstrated stable performance and highly reproducible results across the two laboratories. While further studies with a larger number of laboratories would provide more information on ddPCR's reproducibility, initial evidence from this study showed that ddPCR could significantly enhance our ability to compare and integrate monitoring results across studies/sites/laboratories.

Qualitative Detection of Microcystin (MC) as Early Warning of Water Quality Monitoring Programs

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Cyanobacterial bloom has significant hazard to public health as well as environment due to release of cyanotoxins into water supplies (Ueno et al., 1996) with many fatalities reported (Pouria et al., 1998; Carmichael, 1996). The cyanotoxins comprises hepatotoxins (microcystins and nodularin), neurotoxins (anatoxins and saxitoxins), cytotoxins (cylindrospermopsins), and dermatotoxins (aplysiatoxins and debromoaplysiatoxins), which are produced by about 40 genera of cyanobacteria (Sivonen and Jones, 1999;). Microcystins (MCs), are predominant type of cyanotoxin and produced by different species of cyanobacteria such as Microcystis (unicellular and colony forming), Oscillatoria (filamentous), Anabaena and Nostoc (heterocystous, filamentous and N2-fixing), usually occurring in fresh water bodies throughout the world. Several analytical and biochemical MC detecting methods are currently in use, including HPLC, Immunological (Elisa), and molecular methods (McElhiney & Lawton, 2005). These quantitative methods are limited in terms of their potential application in routine environmental monitoring. Analytical methods often require time consuming sample preparation procedures that usually involve the pre-concentration of considerable water volumes prior to analysis to reach the required sensitivity. Furthermore, the ability of these techniques to identify unknown MCs in environmental samples has been hindered by the lack of analytical standards for many MC variants. Thus a simple, easy-to-use, rapid, robust, specific, sensitive and portable method may account for detecting low concentrations of MCs, which can act as a better monitor for MCs for monitoring programs of water quality (Singh et al. 2012). Generally the detection of microcystin requires quantitative analytical methods such as HPLC or Elisa whose sophisticated analytical requirements have been impacting their insertion in water quality monitoring programs, especially for small and medium-sized communities in Brazil and other developing countries. This study aimed to develop a kit for qualitative assessment of microcystins in water quality monitoring programs. The central idea is to use the qualitative system as an early warning signal of presence / absence (PA) of water cyanotoxin. If the samples reveal the presence of cyanotoxin, samples should be grouped for one complete quantitative analysis to meet the legislation criteria. The methodology for the qualitative kit of microcystin (P/A) is based on inhibition reaction of phosphatase using as substrate phosphate-methyl-umbeliferil MUP in a 2ml reaction medium with the water sample according to Bouiacha et al. (2002). The enzyme PP1A was immobilized on fiberglass membranes. The enzyme activity was determined by fluorescence emitted of methyl umbeliferil (MU) formed with ambient temperature by enzymatic degradation of the substrate (MUP). The water samples were collected from different sources from the Metropolitan urban area of Vit_ria, ES., Brazil and evaluated for the presence of microcystins. Various tests were also carried out using spiked samples with microcystin standards using different water samples at various concentrations of microcystin. The results showed that the proposed system has ability for direct visual determination of microcystin at concentrations above 0.6 to 0.8 mg. L-1 for several water samples evaluated. Under test conditions it was not necessary any cleaning or prior concentration of water samples. The results

shown that proposed qualitative method presented high sensitivity and selectivity for microcystins assessment, with false negative rates below 10% of the total water samples tested.

Bacterial and Viral Indicators of Water Quality in an Urbanized River

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The Chattahoochee River, a major Georgia waterway, is both a drinking water source for nearly 3 million people and a surface water discharge point for 100 public and private wastewater treatment plants serving metro Atlanta and the surrounding areas, as well as being a major recreation site. This project measured water quality at 15 sampling points along a 15-mile stretch of river that runs past the City of Atlanta. This stretch includes two discharge points for wastewater treatment plants serving the greater Atlanta area, and receives stormwater runoff from local communities. Water samples from the river were analyzed for human fecal indicators, including E. coli, male-specific coliphage, and pepper mild mottle virus (PMMoV), a potential indicator of pathogenic human viruses in water. From April to October, water samples were collected at 1-mile intervals from the middle of the river at a depth of 6 inches, with a total of seven sampling rounds. Samples were also taken from the area of two sewage effluent discharge points. Samples were analyzed for E. coli using membrane filtration, male-specific coliphages using two-step enrichment procedure and spot plating, and PMMoV by PCR. E. coli concentrations across all samples ranged from 0.5-2.7 log10 CFU/100mL. The lowest mean concentration (mean of 15 samples taken on the same day) was 1.76 log10 CFU/100mL in May (95% CI 1.66-1.90) and the highest mean concentration was 2.72 log10 CFU/100mL in August (95% CI 2.68-2.76); mean E. coli concentrations were significantly different across sampling rounds (p=0.001). E. coli levels at effluent discharge points were not elevated compared to upstream and downstream samples. Male-specific coliphage was detected in at least 5 samples in every sampling round. PMMoV was sampled at sites one mile upstream and downstream of one of the sewage discharge point; one mile upstream it was detected at a concentration of 52.1 copies/L, and one mile downstream at a concentration of 2.5_104 copies/L. The presence of fecal indicator organisms and PMMoV in the river suggests that the waterway is vulnerable to fecal contamination; given the multiple uses of the river by the surrounding population, effective monitoring and watershed protection is vital for protecting water quality.

Using Protein-Coated Nanospheres to Model Viral Adsorption

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“The World Health Organization estimates that one billion people worldwide drink unsafe water and 3.4 million people, mostly children, die every year from water related diseases. While this is an issue often associated with the undeveloped world, there are also virus outbreaks in the developed world, from water treated in engineered treatment facilities. This is evident in the recent identification of the poliovirus in Israel. Poliovirus was first identified in a wastewater treatment plant in May 2013; by October, more than 140 samples from 25 sites through the country had tested positive for poliovirus. The literature demonstrates that traditional bacterial indicators do not co-locate exclusively with infectious viruses because coliforms respond differently to environmental stressors and engineered treatment processes than protozoan and viral. Given these limitations, alternative indicators for viral

pathogen risk are necessary. This research involves the possible use of an abiotic viral surrogate to model human enteric pathogens, because of the limited ability of bacteria to represent viral transport and the difficulty in the use of surrogate viruses in research. Adsorption removals will be presented in regards to the varied surface characteristics of biotic and abiotic virus surrogates. The water quality community will be provided with new information regarding factors that affect the transport of viral pathogens through filter media at the liquid-solid interface. The presentation will include an analysis of factors that affect pathogen adsoprtion including: properties of the pathogen (i.e. surface charge, size, and morphology), properties of the granular media (i.e. mineralogy, size, texture, angularity) and properties of the water (i.e. pH, ionic strength, and content). The behaviors of bacteriophages coliphages MS2 and ΦX-174 through in bench scale analysis will be compared to 26 nm fluorescent nanospheres (uncoated and protein-coated). Adsorption will be modeled with sand (ANSI/AWWA B100 Filter Sand, 150# with a mean grain diameter of 0.45-0.55 mm) and sand washed through a series of rinses in concentrated acid and base to remove organics and metal oxides. The surfaces of the surrogates will be altered through changes in pH (range 4-8) and ionic strength (including lab water, low ionic strength artificial groundwater (AGW), and high ionic strength AGW.) This data will be used to analyze the impact on surface charge and viral coating on adsorption by demonstrating that protein coated nanospheres will correlate more directly to pathogen adsoprtion than uncoated nanospheres. Transport studies on these surrogates could answer some fundamental questions about pathogen behavior and provide more information about pathogen fate. This work will expand on the current knowledge of viruses through sand by altering surface characteristics of viral indicators to providing appropriate bench scale models. The goal of this research is to demonstrate that the surface charge of a microorganism could be closely mimicked by nanospheres coated with a protein a similar to viral surrogates. Viral indicators will be statistically analyzed (correlation analysis and analysis of variance) to provide a recommendation of the best representation of adsorption of human enteric viruses. Protein-coated nanospheres may provide a new and alternative approach to investigating pathogen transport."

General Esterase Assay for the Detection of Microbial Activity in Water

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The development of a novel enzyme activity assay in this work may contribute to the crucial need for safe drinking water and the improvement of people's health. It is important to ensure the quality of drinking water by regular monitoring in order to protect public health. The greatest need for water testing is usually within communities that have little resources, and it is important to make water testing simpler, cheaper, and more accessible. The general esterase assay is proposed to be a rapid detection technique that can detect and quantify the overall microbial activity in drinking water as an indication of the degree of contamination. The general esterase enzyme activity present in microorganisms is evidenced by the metabolism of the substrate 1-naphthyl acetate to produce 1-naphthol, a chromogenic product that may be correlated with microbial concentration in a water sample. The recent research has suggested the presence of general esterase enzyme in E.coli. Even though the current research is focused on E.coli, it is expected that other biota in water have the general esterase enzyme and would produce the same metabolic products. In this way the general esterase assay can be used to measure the overall microbial concentration present in water. If the assay is found to be an accurate indicator of microbial contamination, it can detect microbial contaminants in water in less than an hour's time since it does not require an incubation period of greater than 24 hours as is required in traditional culture-based techniques.

Examining the influence of urban definition when assessing relative safety of drinking-water in Nigeria: microbial water quality and sanitary risk

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Reducing inequalities is a major issue in water and public health and central to proposals for monitoring of future Sustainable Development Goals. There are periodic calls for differential national and global standards for rural and urban areas, often justified by the suggestion that, for a given water source type, safety is worse in urban areas. The objectives of this study were: (i) to examine the influence of urban extent definition on water safety between rural and urban areas in Nigeria, (ii) to compare the frequency of thermotolerant coliform (TTC) contamination and prevalence of sanitary risks between rural and urban water sources of a given type and (iii) to investigate differences in exposure to contaminated drinking-water in rural and urban areas. We use spatially referenced data from a national randomized sample survey of five Nigerian improved water source types to assess the extent of any disparities in safety between urban and rural areas. We combined the survey data on TTC and sanitary risk with map layers depicting urban versus rural areas according to eight urban definitions. When examining water safety separately for each improved source type, we found no significant urban-rural differences in TTC contamination and sanitary risk for groundwater sources (boreholes and protected dug wells) and inconclusive findings for piped water and stored water, both strongly dependent on urban definition. However, when improved and unimproved source types were combined, TTC contamination was, 1.6 to 2.3 times more likely in rural compared to urban water sources depending on the urban definition used. Our results suggest that different targets for urban and rural water safety are not justified and that rural dwellers are more exposed to unsafe water than urban dwellers. Spatial analyses that differentiate between urban and rural should assess multiple definitions or indicators of urban to assess robustness of findings.

Examination of Next Generation Sequencing (NGS) and traditional methods for detection of fecal contamination in a mixed-use fecal-contaminated waterway

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The level of fecal contamination in a waterway is often determined using fecal indicator bacterial (FIB) species or molecular methods such as quantitative PCR (qPCR) and order specific targets such as the Bacteroidales. Studies with the aim of determining the source of fecal contamination in a waterway have many complicating factors: both agricultural and recreational activities can occur simultaneously within a watershed, both urban and rural-specific wastewater infrastructure might be present, and there are concerns about regeneration of indicator bacteria, the specificity of genetic targets and the "regionality" of those targets. Because the microbial fraction of biota in a watershed is a complex community of organisms and traditional fecal indicator bacteria make up only a small fraction of the total microbial composition of fecal matter, we investigate here the relationship between fecal pollution source and associated microbial communities using traditional methods (qPCR of human and bovine-specific genetic targets, and direct FIB counts) and Next Generation Sequencing from both water and sediment using Illumina sequencing of amplicons from the 16S V4 rDNA region within bacteria. Our aim is to assess how traditional detection methods compare to NGS data under different types and levels of fecal contamination within a mixed-use watershed. Non-traditional genetic indicators or patterns within a microbial community could indicate a specific type of fecal contamination.

Giardia epidemiology among humans and animals in coastal Odisha, India: environmental loading from animals and infection in rural and urban settings

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Giardia lamblia, is an intestinal parasite commonly found in both humans and animals worldwide that can remain infectious for days to months in the environment and cause infection with a relatively low dose. In developing countries, such as rural India, the practice of open defecation and living in close proximity to livestock creates a situation where exposure to Giardia may be high, as unmanaged fecal material containing parasites can contaminate food and water sources through a variety of transmission pathways. The goal of this study was to estimate the environmental loading rate of Giardia cysts into the environment from seven animal host species in a coastal area of Odisha, India, and to evaluate if living in urban versus rural settings was associated with a different Giardia infection risk for humans or animals in order to better understand the epidemiology of Giardia in the study area. From April to May of 2012, 111 pooled animal fecal samples (typically five individuals per pool) from seven animal host species (cattle, buffalo, goat, sheep, chicken, cat, and dog) were collected from urban and rural settings along with 85 human fecal samples from patients at three diarrhea wards in the study area. Samples were screened for the presence of Giardia cysts and enumerated using fluorescent microscopy, with isolate genotypes identified using molecular methods. Overall Giardia shedding prevalence in human samples was 12%, while 32% of pooled animal samples were positive. Loading of Giardia parasites into the local environment, based on observed animal shedding rates and local animal population data for the seven host species residing in the study area was estimated to be 3.39 E + 12 parasites per day. Cattle, representing 66% of the study area's animal population, accounted for >99% of the total daily load. Dogs and cattle were found to shed decisively more Giardia cysts per gram of feces, as much as 2-3 orders of magnitude greater than other animal types (adjusting for location). Substantial support was observed for a location effect on Giardia shedding among animals, with rural animals shedding higher numbers of parasites, while no difference in the presence of Giardia cysts among humans was observed between urban and rural residence. Molecular characterization of isolates identified host-specific Assemblages C and D in dog samples and Assemblage A2 in humans. This study shows exposure from infected cattle and dogs may be an important public health concern in the study area as both cattle and dogs were found to shed relatively high numbers of Giardia cysts per gram and were estimated to contribute the highest loads of Giardia into the environment per day.

Protozoa contamination of community water sources in rural India: associations with fecal source identifiers, water source characteristics, and sanitary conditions

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Contamination of community water sources by the protozoa pathogens, Cryptosporidium and Giardia, is an important public health concern in rural India, where both humans and animal populations share water sources. The goal of this study was to estimate the prevalence of Cryptosporidium and Giardia in

community water sources in a coastal area of Odisha, India, and to evaluate associations between protozoa concentrations and microbial source tracking (MST) fecal markers, fecal coliform concentrations, water source characteristics, and spatial estimates of non-point sources of human and animal fecal loading around each water source to shed light on likely sources and causes of protozoa contamination. Earlier work found evidence that mass fecal loading from populations of cattle, buffalo, goat, sheep, and dog, contribute Cryptosporidium and Giardia into the study area environment at rates of 2.4 E + 10 oocysts and 3.39 E + 12 cysts per day. Public ponds (n=110), public tubewells (n=112), and private tubewells (n=98) across 60 villages were sampled during the 2012 and 2013 monsoon season to assess fecal contamination of the study area. A 20 L water sample was collected from each water source and analyzed by fluorescent microscopy to enumerate Cryptosporidium oocysts and Giardia cysts, membrane filtration culture techniques to quantify thermo-tolerant coliform concentrations, and MST methods to quantify concentrations of human- and livestock/domestic animal- associated fecal markers. Characteristics of water sources including conditions of tubewells and types of use were observed at each water point and GPS locations and related data to estimate the surrounding spatial density of livestock, latrines and people practicing open defecation were collected during site visits. Preliminary analyses indicate pubic ponds were significantly more contaminated with parasites than public or private tubewells ($p < 0.001$, Mann-U test) with a prevalence and mean concentration, respectively, of 33% and 6 oocysts/L of Cryptosporidium, and 74% and 43 cysts/L of Giardia. By comparison, public and private tubewells had mean concentrations below one oocyst and cyst/L, Cryptosporidium prevalences of 12% and 7% respectively, and Giardia prevalences of 9% and 18% respectively. Preliminary analyses indicate a positive correlation in pond water samples of Cryptosporidium and Giardia parasite concentrations with gene copy concentrations of the MST marker BacCow (detecting ruminant fecal sources) ($p <0.05$, Spearman's rank test). Further results from statistical analyses using a mixed effects model to assess associations between multiple predictor variables and parasite counts for Cryptosporidium and Giardia, will be presented and implications discussed. With findings from this study, a better understanding of the primary contributors of protozoan contamination of water sources in the study area will be developed and the knowledge gained made available to practitioners working to improve public health in the region.

Evaluating the severity of illness among water recreators

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Objectives: We evaluated the severity of illness among those engaging in limited-contact water recreation such as boating, fishing, kayaking, and rowing. Methods: Data were obtained from a prospective cohort study which assessed limited contact water recreators and the development of illness following recreation. The severity of GI illness was evaluated using self-reported measures of disease severity such as prescription and over-the-counter (OTC) medication use, visits or phone calls with a health care provider, staying home from work or school due to illness, and visiting the emergency department (ED) or hospital. The total duration of GI illness was also used to evaluate the severity of illness, along with a measure of symptom days, which was created by adding the total number of days with symptoms related to GI illness. Severity was also evaluated using the combined symptom days due to GI illness as well as other non-enteric illnesses such as, respiratory illness, or eye, ear, or skin infections. All measures of severity were evaluated according to the degree of water exposure, which was measured according to the degree of face wetness and the amount of water swallowed during water recreation. Data were analyzed using several statistical methods, including logistic regression and

model-based standardization. Results: There were 11,297 participants (including non-water recreators) who were available for telephone follow-up, of which 1,271 developed GI illness. Of those with GI Illness, approximately 55% took OTC medication, 46% of missed work or school, 16% sought medical care, either on the phone or in person, 8% took prescription medication, and 2% were seen in an emergency room or were hospitalized. Overall, the duration of GI illness ranged from 0 to 22 days, with a mean of 2.25 days and a median of 1 day. The mean duration of GI illness and mean GI symptom-days were statistically significantly higher among water recreators who swallowed water, compared to water recreators who did not swallow water. Additionally, 2,355 participants developed at least one symptom which met any of the case definitions. The total symptom days, for all illnesses, ranged from 0-66, with a mean of 4.1, and a median of 2 days. Participants who got their face wet, or who swallowed water, had statistically significantly larger mean total symptom days than those whom did not get their faces wet or swallow any water. With severity defined as symptoms lasting at least 4 days, among all water recreators (n=5,629) there was an elevated crude relative risk of more severe illness among for those getting their face wet during water recreation (RR 1.55 [1.30, 1.86]), and among those indicating that they swallowed water during water recreation (RR 2.05 [1.57, 2.67]). Conclusions: Increased water exposure, resulting in getting the face wet, or swallowing water could be associated with increased disease severity among water recreators. Further analysis is necessary to determine if fecal indicator bacteria (FIB) may predict the severity of illness among water recreators.

Genotyping Cryptosporidium Oocysts Recovered From Water Regulatory Slides

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While USEPA Method 1622/23 allows the enumeration of Cryptosporidium oocysts in water, it does not determine the species or genotypes of Cryptosporidium detected. This shortcoming is significant with regards to human health risk assessment, since many of the Cryptosporidium species which occur in environmental waters are not pathogenic to humans. In addition, species identification is useful for identifying human and animal sources of fecal pollution and the development of source water protection strategies. Therefore, the development of a method to genotype Cryptosporidium oocysts recovered from Method 1622/23 slides would provide significant added value to the Long Term 2 Enhanced Surface Water Treatment Rule (LT2) monitoring. In Phase 1 of this research we developed a method for recovering and genotyping Cryptosporidium oocysts present on regulatory water microscope slides (WaterRF 4099 Final Report, 2010; http://www.waterrf.org/Pages/Projects.aspx?PID=4099). For the genotyping of human-pathogenic and animal-associated Cryptosporidium oocysts, a single round multiplex PCR targeting the Cryptosporidium genes for 18S ribosomal RNA (18S rDNA) and heat shock protein 70 (hsp70) was developed. The assay detects the 18S gene present in all Cryptosporidium species and genotypes; while the hsp70 target is amplified from only human-pathogenic C. parvum, C. hominis, C. meleagridis and C. cuniculus. These four species of Cryptosporidium are responsible for almost all (>99%) cases of cryptosporidiosis in immunocompetent individuals. The assay is compatible with conventional and real-time PCR platforms/instruments, although real-time high resolution melt (HRM) analysis allows further discrimination of human and animal-associated Cryptosporidium spp. For Phase 2, an international method evaluation was performed with the participation of 12 laboratories located in 6 countries. Diverse surface water field slides negative for Cryptosporidium by microscopy were seeded with single, flow cytometry sorted C. parvum or C. muris oocysts. Single oocyst seeded matrix-free slides and unseeded slides were also included. Based on a total of 990 blind-coded seeded slides analyzed over 5 trials the overall genotyping success rate was 50%. The presence of field matrix did not affect detection, indicating that the method

was sufficiently robust for environmental samples. For Phase 3 of the project, further evaluation of the method using slides with naturally occurring Cryptosporidium was performed with the participation of 11 laboratories located in 5 countries. A total of 220 immunofluorescent assay microscopy (IFA)-positive (i.e. positive for Cryptosporidium oocysts) and 220 IFA-negative blind-coded slides were analyzed. Slides ranged in age from 5 to 20 months old, and the majority of slides were provided by Scottish Water. The number of oocysts on IFA-positive slides ranged from 1 to 78, and 72% of the slides contained 1 to 5 oocysts. The overall genotyping success rate was 65%, and only 6% of the IFA-negative slides tested PCR positive. Based on HRM and DNA sequence analysis, only 6% of samples contained human-pathogenic Cryptosporidium. Genotyping of LT2 Round 2 samples will provide critical information for human health risk assessments and aid the development of effective watershed management plans.

A Comparison of the Relationship Between Regulatory Fecal Indicator Bacteria and Host Specific Genetic qPCR markers in Common Fecal Pollution

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Water quality impairments are commonly associated with elevated concentrations of fecal indicator bacteria (FIB). Microbial source tracking (MST) aims to identify the sources of FIB pollution so targeted remediation strategies can be used to improve water quality. Substrate based bacterial culturing methods are often used when quantifying FIB in MST and determining water quality impairments. Results from these methods, however, do not provide information on the pollution source. Molecular techniques, such as polymerase chain reaction (PCR), offer a quick and sensitive approach for quantifying FIB concentrations and host-specific quantification by targeting genetic markers in the bacteria unique to the host organism. In this study, we conduct a comparison of regulatory FIB (E. coli) to host specific qPCR assays on direct canine and sea bird fecal grab samples from the Grand Strand of South Carolina. Results suggest that inter-specimen variability makes interpretation of qPCR results difficult to attribute a percentage of the FIB load to a particular host. Furthermore, the time series study indicates that the ratio of FIB to genetic marker changes over time. Thus, temporal variability of the addition of waste to the system further complicates interpretation.

Growing Botryococcus spp. on Municipal Wastewater from Puerto Rico for Simultaneous Nutrient Removal and Energy Feedstock Production

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Several approaches have been proposed for the biological treatment of wastewaters. Among these, growing algae in wastewater effluents is one of the most promising, since it represents an alternative for both nutrient load decline and reduction of the costs associated with water treatment. Furthermore, by simultaneously growing algae and remediating wastewaters it is theoretically possible to produce algal by-products, such as oil, bioplastics and nutraceuticals, in a sustainable way. However, few studies aiming to reduce nutrient load in wastewaters from Puerto Rico have been performed with microalgae and none of them have employed native species. We are examining the possibility of using an indigenous microalgal species for the treatment of Puerto Rican wastewaters. A species belonging to the genus Botryococcus has been isolated and identified and a pure culture has been established. Samples

of secondary and tertiary wastewater effluents of a local treatment plant were used to grow Botryococcus spp. Biomass yield, lipid production, fatty acid profiles and nutrient removal efficiency were examined at different growth stages. Cultures were established in five replicates, each one of 250 mL of sterilized tertiary wastewater effluent. The assays were maintained at continuous agitation, room temperature, and constant light. Samples were taken at days 0, 2, 5 and 8, in order to determine the number of cells mL-1, lipid content, fatty acid profile and total nitrogen and phosphates content. Results have shown lower growth rates of Botryococcus spp. cultured in tertiary wastewaters (0.03) when compared with the control (Botryococcus spp. in a minimal salt culture media) (0.09). However, the lipid accumulation rate appears to be higher when culturing cells in wastewaters (0.19 vs. 0.02). Both total nitrogen and phosphates content are being currently assessed in order to determine removal efficiencies. Results of Gas chromatography/Mass spectrometry (GC/MS) analysis performed in control cultures have shown a large quantity of long chain fatty acids, mainly palmitic and linoleic acids (C16 and C18:2, respectively) and large fractions of lighter FAs (e.g. lauric - C12 and myristic - C14), which resemble fatty acid profiles of canola and coconut oil. In order to evaluate changes in lipid composition triggered by wastewater culturing, GC/MS analysis of wastewater grown cultures are being performed. Future experiments will evaluate the effect of inorganic nutrient addition and the involved costs with the end of determining the applicability of microalgal treatment for local wastewaters.

Domestic Dogs: A Significant yet Controllable Source of Fecal Contamination at Arroyo Burro Beach in Santa Barbara, CA

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Coastal beaches in California and around the world receive frequent summertime advisories for surf zone fecal contamination, signaling potential increased health risks to beach users. Elevated levels of fecal indicator bacteria (FIB) including E. coli and enterococci are the most common cause of warnings, but remediating contamination requires the identification of sources. Suburban watersheds are particularly challenging due to their large variety of land uses (urban to undeveloped) and associated fecal sources (human, domestic pets, livestock, wildlife). One such watershed is Arroyo Burro in Santa Barbara, CA whose terminal beach is popular, but chronically FIB-contaminated. To discover FIB sources, a microbial source tracking (MST) study was designed and performed. Initial field reconnaissance indicated that humans, dogs, and birds could be sources; a thorough review of past reports and data suggested that the coastal lagoon discharged FIB when it breached. To test hypotheses related to these sources, surface water, sand, wrack, and groundwater were sampled from the beach, lagoon, and creeks over two years (2012-13). Analyses included quantification of FIB and source-associated DNA markers. Surf zone FIB concentrations were found to be highly correlated with lagoon outlet flow rates and dog fecal marker concentrations. While dogs are allowed on the beach, the primary source of surf zone dog marker was found to be from the creek-fed lagoon. Dog fecal markers were most abundant in a creek reach bordered by the backyards of suburban homes, just upstream of the lagoon. The load of DNA-based dog marker in this reach was estimated to be high enough to account for FIB entering the lagoon. A short-term pet waste public education program was conducted by the City of Santa Barbara, and subsequent dog marker concentrations in the creek significantly decreased. Results from this study illustrate the impact that domestic dogs can have on coastal water quality, and that education may be effective in controlling this source.

Evaluation of two hand hygiene methods on farm worker hands during fresh produce harvest

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Produce-related foodborne disease outbreaks lead to economic losses, illness, and death. To prevent produce-related outbreaks, it is imperative to identify the risk factors for produce contamination on farms. Since 2011, we have worked with produce farms in Mexico to quantify fecal contamination on over 200 matched samples of produce and farmworker hands. Concentrations and prevalence of fecal indicator bacteria were significantly correlated between matched produce and hand samples (e.g. for E. coli rho = 0.55, $p < 0.0001$, adjusted OR 8.9, 95% CI: 3.3-24.2). Based on these results, the goal of this study was to evaluate two hand-hygiene methods to assess their ability to reduce organic matter and fecal contamination on farmworker hands during harvest. In May 2013, we recruited 159 farmworkers at two farms from the parent study. We compared two methods of hand-hygiene: hand washing with a foam cleanser, and the SaniTwice method using an ethanol-based hand sanitizer gel. Farmworkers were randomly assigned to one of the intervention groups or to a control group. The intervention groups were trained in their hygiene method before harvesting produce, while the control group received no hygiene training. All groups harvested produce for 30 minutes before sampling (control group) or intervention. The intervention groups practiced hand-hygiene, and then gave hand rinse samples either immediately, or after continuing to harvest produce post-hand-hygiene. We measured absorbance of the hand rinsate with a spectrophotometer at 600nm as a proxy for quantifying organic matter on hands and enumerated E. coli, fecal coliforms, and enterococcus as indicators of fecal contamination. The foam cleanser group had significantly less organic matter than the SaniTwice group, and both groups had less organic matter than the control group ($p<0.05$). The foam cleanser sub-group who harvested produce between hand-hygiene and sampling had significantly less organic matter compared to the similar SaniTwice sub-group and the control group. E. coli was non-detectable in almost all groups. The control group had, on average, greater than 3 log10 fecal coliforms and 4 log10 enterococcus per hand. The foam cleanser group had levels of fecal coliforms and enterococcus that were not significantly different from the control group. The SaniTwice group had, on average, approximately one log10 fewer fecal coliforms and enterococcus per hand than the control group immediately after practicing hand-hygiene ($p< 0.05$). In intervention sub-groups who harvested produce between hand-hygiene and sampling, levels of fecal coliforms and enterococcus were not significantly different from the control group. While hand washing using foam cleanser produced both immediate and prolonged reductions in organic matter, it did not reduce fecal indicator bacteria, and may not be an appropriate hand-hygiene product for this environment. The SaniTwice method appears to reduce both organic matter and fecal indicator bacteria, and may be an appropriate hand-hygiene method when water isn't available. The hands of field workers became re-contaminated with both organic material and fecal indicator bacteria after harvesting produce for 30 minutes, meaning hand-hygiene, under currently practiced methods, would ideally need to be practiced frequently throughout the day to minimize fecal exposure of produce.

Optimizing enrichment time for ANSR assay for Salmonella detection in water matrices

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The current US detection technique for Salmonella in water (EPA1682 method) is culture based and can take up to full week to process a single batch of samples. While this assay has been shown to be reliable in the detection of Salmonella, it does not allow for rapid screening of a large volume of samples and does not include molecular techniques for detection and/or characterization of Salmonella spp.. Surface waters provide a unique challenge for pathogen detection due to changing environmental conditions and microbial populations that may affect method chemistry. Due to the importance of these pathogens with regard to human health, rapid detection and screening of water samples is necessary to determine the possible risks of potential pathogens present at any given time. The ANSR_ isothermal assay, using Nicking Enzyme Amplification Reaction (NEAR?) technology, originally developed for food safety, may be able to provide rapid detection in water. Time trials were performed to examine the effects of shortening the initial enrichment time of 24 hours. Samples from Grand River, Red Cedar River, and the East Lansing sewage treatment plant along with laboratory reagent water were seeded with Salmonella typhimurium (ATCC #14028), examined over seven time points, and recovery efficiency was used to evaluate equivalency with the results of the 24 hour recommended incubation time. Laboratory reagent water results indicate that detection of Salmonella peaked after 8 hours of incubation and matched results from the 24-hour incubation from this point forward. Recovery remained constant at 82% for hours 8-24. This data indicates that incubation time for the ANSR? assay could be decreased by 16 hours, improving the usefulness of the assay as a rapid detection technique. Future assays should be performed on sample matrices from a diverse geographical area in order to provide more evidence for this finding, and to investigate any matrix-specific efficiency problems.

Choosing the Right Microbiological Method: The Pitfalls of Comparison Studies

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The comparison of microbiological methods for the detection of organisms in water is an essential part of improvement of microbiological methods and consequently it is essential that such comparisons are undertaken carefully and that due consideration is given to all relevant parameters. In many instances a reference or standard procedure exists and new methods are often compared to such a procedure in order to determine the suitability of a new method. In many countries protocols exist for such comparisons and in order for a new method to be accepted it must perform as well or better than the reference procedure. Unfortunately many of these "standard" procedures are either poorly characterized or out-dated. In such cases it is even more important that comparisons are designed appropriately. The first parameter that must be carefully controlled is the definition of the target organism. This is of particular importance when comparing methods for the detection of total coliforms and E.coli. The majority of comparison studies that have been published in the last decade have compared methods based on the detection of enzyme activity (_-galactosidase and _-glucuronidase) with "traditional" methods based upon fermentation of lactose. Unless appropriate confirmation procedures are employed such comparisons are invalid resulting in inaccurate false positive and false negative data. We have performed many studies comparing methods for total coliforms and E. coli using

standard procedures and have investigated various aspects in order to better understand critical parameters. Where definitions differ between methods, it is essential that full identification of target organisms is undertaken. For example, in one comparison where two coliform methods were being compared the false positive rate, as determined by the reference procedure was 22.8%, whereas when full identification of the target organisms was undertaken, the false positive rate fell to 0.4%. This was largely due to the fact that the reference procedure involved fermentation of lactose as a confirmation procedure but the alternative method was based upon detection of _-galactosidase activity. Therefore some samples that were positive in the test method contained only organisms that were positive for _-galactosidase production but failed to ferment lactose, meaning that they were recorded as false positives. However, these false positive rates are impacted by study design and the sensitivity of the reference procedure. When comparing two presence absence systems, some study protocols require that a minimum and maximum proportion of the spiked samples used for the comparison must be positive. This is clearly a necessary requirement in order to be able to apply appropriate statistical tests but, if the reference procedure lacks sensitivity, then more organisms are required to give a positive result. When this situation occurs, the false positive rate of the test method is lowered because most samples that contain organisms that are _-galactosidase positive but lactose negative also contain lactose positive organisms. In fact the same medium that had a 22.8% false positive rate in a comparison of two quantitative techniques had a false positive rate of only 3.7% when used as a presence absence test. False negative rates are of concern to laboratories and regulators alike but the numbers need to be taken in context and not in a simple list of performance characteristics. In one comparison study that was undertaken the false negative rate of the test method was determined to be 14.5% which to many would be considered to be unacceptably high. However, the method had detected 1.75 times as many positive E. coli samples as the reference procedure and as such was far more protective of public health. Thus in this case, where the test method had failed to detect the presence of E. coli in a significant proportion of samples, the false negative rate was irrelevant. The medium had been designed with the recovery of injured organisms as a primary focus. Thus while this medium recovered and detected many more organisms than the reference procedure, some organisms were recovered but remained undetected. The comparison of new methods with reference procedures is fraught with difficulties and is more complex than many realise. This presentation will use data from actual comparison studies to highlight the problem areas when designing such studies. The adoption of more harmonious procedures for comparing methods should lead to less conflicting data appearing in the literature.

Application of a new integrative process-based nearshore water quality modeling system to evaluate the contribution of river discharge to observed E. coli concentrations

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Stormwater runoff is often implicated as a source of elevated bacterial concentrations observed at beaches. However, the relationship between river discharge and bacterial concentrations at nearby beaches is not consistent, due to wash-off and accumulation processes on the landscape and variability in nearshore currents. This complex relationship complicates the use of river discharge near beaches as a predictor of beach water quality. Process-based predictive models linking watershed hydrology to nearshore fate and transport offer an opportunity to investigate this complicated relationship between river discharge and beach water quality. We will present results from an application of a novel linked hydrology-hydrodynamics-bacterial water quality modeling system developed to predict the fate and transport of E. coli from watersheds to beaches. The lumped parameter rainfall-runoff model, IHACRES

(Identification of Unit Hydrographs And Component flows from Rainfall, Evaporation and Streamflow data , Jakeman et al., 1990), simulates river discharge and effective precipitation, which is used to drive a landscape wash-off and accumulation model. The resulting river E. coli loads are then input to a 3D Lagrangian particle model driven by currents derived from the Finite Volume Coastal Ocean Model (FVCOM, Chen et al., 2003) adapted for the Huron to Erie Connecting Waterways Forecasting System (HECWFS, Anderson et al., 2010). This presentation will share results from a modeling study conducted to evaluate the potential impacts of Michigan's Clinton River on nearshore water quality at Lake St. Clair. Results demonstrate the utility (and challenges/limitations) of such integrative modeling frameworks for improving our understanding of river impacts on beach water quality, and how this may be translated into predictive beach water quality modeling.

Biodegradation of Textile industry Effluents Using Selected Bacterial species in Challawa, Nigeria

Adamu Galadima Dagona

BIODEGRADATION OF TEXTILE INDUSTRY EFFLUENTS USING SELECTED BACTERIAL SPECIES IN CHALLAWA, NIGERIA A.G. Dagona-1a aDepartment of Biological Sciences Yobe State University, Damaturu bCorresponding Author E-mail: agdagona@gmail.com Tel: +234(0)8039147822 Abstract This study was carried out to determine the bioremediation potential of bacterial species isolated from Challawa industry in Kano metropolis. Effluents release from textile industry can cause serious environmental effects due to the presence of toxic dyes, which eventually affects entire life of humans, plants and animals, thereby limiting its utilization. Physicochemical characteristic of the effluents was carried out. The result indicated high rates of electrical conductivity (EC), total dissolved solids (TDS), total suspended solid (TSS), dissolved solids (DO), chemical oxygen demand (COD), However heavy metals: chromium (Cr), cadmium Cd), copper (Cu), iron (Fe), nickel (Ni), zinc (Zn) and lead (Pb). The result shows that the parameters were high above the prescribed water limit. Based on morphology and biochemical characterization, seven (7) bacterial isolates were identified, of which five (5) were selected based on their ability to degrade textile effluent and grow on minimum basal medium efficiently and rapidly. The biodegradation/decolourisation ability of the isolates from textile effluent was carried out for ten day. Degradation/decolourization were expressed in simple percentages with Bacillus lichniformis having (91.60%), Bacillus subtilis (99.60%), Alcaligenes feacalis (95.00%), Pseudomonas fluorescence (96.00%) and Bacillus brevis with (95.60%). Three microbial consortia were developed and tested for their effectiveness in bioremediation. Consortia 1 degrade 99% of textile effluent within ten days. Analysis of variance ANOVA result revealed that, there were statistically significant differences at ($p < 0.05$), in reduction and adsorption of DO, pH heavy metals (Cr, Cd, Cu, Fe, Mn, Ni, Pb, and Zn) with the exception of BOD, COD, TSS, TDS and EC. The study underscores the need to adjust immediate bioremediation programme using such bacterial species to control discharge of textile effluent before discharge to the environment. Keywords: Bacteria, effluent, biodegradation, decolourization and isolates.

Impact of Soil Column Length on Predicting Virus Removal

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Laboratory studies on virus transport through soil have usually been conducted with columns of less than one meter. A recent review of virus transport suggested that virus transport cannot be modeled by

filtration theory, and that using soil columns less than one meter in the laboratory are limited in mimicking virus transport in the field. We conducted a laboratory study on the transport of MS-2, enteroviruses, adenoviruses, and pepper mild mottle virus (PMMoV) using 4.4 m long columns filled with uniform soil under saturated flow conditions. MS-2 was spiked in secondary treated wastewater and fed to the columns over 24 days. PMMoV occurs in higher concentrations than human enteric viruses in untreated and treated wastewater and has been suggested as an indicator of fecal contamination in surface waters. MS-2 was determined by infectivity assay; enterovirus, adenoviruses PMMoV by quantitative PCR. MS-2 seeded wastewater was removed by ~99.9% within the first 60 to 90 cm, but little additional removal occurred during travel through the remaining 350 cm. The removal as a function of depth exhibited an exponential-linear relationship similar to what has been observed in field studies. PMMoV, while exhibiting lower removals near the top of the columns was eventually reduced by ~99.99% after travel though the length of the column. It also demonstrated an exponential-linear removal. All of the enteroviruses and adenoviruses were removed below detection within the first 60 cm of the column. The results demonstrate that longer soil columns are more suitable to appropriately mimic virus transport under field conditions and that PMMoV might be a good tracer for viruses during aquifer recharge operations with reclaimed water.

The effect of storage time on Vibrio spp. and fecal indicator bacteria in estuarine water samples in an ISCO autosampler

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Monitoring bacterial concentrations during major storm events in coastal and estuarine ecosystems is critical to avoid adverse effects to public health associated with fecal contamination, stormwater, or naturally found bacterial pathogens such as Vibrio species. We have implemented the use of an autonomous vertical profiler (AVP), a water column profiling instrument for in situ sampling of the Neuse River Estuary in eastern North Carolina, during storm events that preclude sampling by boat. In addition to housing an anemometer, LISST particle analyzer and YSI Multi Parameter Sonde, the AVP also houses an ISCO automated sampler, all of which can be remotely triggered to collect and store water samples without compromising the safety of the researcher. Little data exist regarding short-term (less than 24 h) bottle effects on bacterial concentrations. "Bottle effects" are described as the change in water quality parameters as a consequence of confinement rather than planned manipulation (1,2). When using the AVP's rosette style autosampler there has been concern as to the impact of bottle effects on bacterial concentrations in estuarine water samples and thereby potential misrepresentation of in situ conditions. We conducted three experiments of different time lengths denoted as short (9 h), long (21 h), and full (29 h). Over the course of the three studies we observed no significant change in total Vibrio spp. (VIB), V. parahaemolyticus (VP) or Enterococcus spp. (ENT) concentrations in our short (pVIB=0.189, pVP=0.521, pENT =0.080), long (pVIB=0.521, pVP=0.509, pENT=0.509) and full (pVP=0.109, pENT=0.053) term experiments. While V. vulnificus showed significant change during the short-term experiment (p=0.001), neither the short, long (p=0.334) or full (p=0.415) term experiments yielded any notable bottle effects. Our findings suggest that bottle effects are negligible over short time scales in relation to total Vibrio spp. abundance and V. vulnificus, V. parahaemolyticus and Enterococcus spp. concentrations. We did, however, find that sampling during a specific time of day was associated with increased variability in V. vulnificus concentrations regardless of storage time. Our report advises against analyzing samples for V. vulnificus at times associated with this increased variability, typically midday and evening. 1 Pernthaler, J., & Amann, R. (2005). Fate of heterotrophic microbes in pelagic habitats:

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Occurrence of Enterococcus and vibrios in seawater of Singapore

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This study investigated the occurrence and distribution of Enterococcus and vibrios in seawater surrounding Singapore. Water samples were collected from 8 sampling sites in April, May, October, November and December 2013. Enterococci were detected and enumerated using EnterolertTM (IDEXX Laboratories, Westbrook, Maine) and reported as MPN. Detection of vibrios involved concentration of 300-mL of water samples through 0.45_m cellulose acetate membrane filter and enriched in alkaline peptone water. PCR was applied to detect Vibrio spp., V. parahaemolyticus, V. cholera, V. vulnificus and V. parahaemolyticus toxic gene in enriched samples. Primer sequences were adopted from published studies. The geometric mean of Enterococcus cell concentration in water samples collected from all sampling sites ranged from 43 MPN/100ml to 1045 MPN/100ml. Generally all sampling sites achieved Singapore recreational water guideline value of 200 MPN/100ml except for two sampling sites at the northern part of Singapore, located along the Johor Straits. The detection frequency of vibrios at all sampling sites showed that of the 67 samples tested, 100% of water samples were positive for Vibrio spp. and V. parahaemolyticus; 67.16% of water samples were positive for V. vulnificus; 8.96% of water samples were positive for V. parahaemolyticus toxic gene; and 7.46% of water samples were positive for V. cholera. MPN-PCR was conducted to quantify the cell concentration of V. parahaemolyticus in water samples collected in December 2013. The MPN-PCR results showed that the V. parahaemolyticus count ranged from 0.03 MPN/ml to 2.4 MPN/ml, with the highest counts at one of the sampling points in Johor Straits. V. vulnificus was present in all samples collected along the Johor Straits. Overall, a high prevalence of V. parahaemolyticus and V. vulnificus was found in Singapore coastal waters, perhaps attributed to the warm temperature in Singapore coastal waters or the lack of water flow along the Johor Straits which subsequently caused the accumulation of bacterial indicators and pathogens. Quantitative microbial risk assessment (QMRA) was performed to assess the potential risks to swimmers and recreational water users by using Enterococcus and V. parahaemolyticus models. The ingestion rate for swimmers was adopted from Dufour et al.'s study (2006) while the ingestion rate for recreational water users was assumed to be proportional to the risk of 10 minutes of swimming duration. The exponential dose-response model developed by Haas et al. (1999) and Stone et al. (2008) was used to estimate the risk of gastrointestinal illness from Enterococcus. The risk of infection from V. parahaemolyticus was estimated by the β-Poisson model (Dickinson et al. 2013, US.FDA 2005). Point estimation of probability of illness risk was carried out based on the geometric mean of Enterococcus cell concentration, as well as V. parahaemolyticus cell concentration for a single sampling event using the maximum ingestion rate. The results suggested that the illness risk from V. parahaemolyticus model was below the U.S. EPA guideline of 8 illnesses in 1000 bathers. Nevertheless, the Enterococcus model showed a few sampling sites in the Johor Straits posed higher risk of gastrointestinal illness and had exceeded the U.S. EPA guideline value.

Using Time-Frequency Analysis to Understand Long Term Bacterial Dynamics in eastern North Carolina Waters

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Long term studies on anthropogenic and native bacteria in coastal waters are limited. North Carolina is data rich, with many state-monitored and long term bacterial datasets that span from 10 to 50 years. Many biological and environmental process are cyclical in nature; therefore, using short term data is not effective in describing some potentially important bacterial relationships. Our research has focused on the relationship between monitored bacteria and related environmental variables over time using a 10 and 20 year fecal indicator bacteria (FIB) dataset and a 10 year total Vibrio spp. dataset. The central objective of this study was to examine multiple time scales of data from an estuary using time-frequency analyses in order to understand the microbial dynamics of water quality across a range of hydrodynamic and meteorological conditions, which will aide in variable selection during future model development. To do this, we first employed simple ordinary least squares (OLS) regression analysis to look for trends in the FIB time series data and then used periodogram analysis to examine which time periods comprised significantly large proportions of the time series variation. As a contrast to the FIB data, we monitored and modeled levels of native Vibrio spp., a bacterial genus that contains potential human pathogens, but is not related to fecal contamination. We found that the fecal coliform time series had no patterns, trends, or periods that were detectable above white noise levels. However, there was a significant, but weak linear trend in the total Vibrio spp. dataset, which was detected using OLS regression. Using periodogram analysis, we found no important periods in the FIB dataset, but we were able to detected regular periods in the total Vibrio spp. dataset that accounted for large amounts of the time series variance. These periods were found at 11.6 and 104 months and accounted for 28.5 and 16.8% of the data variance, respectively. Cross spectral analysis was then used to determine the relationship between total Vibrio spp. and the time series of various environmental parameters (salinity, temperature, dissolved oxygen, and pH) with respect to particular frequency bands. The results found in this study can have important management implications. The Neuse River Estuary watershed encompasses one of the fastest growing population centers in the US. However, the time-frequency analysis in this study showed that fecal coliforms and the pathogens they represent, might not be the largest public health problem in NC coastal areas, as they are not increasing linearly over time. However, there does seem to be a linear trend in total Vibrio spp. over time, indicating that this genus might represent more of a public health concern in the coming years as climate change and other anthropogenic factors continue to change densities of native pathogens within the genus.

Identification of bacterial specialists among members of Aves, Mammalia, and Pisces

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Background: To accurately assess human health risk under an indicator paradigm, an understanding of the microbial populations within sources of contaminants is essential. Advances in sequencing not only offer benefits for direct detection of allocthonous aquatic bacteria, but also for investigating the distribution of these bacteria within their animal hosts. In particular, bacteria that have achieved evolutionary success and maintain high abundances within hosts (specialists) have been targeted for

source-identification purposes, but it is likely that deep sequencing may reveal additional taxa that offer significant diagnostic potential. Because a much greater proportion of communities are sampled, deep sequencing may also reveal large-scale trends in host-associated microbial biogeography, such as an abundance-occupancy relationship, that contribute to the specificity and sensitivity of all microbial molecular detection methods. By analyzing over 44 million sequence reads collected from birds, mammals, and fish with species distribution models typically used in macroecology, we determine the taxonomic identity of bacterial specialist found within animal hosts and investigate the relationship between bacterial abundance and occupancy (distribution among host species). Methods: We sequenced and analyzed the V6 region of the bacterial 16S rRNA gene from 73 fecal samples using VAMPs (vamps.mbl.edu), the vegan package in R, as well as custom scripts. To identify specialist bacteria, we used the CLAM test, which has been previously used to describe tree distribution patterns in Costa Rican rainforests. The relationship between operational taxonomic unit (OTU) abundance and occupancy was investigated under various measures of abundance. Results: CLAM tests on 68,424 OTUs resulted in the identification of 14,031 specialists (20.5% of all OTUs and 92.7% of all sequences in the final dataset). Bacteria from a wide taxonomic range were identified as specialists; however, across all animals a high proportion of Lachnospiraceae, Ruminococcaceae, and Bacteroidaceae were identified as specialists relative to all OTUs within respective taxa. We also found a highly significant relationship between OTU abundance and occupancy. Conclusion: Results reinforce the assertion that bacteria within Firmicutes and Bacteroidetes may have formed the most ancient relationships with vertebrate hosts and that macroecological models can be helpful for identifying specialists in large diverse microbial sequence datasets. Newly identified specialist taxa identified herein offer significant potential as indicators of specific sources of fecal contaminants and could be targeted for detection in the environment using more sensitive methods. Although the relationship between abundance and occupancy suggests a negative relationship between the specificity and sensitivity of more specific methods, such as qPCR, this assessment was made through the analysis of short homologous 60 base pair sequence reads and the relative sequence conservation of targeted regions likely plays a large role in method specificity and sensitivity as well.

Assessing the impacts of nutrient loading on culturable E. coli fate in a re-created natural stream mesocosm

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Fecal indicator bacteria such as Escherichia coli are used globally as a surrogate measure to estimate the potential for human pathogen presence in surface waters. It is presumed that their presence in water signifies recent fecal contamination as E. coli and other fecal indicator bacteria were once thought to survive poorly outside of the large intestine. Recent findings have proven otherwise; however, knowledge and understanding regarding the mechanisms influencing the fate and transport of these organisms in soil and water environments remains incomplete. Literature documents a number of external factors that influence the survival of E. coli in the environment including temperature, moisture, ultra-violet light exposure, pH, salinity, nutrient supply as well as competition and predation from the larger microbial community. The interplay between these factors and the fate of E. coli in the environment remains somewhat of an enigma though. This disconnect in knowledge has likely led to inappropriate labeling of numerous water bodies as impaired and has subsequently spurred the ill-advised expenditure of private and public funds to plan and carryout remedial actions required by law.

An improved understanding of E. coli fate and transport in the environment will provide useful information that can potentially minimize erroneous water body impairments and improve water quality managers' efforts to understand and manage bacteria levels at a watershed scale. This paper presents preliminary findings of an ongoing effort to evaluate the impacts of nutrient loading on E. coli fate in a re-created stream environment. A laboratory based stream mesocosm system is being used to simulate the in stream environment while being able to control microbial inputs and hold certain water quality parameters constant. High and low level nutrient treatments will be applied to simulate nutrient loading to the stream that may occur due during a runoff producing rain event. The response of E. coli to applied treatments will be documented along with changes in other ambient water quality constituents. Through this effort, data will be collected that can be used to improve the ability of in stream and watershed based water quality models to predict in stream E. coli fate as it results to ambient water quality and will in turn lead to improved bacteria contamination assessment, modeling, managing and regulation.

Identifying sources of and quantifying differences in E. coli occurrence in soils from relatively unimpacted catchments under varying land uses

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Pollution of water resources with fecal matter is a critical problem that is partly responsible for the millions of water-borne illnesses contracted by humans globally each year. Escherichia coli and other organisms are considered fecal indicator bacteria and are used as a surrogate measure to estimate the potential for human pathogen presence in surface waters. When problematic levels of E. coli are documented in a water body, managing fecal contributions from its sources identified throughout the watershed is common; however, identifying these sources and documenting their respective portion of the total E. coli load can be challenging. In some watersheds, bacterial source tracking (BST) has been utilized to identify contributing sources of E. coli and has greatly aided water quality managers in addressing potential pollution issues. This approach has not proven perfect though as unidentified sources are almost always noted. Additionally, an increasing number of cases in the published literature are documenting the presence of endemic or naturalized species of fecal indicator bacteria present in soil environments that cannot be traced back to fecal contamination. As such, management measures designed to mitigate known sources of E. coli may be misguided or there may be no fecal pollution problem at all. This study was designed to quantify and identify the sources of E. coli in soil and surface runoff from three intensively managed catchments of various land uses. Known sources of fecal material will be collected with a focus on small mammals as they are currently under-represented in the Texas E. coli BST Library and could be at least partly responsible for the unidentified sources of E. coli. Preliminary findings of this work will be presented and will illustrate the differences in documented E. coli sources between land uses as well as the overall abundance of E. coli in each soil environment. Once complete, findings from this project will improve the understanding of E. coli sources in the landscape. This knowledge will supplement future watershed planning and assessments by enhancing the number of known sources of fecal material in the current Texas E. coli BST Library and by providing a better understanding of E. coli dynamics and sources in sampled soils. Ultimately, results will aid planners and managers in making more informed E. coli load management decisions in their watersheds.

Influence of Animal Agriculture on Occurrence and Distribution of Zoonotic Bacterial Pathogen Genes in Small U.S. Watersheds

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Although many studies of bacterial pathogen fate and transport have been conducted at the field, or large watershed scale, it remains unclear whether differing animal agricultural practices influence pathogen transport and occurrence in water under typical watershed conditions. Animal waste, baseflow and runoff water, and sediment samples from 19 small (<32 km2) watersheds in 12 U.S. states having either no major animal agriculture (control), or a single dominant animal type (e.g. beef, dairy, swine, or poultry) were tested for: 1) concentrations of the chemicals cholesterol, coprostanol, and estrone; 2) concentrations of the fecal indicator bacteria (FIB) Escherichia coli (EC) and enterococci (ENT); and 3) the zoonotic pathogens shiga-toxin producing and enterotoxigenic EC, Salmonella, Campylobacter, and pathogenic and vancomycin-resistant ENT by presence/absence polymerase chain reaction (P/A PCR) for viable organisms and/or quantitative PCR. Animal-waste gene profiles influenced the majority of watershed samples (as determined by discriminant analysis). Pathogens were not abundantly found, but were viable by P/A PCR. Two of 3 dairy waste samples had both the ENT esp gene (human pathogenicity) and the vanA gene (vancomycin resistance). FIB and cholesterol concentrations, and total numbers of pathogen genes, were correlated in runoff samples, suggesting fecal sources. Watershed soil or hydrologic characteristics had little influence on study results. Poultry and swine runoff results varied across sites. Animal access to streams in a beef watershed increased all chemical and microbiological indicators at that site. Dairy watershed runoff samples in 3 states had greater concentrations of EC, ENT, cholesterol, and coprostanol and greater numbers of dairy-waste pathogen genes compared to samples collected during baseflow conditions. Typical animal agricultural practices appear to influence zoonotic pathogen types or transport, and impart signature pathogen gene profiles in small watersheds throughout the U.S.

Fecal Source Identification in Impaired Waters in the National Capitol Region

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The Anacostia River is a brackish tidal tributary that flows from the Maryland suburbs, through the District of Columbia, then to its confluence with the Potomac River. Its watershed includes numerous urban/suburban tributaries that can be impacted by infrastructure problems, homeless encampments, and birds and wildlife populations in greenways and woodlots. The project began in 2007 with the goals of monitoring tributaries throughout the watershed and determining what fecal sources might impact each location. Research objectives were to 1) identify "hot-spots" of human fecal contamination wherever they might occur; 2) compare and evaluate several human-specific Bacteroidales qPCR genetic markers for their suitability to the region; 3) use the selected markers to establish the relative contribution of human waste to overall bacterial loading; and 4) determine any spatial and temporal relationships that may be reflected in human-source pollution. The Anacostia Watershed encompasses 456 sq. km in Montgomery and Prince George's Counties in Maryland and southeast Washington, DC. Both the Anacostia and Potomac are ranked among the most threatened rivers in the US, and the

Potomac is a major river system that impacts the Chesapeake Bay. Water quality data has been collected seasonally from 42 sites of varying degrees of impairment within the Potomac and Anacostia River watersheds from 2007 to the present, and a clear relationship has been established between precipitation and fecal indicator bacterial (FIB) levels, along with the relative contribution of human-source pollution when present. Extensive field and sewer investigations at sites with both high FIB levels and human-specific markers identified potential sources of the fecal pollution in most, but not all cases. Steps are currently being taken on a site by site basis to confirm the suspected sources, and then perform whatever repairs are necessary to fix the identified problem. Water quality is slowly improving as additional problems are located and remediated. Watershed monitoring will continue through 2015 to identify and aid in the elimination or reduction of additional human fecal contamination "hot-spots."

Effects of precipitation events and land use on Salmonella concentrations in ponds used for fruit and vegetable irrigation

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In south Georgia, high incidences of Salmonella in natural waterways have raised questions about the safety of irrigation water sources fed by storm runoff. Using dammed waterways to collect and store water for use in crop irrigation is common in this region, but the potential risks for bacterial contamination are not well understood. We measured Salmonella in storm runoff entering irrigation ponds, as well as concentrations of Salmonella in pond water before and after storms. Ponds generally contained higher concentrations of Salmonella after storm events. Salmonella concentrations in runoff from agricultural fields and forested areas were lower than pond concentrations after storms. Concentrations of Salmonella in small streams entering the ponds were highest. 100% of stream samples contained detectable Salmonella, while 38-40% of field or forest runoff samples of similar volumes contained Salmonella. At least 18 different Salmonella serotypes were present, including several serotypes commonly implicated in human illness. Neither of the watersheds involved in this study contained livestock operations. These findings highlight the need for evaluation of actual crop contamination risks associated with irrigation water.

Custom Microarray for Pathogen Detection and Microbial Source Tracking

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Animals and human waste contributes a broad array of pathogens to receiving waters that can endanger human health and degrade ecosystem services. Fecal contamination threatens the safety of source waters used for potable water and irrigation, as well as recreational waters and waters needed for ecosystem functions. Fecal indicator bacteria such as Escherichia coli and enterococci have been used for over a century as surrogates for pathogens in microbial water quality testing; however, it is now well established that one or two bacterial indicators cannot adequately reflect risk of the myriad of allochthonous and autochthonous waterborne pathogens that may infect humans in a given water body. The obvious solution, that of testing directly for all possible pathogens, has been unachievable in practice due to the logistical difficulties and expense of such an effort on a one-test-per-pathogen basis. The sporadic distribution of pathogens in host populations and the association of many pathogens with

a specific host make the choice of a group of representative pathogens that is appropriate for all or even most watersheds impossible. Microarray technology provides a platform to simultaneously test for thousands of DNA targets. We have developed a custom microarray on an Agilent platform that is specialized for detecting waterborne pathogens (including a total of 322 viruses, bacteria, protozoa, and microbial source tracking markers) from a diversity of host species. Mismatch sequences are included for quality control. In order to overcome the disadvantages of small sample size and sparse distribution of pathogens in water, we use whole genome amplification to non-selectively amplify DNA and cDNA (derived from RNA viruses), thereby avoiding the requirement for target-specific primers associated with PCR. We have tested domestic wastewater (human sewage), livestock feces (cattle and swine), and positive controls (pure cultures or plasmids with specific targets) by whole genome amplification and detection by the microarray. Results to date are highly encouraging, with pathogen and MST marker detection in the expected samples. Furthermore, results are semi-quantitative, i.e. ten-fold diluted samples provide 45-90% less fluorescent signal than undiluted samples. Further work on sensitivity, specificity, and limits of detection will establish the usefulness of the microarray for pathogen and pollution source detection in environmental waters, providing an invaluable tool in the arsenal available to scientists, managers, and regulators concerned with water quality, human health, and ecosystem health.

Multidrug- and methicillin-resistant Staphylococcus aureus are present in surface waters near industrial hog operations in North Carolina

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Use of sub-therapeutic antibiotics in industrial animal production could potentially lead to dissemination of new antibiotic resistant strains of clinically relevant bacteria such as Staphylococcus aureus. Although nasal carriage of multidrug- and methicillin-resistant S. aureus in industrial animal workers has been documented, transmission of S. aureus to the water environment in areas where waste products are sprayed has not been characterized. We investigated whether methicillin-resistant S. aureus (MRSA) is present in surface waters near industrial animal operations that manage liquid waste with lagoons and spray fields. Surface water samples (n = 183) were collected over the course of approximately one year from six locations in southeastern NC and analyzed for the presence of MRSA. A total of 698 presumptive Staphylococcus isolates exhibiting phenotypic antibiotic resistance were recovered and archived. Culture-based, biochemical, and molecular tests, as well as matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry were used to confirm the identity of presumptive Staphylococcus isolates. Susceptibility to 15 antibiotics was measured using Kirby-Bauer disk diffusion. To better understand the potential origins of the isolates, spa typing along with PCR confirmation of the scn and tetM genes were performed. A total of 24 isolates were confirmed S. aureus, 12 of which were MRSA. The 24 S. aureus isolates were recovered from 5 sites and 16 distinct samples, with an overall percent detection of 0.09 (16/183 samples). Fifteen isolates were resistant to at least one antibiotic and eleven were multidrug-resistant (defined as complete resistance to three or more antibiotic classes). The most common spa types represented in this study were t008 (12/24) and t021 (7/24), which have been associated with clonal complexes 8 and 30, respectively. Other spa types represented include t190, t216, t338, and t267. No spa types detected in this study have previously been associated with livestock, but lack of the scn gene was observed in seven isolates, which has been

considered a marker of livestock-association. This study demonstrated that multidrug- and methicillin-resistant S. aureus were present in surface waters adjacent to industrial hog operation waste spray fields in southeastern NC. To our knowledge, this is the first documentation of waterborne S. aureus and MRSA collected from surface waters near industrial hog operations.

Comparison of EPA guideline-based beach action decisions from enterococci qPCR target sequence-adjusted cell density estimates and culture methods

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The U.S. Environmental Protection Agency (EPA) has recently released beach action values for recreational water quality monitoring results from enterococci and E. coli culture methods as well as for a quantitative polymerase chain reaction (qPCR) method for enterococci. Reported values for single sample analyses are 235 CFU or MPN for E. coli culture and 70 CFU or MPN for enterococci culture and 1000 calibrator cell equivalents (CCE) for enterococci qPCR, based on a NEEAR epidemiological study-estimated gastrointestinal illness (NGI) rate of 36 NGI/1000 recreators. Studies were conducted in which beach action decisions resulting from standard culture-based analyses for both enterococci and E. coli were compared to those based upon enterococci CCE estimates, adjusted to a benchmark target sequence to cell ratio corresponding to the EPA beach action values, for 234 water samples from eight major midwestern U.S. rivers. Beach action decisions from enterococci qPCR agreed with those from the enterococci culture methods in 87% of the method comparisons and with those from the E. coli culture methods in 92% of the method comparisons.

Application of predictive and space/time interpolative models of fecal contamination in an urbanizing watershed

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B. Everett Jordan Lake functions as a drinking water supply in the rapidly urbanizing Research Triangle Area of central North Carolina, and its watershed is primarily impacted by nonpoint source (NPS) pollution. Expense and time constraints limit the utility of traditional sampling strategies in managing watersheds afflicted by NPS pollution, presenting the need for tools with greater flexibility in monitoring applications. This study constructed two models of fecal coliform (FC) concentration, one interpolative and the other predictive, to aid in identifying locations and conditions of particular concern for management and remediation purposes. Bimonthly dry-weather samples were collected over the course of one year from fifteen sites on creeks in the Jordan Lake catchment and assessed for FC concentration by standard membrane filtration. Additional samples collected during three storm events and drawn from the public EPA STORET database at eight additional locations were also assessed. A Bayesian maximum entropy (BME) framework was used to build an interpolative model drawing on the autocorrelation in space and time of the collected FC concentration data. The model output daily maps of FC estimates across the catchment, which described distinct spatial patterns of seasonally elevated FC levels and indicated routine impairment of more than 90% of the study area river miles during late spring and early summer. A statistical predictive model of FC concentration was created incorporating

antecedent rainfall, intensity of watershed development, and streamflow, all of which exhibited statistically significant correlation with FC concentration. The two models are complementary for management purposes: the interpolative model describes the extent of impairment and spatial/temporal trends to target interventions, and the predictive model provides real-time FC estimates to inform timely action. It may be possible to integrate predicted FC estimates in the interpolative model to estimate the spatial extent of elevated FC levels in real time, though the compounded uncertainty may prevent meaningful results from being obtained.

Hydrodynamic modelling and fecal indicator dispersion in current and future climates

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After intense rainfalls, overflows can occur from sewer systems to receiving waters. The effluents are composed of sewage and stormwaters. The objective of this research is to build a predictive model for the transport and propagation of fecal indicator bacteria (Escherichia coli) and pharmaceuticals in the Des Prairies River during critical periods for drinking water treatment plants. More specifically, we are evaluating the dilution capacity of a large river serving as a drinking water source during extreme events and affected by climate change. Des Prairies River is located in an urban area in the Greater Montreal Region (Quebec, Canada). It is fed by the Ottawa River via the Lake of Two Mountains. It is directly influenced by the hydrology and water resources management of the Great Lakes and the Ottawa River. The only sources of fecal contaminants along the river are overflows from combined sewer (CSO) and sanitary sewer systems (SSO), during heavy rainfall or snowmelt, and stormwaters. Two of the three water intakes on the river are downstream of overflow infrastructures, which could present a risk to human health in the case of drinking water treatment failure. E. coli were studied because in Quebec, Canada, standards for drinking water are based on the annual average concentration of E. coli in raw water. Our approach combines hydrology, hydraulics and water quality. Hydrology is used to set critical scenarios following frequency analysis of the current climate and predicted in order to introduce climate change effects. The water quality at the water intakes is determined from modelling the dispersion and attenuation of contaminants from CSO discharge points, and the impacts of climate change on river flowrates. The impact of climate change on urban CSO hydrology was not simulated in this portion of the study. To build the des Prairies River model, we used Hydrosim, developed by INRS-ETE (National Institute of Scientific Research, Water, Earth and Environment), following the work done by Centre d'Expertise Hydrique du Quebec (CEHQ). Dispersim, coupled to Hydrosim via Modeleur, is used to model the dispersion of contaminants. The dispersion and diffusion of E. coli were simulated using first order reaction kinetics. Our model was calibrated following several sampling campaigns of water level and concentrations in the des Prairies River. The impact of climate change is represented by the change of flow in the river using the Hydrotel model. The predicted impacts of climate change on water quality will be presented. During low flow events a decrease of flows (-10%) by 2070 is expected, which would decrease the dilution capacity of the river. In general, for this river with a large watershed, the local peak contaminant load from the snowmelt period precedes the peak flow of the river. Thus, dilution capacity in the spring period with low flow is also of concern to the drinking water treatment plants because there may be high local contaminant loads with only moderate dilution, and low water temperatures affecting water treatment plant efficiency. Thus, the vulnerability of water intakes in the future climate will be assessed using conservative hydraulic scenarios. Recommendations integrating climate change into source water protection planning will be presented.

Comparison of Cryptosporidium spp. detection by biofilms versus filtration in an urban water supply

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Cryptosporidium is a protozoan parasite responsible for a waterborne gastrointestinal disease that is potentially fatal for immunocompromised individuals. Monitoring Cryptosporidium contamination in watersheds that serve as sources of municipal drinking water is necessary to identify public health risks and determine where limited resources should most effectively be targeted to protect consumers from waterborne exposure to pathogenic oocysts. Current sample methodology for Cryptosporidium monitoring in water supplies (EPA Method 1623.1) relies on filtering and processing 10 L of water, providing a snapshot of river conditions at the time of filtration. Because oocysts are discrete particles that can be present in water at low concentrations, a sample deemed negative by Method 1623.1 does not mean that oocysts are not (or have not recently been) present in the water supply, only that no oocysts were captured in that 10-L water sample. We have field data showing that biofilm sampling detects oocysts in water supplies more often than filtration. Biofilms grow on solid substrates in water, and oocysts traveling along water flow paths which intersect with biofilm surfaces will attach. Biofilms thus provide an integrated look at stream conditions over the time that the biofilm has been growing in the water. We deployed glass slides (the substrate for biofilm growth) continuously at one urban water treatment plant (WTP) intake from Sept. 2009-Aug. 2010, recovering and processing slides (and deploying fresh slides) on each day that samples were filtered at that location. Glass slides were processed by scraping the biofilm, isolating oocysts from the scraped biofilm with immunomagnetic separation (IMS), and quantifying oocysts using a combined immunofluorescent/fluorescent in-situ hybridization (IFA/FISH) assay; filtered samples were processed by elution, IMS, and either IFA/FISH (for quantitative detection) or polymerase chain reaction (PCR; for oocyst genotyping) . The glass slides were left in the water for varying lengths of time spanning 7 to 48 days, and oocysts were detected in every batch (21/21) of biofilm slides collected and analyzed, compared to oocyst detection in just 20% (3/15) and 75% (6/8) of filters collected and analyzed by PCR or IFA/FISH, respectively. These data will be supplemented with an additional year (spanning July 2013 through June 2014) of biweekly sampling at two sites in this same urban watershed (the same WTP intake sampled in 2009-2010 and an additional stream site influenced by misconnected sanitary sewer lines), comparing Cryptosporidium detection by filtration versus biofilms at each site. Filter and biofilm samples are being processed by elution and scraping, respectively, followed by IMS and IFA/FISH. So far, oocysts have been detected in 36% (4/11) and 64% (7/11) of filter and biofilm slides, respectively, at the WTP intake and 60% (3/5) and 50% (2/4) of filter and biofilm slides, respectively, at the stream site. These data suggest that analyzing environmental biofilms for Cryptosporidium oocysts may provide a more accurate assessment of recent oocyst contamination in a water supply than can be determined with filtration alone. Furthermore, biofilm collection is significantly cheaper than filtration ($3 per set of glass slides compared to $110 per filter) and could thus be performed more frequently and at more environmental locations by water utilities to monitor for oocyst contamination in drinking water supplies.

Investigating sources of fecal contamination and pathogen exposure using advanced molecular methods to understand pathways of diarrhea disease transmission in rural India

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Diarrhea and related enteric diseases caused by fecal-oral transmission of microbial pathogens remain a major cause of global morbidity and mortality, particularly in resource poor settings where safe drinking water and basic sanitation are lacking. The Millennium Development Goal (MDG) 7c aims to address this situation by increasing access to safe drinking water and basic sanitation. The MDG definition of safe drinking water access as using an improved source, however, does not include assessment of microbial safety while questions remain regarding the effectiveness of basic on-site sanitation facilities, without additional interventions, to reduce pathogen exposure and thereby improve health. Studies in low-income countries have shown improved drinking water sources are often contaminated with feces, and household stored drinking water can have higher levels of fecal contamination than source water, through handling and contact with dirty hands. Thus, in settings where personal and domestic hygiene practices are poor or drinking water is microbiologically contaminated, providing simple on-site sanitation facilities may be insufficient to impact health. Furthermore, where animal sanitation is lacking, zoonotic fecal-oral pathogens such as Cryptosporidium and Giardia may be significant causes of disease which remain uninterrupted by simple latrines. In India, where 626 million mostly rural people practice open defecation, and 535,000 children under age five die each year from diarrhea, improving sanitation and reducing fecal exposure remain essential public health tasks. A large cluster randomized controlled trial in the Indian state of Odisha led by the London School of Hygiene and Tropical Medicine is underway to evaluate the health impacts of improving rural sanitation through the provision of basic household latrines. In conjunction with the health impact study, a team of environmental and clinical microbiologists, environmental engineers, and parasitologists from the University of California Davis, the Asian Institute of Public Health and the Kalinga Institute of Industrial Technology's School of Biotechnology in Bhubaneswar, India, and the University of Nebraska Medical Center are undertaking research using advanced molecular microbial source tracking (MST) tools that distinguish human from non-human animal sources of fecal contamination and molecular genetic detection methods to investigate fecal environmental contamination and pathogen exposure in the public and domestic domains of diarrhea disease transmission. The aims of this research are to shed light on sources and key routes of fecal-oral pathogen transmission, including zoonotic transmission, and to improve understanding of the role of household latrines in reducing human fecal contamination and pathogen exposure in Indian communities where both human and animal fecal pollution are widespread. Following validation of sensitivity and specificity of host-associated Bacteroidales MST qPCR assays to distinguish human from non-human animal fecal sources in India, we collected environmental samples in the public (community) and domestic (household) domains of disease transmission in 60 study villages during the 2012 and 2013 monsoon seasons. Sampled routes of transmission in the public domain included improved public and private tubewells (deep and shallow groundwater, respectively) used for drinking (n=210) and public open ponds (n=110) used for non-drinking domestic and personal hygiene activities, while routes sampled in the domestic domain consisted of mother and child hands rinses (n=692) and household stored drinking water (n=353) collected from a sample of 5-6 households in each study village. Samples were tested for fecal coliform using membrane filtration culture methods on the day of collection, and processed, stabilized and stored for further MST qPCR analyses and molecular testing of bacterial (Vibrio cholerae, pathogenic Escherichia coli) and viral (rotavirus,

adenovirus 40/41) pathogens. Public domain samples were also analyzed to enumerate Cryptosporidium and Giardia by florescent microcopy following concentration by IMS-DFA methods, within one week of collection, and high count samples preserved for further genotyping. The presentation will describe the study design, sampling approach, and analytical methods and present initial findings and implications from analyses of the 2012 monsoon season samples from 24 villages to (1) compare the levels of human and livestock animal fecal pollution in improved (public and private tube wells) and unimproved (public ponds) water sources, household stored drinking water, and on hands in study communities, using the validated MST assays, and (2) examine pathogen levels to assess the microbial safety of improved and unimproved water sources across the study area.

Vegetated floodplain as best management practice in storm water treatment to remove fine contaminants including phytoplankton (algae) and sediment

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Lake Tahoe is a major tourist attraction in both Nevada and California. Monitoring data indicate a decline in the water quality of both deep-water zone and nearshore zone. Phytoplankton (algae) and fine sediment particles are the two main causes for the impairment of Lake Tahoe's water clarity. The common property of fine sediment particles and microalgae is the fine size and low mass. Due to their low mass, fine contaminants settle poorly in the floodplain before passing into the lake. The objective of the study was to assess the ability of biofilms in the flood plain to remove fine contaminants because internal water channels and other architectural elements can trap them within. The study was conducted under both controlled laboratory condition and field condition. Artificial storm water was prepared by mixing Kaolinite (surrogate of fine contaminants) and tap water as and pumped through biofilm pre-grown on plastic slides mounted on glass plates in the laboratory. The biofilm reached maximum capacity of fine contaminants removal after 25 h and the amount of biofilm mass decreased by 88% after 6 d. This loss of biomass was likely caused by sloughing of biofilm due to a nutrient deficiency in the storm water. The biofilms in the floodplains surrounding Lake Tahoe often experience dehydration during the dry season prompting us to examine the effect of dehydration on removal of fine contaminants in the field. The dehydrated biofilm removed three-fold less fine materials than the nondehydrated biofilm. This observation can be explained by the fact that biofilms tend to shrink after dehydration and, therefore, the number of internal water channels are diminished reducing space available for trapped fine contaminants. Accordingly, there was a decrease in thickness of the dehydrated biofilm to 32.1 _m, which is only half of the thickness of the nondehydrated biofilm. In contrast, the biovolume and roughness values were about 9.8 x 10^5 and 0.5, respectively, for both dehydrated biofilm and nondehydrated biofilms. Because the biofilm biovolume did not change while the thickness decreased by half the dehydrated biofilm had a higher density, which was verified microscopically. Field monitoring confirmed that biofilms did remove more fine contaminants than control plates. Water quality in the floodplain was improved possibly involving biofilms. In summary, biofilms in floodplains can remove fine contaminants. The results of this research may contribute to water quality management in Lake Tahoe and beyond if biofilms in vegetated floodplains become a general tool for storm water treatment.

Decay of fecal indicator bacteria and human-associated microbial source tracking markers in the upper Mississippi River is differentially affected by ambient sunlight and indigenous river microbiota

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Fecal indicator bacteria (FIB) such as enterococci and Escherichia coli have been used for over a century to assess the microbial quality of recreational waters in the United State and worldwide. However, quantification of FIB provides no information about the pollution source(s) as they are not restricted to human (or other important animal) gastrointestinal tracts. As a consequence, microbial source tracking (MST) has evolved in response to a need to identify water pollution source(s), but relatively little is known about the factors influencing the relative decay of MST markers in the environment. An in situ mesocosm device was constructed and deployed at a recreational beach in the Mississippi River to evaluate the effect of select environmental variables on decay of culture-based FIB (E. coli and enterococci), as well as molecular-based FIB (Entero1a, GenBac3) and human-associated MST genetic markers (HF183, HumM2) measured by quantitative real-time PCR (qPCR). Dialysis bags contained in the mesocosm were filled with mixtures of primary sewage effluent and ambient river water (1:1 v/v ratio) and collected approximately every other day for seven days. The experimental design was divided into following treatments: 1) exposure to sunlight and natural microbiota, 2) exposure to sunlight in sterilized ambient water, 3) no sunlight exposure in the presence of natural microbiota, and 4) no exposure to either. Overall, we noted a largely biphasic decay patterns for FIB (irrespective of the detection method) and MST markers with little change within the first 72h, followed by a more pronounced decrease in concentrations (between 72h and 120 h), reaching an apparent plateau during the last 48 h of the 7-day experiment. In general, decay of culture-based FIB was the fastest while molecular-based FIB measurements and human-associated markers decayed at a slower rate. A strong correlation was noted in decay patterns of molecular FIB and MST markers (r2 0.96-0.98, P< 0.0001), but not between culturable FIB and any qPCR measurement. Exposure to sunlight was an important determinant of decay in the early stages (first 72h) for enterococci, E. coli, Enteroa1, HF183 and HumM2 (P value and % contribution to variability ranges: 0.01 - < 0.0001 and 15% - 80%, respectively). For GenBac3, the effect of sunlight after 120h of exposure was dependent on the indigenous river microbiota as indicated by the significant interaction between the two variables (P < 0.0001, 35% contribution to variability). After extended exposure (120h), the presence of indigenous river microbiota was a more influential determinant of decay for enterococci, Entero1a, GenBac3 and HF182 (P value and % contribution to variability ranges: 0.003 - < 0.0001 and 28% - 70%, respectively). For E. coli, exposure to sunlight remained the only significant determinant of decay, while neither variable affected decay of HumM2 after 120h. Our findings indicate that biotic interactions from indigenous river microbiota and exposure to ambient sunlight are both important factors in the decay of sewage-borne culturable and qPCR FIB, as well as human-associated MST genetic markers. In general, sunlight-induced decay was often a key factor in the early stages of decomposition (< 72 h), while indigenous river microbiota played a larger role after some 120 h of mixing raw sewage with river water.

Disentangling meteorological, terrestrial, and nearshore aquatic drivers of beach water quality by combining statistical and physically-based models

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Bacterial water quality for recreational waters is monitored using fecal indicator bacteria (FIB) to protect public health. However, FIB quantification takes 18-24 hours using approved regulatory methods, and the potential lag time between risk to human health and the realization of that risk has motivated development of predictive and real-time beach water quality models to support beach closure management decisions. Some models use statistical regression analysis to determine relationships between observed FIB concentrations and environmental variables such as precipitation, wave height, and river discharge. However, we find these models often have limited skill in predicting FIB concentrations due to the interconnected and dynamic nature of environmental systems. Linked hydrological-hydrodynamics modeling systems, on the other hand, can provide new insight into some of these dynamic processes, and with a new understanding comes an opportunity to inform statistical models. We will present results from an assessment of the drivers of variability in beach water quality, considering output from a linked hydrologic-hydrodynamic modeling system that simulates transport of river water in nearshore zones in addition to more conventional drivers of beach water quality. Specifically, we present results of water samples collection and analysis (for E. coli concentration) from a 20 km stretch of Lake St. Clair shoreline (Michigan) and 3 locations from the contributing watershed over a two year period, with an emphasis on characterizing variability in E. coli concentration among and within sampling sites. Incorporating both observed and modeled environmental variables, we applied our data in regression- and ANOVA-based statistical methods to determine the drivers of variability in E. coli concentrations across space and time. Using process-based model output as an input to statistical analyses will, in turn, inform the further development of linked process-based models. This unique approach will lead to a more complete understanding of how FIB move within complex environmental systems, and, potentially, provide improved predictive capabilities.

Hepatitis A Outbreak Caused by Contaminated Centrally Piped Water in Aspindza, Georgia, August-November 2013

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BACKGROUND: On October 10, 2013 Georgia's National Centers for Disease Control (NCDC) was notified by Aspindza Public Health Center regarding 17 persons with hepatitis A (HA) symptoms occurring since August 18th. We investigated to assess the outbreak, identify risk factors and implement preventive measures. METHODS: We conducted a case-control study. Data was collected with the standard questionnaire regarding demographics, clinical presentation and exposures (food: gathering, catering service, celebration; water: centrally piped water and individual well-water). Water distribution in the village via reservoirs and pipes was investigated. Probable Hepatitis A (HA) case was defined based on reviewed medical records: Aspindza resident, with dark urine, general weakness and one of the following: jaundice, scleral icterus, high fever, vomiting in August-November, 2013. The door-to-door case-finding was conducted to identify new cases. We collected blood samples from suspect cases; Hepatitis A (HA) was confirmed by ELISA IgM (NCDC). Taking into consideration homogeneity of water

consumption, we considered households with at least one sick person as case-households, and households with no cases as control-households. RESULTS: Enhanced surveillance detected 13 additional case-patients. We interviewed 50 persons: 30 cases (from 12 case-households) and 20 controls (from15 control-households). Six of 30 case-patients were lab confirmed. Case-patient were 2-24 years of age (median=14 years); 16 (53%) were females. No food items were associated with HA. Drinking water from centralized system was associated with the disease (OR =12.6; 1.3 - 124 95% CI); 11 (92%) of 12 case-household used pipe water and 7(47%) out of 15 control-households used the same water source. The village uses piped water from reservoir containing underground water. The water is not pre-treated or filtered priory to distribution in households. Coliform bacteria were found in central reservoir-supplied water. The test result shows existence (presence/absence test) of the coliform bacteria from 300cm3 and Streptococcus faecalis from 250 cm3 of water. CONCLUSIONS: HA outbreak was most likely due to contaminated centralized water; contamination occurred within the reservoir where the underground waters are collected. Subsequent chlorination initially eliminated coliform contamination within one day, however, repeat water testing one week later again found contamination. We advised the community to boil reservoir-supplied water before drinking and food preparation; we advised participants in hand-washing hygiene. Enhanced disease surveillance continues. Since the last case was revealed in December 4, we continue observing the village. After 2 incubation periods from the last case we will announce the outbreak finished. Keywords: Hepatitis A, outbreak, case-control, Georgia, piped water.

Quantitative analysis of fecal contamination in stormwater conveyance systems in Wrightsville Beach, NC

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Stormwater conveyed to recreational beaches can represent a public health risk due to the presence of fecal contamination. The threat of pathogen exposure through this contamination greatly increases during and after a storm event due to both overland scouring and subsurface connections with groundwater, sewage infrastructure, and on-site wastewater systems. In this study we aimed to quantify and determine the source of fecal contamination in storm drains leading to the degradation of receiving waters used for primary recreational water activities in coastal southeastern North Carolina. Two watersheds - Iula Street and Snyder Street, located within the Town of Wrightsville Beach (North Carolina, USA), have historically been associated with significantly increased concentrations of fecal indicator bacteria (FIB) in coastal receiving waters following precipitation events. We conducted a multi-tiered study of both watersheds via a storm event based sampling design, and through the use of defined substrate technology and qPCR-based analysis. Water samples were collected from these watersheds between July 27, 2011 and August 15, 2013 following storm events (n=16) with antecedent precipitation ranging from 0.51 to 128.78 millimeters. Samples were analyzed for Escherichia coli and Enterococcus spp. using defined substrate technology, and human, dog, and gull molecular markers using qPCR. Additionally, we studied the potential of bacterial regrowth occurring in accumulated sediment inside the storm drains. Sampling of multiple locations within the storm drain systems of these small watersheds continued to reveal extremely high concentrations of FIB. For Snyder Street Watershed, FIB concentrations during storm events averaged 2,807 MPN/100mL and 1,943 MPN/100mL for E. coli and Enterococcus spp. respectively. Twenty-four hour total rainfall amount had a strong correlation to Fecal Bacteroides spp. concentrations at the origin of the Snyder Street storm drain (r=0.857). Detection of multiple human specific markers (i.e. BacHum, HF183) confirms the presence of

human fecal contamination in both watersheds. HF183 concentrations were high during specific storms (612,276 CE/100mL in the Snyder Street storm drain on July 27, 2011), but the signal was ephemeral in nature as related to sampling locations and only quantified in 7 of 16 storms. Storm drain infrastructure needs inspection and replacement as necessary in order to reduce human fecal contamination in these watersheds. Additionally, gull and dog fecal contamination have both been quantified in the system. The gull fecal contamination signal, quantified in 11 of 16 storms, indicates the need for a future bird fecal contamination control program. The storm drains are typically laden with a high level of muck and sand sediment, which was found to be a source of high concentrations of plant-associated Enterococcus spp. during warm summer months (up to 13,903 CE/g of dry sediment). This indicates that perhaps such high concentrations of Enterococcus spp. in receiving waters may not be completely attributed to fresh fecal contamination sources. Ongoing work in this area will indicate whether or not bacterial regrowth in these watersheds is a significant contributor to the measured Enterococcus spp.

Determination of Enterovirus, Giardia and Cryptosporidium Limits in Reclaimed Domestic Wastewater for Unrestricted Urban Reuse

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Introduction and objectives: Strategies to minimize public health risks associated with the reuse of treated municipal wastewater have been a challenge for authorities in developing countries. In metropolitan regions, like Sao Paulo city, where there is a high demand for water and water resources are each day more scarcely, the urban reuse of wastewater is already a reality. Street washing is one of the most common urban reuse in the city of Sao Paulo; however there is no Brazilian regulation establishing quality standards for this use. Our purpose was to develop an approach to determine maximum concentration limits for Enterovirus, Giardia and Cryptosporidium in treated wastewater, based on tolerable infection risks for workers involved in the street washing activity. Methodology: Risk assessment was conducted as follows: i) Worker exposure - respiration intake (R): 0.830m3/h; daily exposure time (T): 2h/d; work day per year (WD): 242. ii) Plume model - water flow rate (F) - normal distribution: 15_3 m3/h; efficiency of aerossolization (A) - triangular distribution: 0.001, 0.003 and 0.018; impaction factor (I) - triangular distribution: 0.08, 1.0 and 1.2; distance between worker and water flow (d) - triangular distribution: 4.0, 5.0 and 6.0 m; distance between worker and spray plume centerline in cross-wind direction (y): 0.0m; worker's height mouth/nose (z): 1.6m; height of plume formation (H) - triangular distribution: 0.4, 0.5 and 0.8m; wind velocity (u) - triangular distribution: 2, 3 and 4m/s; dispersion (D): estimated via Gaussian plume model (H_glund et al, 2002) as a function of d, y, z, H and u. iii) Dose-response parameters - Enterovirus: Beta-Poisson, alpha=1.06 and beta=998.8; Giardia: Exponential, r=0.01982; Cryptosporidium: Exponential, r=00467. iv) Daily risk of infection (Pd) - Daily dose was computed as: Dose = C x D x F x T x A x IF x R, where C denotes the pathogen concentration in the treated wastewater (PFU/L or (oo)cysts/L). Pd was computed by the dose-response function, here denoted as Pd = P(Dose). v) Annual risk of infection (Pa) - Pa(C) = 1-[1 - Pd(C)]^(WD) was computed over K=100,000 Monte Carlo simulations, yielding a distribution of annual risks and allowing quantile estimation. The q-quantile of Pa(C) is denoted by Paq(C). vi) Determination of concentration limits - given a tolerable annual risk (Ta), the corresponding daily risk (Pd) and concentration (C) were computed by simple inversion of above equations (substituting Pa by Ta): Pd = 1 - (1 - Ta)^(1/WD), Dose = InvP(Pd) (InvP = inverse of Pd) and C = Dose / (D x F x T x A x IF x R). Monte Carlo simulations resulted in a collection C1, C2 ... CK of concentrations, each one yielding a different risk distribution. In order to choose the most suitable of these concentrations, besides the parameter Ta, we also took into account

the desired confidence level (q) such that Paq(C) < Ta. Given the pair (Ta, q), which we denoted by Taq, the upper limit for the q-quantile concentration, denoted by Cq, was the maximum value of C among C1, C2 ... CK such that Paq(C) < Ta. For example, if Ta0.5 = 1x10-4 (meaning that the median tolerable risk is 1 x 10-4), the upper limit for the median concentration should be C0.5 = max {C | Pa0.5(C) < 1 x 10-4}. vii) Tolerable annual risks - For each scenario, we defined values for Ta0.5 and Ta0.95 in order to compute the upper bounds for 0.5 and 0.95 - quantiles of pathogen concentrations in treated wastewater (C0.5 and C0.95). Three scenarios were considered, in descending order of conservativeness: (1) Ta0.5 = 1 x 10^(-4) / Ta0.95 = 5 x 10^(-4); (2) Ta0.5 = 1 x 10^(-4) / Ta0.95 = 1 x 10^(-3); (3) Ta0.5 = 5 x 10^(-4) / Ta0.95 = 5 x 10^(-3). Results and Conclusions: For scenario (1), concentration limits (C0.5, C0.95) were (0.375, 0.750) PFU/L for Enterovirus, (0.020, 0.040) cysts/L for Giardia and (0.085, 0.171) oocysts/L for Cryptosporidium; for scenario (2), concentration limits were (0.375, 1.501) PFU/L for Enterovirus, (0.020, 0.080) cysts/L for Giardia and (0.085, 0.341) oocysts/L for Cryptosporidium and for scenario (3), concentration limits were (1.877, 7.520) PFU/L for Enterovirus, (0.101, 0.403) cysts/L for Giardia and (0.427, 1.709) oocysts/L for Cryptosporidium. The obtained results must be seen as preliminary, since some issues regarding the model assumptions, particularly the Gaussian plume model, must be further addressed. Nonetheless, the proposed approach tried to offer intuitive quality standards to comply with risk criteria, considering the uncertainties involved in this process. This study aimed to be a contribution to the urgent need of setting minimum standard for quality of treated wastewater to protect workers' health as well passers-by and neighborhoods during the street washing process.

Arcobacter Transmission Dynamics at an Avian-Dense Wetland and a Lake Erie Beach: Understanding the Role of Wetland Ecoservices in Protecting Beach Water Quality

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Arcobacter has been recognized as an important gastrointestinal pathogen and a potential zoonotic agent. Arcobacter-contaminated water is considered a route of transmission to humans. In the Lake Erie region, it was isolated in a gastrointestinal illness outbreak and the outbreak was attributed to the transport of microbiological agents from wastewater treatment facilities and septic tanks to the lake and the subsurface in South Bass Island. Arcobacter was prevalent in the water at two urban central Lake Erie beaches, and microbial source tracking and phylogenetic analysis showed that Lake Erie beach water may serve as a potential reservoir for Arcobacter species originated from human and animal sources. In this study, we investigated Arcobacter density and beach water quality at a beach in western Lake Erie, which is located adjacent to a near shore wetland (Ottawa National Wildlife Refuge (ONWR), Ohio) that has densely populated avian flocks and is also under the influence of agricultural runoff. The aims of this study are: 1) to understand the ecosystem service of the wetland and its impact on beach water quality; and 2) to identify the major contamination sources of Arcobacter at ONWR. During the 2012 swimming season, a total of 64 water samples were collected once a week from each of the four sites (Site 1-3 along Crane Creek, site 4 from the Lake Erie beach). Crane Creek approximately bisects ONWR, which has an area of 6,704 acres and provides an important avian habitat in the Great Lakes region, especially for birds that depend on the wetlands when traversing the Mississippi and Atlantic flyways during their migrations. Site 1 is located where water typically flows into the wetland from agricultural fields; site 2 is approximately midway through Crane Creek's passage across ONWR; site 3 is where Crane Creek and Lake Erie meet; and site 4 is at the adjacent swimming beach approximately. Since Branta canadensis (Canada Goose) was one of the most predominant birds at ONWR, we collected

their fecal samples along the four sites (total 62 samples) in order to investigate whether they shed Arcobacter. With the water samples, a total of 200 ml of each water sample was filtered through a 47 mm diameter membrane with a 0.45 _m pore size. After DNA extraction, real-time quantitative PCR (qPCR) assays were carried out in duplicate targeting Arcobacter 23S rRNA genes for Arcobacter (Arco). Microbial source tracking for human-specific fecal contamination was conducted targeting 16S rRNA genes of Bacteroidales (HF183). Physicochemical parameters (water temperature, turbidity, pH, dissolved oxygen DO, conductivity) were measured in situ. The data for wave height and precipitation were obtained from the National Oceanic and Atmospheric Administration's National Weather Service. For faecal indicator bacteria, E. coli densities were determined with modified m-TEC agar method. Total phosphorus and total nitrogen were each measured using U.S. Environmental Protection Agency approved methods; method 8190 for total phosphorus (Hach PhosVer 3 acid persulfate digestion method) and method 10206 for total nitrogen (Hach dimethylphenol method). The levels of Arcobacter spp. showed temporal and spatial variations, ranged from 8.9 x 103 to 2.90 x 106 gene copy number/100ml. Arcobacter concentrations and prevalence were highest at sites where water enters the wetland and levels were reduced by the wetland. Additionally, Arcobacter levels display a small increase at the beginning of the summer, but then remain steady until late July-early August, when their levels begin to rise substantially. The monthly mean of Arco levels were higher in August at (Site 2, 3, 4) than other months. When compared with E. coli densities, there is a significant correlation between Arcobacter and E. coli levels (Spearman correlation, 0.466 ($p<0.001$)). Contrary to our expectations, Arcobacter spp. was not detected from any of the goose faecal samples. Thus, the possibility of Arcobacter shedding from the densely populated geese was ruled out. The result of HF183 assay demonstrated that all four sites did not have human-specific fecal contamination, so the association of Arcobacter at the study sites with human sources was minimal. The results indicate that all the Crane Creek sites experienced faecal contamination at various levels from agricultural and wildlife sources, and the ONWR wetlands reduced E. coli, Arcobacter spp. and nutrient levels considerably before the water reached Lake Erie and nearby beach waters. The sources of Arcobacter at the study sites are probably from other avian wildlife forms or agricultural run-off; as expected not from humans and interestingly not from geese. More microbial source tracking is warranted to pinpoint the major source of Arcobacter. Keeping the healthy near shore wetlands is important to maintain safe surface water and may contribute to controlling spread of infectious agents.

Building local capacity for microbial source tracking in the Myrtle Beach Urbanized Area

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Recent advances in microbial source tracking (MST) hold out the promise for more effective remediation of fecal bacteria contamination arising from nonpoint runoff. To bring these new technologies into the management realm requires an interdisciplinary effort between municipal staff, particularly the stormwater program managers, and laboratory staff involved in the field science. This has been done in the Myrtle Beach Urbanized Area where stormwater management is required under the Clean Water Act's NPDES Phase II program. This area is located in northeastern South Carolina and comprises the Grand Strand that hosts 14 million tourists each year who are seeking recreational and fishing opportunities in the coastal waters and rivers. Over 80 sites in this region are impaired due to contraventions of water quality standards for fecal indicator bacteria (FIB). Decentralization of stormwater management onto the local municipalities has required development of local capacity for MST. To address this, the municipalities have partnered with Coastal Carolina University's Environmental

Quality Lab (EQL) to develop a MST protocol tailored to the unique needs of the region. This MST protocol is now being used by the municipalities to inform remediation strategies. The protocol employs multiple tracers, including qPCR assays, culture-based FIB enumeration and chemical constituents, such as caffeine and optical brighteners, to provide a weight-of evidence approach to host animal source identification. Sample site selection is based on a watershed approach that isolates geographic sources. Sites are selected collaboratively using GIS maps of land-use/land-cover, soils, topography, natural water features, and stormwater infrastructure, such as pipes, ditches, and retention ponds. A sanitary survey is conducted and extant FIB data are scrutinized. Wet weather samples are collected with Nalgene first-flush stormwater samplers whose use is optimized from site-specific storm hydrographs. The EQL has adapted and validated Bacteriodales qPCR assays from the peer-reviewed literature that are specific for human, canine and bird hosts. Quantification was challenging due to the lack of commercial standards and uniformity in their preparation and use. To characterize qPCR marker concentrations, a percentile-based ranking system was developed. This system is concurrently applied to the FIB and chemical measurements to realize the weight-of-evidence approach. The results from all the parameters obtained at a site are combined to generate an index value that enables comparisons among sites and sampling events. General insights obtained from application of this MST protocol include: (1) wildlife and dogs are a significant widespread source and (2) the weight-of -evidence approach is complicated by the potential for differential persistence of the tracers. Contributions from wildlife can reflect anthropogenic influences including altered hydrology, increased populations, and restriction of habitat to margins around waterways. Modification of the fecal bacteria tracers following release from the host animals is suspected to result from inoculation of sediments and soils, ensuing possible loss of the qPCR host animal marker, and resuspension during high flow events. The results from the qPCR assay (BacCan) meant to target dogs could be obscured by increasing populations of coyote as the latter are canines and hence potentially reactive to the BacCan assay.

Associations between marine phytoplankton and symptoms of illness among recreational beachgoers in Puerto Rico, 2009

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While phytoplankton generally have crucial roles in marine ecosystems, a small subset can release toxins and produce harmful algal blooms (HABs). HABs can be a threat to human health as symptoms from exposure range from neurological impairment to gastrointestinal (GI), dermal, and/or respiratory illness. The objective of this study was to evaluate the association between phytoplankton in marine water and symptoms of illness. During the summer of 2009, beachgoers were recruited into a prospective study at Boquer_n Beach, Puerto Rico. On the day of the beach visit, study participants were interviewed for baseline health conditions and activities engaged in at the beach. Also, water samples were quantitatively assessed for phytoplankton (total and group cell counts), and two algal toxins (lyngbyatoxin and debromoaplysiatoxin). A follow-up telephone interview was conducted 10-12 days later to determine if exposure had resulted in the development of any symptom of illness. Logistic regression models were used to describe the association between exposure to different categories of phytoplankton and incidence of illness among participants who reported immersing their body in the water. Cell counts were categorized as high (≥75th percentile), medium (25th-75th percentile), and low (≤25the percentile). The lowest category served as the referent in the regression models. All results were adjusted for potential confounders. Of 15,726 study participants, 12,111 (77%) reported immersing their body in the water and were included in the analysis. Daily total phytoplankton cell

counts ranged from 346 to 2,012 cells/mL (median: 712 cells/mL). The 3 most frequently identified algal groups were bacillariophyta (median: 386 cells/mL), cyanobacteria (132 cells/mL), and pyrrhophyta (37 cells/mL). Concentrations of lyngbyatoxin and debromoaplysiatoxin were below the limit of detection. Respiratory illness was the most commonly reported symptom of illness (7%), followed by GI illness (5%), rash (5%), eye irritation (3%), and earache (2%). The high category of total phytoplankton cell count was associated with eye irritation (Odds Ratio=1.32; 95% confidence interval: 1.03-1.69) and rash (OR=1.28; 95% CI: 1.03-1.59). The medium category of bacillariophyta was associated with respiratory illness (OR=1.27; 95% CI 1.06-1.52), however, the odds ratio did not increase with the high category (OR=1.09; 95% CI: 0.88-1.35). The associations for respiratory illness, eye irritation, rash, and earache all appeared to rise with increasing category of cyanobacteria. The most apparent association was for respiratory illness, with the high cyanobacteria category having OR=1.37 (95% CI: 1.12-1.67) and the medium category having OR=1.29 (95% CI: 1.07-1.55). Symptoms of illness were not associated with exposure to pyrrhophyta. Our findings suggest that there may be an association between marine phytoplankton and illness. In addition, these risks may vary by algal group. However, because cell counts were so low, we cannot determine whether the association was a result of the algal exposure or if the phytoplankton were markers for other causative factors we did not measure. This abstract does not necessarily represent EPA policy.

Exceeding the Level 1 and 2 Assessment Requirement Under the RTCR for Groundwater Wells

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Pathogens associated with fecal contamination are the primary cause of waterborne disease outbreaks in the United States. Water supplies are expected to become increasingly vulnerable to waterborne pathogens as a result of global climate change, and Wisconsin's groundwater is no exception. Current groundwater monitoring regulations are relatively successful at detecting potential fecal contamination, but do not provide information on its source. Under the Revised Total Coliform Rule (RTCR), unsafe sample results in transient non-community wells could result in financially burdensome retesting, follow-up monitoring, and completion of Level 1 and Level 2 assessments. To prevent a dramatic increase in the number of total coliform-positive follow-up samples as a result of the new requirements, the PI's research group in coordination with the Wisconsin Department of Natural Resources (WDNR) is implementing an alternative RTCR unsafe testing follow-up and comprehensive assessment program that will be used to satisfy both retesting and Level 1 and 2 requirements. This program focuses on source tracking and corrective actions, and is more aggressive than the Level 1 and Level 2 assessment protocols described in the RTCR. The WDNR well assessment protocol will include three basic components: (1) sanitary surveys, (2) measurement of a suite of microbial indicator organisms, and (3) corrective action. The source remediation achieved through implementation of this protocol is intended to provide a long-term solution that is sustainable, holistic, and economical for each public water supply investigated. The goal of this project is to develop, test and deploy a scientifically-based well assessment protocol. This protocol is envisioned to consist of: large volume (100 liter) sampling capabilities among the WDNR and Wisconsin Public Health Department communities with a sanitary survey component; and development and testing of a suite of microbial indicators that can be standardized to accurately and efficiently track the sources of coliforms in public water supply groundwater wells in Wisconsin. To accomplish the project goal, the following objectives must be met: train and coordinate with WDNR staff to develop hollow fiber ultrafiltration (HFUF) sampling hardware and capabilities; integrate a sanitary survey component to unsafe sample follow-up activities; conduct bi-weekly analysis of RTCR positive

samples (unsafes) using HFUF concentrates for the full-suite of analytes; and perform a critical analysis of sanitary surveys, monitoring data and success of remedial designs. All FST methods have limitations, suggesting that a toolbox approach utilizing multiple methods is required to consistently determine contamination sources. In addition to re-testing RTCR unsafe wells for general fecal indicators (total coliforms and generic E. coli by the enzyme substrate method), the proposed initial toolbox for this project includes indicators to achieve the following: (1) identify general microbial community size using adenosine triphosphate (ATP) measurements to help discriminate among water stagnation, biofilms and aquifer contamination, (2) identify shiga toxin-producing bacterial contamination (toxigenic E. coli by qPCR) as an assessment of bacterial pathogen potential, (3) differentiate between human and animal contamination sources (F coliphage genotyping by PCR), (4) identify human-specific contamination sources (sorbitol-fermenting Bifidobacteria sp., human adenovirus, and human Bacteroides sp. by qPCR), (5) identify grazing animal-specific contamination sources (Rhodococcus coprophilus by qPCR), (6) identify bovine-specific contamination sources (bovine adenovirus, bovine polyomavirus, bovine Bifidobacteria and bovine Bacteroides sp. by qPCR), and (7) identifying other important agricultural animal sources (swine and poultry Bifidobacteria by qPCR). The well assessment protocol will help to identify sources of microbial contamination entering Wisconsin's public water supplies that rely on groundwater. The information gathered for an individual well will provide the scientific basis for developing measures to prevent contamination from recurring. If demonstrated to be successful, the developed well assessment protocol will help to protect public health in Wisconsin. WDNR staff is also working with staff within United States Environmental Protection Agency (EPA) Region 5, and if successful, this well assessment protocol may become a model for the region. Pilot testing on approximately ten wells returning unsafe RTCR results will be completed by the end of March 2014. This paper will report on the successes and lessons learned from the pilot work and plans for implementation of the full-scale program.

The Wisconsin Emergency Preparedness qPCR Assay for E. coli in Drinking and Recreational Waters

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Public health risks associated with waterborne disease outbreaks still exist throughout developed countries, and Wisconsin is no exception. Recent research has indicated that the standard culture-based methods used today to determine the risk of waterborne disease in drinking and recreational waters are less than perfect for protecting public health. Utilizing molecular methods, specifically quantitative polymerase chain reaction (qPCR), may be one approach to improving the protection of public health by reducing analysis times. Standard culture-based methods require at least 24 hours to complete, whereas qPCR methods can provide same-day results in a matter of hours, helping to reduce public exposure to contaminated waters. Therefore, the objective of this research project was to optimize qPCR assays for generic E. coli, a general indicator of fecal contamination, and toxigenic E. coli, a known fecal pathogen, to provide rapid and unbiased results to allow for inclusion of these assays in the State's emergency preparedness program. Molecular assays were optimized for the detection of generic E. coli and toxigenic E. coli in water samples. For analysis of drinking waters, which generally have a low microbial content, initial hollow fiber ultrafiltration (HFUF) is required to concentrate microorganisms present in the sample. Next, turbidities are measured for both high microbial content samples and concentrated low microbial content samples to determine the need for sample clean-up, which removes substances that may inhibit qPCR reactions. Next, up to 250 mL of each sample is membrane filtered to concentrate microorganisms. For samples with turbidities less than five NTU, membranes are prepared for nucleic

acid extraction using a direct bead-beating protocol. For samples with turbidities greater than or equal to five NTU, membranes are prepared for nucleic acid extraction using the Zymo ZR Soil Microbe DNA KitTM for sample clean-up. Next, membranes are frozen at -80_C for at least one hour for expedited samples, and for up to several days or weeks for samples that can be batched. Following membrane thawing, nucleic acids are extracted and triplicate extract aliquots of five _L are used as template in the qPCR reaction. Mastermix for the qPCR reaction is prepared using 2X Taqman_ Environmental Master Mix 2.0. Depending on the desired analysis (generic E. coli, toxigenic E. coli, or both) one or both qPCR assays are then performed. The generic E. coli qPCR assay targets the uidA gene, which encodes the β-glucuronidase enzyme. The toxigenic E. coli assay includes four gene targets: the Shiga-toxin genes, stx1 and stx2, an O157:H7 specific open-reading frame (ORF) Z3276, and the E. coli/Shigella 16S gene. The combination of these targets in a single assay allows for the quantification of toxigenic (stx-producing) organisms, E. coli O157:H7 (a toxigenic E. coli strain particularly threatening to public health), and total E. coli simultaneously. The detection limits and efficiencies of the qPCR reactions were evaluated on a suite of synthetic waters spiked with known numbers of target organisms. Overall assay performance was evaluated by comparing qPCR results with standard culture-based methods on a variety of drinking and beach waters. The effects of water quality parameters including pH, conductivity, total organic carbon, and hardness were assessed with respect to assay performance with environmental waters. A synopsis of these results and recommendations for assay application will be presented.

Escherichia coli associated with macroalgae in the Venice Lagoon

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Recent studies provided evidence that the macroalga Cladopohora in lakes can host attached populations of Escherichia coli, with consequences on the environmental quality and the human health. To expand the knowledge to other widely-diffused macroalgae, we collected samples of autochtonous (Ulva spp.) and invasive (Sargassum muticum and Undaria pinnatifida) macroalge and their overlying water in the lagoon of Venice (Italy). Samples were analyzed to i) quantify E. coli attached to the macroalgae; ii) assign isolates to the phylogenetic group or to cryptic Escherichia clades iii) describe the genotypic diversity of the attached isolates and iv) investigate, by means of laboratory experiments, whether macroalgae can favour the bacterial growth and persistence. Attached E. coli populations were abundant and significantly related with the abundance in water. The analysis of 400 isolates showed that macroalgal-associated E. coli belonged to all known phylogroups, including extra-intestinal, potentially pathogenic strains and a low fraction of cryptic clades. Most of attached E. coli were able to grow on macroalgal extracts as the only source of carbon, suggesting that the macroalgal habitat can potentially support their growth. The genotypic diversity of attached isolates was very high, with significant differences between macroalgae and water. We report the existence of a new, under-recognized reservoir of E. coli in the lagoon of Venice, deserving further investigations from either the public health and the ecological perspective.

Epidemic Escherichia coli ST131 and Enterococcus faecium ST17 in Coastal Marine Sediments from an Italian Beach

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Fecal indicator bacteria (FIB) are used worldwide to assess the water quality in coastal environments, but little is known about their genetic diversity and pathogenicity. This study examines the prevalence, antimicrobial resistance, virulence, and genetic diversity of FIB isolated from marine sediments from a central Adriatic seaside resort. FIB, recovered from 6 out of 7 sites, were significantly more abundant at sampling stations 300 m offshore than close to the shore. Escherichia coli accounted for 34.5% of fecal coliforms, and Enterococcus faecalis accounted for 32% of enterococci. Most isolates (27% of E. coli and 22% of enterococci) were recovered from the sediments that had the highest organic content. Multidrug-resistant E. coli (31%) and enterococci (22%) were found at nearly all sites, whereas 34.5% of E. coli and 28% of enterococci harboring multiple virulence factors were recovered from just two sites. Pulsed-field gel electrophoresis typing showed wide genetic diversity among isolates. Human epidemic clones (E. coli ST131 and Enterococcus faecium ST17) were identified for the first time by multilocus sequence typing in an area where bathing had not been prohibited. These clones were from sites far removed from riverine inputs, suggesting a wide diffusion of pathogenic FIB in the coastal environment and a high public health risk.

Evaluation of PMA-qPCR method to detect infectious enteric viruses to inform risk assessment and drinking water management

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INTRODUCTION Enteric viruses have been detected in surface and groundwater sources, and water utilities have a responsibility to ensure public health is protected from exposure to enteric pathogens through drinking water distribution. Molecular-based methods for the detection of pathogenic enteric viruses, such as quantitative PCR (qPCR), can be sensitive, specific, less labor-intensive, and more rapid than culture-based methods. However, qPCR is currently limited by its inability to differentiate between infectious and non-infectious viruses. Therefore, there is considerable interest in improving detection and differentiation of the infectious fraction of enteric viruses in water by qPCR to inform microbial risk assessments in drinking water treatment. Pre-treatment of microbial suspensions with propidium monoazide (PMA, a nucleic acid intercalating dye) and other enzyme treatments such as proteinase K and RNase, have shown variable success with detecting several infectious viruses and surrogates (Kim & Ko 2012; Pecson et al. 2009). PMA can penetrate damaged viral protein coat and form linkages with accessible nucleic acid when exposed to light. PMA-linked nucleic acid is then prevented from amplification during qPCR; theoretically resulting in detection of amplicons from whole or un-damaged viruses representing the infectious fraction. The limitation of PMA-PCR for differentiating infectious and non-infectious viruses is that it is assumed that cells with damaged protein coat are non-infectious; which is not always the case for viruses. This approach theoretically proves more useful for preventing detection of viruses inactivated by chlorine, rather than UV; as UV may only cause damage to the viral genome and not the protein coat. Therefore, this pretreatment method may only be effective for viruses that have sustained significant damage as a result of disinfection processes (e.g. hypochlorite and

heating/pasteurization). The objectives of this study are to evaluate and optimize a PMA-qPCR assay to detect the infectious fraction of adenovirus type 40 (Ad40) inactivated by Ultraviolet light (UV) and hypochlorite in a range of environmental water matrices. Strains of adenovirus have been found in North American drinking water sources; primarily those impacted by sewage and wastewater discharge (Vidovic et al. 2011). Ad40 causes gastroenteritis, particularly in children, and is on the USEPA Contaminant Candidate List (CCL) 3 (USEPA, 2009). Adenovirus is of interest to inform drinking water microbial risk assessment as it is highly resistant to UV disinfection, and has been proposed as a source tracking tool. METHODS Ad40, having a double-stranded linear DNA genome, is known to be resistant to inactivation by UV light. An amplicon for the 96 bp hexon gene was evaluated to determine the sensitivity of the PMA-qPCR assay. The concentration of PMA was optimized for various water matrices, including: groundwater, surface water and tap wate; for the anticipated viral load within a sample; along with the appropriate controls (e.g. no PMA and PMA no light) with replications. A PMA-qPCR method was assessed using pure cultures of viable Ad40, as well as in spiked samples after inactivation by UV (20 and 40 mJ/cm3 using UVPure technology) and hypochlorite (0.5-2 mg/L) with contact times representative of disinfection in drinking water treatment (USEPA, 2009). The infectious fraction of Ad40 was enumerated with a cell culture assay using HEK293 cells. Inactivated samples were imaged using transmission electron microscopy (TEM) to visualize damage to the protein coat, and to provide evidence for the application of PMA-qPCR relative to each inactivation method. IMPLICATIONS Evaluation and validation of PMA-qPCR to quantify the infectious fraction of genomic material in samples of Ad40 will provide the basis with which to apply this method to other enteric viral pathogens relevant to water contamination and human health risk. REFERENCES Kim SY, Ko G. 2012. Using propidium monoazide to distinguish between viable and nonviable bacteria, MS2 and murine norovirus. Letters in Applied Microbiology, 55: 182-188. Pecson BM, Martin LV, and Kohn T. 2009. Quantitative PCR for determining the infectivity of bacteriophage MS2 upon inactivation by heat, UV-B radiation, and singlet oxygen: advantages and limitations of an enzymatic treatment to reduce false-positive results. Applied and Environmental Microbiology, 75: 5544-5554. USEPA. 2009. Final Microbial Contaminant Candidate List (CCL) - 3. Accessed on January 21, 2013 at: http://water.epa.gov/scitech/drinkingwater/dws/ccl/ccl3.cfm. Vidovic S. Mahmoud A, Flemming C, Sprinthorpe S, Sattar SA. 2011. Genetic analysis of infectious human adenoviruses from wastewater of two urban communities in Canada shoes first evidence of genotypes Ad3a16 and Ad3a18 in North America. Applied Environmental Microbiology, 77 (12): 4256-4259.

Bacteroides fragilis GB-124 bacteriophages, novel indicators of human fecal pollution for the US?

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Testing water directly for pathogens is difficult because of the broad phylogenetic diversity (encompassing bacterial, viral and protozoan groups), an overwhelming number of organisms from each group, and the lack of appropriate, sensitive and cost/time effective methodologies. Fecal indicator bacteria (FIB), such as Escherichia coli and enterococci, are used worldwide to signify the presence of fecal contamination; however, there is little correlation between the presence of pathogens and that of fecal indicators. Recently, researchers in Europe have developed an assay using bacteriophages that infect a human-associated strain of Bacteroides fragilis (GB-124). In their studies, GB-124 phages were shown to be human specific and to have densities comparable to that of traditional fecal indicators. Furthermore, GB-124 phage was co-detected with adenoviruses and noroviruses in sewage and human impacted waterways. In order to determine the suitability of GB-124 phages for the continental United

States, here we describe the results from seven municipal wastewater treatment plants (WWTPs) across the US, scats from 13 different animal species and more time-intensive sampling at several local WWTPs. Thirty-six primary sewage effluent samples and > 250 fecal scats from 13 different animals species were analyzed for plaques of GB-124 phages, somatic and FRNA coliphages, as well as culturable FIB. For the seven WWTPs, samples were also analyzed for adenoviruses by qPCR. In order to establish temperature stability of the GB-124 phages, along with both coliphage types, we followed their plaque-forming units (PFU) at 4_, 20_ & 35_C to represent likely ranges in continental climates. GB-124 phages were consistently detected in primary sewage effluents with concentrations ranging from 102-104 PFU per 100 mL. There were no statistically significant differences in concentrations by season or by the geographical region in which samples were collected. Concentrations of FIB and both coliphage types in primary treated sewage were approximately one order of magnitude higher than that of GB-124 phages. Adenoviruses were detected in the majority of primary sewage effluent samples, but their concentrations were not correlated with any of the indicator organisms tested. Importantly, GB-124 phages were not detected in any of the animal fecal samples tested, while both coliphages were randomly detected at concentrations ranging from 102 to 105 per gram (DW) of feces. The temperature stability of GB-124 phages (along with coliphages) was inversely proportional to temperature; phages were most stable at 4_C, persisting for up to two weeks. At higher temperatures (20_ & 35_C), all phages deteriorated relatively rapidly and no plaques were detected after one week of incubation in the dark. In summary, GB-124 phages appear to be indicative of human fecal pollution source(s) in the continental US and the assay is quick, inexpensive and easy to use culture-based method that not only relays phage quantitative but also an assessment of infectivity.

Water Quality Assessment for Southern Arizona on the Upper Santa Cruz River

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Utilization of areas close to rivers for agricultural and industrial purposes is beneficial for each respective trade, but can have detrimental effects on water quality through deposition of animal wastes and discharge of heavy metals. Point and non-point pollution sources can limit water uses and potentially impact human health downstream. In this study we tested water quality along a stretch of the Santa Cruz River near Nogales, AZ both upstream and downstream from the Nogales International Wastewater Treatment Plant (NIWTP). This stretch of the river is effluent-dependent and animals heavily graze surrounding areas. Our work endeavored to assess water quality according to the Arizona Department of Environmental Quality (ADEQ) standards and identify sources of pollution entering the river system. Dissolved copper and cadmium were analyzed via ICP, and bacterial contributions were assessed using the IDEXX Colilert and Enterolert systems. Sources of fecal contamination were analyzed by microbial source tracking (MST) methods using quantitative PCR (qPCR) targeting the 16S rRNA gene of Bacteroides using the HF183 and Allbac markers for human and total Bacteroides respectively. The CowM2 marker was also utilized in this study to estimate fecal contributions originating from cattle. Results show that of the seven sampling locations evaluated, the site located directly at the wastewater outfall contained the lowest mean E. coli counts (mean = 5 cfu/100ml) while the highest counts came from the Santa Cruz tributary Nogales Wash (mean = 348 cfu/100ml). The Nogales Wash exceeded the ADEQ standards for chlorine, copper, and E.coli on multiple occasions. Recent technology upgarades at the NIWTP proved effective at removal of regulated chemical and biological contaminants. Quantitative PCR results indicate the percent of samples testing positive using the Allbac marker was 100%, while over 80% tested positive for HF183 human marker, and approximately 31% tested positive for the

CowM2 marker. Continuing to evaluate sources of water quality degradation will lead to improved management guidelines for Arizona's riparian areas, ultimately leading to sustainable water supplies that satisfy ADEQ standards.

Health risk of contamination during drinking water mains repair
Traditional coagulants and their potential in treating domestic water supply

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A Quantitative Microbial Risk Assessment (QMRA) model was developed for contamination events after mains repairs. This model involves many steps that were all quantified, including their variability and uncertainty. The frequency of such events was estimated based on the history of E. coli measurements in mains following repair (and flushing). The pathogen concentration in case of such an event was estimated based on pathogen measurements in contamination sources in combination with the estimated ingress of contaminated material. The time of the event was estimated based on the registration of opening of valves after repair. It was assumed that the entire volume in the isolation section was contaminated. A hydraulic model was used to determine how the contamination is transported through the network after the valves are opened. The case study considered was an all pipes network with detailed validated probabilistic demand patterns. The contamination leaves the system only through the customers tap. The consumption of tap water (without heat treatment) was assumed to take place around breakfast, lunch and dinner times, and not just proportional to the total consumption pattern, since this is determined mainly by non-drinking use of the water. Drinking consumption followed a Poisson distribution on number of tap water consumptions and a lognormal distribution of total volume per consumption. The timing of the contamination, the hydraulic transport model and the timing of opening the tap for drinking determined the probability of coincidence of opening the tap at the time that contaminated water in the network passes the house connection. Specific dose response relations were used; the pathogens considered are Campylobacter, Cryptosporidium, Giardia and Enterovirus. The annual probability of infection was calculated using a stochastic model. The sensitivity analysis showed that the contamination frequency and concentration are very important parameters. The time of opening the valves and the time of consumption are important parameters. The event location within the network and the volume of consumption are of smaller importance. Opening only one valve before "releasing" the entire isolation section is an effective measure to contain the possible contamination and reduce the risk considerably by minimising the exposed population.

Assessment of the Microbial Community in the Siem Reap River, Siem Reap, Cambodia

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Worldwide, 1.6 million deaths due to diarrheal disease are linked to a lack of safe drinking water. In Cambodia, approximately 40% of the population does not have access to safe drinking water. The current protocol set forth by the World Health Organization only assesses for the bacterium Escherichia coli and/or Campylobacter jejuni to determine if water is potable (safe to drink) or not. While certain types of E. coli have been shown to cause disease, there are many other organisms, belonging to the

families Enterobacteriaceae and Vibrionaceae, which are known to cause gastrointestinal (GI) disease. In Siem Reap, Cambodia, the Siem Reap River serves as a water source for many people. This river, however, is known to contain microbiological contaminants, which can cause GI disease. Little, however, is known about the total microbial community found is these waters, including those pathogenic bacteria. In January 2014, a group of researchers from Bridgewater State University, Bridgewater, Massachusetts traveled to Siem Reap, Cambodia to expand upon previous research by assessing the total microbial community found in a known water collection area of the Siem Reap River. Briefly, water samples were filtered onto cellulose acetate filters (Millipore). Filters were then fixed in ethanol, frozen and shipped to Bridgewater State University. Here, the filters were aseptically transferred to lysis buffer and total community DNA extracted using the PowerSoil DNA Extraction kit (MoBio). Extracted DNA was used in PCR amplification of the 16S rRNA gene (specific to bacteria). These bacterial PCR amplicons were then cloned into plasmid vectors, the plasmids purified and the 16S rRNA gene insert sequenced. Sequences were compared to sequences in the NCBI database to identify organisms present in the water samples. Of a total of 137 clones sequenced, recovered sequences belonged to the phyla Proteobacteria (43.2%), Firmicutes (36.7%) and Bacteroidetes (20.1%) and were found to have a high similarity to uncultured human fecal bacterium clones indicating the presence of high concentrations of human and/or animal waste. A better understanding of the microorganisms present in these waters could ultimately lead to better treatment of the gastrointestinal diseases.

The Affect of Pause Time on the Efficiency of Light Biosand Filters in Siem Reap, Cambodia

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Worldwide, approximately 780 million people lack access to any improved water source, leading to 1.6 million deaths annually due to microbial associated diarrheal disease. Sadly, 90% of these deaths occur in children under five years of age. In Cambodia, 40% of the population does not have access to safe drinking water, however it has been shown that Point of Use (POU) water filtration systems, including biosand filters are an effective way to make water safe to drink. Biosand filters have been shown to remove approximately 98% - 99% of bacteria, including pathogens that cause diarrheal disease. In Siem Reap, Cambodia, thousands of these biosand filters have been installed in remote Cambodian villages. These filters have been shown to decrease the number of Escherichia coli cells (an indicator species of fecal contamination in water) to a safe level. The filters currently being installed are made of concrete, which makes installation nearly impossible in the floating villages of Cambodia. An alternative to the Concrete Biosand Filter (CBSF) is a lighter, PVC based filter, (light biosand filter: LBSF). In January 2013, a group of researchers from Bridgewater State University in Bridgewater, Massachusetts traveled to Siem Reap, Cambodia to begin testing the efficiency of these new LBSFs. Water samples were collected from the Siem Reap River, a known microbiologically contaminated water source, and treated by HBSFs or by LBSFs. Overall, the LBSFs did not perform as well as the HBSFs, however they did decrease the amount of biological contaminants, indicating the potential for these filters to be installed in remote and floating villages, providing safe drinking water for families living there. This past January, we again traveled back to Siem Reap, Cambodia. Specifically, this year we assessed how pause time, or the time the water remains in the filter prior to being collected, affects the efficiency of these LBSFs. Again, water samples were collected from the microbiologically contaminated Siem Reap River. Water was left untreated (control), or treated by LBSFs. Water was allowed 1, 2, 4 and 6 hour pause times. Treated water was then filtered onto cellulose acetate filters (Millipore) and the filters transferred to the coliform-selective, E. coli differential media, RAPID' E. coli 2 (BioRad). Plates were run in triplicate, incubated at 44.5_C for

22 - 25 hours, colonies counted and colony forming units (CFUs) per 100 milliliters (mls) of water calculated from each of the three treatments. Data was analyzed using the Independent t-test to assess for statistical differences between the pause times. As predicted we did see an increase in efficiency of pathogen removal for the longer pause times, however, the most efficient pathogen removal (fewest number of CFUs per 100 mls of water) was seen with the 1-hour pause time. A plausible explanation for this result is we did not achieve 100% displacement with the initial pour. As such, the water collected for the 1-hour pause time was a mix of water that had been in the filter overnight and water, which had sat in the filter for 1 hour. Additional tests will be conducted to assess the volume of water needed to achieve 100% displacement for these filters in order to ensure maximum efficiency of these POU filters.

A community based approach to eliminate water-borne diseases in Lower Nyakach, Kenya

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BACKGROUND Lower Nyakach, a region near Lake Victoria in western Kenya, is divided into 12 locations, with a population of 70,000.The absence of improved water sources requires the use of highly contaminated ponds, rivers, streams and shallow wells for drinking water, leading to a chronically high incidence of water-borne diseases. Friends of the Old (FOTO), a community-based organization working in Lower Nyakach, has made the elimination of water-borne diseases a top priority. FOTO's approach is to: 1) use practical field methods involving FOTO staff and community members to evaluate the bacterial quality of drinking water; 2) educate communities about the relationship between fecal contamination of water and disease; 3) introduce practical household water treatment and storage (HWTS) methods. METHODS Water quality testing was performed using two commercially-available tests specific for Escherichia coli: the Colilert_ 10 ml presence/absence test (IDEXX, Westbrook, ME) and the E. coli/Coliform Count PetrifilmTM (3M, St. Paul, MN), a quantitative test for 1 ml. FOTO staff members are the links to their communities. They were trained in basic microbiology, how to perform the two microbiology tests and correlate the results with WHO disease risk categories: low, moderate, high, or very high. Two HWTS methods were used. The main method used commercially available 1.2% solutions of sodium hypochlorite that come in a 150 ml bottle. One bottle will treat 850-1,000 liters of water, sufficient to last most families at least two months. The second method is the use of a simple Cookit solar cooker that uses sunshine to pasteurize water at 65_C. RESULTS Colilert and Petrifilm tests performed by FOTO staff repeatedly demonstrated that community water sources were highly contaminated and posed a high or very high disease risk. The visual nature of E. coli positive Colilert and Petrifilm tests before chlorine or heat treatment, and the absence of E. coli after treatment provided striking evidence-based microbiology to community members. This information was essential for dispelling commonly held myths about water, such as clear water is always safe to drink. In February, 2012, FOTO started distributing chlorine solutions to 9,600 families over two months. This quantity was increased to 14,400 in August, 2013, to reach all families in Lower Nyakach. The cost of purchasing and distributing each chlorine bottle is approximately 25 cents. Lower Nyakach clinics reported a 41% decrease in diarrhea cases (441 to 260) from January, 2012, before chlorine distribution, to January, 2013. In 2013, 50 families per month received a Cookit set and ceramic safe storage container, enabling solar water pasteurization of ~10 liters on sunny days. Diarrhea data from January, 2014, will be included. CONCLUSIONS The strategy of involving communities in evidence-based microbiology testing of water sources and providing inexpensive treatment options to impoverished families has reduced the burden of water-borne diseases in Lower Nyakach, with the goal of eliminating water-borne diseases.

This strategy could be replicated throughout Kenya and in other developing countries to extend the human right to safe water.

Traditional coagulants and their potential in treating domestic water supply

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Abstract Rain water is believed to be pure by majority of the rural populations, but such harvested water is usually contaminated and polluted by bird droppings, dust, algae, bacteria grow and other pollutants. MWODA through sharing ideas with the community developed an idea of using coagulation seeds including moringa and mucana seeds to purify small domestic water supply. Analysis On average a household purifies one to three 20 to 60 litres containers a day of drinking and cooking water, depending on the size of the family. The rapid and slow mixing followed by settling sedimentation of treated water done by the villagers is analogous to conventional surface water treatment process that are commonly practiced by many country and during the performance jar tests to determine optimum dosage of chemical coagulations. Method MWODA teaches women to use coagulation flocculation process to remove suspended and dissolved matter immediately. Seeds are grounded to fine powder and kept in plastic bags in dry places 1 to 2 months for daily use. The 20 litre bucket of drinking water is dosed with 2 to 5 teaspoons (about 20 to 50 grams) of seed powder, while 500 grams to 1 Kilogram of wood ashes is used to purify the same quantity of water for bathing and washing purposes. The water is subjected to rapd mixing speed of about 100-150 revolution per minutes and allowed to settle itself for about 5 to 10 minutes after which the formed focus are allowed to settle for 20 to 25 minutes. The purified water is then stored for drinking and cooking purposes. Expected results Clean and safe water for drinking Clean water for cooking. Clean water for washing clothes Reduction in opportunities of water related diseases Increased access to safe domestic water supply. Conclusions Local water treatment knowledge has evolved and propagated among rural populations due to inadequate water supply sources. The installed potable water supply sources are insufficient and also unsustainable due to poor governance, poor operation and maintenance of water scheme and insufficient investments by the government in rural water supply. Way forward I call upon the donors, alliances and networks to support this project to promote this idea which is appropriate for the rural communities. The areas of support include i) Conduct scientific research on the use of coagulated seeds in purifying drinking water. ii) Promote rain water harvesting among the communities. iii) Train them in operation and maintenance of water facilities.

Can we predict the behavior of fecal indicator and host-specific qPCR markers using environmental parameters and predictive modeling in headwater streams?

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Inadequately managed agricultural watersheds may be one of the most important contributors to high levels of microbial contaminants in surface waters. Little is known about the effect of environmental determinants on the fate and transport of molecular markers of fecal contamination originating from agricultural non point sources. We investigated two cattle farms with differing management schemes to compare how physicochemical and meteorological parameters influence fecal qPCR marker loadings and transport dynamics in headwater streams. Farm A employs a high-intensity cattle rotation with intermittent direct stream contact. Farm B allows unrestricted cattle access to the stream. Rain event

and bimonthly base flow samples were collected along the stream for each farm. Samples were analyzed for culturable E. coli and enterococci, enterococci qPCR marker (entero), cow and ruminant specific markers (CowM3 and Bobac, respectively), UV, total suspended solids (TSS) and turbidity. During baseflow, the stream in Farm B exhibited a significantly higher ($P<0.0001$) daily loading rate than the stream in Farm A for culturable enterococci (19.1 and 6.9 x 10e9 enterococci/day, respectively) and CowM3 (167 and 3.95 x 10e9 gene copies (GC)/day, respectively). No significant difference ($p>0.05$) was identified in the loading rates during rain events. Using a predictive modeling approach, (Virtual Beach Software tool) we were able to model molecular and culturable fecal indicator bacteria (FIB) concentrations. The models indicated that discharge and turbidity were the most important parameters with adjusted r2 of 0.714 (Farm A) and 0.426 (FarmB) for E. coli; and 0.767 (Farm A) and 0.527 (FarmB) for enterococci. Temperature, turbidity, total suspended solids and rain intensity showed significant ($p<0.0001$) relationships with culturable enterococci, CowM3, and entero marker concentrations. In farm A, CowM3 showed a strong positive correlation with the entero marker($r2= 0.68$) and E. coli($r2= 0.71$), but not with culturable enterococci ($r2= 0.26$). The results of this study elucidate and compare environmental parameters that affect the fate and transport of culturable FIB, cow-specific, and entero qPCR markers in headwater streams and help validate the use of predictive modeling tools as an alternative methodology to assess water quality conditions in inland waters.

A comparison of culture-based and qPCR methods for monitoring indicator bacteria at freshwater swimming sites in the Los Angeles River Watershed, California

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Popular freshwater swimming sites in the Los Angeles River Watershed were sampled during summer 2012 to compare the relative extent of bacterial contamination as measured by conventional culture-based methods and qPCR methods. Samples were analyzed for Escherichia coli, enterococci, and Clostridium perfringens (vegetative cells and spores) to characterize how well indicators correlated with each other, with respect to ambient levels and to "elevations" from background, possibly indicative of a pollution input. A secondary objective was to evaluate the economic impact of implementing qPCR in the long-term ambient monitoring program. Results showed that indicator species did not correlate well with each other ($R2 < 0.1$) both spatially and temporally, though C. perfringens vegetative cells and spores were moderately correlated ($R2 = 0.31$, $p = 0.07$). Elevated concentrations were most common on holidays and weekends, although these were not strongly correlated to the number of bathers. Clostridium perfringens may be a useful indicator at our study sites, as a comparison of vegetative to endospore forms of this organism may be used to understand how recently a contamination event or input occurred. Results from the economic analysis demonstrate that qPCR should be allocated to swimming sites where public health costs exceed the public's willingness to pay to use that site and to identify the source of contamination. This is the first study evaluating the utility and economic viability of employing qPCR in freshwater systems in an urban watershed of the western United States.

Circulation of bla-genes in Escherichia coli isolates from hospital sewage in Nicaragua

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Sewage from hospital is an important route for dissemination of antibiotic resistant bacteria among humans, animals and the environment. Escherichia coli, part of the normal flora of human's gastrointestinal tract, is one of the main representatives of bacterial communities found in hospital effluents, and they could be a source of spreading and transmission of genes encoding betalactamases to other bacteria present in sewage and the environment. We studied the antibiotic resistance patterns among 96 E. coli isolates, from three sewage effluents, collected through October 2008 to May 2009 in León, Nicaragua. High levels of antibiotic resistance were found in E. coli isolates in hospital sewage water. Among these isolates, ampicillin, chloramphenicol, ciprofloxacin, nalidixic acid, trimethoprim-sulfamethoxazole was the most common multi-resistance profile. ESBL-producing E. coli were identified in 33% of the isolates. Detection of bla-genes, in ESBL-producing E. coli, showed gene encoding for blaSHV-11/12 enzyme (53%), blaTEM-1 enzyme (14%), blaCTX-M-9 (65%), blaCTX-M-15 (34%). Even though we did not perform a longitudinal study of environmental water samples, our results suggest that multiresistant ESBL-producing E. coli were widely spread in hospital sewage water.

Solar Energy Secures Safe Drinking water for Schools in Southern Uganda

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INTRODUCTION AND OBJECTIVES Poor water conditions are one of the biggest challenges in Uganda, like any other developing country with nearby 2 million children dying from water related diseases every year. Kawuku Women's Development Association, a Community Based Organization promotes water harvesting and solar water purification to provide safe drinking water to schools in Uganda. The project has reduced on water related diseases mainly typhoid and diarrhea among the school children due to drinking dirty and contaminated water. If we are to work together to save millions of children from dying of water related illnesses, a regular water supply and power supply must be secured. METHODOLOGY APPROACH The solar power serves as the water purification system for the project. Usually a regular water and power supply must be secured. In this case Kawuku Women's Group together with school promotes Water harvesting by collecting water from the school building roof to the storage tank and installing a solar panel, battery pack to the supply, and the pressure pump. This way the school is completely self supporting in creating its own safe drinking water supply for the school population and replication by the neighboring schools. Analysis The process is a plug and play installation that includes a storage tank, solar panels, battery packs, pressure pump, pre-filters and of course the water purification system. Water is pumped from the water harvesting storage tank to the water purifier through which it is purified by use of the solar power to the stainless tank and ready to drink by school children. The process is cost effective and efficient for safe drinking water because solar power is a free resource and harvested water is a free gift from God. Using solar power reduces the depletion of the water resources where hydro power is generated For example in Uganda, the once filled river Nile where hydro power is generated is reducing its water level which is due to the enormous climate change and using solar power is a climate change mitigation strategy. Results The project creates a sustainable link between water and energy because with water harvesting and solar water purification. No threat for continued climate change. There is increased access to safe drinking water in

a total of 47 schools. There is reduction in illnesses related to water diseases due to presence of solar water purifiers in schools. Reduced costs on expensive firewood and electricity bills. Conclusion Given the many connections between energy and water, the choices we make in the near future about how we produce and use energy will determine not only the extent to which we mitigate the worst impacts of climate change, but also how resilient our energy system is to the variability of our water resources and the many competing demands for it. I call upon World Water Week participants to come up and support such an initiative to save the ever increasing death among the children in schools and the entire community.

Use of Bacteriophages to Evaluate Ambient Water Quality

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In November 2012, EPA released final recreational water quality criteria (RWQC) recommendations. EPA's process for developing the 2012 RWQC included consideration of a series of foundational elements that include the illness rate, health endpoint, waterbody type, target population, source of contamination, indicator method, and expression of criteria. Specifically, the 2012 RWQC recommend culturable bacterial indicators of fecal contamination (enterococci and E. coli). Notably, the recommended geometric mean of enterococci or E. coli water quality levels are linked to identified levels of public health protection (32 and 36 gastrointestinal illnesses per 1,000 recreation events). In addition, the 2012 RWQC provided supplemental elements for enhanced protection of recreational waters, namely, a rapid analytical method, quantitative polymerase chain reaction (qPCR) which can be adopted on a site-specific basis. Microbial monitoring, quantitative microbial risk assessment (QMRA), and epidemiological studies over the past thirty years indicate that viruses are the microorganism group predominately driving the illnesses associated with primary contact in recreational waters impacted by human sources. This is important because traditional culturable fecal indicator bacteria do not persist in the environment or through disinfection treatments as long as viruses; do not show consistent correlations with viruses; and do not contribute to the majority of illnesses associated with primary contact recreation. Moreover, wastewater treatment has advanced over the last few decades, and currently more wastewater treatment facilities are employing secondary treatment and disinfection processes. These disinfection processes are more effective at reducing bacterial microorganisms than viruses or protozoa. Bacteriophages, a viral indicator, have great potential to be useful for the evaluation ambient water quality and wastewater treatment efficacy. Bacteriophages are of fecal origin; are present in high numbers in sewage; are physically similar to enteric viruses of concern; are similarly persistent to enteric viruses of concern; are significantly correlated to pathogens; are non-pathogenic; have EPA approved Part 136 methods with rapid methods under evaluation; can be enumerated easily, cheaply, and quickly; and are more resistant to sewage treatment than bacterial indicators. Moreover, the U.S. Food and Drug Administration (FDA) is also considering the potential use of bacteriophage as a viral indicator of wastewater treatment for the protection of shellfish waters. In this presentation, we review available scientific information to consider whether and how bacteriophages could be used to provide improvements in water quality and public health protection for recreational waters and other CWA applications.

The Perfect Microbial Source Tracking Experiment: A sewage spill in an estuarine creek during Superstorm Sandy

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During Hurricane Sandy, a force main break occurred in a 30-inch concrete reinforced pipe near Shingle Creek in Suffolk, VA. In order to prevent washout of the road and reduce pressure on the damaged area of the pipe, flow from the adjacent sanitary sewer pump station was diverted into Shingle Creek. Shingle Creek flows into the Nansemond River, a high priority area for recreation and shellfish harvesting. The spill started on October 30, 2012 and was finally terminated on November 8, 2012. The total estimated discharge for the duration of the spill was 18 million gallons. It was immediately recognized that the sewage spill offered a unique opportunity to study fate and transport of fecal indicator bacteria, and molecular markers of human fecal contamination and pathogenic viruses for microbial source tracking (MST), over time and space. Water quality monitoring was conducted from onset of spill through the middle of December 2012, and included grab samples for laboratory analysis of E.coli and Enterococcus plus field measurements for dissolved oxygen (DO), temperature, salinity, and pH. Additional water samples were collected and filtered onto polycarbonate and HA filters for qPCR based quantification of Fecal Bacteroides spp., BacHum, HumM2, and HF183, and human pathogenic viruses such as adenovirus. During the period of active discharge, the FIB and MST based molecular markers were remarkably strongly correlated (r=0.99), even though sampling was conducted at multiple locations and during multiple tidal stages. Even over the entire study period, the FIB and human fecal contamination markers remained strongly correlated (r=0.80-0.93) to the FIB concentrations. Even during the active discharge, heightened levels of FIB were not observed more than 10 km downstream in the Nansemond River. Furthermore, once discharge was terminated, FIB concentrations in Shingle Creek dropped rapidly, and returned to safe levels according to existing criteria in the Nansemond River within 7 days. No markers of human contamination were found in the Nansemond River after the discharge was terminated. This sewage spill offered an important opportunity to characterize relationships between FIB and MST-based quantification of markers of human fecal contamination. Finally, our findings show that future MST-based studies incorporating fate and transport and degradation concepts will be vital to appropriate application of quantitative MST for stormwater and extreme storm event mitigation strategies.

Validation of Bacteroidales quantitative PCR assays for microbial source tracking in India to improve understanding of fecal sources and microbial exposure risks.

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Microbial source tracking (MST) based on host-associated Bacteroidales genetic markers is an emerging approach to distinguish and measure human and non-human animal contributions to fecal contamination. Bacteroidales quantitative PCR (qPCR) assays developed for application in North America and Europe are increasingly being used across the developed world, including in countries in Asia and Oceania to complement standard fecal indicator bacteria methods in order to identify environmental sources of fecal contamination and assess exposure risks. Recent studies have also shown the relevance

of MST using Bacteroidales genetic markers to identify fecal sources and assist in targeting public health interventions in less developed countries. To our knowledge, however, no MST Bacteroidales assays have been applied to address fecal pollution problems and assess exposure risks in India where both human open defecation and livestock animal fecal loading are widespread and microbial contamination and associated disease burdens are high. Because geographical differences significantly affect sensitivity and specificity of host-associated Bacteroidales assays, performance assessment is necessary prior to application of these assays in a new region of interest such as India. As part of a larger study in Odisha, India to apply advanced molecular microbial detection methods to evaluate sources of microbial contamination and pathways of fecal-oral pathogen transmission in rural communities, we undertook research to (1) evaluate the performance of existing Bacteriodales qPCR assays for application in India, in terms of sensitivity and specificity to distinguish human and major animal fecal contamination and (2) identify a combination of up to five of the best performing host-associated MST assays, based on testing results, for large-scale application in the evaluation of the impacts of improved sanitation in Odisha, India. We selected ten candidate Bacteroidales qPCR assays based on the literature, and tested them in India against fresh fecal samples collected in coastal Odisha, India from individual humans (n=30), sewerage systems (n=5), and individual animals (cattle, buffalo, goat, sheep, dog and chicken) (n=346) pooled to create composite samples (n=60) of fecal materials from 5 (cattle, buffalo, goat, sheep, dog) or 10 (chicken) individuals animals. The two universal/general Bacteroidales assays tested (BacUni, GenBac3) both achieved 100% sensitivity on the test set of 95 samples. Of the five human-associated assays tested (HF183 Taqman, BacHum, HumM2, BacH, HF183 SYBR), BacHum had the highest combined sensitivity (49%) and specificity (78%) and did not cross-react with any fecal samples from cattle, the most populous livestock animal in communities across India. Of the ruminant- and cattle-associated assays tested (BacCow, CowM2), BacCow was more sensitive than CowM2 in detecting the full range of common Indian livestock animal fecal sources, and neither cross-reacted with human sources. BacCan, the dog-associated assay tested, showed no cross-reactivity with human sources, and high sensitivity (90%) for dog fecal samples. Joint sensitivity and specificity for pairs of tested human-associated assays indicates application of BacHum and HumM2 together would increase detection of individual human fecal contamination by 17% over BacHum alone, demonstrating value in using HumM2 and BacHum in combination, on environmental samples where higher individual human sensitivity may be required, such as those collected within the home environment. Overall, our results indicate BacUni, BacHum and BacCow would be the most suitable set of currently available MST Bacteroidales qPCR assays to distinguish and quantify relative amounts of human-associated and non-human animal-associated contributions to environmental fecal contamination in India, until new human-associated qPCR assays specific for the Indian context can be developed.

UV Influence on the Re-growth of Pathogens in Cow Fecal Extract

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A significant amount of work has been done to elucidate the environmental factors that determine the survival of pathogenic microorganisms. Most of these studies have focused on the die-off of pathogens in water microcosms, irrigation water, manure amended soils/compost manure and intact cattle feces. It is interesting to note that a significant fraction of these die-off studies report an initial growth phase of the microbe in question before the start of a decline phase. However, few to no studies have investigated the factors influencing this re-growth phase of pathogens in these matrices. Preliminary studies in our laboratory have shown that several factors including UV, nutrients, growth phase,

temperature and pH play vital roles in the re-growth of Escherichia coli strain C3000 in cow fecal extracts. Of these factors, UV irradiation appears to be one of the most significant. Contrary to the notion that ambient UV is mainly responsible for the killing of pathogenic microorganisms, we observed that under certain conditions, UV can aid in re-growth. We have found that under oligotrophic conditions, UV may aid in the conversion of non-labile nutrients into labile ones that are readily available for uptake by microorganisms. E. coli populations inoculated into previously irradiated and non-irradiated manure extracts had growth rates of 0.48 and 0.29 generations/hour, respectively. However, E. coli populations inoculated post UV irradiation into high to medium concentrations of manure extracts experienced a lag phase before a sudden growth up to 4 logs. Conversely, E. coli populations in non-irradiated manure extract did not show a lag phase, but had a similar growth rate. We also observed that re-growth was dependent on UV dose and manure extract concentration. Our study indicates that under certain conditions, re-growth of fecal indicators can occur either after manure application or in a receiving body of water after a run-off producing rain event. In addition, our results will have implications for models predicting die-off rates of bacteria in the environment. Further research is under way to test these hypotheses with pathogens of public health importance such as the Shiga-like toxin producing E. coli and Salmonella Typhimurium.

Environmental aspects of the bioaccumulation of viral and bacterial indicators in mussels (Mytilus edulis)

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Introduction: Shellfish are filter-feeding aquatic animals that can bioaccumulate pathogens from contaminated water, thereby posing a potential risk of infection to human consumers. Therefore, the prevention of these infections is of significant public health importance. An environmental study was carried out to investigate the seasonality of bioaccumulation of bacteriophages and fecal bacterial indicators in mussels (Mytilus edulis) and their overlying waters at a site in Southeast England, United Kingdom. Methods: Freshly collected mussels and samples of overlying water were analyzed using standard microbiological methods of a period of twelve months. The concentration of fecal coliforms, enterococci and E.coli were determined by membrane filtration and most probable number methods (MPN), while the concentration of somatic coliphages (WG5), F RNA phages (WG49) and human-specific Bacteroides fragilis phages (GB124) were determined by direct plaque assay using a standardized double agar method. The accumulation factor of each parameter was obtained by dividing the log concentration of each organism in mussels (PFU or MPN/100g) by the corresponding log concentration of organism in the overlying water (PFU or CFU/100ml). Results: E.coli, fecal coliforms, enterococci and somatic coliphages showed high accumulation (2.31 - 6.04) in sampled mussels and the overlying water. The accumulation factor of human specific Bacteroides fragilis phages ranged from 1.24 - 2.12; somatic coliphages 1.29 - 2.31; F RNA phages 1.08 - 1.74; E.coli 0.79 -2.39; fecal coliforms 0.77 - 2.32; and enterococci 0.88 - 6.04. However, the mean of these values showed no significant differences ($P < 0.05$) during the period of study, except during July and August. In general, the average accumulation factors of all target indicators correlated positively with water temperature. These results are in agreement with other studies that have shown seasonal variations in uptake, which suggest that ambient temperature may be a useful predictor of shellfish hygiene in temperate climates. Conclusion: The work has highlighted the seasonality of fecal indicator bioaccumulation in mussels in temperate climates. This work will form the basis of a more detailed study over the next two years, during which time the seasonality of levels of pathogenic viruses in shellfish will also be investigated. The study will provide

comprehensive data on the temporal dynamics of fecal indicators, viral pathogens and a human-specific bacteriophage marker in shellfisheries and their relationship with environmental parameters. This new information should provide simple but effective monitoring tools and protocols for improved public health protection. Keywords: exposure, monitoring, human health, microbial source tracking, shellfish, surrogates, viruses

Perception and reality: an integrated and systematic study of microbiological risk to groundwater in Makoko slum, Lagos Lagoon, Nigeria.

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This study investigated the health risks from groundwater sources to residents of the Makoko slum area neighbouring Lagos Lagoon in Nigeria. Results from bacteriological analyses of water samples taken from boreholes and wells in Makoko were compared with sanitary risk assessments for the water sources, and records of water borne infections within the local community. Escherichia coli were used as faecal indicator organisms, and sanitary inspections were made using forms based on those developed by the WHO for boreholes and hand-dug wells. Records of cases of typhoid fever reported to hospitals, clinics and medical laboratories provided health data. The sanitary inspections indicated that the water sources had high levels of sanitary risk, and this was consistent with Escherichia coli counts for water samples that were well in excess of the WHO guideline of 0 CFU/100 ml. Despite the evidence of significant faecal pollution of the aquifers, indicating the probable presence of pathogens, health records indicated a surprisingly low incidence of reported typhoid fever. The paper suggests what coping strategies the slum dwellers have developed to minimize the risk of infection from groundwater, and proposes approaches that could be promoted within the community by individuals and government agencies to improve access to safer water sources.

Quantitative Assessment of Bacterial Pathogens at Great Lakes Beaches

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Great Lakes beaches are often closed due to poor water quality resulting from fecal bacterial contamination. Determining the abundance, sources, and persistence of pathogenic microorganisms may improve our understanding of beach recreational risk to human health. Quantitative PCR (qPCR) assays for Shigella (ipaH gene), Campylobacter jejuni (mapA gene), E. coli (stx2 and an eae sequence specific for E.coli O157:H7), and a general Salmonella enterica sequence were optimized and used to estimate the abundance of these pathogen genes at Great Lakes beaches. Approximately 300 samples from three environmental matrices including water, sediment, and Cladophora glomerata (a nuisance macro-alga common to the Great Lakes) were compared at seven beaches sampled from June through September 2012. Beaches were located throughout the Great Lakes including lakes Michigan, Huron, Erie, and Superior. The detection of each pathogen gene was site-specific and Cladophora and sediment were identified as potential reservoirs for microorganisms carrying the genes studied. The abundance of ipaH, mapA, stx2, and eae from Cladophora and sediment samples with quantifiable detections were two to three orders of magnitude greater than in the equivalent volume of water. Fecal indicator bacteria (FIB) concentrations were correlated with average Campylobacter jejuni (mapA) abundance in

water among the seven beaches. The quantity of stx2 genes was also related to E. coli abundance at one beach, but none of the other genes were correlated with FIB concentrations. A quantitative microbial risk assessment (QMRA) tool developed by Michigan State University's Center for Advancing Microbial Risk Assessment was used to evaluate the significance of the gene abundances in water. The tool indicated a moderate risk for illness from Campylobacter jejuni at most beaches, even when only 10% of the gene copies were assumed to be from viable infective cells and when recreational water quality criteria were met. This study is thought to be the first comprehensive quantitative geographic assessment of bacterial pathogens in a variety of matrices at Great Lakes beaches and will be important for advancement of QMRA.

Sewage Impacts on Water Quality and Antibiotic Resistance in Beach Waters in the Galápagos Islands, Ecuador

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Tourism and residential population growth are increasing on the Galápagos Islands, yet the effects on environmental quality are not well understood. This study provides a baseline characterization of water quality on one of the inhabited islands of the Galápagos (San Cristóbal), and evaluates the potential relationship between human activity and bacterial antibiotic resistance in recreational waters on this island. Five sample beaches and the mouths of two sewage effluent pipes were selected to represent recreational water with and without the presence of sewage effluent. Enterococci concentrations were quantified using the IDEXX Enterolert kit. E. coli was isolated from samples and tested for susceptibility to five antibiotics using Kirby- Bauer disk diffusion. These measurements were compared ON beaches with sewage effluent and beaches without sewage effluent. Significantly higher enterococci concentrations were found near sites subjected to sewage discharge ($p < 0.01$). Sites of direct sewage discharge and land drainage from the highlands had significantly higher levels of antibiotic resistant bacteria though it is unclear whether sewage impacts the level of resistance at nearby beaches ($p < 0.05$). The high levels of enterococci and antibiotic resistance observed in this study indicate that the release of raw sewage into recreational waters could be putting the environment as well as public health at risk. This study provides insight into how humans impact.

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Microbial pathogen-sediment interactions in the Conwy Estuary, North Wales, UK

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The contamination of estuarine environments with pathogenic bacteria and viruses derived from both point and diffuse sources of microbial pollution can impact upon bathing water quality and shellfish hygiene, posing a risk to human health. Estuarine sediments represent a significant reservoir for pathogenic bacteria, as sediment attachment enhances the survival and persistence of pathogens. However, there is a paucity of information on the potential for pathogen resuspension and transport to

other estuarine and coastal zones where they may impact upon the quality of beaches, bathing and shellfish waters. This study investigated the relationship between the composition of estuarine sediments (e.g. particle grain size and organic content) with bacterial pathogen abundance in the Conwy estuary, a biologically productive estuary in North Wales, UK. The abundance of culturable E. coli, total coliforms, enterococci, Salmonella, Campylobacter and Vibrio spp. (used as a proxy for pathogen abundance and described as pathogen indicator bacteria (PIB)) were determined across 4 transverse transects of the Conwy estuary. The abundance of all cultured bacterial groups showed a significant positive correlation with sediments comprising high organic matter content and greater proportions of silt and clay, highlighting "hotspots" of PIB contamination within the estuary. Enumeration of cultureable E. coli, total coliforms and Vibrio spp. form the overlying water column revealed greater densities of PIB in the sediment, with a 281, 433 and 58-fold difference in abundance (colony forming units (CFU/100 g) vs. (CFU/100 ml)), respectively. To determine if PIB are a suitable surrogate for the behaviour of actual pathogenic bacteria in the Conwy estuary, both end-point and quantitiative PCR techniques are being applied to detect and enumerate the presence of specific bacterial pathogen groups and their virulence factors in order to characterise their interactions with estuarine sediments. Laboratory-based microcosm experiments will address the attachment, persistence and potential resuspension of sediment associated pathogens from the bed sediment. The identification of pathogenic bacterial hotspots within estuarine environments is important in safeguarding human health, as these areas provide a wealth of ecosystem services such as food, tourism and recreation. The findings from this study suggest that the geomorphology and composition of estuarine sediments can be used as a predictor to model potential hotspots of pathogen contamination, in addition to providing data that will enhance the modelling and prediction of public health risk in relation to sediment transport and resuspension under storm flow and other hydrodynamic conditions.

QMRA for water safety management: two Nordic norovirus case-studies from the EU-VISK project

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Water Safety Planning (WSP) aims to systematically assess the risks associated with a water supply from catchment to tap in order to support risk management and the provision of safe drinking water. Quantitative Microbial Risk Assessment (QMRA) is a potentially powerful tool to support WSP. This was applied as an objective in the EU-VISK project (www.visk.nu) to provide recommendations for the catchment and treatment related mitigation of the risk of norovirus infection (via drinking water) in the Nordic region. Sampling for norovirus were therefore performed during 2011/2012 and the data assessed and interpreted using QMRA for two case systems (1) the Nedre Romerick Vannverk (NRV) with the Glomma River (Norway) as source and (2) the _verby Water treatment plant sourced from the river G_ta _lv (Sweden). Source water: the baseline and event driven norovirus concentration was evaluated based on (a) catchment surveys of likely norovirus sources; (b) qPCR virus enumeration (Glomma: n=41, 20L weekly samples analysed in triplicate with paired mengovirus spiked recovery results and G_ta _lv: n=15 fortnightly samples); (c) Source based modelling relying on historical monitoring of faecal indicators (Glomma: E. coli n=505, C. perfringens n=271. G_ta _lv: E. coli n=212) and (d) Hydrodynamic modelling of norovirus concentrations in the river under baseline and specific upstream loading events (pumping station overflow, sewage bypass events, onsite sewer overflows). Treatment performance: (a) For NRV (conventional treatment/GAC/UV and free chlorine disinfection) treatment performance was evaluated at pilot scale for baseline and three event conditions (hydraulic

step, poor coagulation, inappropriate pH) from spiking trials with MS2 bacteriophage. The results were translated to full-scale using a process flow model constructed in AnalyticaTM. For _verby (conventional treatment/slow sand filtration /free chlorine disinfection) treatment performance was evaluated by statistical models fitted to historical monitoring data of E.coli from the intake (n=212) and after each of the four rapid sand filters (n=44 each filter line). (b) At both plants, free chlorine inactivation was quantified using a novel modelling approach relying on (i) a 'tanks-in-series' model to describe hydraulic residence time, (ii) historical measurements (_verby) and regulatory requirements(NRV) for chlorine concentration, and (iii) literature based chlorine sensitivity survival functions for murine norovirus and feline calicivirus. Keeping all other components constant, the influence of source water concentration and the two modelled treatment barriers on consumer risk was investigated. For the Glomma River case, incorporation of recovery into the prediction of the norovirus concentration led to more than an order of magnitude increase at the mean level(mean mengovirus recovery = 2.97%), and up to 4 orders of magnitude increase at the upper 95th percentile. Modelling of virus loading relying on measured faecal indicator concentrations was shown to be consistent with enumeration data for surface waters (Glomma River) and for sewage (G_ta _lv). Hydrodynamic modelling of the G_ta _lv showed that the regular discharge of treated sewage to the river was an important source of viruses, and of a similar magnitude to wastewater treatment by-pass events, challenging the assumption that water was of high quality under baseline conditions. Under expected, average operation both systems were able to provide safe drinking water. Accounting for fluctuations led to much higher risk estimates. Hydraulic steps during the filtration cycle (as occurs routinely when a filter goes to backwash) may influence the consumer risk by more than 10 times until the filter restabilises. Failure of coagulant dosing was the most important factor influencing conventional treatment performance, and in the case of NRV, increased the consumer risk 100 to 200 fold. For both systems, meeting safe drinking water targets was strongly dependent upon effective free chlorine inactivation. For NRV, when hydraulics were assumed to be poor (2CSTRs), the annual risk target (1 _ 10-6 DALY) was exceeded more than 95% of the time. An improvement in the hydraulic performance of the contactor (6 CSTRs) reduced this predicted exceedence to 5%. Characterisation of the hydraulic residence time of the disinfection contactor is therefore important for evaluating the robustness of the disinfection step at any water treatment plant. This study demonstrated the value of QMRA as a risk management tool within the WSP context of catchment and water treatment performances for interpreting the health significance and system management implications of new scientific data.

The Development of Novel Microbial Indicators Used for Environmental Tracking of Biosolid Contaminants

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Background: Biosolids are generated from the treatment of human waste. Upon proper treatment they can be land applied on fields, adhering to regulations and guidelines established by the U.S. Environmental Protection Agency (USEPA). This is the primary means of managing and disposing biosolids. However, this practice generates concern due to the lack of evidence about the fate and transport of associated contaminants and its potential impact on the environment and human health. To investigate this impact it is important to identify indicators that can be used to trace a contaminant to its original source, distinguishing biosolid runoff from other sources of pollution. Hence, it was the goal of this research to examine the diversity of the microbial community present in biosolid samples and to develop novel indicators unique and dominant in biosolids that can be markers for tracking pollutants in

the environment. Methods: Influent and biosolid samples were collected from two wastewater treatment plants that used two different treatment processes to produce its biosolids (mesophilic anaerobic digestion versus thermophilic anaerobic digestion). Total community DNA was extracted and high-throughput 454 pyrosequencing was performed to examine the microbial communities present in all samples. This approach allowed deep sequencing of the samples, including microorganisms that cannot be cultured and are not usually monitored. Results: The pyrosequencing analysis identified several candidates within the Bacteria and Archaea kingdom that could potentially serve as microbial indicators, specific for biosolid materials found in the environment. Conclusion: This research provides tools essential for understanding the potential impact land application of biosolids has on the environment and human health. Traditional microbial indicators (e.g. fecal coliforms, total coliforms, Salmonella) are not source specific and the development of novel biosolid specific indicators could contribute to science and provide a useful tool for the USEPA and wastewater treatment plants to better regulate and monitor the land application of biosolids. Keywords: pyrosequencing, biosolids, pathogens, fate and transport, microbial indicators

Microbiological quality of source, tap and bottled water samples collected from Suzhou, China

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The microbial quality and safety of drinking water is an important public health issue. Microbial contamination in drinking water have been linked to transmission of various waterborne illnesses and diseases. The urban water systems in China face various environmental and health issues. The specific aims of this research were i) to study the microbial quality of source, tap and bottled water samples in Suzhou region (China) by culture independent molecular methods and ii) to detect and quantify the hygienic relevant bacteria by conventional and quantitative PCR methods. The source water samples were collected from source water protection area within Taihu Lake as this water is used as drinking water source for Suzhou and nearby cities. The tap water samples were collected from different parts of Suzhou which covers both relatively new and very old drinking water distribution networks. In addition, bottled water samples were also collected to assess the microbial quality. Five brands of small (1-3 liter bottles/cans; Brands A-E) and large containers (20 liters; Brands 1-5) were collected on two different time points for microbiological assessment. All the water samples were subjected to physico-chemical analysis and the parameters were selected based on World Health Organization (WHO) guidelines. The total cell counts were determined by DAPI staining and microscopy. Microbial community was analyzed by PCR amplification of 16S rRNA gene and molecular cloning followed by phylogenetic identifications. The specific detection of hygienic relevant bacteria was determined by conventional and qPCR methods. The results showed that the source water samples were dominated by Bacteroidetes (45%) followed by Actinobacteria, β, and α-proteobacteria. The tap water samples collected from area which has relatively new water distribution network (e.g. Suzhou Industrial Park, SIP) contained significantly low total cell count as compared to the tap water from the area which has very old distribution network (e.g. Old town, OT). The OT samples had high bacterial diversity with 14, 14 and 12 % of α, β and δ-proteobacteria, respectively and 8 other bacterial groups each represented in 2-7%. Interestingly, some of the hygienic relevant bacteria were detected in OT samples which were further confirmed by conventional and qPCR. Among the bottled water samples, the water stored in large containers (tanks) had highest microbial content as compared to small bottles/cans. In the tank water samples, Alphaproteobacteria (63-79%) were dominant with high proportion of Methylobacterium, Sphingomonas and Novosphingobium, which are known to form biofilms on surfaces. In some of the

samples, hygienic relevant bacteria were detected. The overall results indicate that culture-independent methods are useful in detecting microbial content in drinking and bottled water samples and the microbial quality varied among sampling location/distribution network and brands, respectively.

Opportunities for the use of QMRA to implement EPA's 2012 Recreational Water Quality Criteria

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EPA's 2012 Recreational Water Quality Criteria discusses tools that can be used by States to develop site-specific alternative water quality criteria for inclusion into water quality standards. EPA's criteria are nationally recommended values that are broadly applicable to all ambient waters with the recreational designated use. However, there are waters with conditions that differ from those studied and used to inform the 2012 criteria. States may want to consider criteria reflective of those specific conditions or may want to employ a different indicator of fecal contamination and/or enumeration method. Any site-specific alternative water quality criterion would need to be scientifically defensible and protective of the designated use. Quantitative Microbial Risk Assessment (QMRA), one of the tools for evaluating and managing recreational waters identified in the 2012 criteria, compiles information pertaining to specific microorganisms - particularly pathogens of concern, but can also include an evaluation of fecal indicators in a risk-based context - the potential human health impacts from specific pathogen(s), and the routes of human exposure, in a logical and scientifically defensible manner. The QMRA framework is a formal investigative process that specifically addresses the etiologic agent(s) of illness. EPA is preparing technical support materials that discuss the use of QMRA in the development of site-specific water quality criteria. This guidance encourages consistency and transparency in risk assessment and is designed to support risk management and decision making. QMRA methodologies have been applied to evaluate and manage pathogen risks for a range of scenarios including those associated with exposures through food, sludge/biosolids, drinking water, recycled water and recreational waters. In recent years, the use of QMRA has emerged as a beneficial tool in the understanding and management of surface water quality. QMRA-based approaches have been featured in studies that explore in more detail the human health effects reported in epidemiology studies, identify pathogens of concern from sources of fecal contamination affecting recreational waters, characterize relative potential human health risks from different sources of fecal contamination, describe the development of water quality targets for waters impacted by non-human fecal sources and develop effective water quality management plans. A major benefit in using a QMRA-based approach is that it allows risk managers to explore important practical questions that have been heretofore difficult to address solely with field- or laboratory-based studies. Incorporating the QMRA framework with the traditional approaches to characterizing human health risks in surface waters creates a powerful tool for risk managers to examine the etiologic agents of illness and how to control those sources to protect public health. QMRA shows promise as a tool that can translate water quality targets among the different regulatory applications to promote consistent public health protection across different scenarios and provide a consistent evaluation metric for maintaining and restoring our nation's waters. Herein, we summarize and discuss the application of QMRA in developing site-specific alternative criteria. Example QMRA-based studies addressing specific risk management questions are provided to demonstrate the robust nature and usefulness of the QMRA framework included in the technical support materials.

Quantitative Microbial Risk Assessment (QMRA) of Freshwater Impacted by Animal Fecal Material

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We evaluated the potential for human illness from a hypothetical exposure to freshwater that was impacted by land-applied, animal fecal material. The scenario included 1) fresh cattle manure, pig slurry, or chicken litter (fecal material) is land-applied adjacent to a freshwater waterbody at standard agronomic rates; 2) the fecal materials contain fecal indicator bacteria (FIB) and pathogens of public health concern (reference pathogens) at levels reported in peer-reviewed literature; 3) FIB and reference pathogens are mobilized via runoff at rates estimated from our rainfall simulation experiments; 4) primary recreational contact (e.g., swimming) occurs in undiluted runoff at the edge of the waterbody or in diluted runoff containing specific reference levels of FIB in the waterbody; and 5) exposure to reference pathogens occurs through ingestion of water during recreation. Epidemiology studies link swimming-associated illnesses with fecal indicator bacteria (FIB) densities in sewage-impacted recreational waters, where FIB represent the potential presence of human fecal contamination. QMRA is emerging as a complement to epidemiology for understanding risks in recreational waters, developing recreational water standards, and making beach management decisions. To further this science, we have conducted a series of QMRA-based studies and developed an approach to compare the potential health risks associated with various fecal contamination sources in recreational waters. Our results indicate that GI illness risks associated with exposure to recreational waters directly impacted by fresh cattle feces may not be substantially different from waters impacted by human sources, but the risks associated with exposure to recreational waters impacted by the other sources evaluated appear substantially lower than waters impacted by human sources. This study considers indirect contamination in which FIB and pathogens from land-applied manure are mobilized into surface water via a rainfall event. Prior studies of pathogen and indicator mobilization via overland flow from land applied manures have explored the influence that numerous factors have on mobilization. Not surprisingly, mobilization fractions reported in the literature vary widely. Hence, rather than exploring all the possible important conditions and factors, we conducted a series of pilot-scale experiments to characterize mobilization of indicator organism and zoonotic pathogens from an intense rainfall event for one pasture condition. In this study we used a "forward" QMRA to estimate risk associated with recreational exposure in undiluted runoff from freshly-applied livestock wastes. The forward QMRA is the familiar application of QMRA in which pathogen exposure is estimated based on source pathogen density and a fate and transport model and risk is estimated using estimated pathogen doses and vetted dose-response models. We also used a "relative risk" QMRA model to compare risks from exposure to livestock-impacted waters to those associated with human sources. In the relative risk model, each fecal source is assumed to contribute enough contamination such that the hypothetical waterbody contains FIB equal to a predetermined reference density. In this way we use the QMRA results to draw inferences about potential risks associated with recreation in water impacted by land-applied livestock wastes. Finally, we discuss management considerations and implications for site-specific water quality criteria.

Quantification and genotypes of Giardia cysts and Cryptosporidium oocysts in drinking water supplies

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Introduction and objectives: Contaminated drinking water supplies are a public health concern worldwide especially in areas with poor sanitation. This kind of scenario exposes the population to pathogens that cause waterborne diseases often causing severe health problems. The protozoa parasites Giardia and Cryptosporidium have been described as important waterborne disease pathogens, and are associated with severe gastrointestinal illnesses. The most concern about these protozoa is the fact they are resistant to disinfection process usually used in Water Treatment Plants (WTPs). The purpose of the present study was to quantify and genotyping Giardia cysts and Cryptosporidium oocysts in water catchment area in the Metropolitan Region of Sao Paulo. Methodology: Sampling was performed in a catchment point, before Water Treatment Plant (WTP), located at the Metropolitan Region of Sao Paulo (MRSP), which is responsible to supply water to a population of 64,000 people, approximately. The samples collection was carried out weekly from May to November/2013, totalizing 26 samples of surface water. Giardia sp and Cryptosporodium sp were detected by IFA (immunofluorescence microscopy assay) using the USEPA 1623.1 Method, 2012. For molecular assays the concentrated of the samples were used. For both parasites genomic DNA was extracted using commercial kit QIAamp DNA Stool Mini _ (Qiagen, Germany) and kept at -20_C. Molecular characterization was carried out through nested PCR using specific primers based on 18S rRNA gene for Cryptosporidium and gdh gene for Giardia, followed by sequence analysis product amplified. Results: Giardia sp and Cryptosporidium sp were detected in 80.8% and 38.5%, respectively, of the samples from the point catchment before treatment at the conventional WTP. The concentration varied from <0.1 to 4.9 cysts/L for Giardia and from <0.1 to 0.3 oocyst/L for Cryptosporidium. Giardia cysts were more abundant than Cryptosporidium oocysts in all samples examined. Out of 20 samples screened for the presence of Giardia that resulted in 90% (18/20) of samples positive for the presence of Giardia intestinalis, demonstrated by the presence of the fragment of 155 base pairs expected for the positive reaction. Molecular difference of Giardia genotypes considered the importance for human clinics, it_s being developed. Until now the genotypic characterization of Cryptosporidium samples revealed the occurrence of C. hominis in 23% (3/13) and 7.7% (1/13) of C.parvum in the sites analyzed. Conclusion: These results revealed an elevated percentage of the water samples that were positive for Giardia sp (80.8%) and Cryptosporidium (38.5%) in concentrations from <0.1 to 4.9 cysts/L and <1 to 0.3 oocyst/L, respectively, showing that this supply of drinking water is impacted with fecal matter. The presence of Giardia intestinalis is strong evidence that assemblages A and B which are associated with human giardiasis can be present. The same was observed for Cryptosporidium where human genotypes are present in water samples confirming the presence of anthropogenic source of pollution. As these protozoa are resistant to disinfection process their presence in water poses a health risk to its consumers by the presence of human genotypes.

Microbial Source Tracking Coupled with Education to Promote Water Quality Improvements by Encouraging Community Stewardship in Clifton, Arizona

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Fecal contamination of ground and/or surface water can result from several sources ranging from human sewage, agricultural or livestock operations runoff, and local wildlife. The Arizona Department of Environmental Quality (ADEQ) is a regulatory agency that maintains a 303d list of surface waters that do not meet clean water regulatory standards in the state of Arizona. As of 2010, ADEQ listed 21 impaired watersheds throughout the state of Arizona on the 303d list due to E. coli presence higher than the US EPA set standards. The San Francisco River (SFR) is listed on the 303d list and it is comprised of the Upper Gila River Watershed from Coolidge Dam to the Arizona-New Mexico border and covers about 6,000 square miles; which 17 percent is privately owned and the remainder is under the stewardship of state, federal and tribal governments. In the first phase of this study our team used microbial source tracking (MST) techniques designed to target specific diagnostic sequences within the Bacteroides genome present in feces from different animals; coupled with conventional microbial methods to determine the dominant sources of fecal contamination in the SFR. The objective was to differentiate between human and bovine fecal contamination by targeting 16S rRNA Bacteroides molecular markers found in the literature. Results indicate that 30% of total samples assayed (n=74) show high levels of total coliforms and E. coli, 43% show contributions of human molecular markers, and 52% show bovine molecular markers. The second phase of our study was to evaluate community perception on water quality of the SFR in Clifton, Arizona, composed of about 56% Hispanic population. Preliminary survey results on public perception regarding water quality of the SFR consisted of 48% of people surveyed think the SFR has poor water quality for swimming and the majority of respondents concerned with poor water quality and their health. Sixty percent of people get information from the newspaper, factsheets or brochures and 52% of people get information from conversations with others. The survey information collected will be coupled with MST results to develop a one-day workshop on basic microbiology sources in the area along with resources such as fact sheets/brochures on water quality and human health. This data will be used to understand the interaction between the research and the public to promote greater understanding of the issues that impact water quality and human health. In addition, this research will highlight the impact community stewardship has had on the SFR to encourage behavior change.

Development of a Propidium Monoazide - Quantitative PCR Method for the Detection and Quantification of Viable Enterococcus Faecalis Bacteria in Marine Recreational Waters

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AIMS: The objective of the study was to develop a modified propidium monoazide - quantitative PCR method for the detection and quantification of viable Enterococcus faecalis cells in marine recreational waters. This was achieved through three main aims. The first aim was to optimize the treatment of propidium monoazide (PMA) to determine the optimal conditions which achieve the maximum differentiation between viable and dead E. faecalis cells in phosphate buffer saline (PBS) and marine waters. The second aim was to develop quantitative PCR (qPCR) and PMA-qPCR standard curves to

quantify total and viable E. faecalis cells in marine waters. The third aim was to compare the E. faecalis measurements in marine waters by the membrane filtration (MF) method, quantitative PCR, and PMA-qPCR. METHODS: To optimize the treatment of PMA, PBS and marine water samples (10 mL) were spiked with defined concentrations of either viable or heat-killed E. faecalis cells and concentrated through membrane filtration on polycarbonate filters. The filters were subjected to PMA treatment where five concentrations of PMA (10, 15, 25, 50, or 100 _M), three incubation periods (5, 10, or 20 min), and five light exposure periods (10, 20, 30, 40, or 60 min) were tested. Blue LEDs were used as a light source to photo-activate PMA. To develop quantitative PCR and PMA-qPCR standard curves, marine water samples (10 mL) were spiked with serially diluted concentrations of viable E. faecalis cells, ranging from 10^1 to 10^7 colony-forming units per mL. To compare the developed PMA-qPCR assay with qPCR (no PMA treatment) and the MF method, marine water samples were spiked with defined concentrations of viable E. faecalis cells and transferred to transparent sterile tubes (50 mL), which were exposed to the daily solar radiation and placed under constant shaking for a period of three days. During these three days, 10 mL samples were taken at specific time intervals and tested by the three methods. RESULTS: The optimal treatment conditions of PMA in PBS were at a PMA concentration of 15 _M, an incubation period of 5 minutes, and a light exposure period of 30 minutes where as the optimal treatment conditions of PMA in marine waters were at a PMA concentration of 50 _M, an incubation period of 10 minutes, and a light exposure period of 30 minutes. The optimal PMA treatments exhibited variable success in significantly reducing the qPCR signal of dead E. faecalis cells, whereby the qPCR signal of dead E. faecalis cells was reduced by 4 log units in PBS and by 3 log units in marine waters. The generated quantitative PCR and PMA-qPCR standard curves were linear over a 6-log dynamic range and had a minimum detection limit of 100 cells per mL. The E. faecalis measurements obtained by the developed PMA-qPCR assay did not match that of the developed qPCR assay and the standard MF method. CONCLUSIONS: The developed PMA-qPCR assay is a rapid and accurate method for the detection and quantification of viable E. faecalis cells in marine recreational waters though the corresponding detection limit can benefit from additional improvement. The reduced success of the optimal PMA treatment in marine waters indicates an inhibitory effect of the marine water matrix on the activity of PMA. Therefore, the effects of the marine water matrix need further examination.

Coliphage, a fast and sensitive fecal indicator

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Coliphage, as a fecal indicator, are frequently overlooked because they are outside of the bacterial target organism paradigm of most public health regulations. Public health officials debate coliphage detection and its correlation with microbial contamination, but most can agree that as a viral surrogate coliphage detection provides complementary contamination data with other microbial contaminates. The most interesting property of coliphage, that should get the attention of water-researchers and public health stakeholders, is their prolific growth amplification and expression. In numerous applications a single plaque forming unit can be detected in large volumes of water, 100mL-10L, in less than 8 hours. It is likely that with further development the time of detection can be reduced to only a few hours. Coliphage, viruses to coliform bacteria, are a fecal indicator in the Ground Water Rule. Some beach studies with limited data epidemiological data suggest that coliphage could have correlation to disease occurrence. More study is needed to understand the correlations of coliphage to water-borne human disease risks. As a viral surrogate coliphage diffuse farther distances and have greater chemical and environmental robustness than the bacterial fecal indicators, E. coli and enterococci. Therefore coliphage are interesting for drinking water quality, beach water testing and as waste water recharge process indicators. Agriculturally, coliphage are interesting in quality assessment of shellfish aquaculture

areas and in irrigation and process waters for fruit and produce. Coliphage detection methods have rapidly advanced in the last 10 years. Coliphage detection methodology described in Section 9224 of Standard Methods of the Examination of Water and Waste Water needs updating. Recently developed rapid quantitative methods that work in a working shift take advantage of _-Galactosidase expression in coliphage lysing host cells. These methods utilize filters and/or (most probable number) MPN to concentrate and quantify plaque forming units (pfu) of the organism in water samples. In these methods, the coliphage lysis of the host bacteria triggers galactosidase operon expression. The expressed enzyme produces distinctive color with the use of colorimetric or fluorometric enzyme substrates. These rapid quantitative results may under estimate the actual coliphage plaque forming units due to host lysogenic coliphages that do not induce lysis and enzyme production. Qualitative coliphage methods utilize host pre-enrichment to amplify coliphage in host prior to detection by enzyme or plaque detection methods. Qualitative methods have been shown to detect as low as 1-2 plaque forming units (pfu) in large volumes 100mL to 10L of water and correlate to plaque enumeration of the waste water spiking sources. Recently electrostatic filters in combination with qualitative coliphage detection methods have been shown to detect as few as 2 pfu/10L in 8 hours. These new coliphage methods, and some commercially available variants, offer the possibility for more extensive study, development and research. The genetic code triggering host lysis and the resulting biochemistry have the potential to be tied with rapid detection instrumentation and automation. For example an instrument that uses fluorescence to continuously monitor a flow of water for the presence of coliphage might be applicable to drinking water produced from ground water from aquifers supplemented with reclaimed brown waters. More public health research is needed to understand the detection of coliphage in water sources as it relates to the incidence of water and food borne disease. However, microbiologists may want to consider coliphage genetics and biochemistry in the development of real time detection systems for the quality of drinking and bathing water. Coliphage as a process control indicator in water management systems including filtration, waste water recharge, and water used in food production offers great promise in achievable sensitivity and speed to detect in macro-scale volumes of water.

Pathogen removal during wastewater treatment and the impact of the effluents on the water quality in a rural watershed in Southeastern Georgia

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Statesboro, Georgia is a rural town with an increasing population trend due to the presence of Georgia Southern University. In addition to the increasing population, GA has been experiencing extreme precipitation events in the last years causing devastating effects on the wastewater treatment plant (WWTP) operations. Two of the five record annual precipitation amounts since the beginning of the century were detected in 2009 and 2013. The Statesboro WWTP disposes of its effluents into Little Lotts Creek, a tributary to the Ogeechee River Basin. This basin serves approximately 400,000 people in GA by providing drinking water through municipally or privately owned public water systems and irrigation water for over 76,000 acres of agricultural use. Ogeechee River Basin also acts as a source of recreational activities for local residents and tourists. Runoff, septic systems, sanitary sewer overflows, non-point sources and/or animal wastes are main problems related to the basin (EPD, 2001). Escherichia coli (E. coli) has been recommended by the United States Environmental Protection Agency (U.S. EPA) and some states have started using this bacterium as a better indicator of health risk from freshwater contact. However, fecal coliforms are still being used in many states as the indicator bacteria,

particularly for river water quality detection purposes. Currently, the National Pollutant Discharge Elimination System (NPDES) permits rely on fecal coliform concentrations in GA. This study examines the efficacy of bacterial removal in a rural wastewater treatment facility over time. Indicator bacteria (E. coli) and pathogenic E. coli O157:H7 has been analyzed in samples collected monthly from influent, secondary treatment effluent and UV disinfected effluent as well as an upstream point at the Little Lots Creek and downstream point. Fecal coliform data (APHA, 2005) was obtained from the WWTP database for the same day of sampling events. E. coli was detected by using both chromogenic substrate test (Colilert, IDEXX Laboratories, Inc., Westbrook, ME) and quantitative polymerase chain reaction (qPCR) method (Noble et al., 2010). Shiga-toxin producing E. coli O157: H7 was detected by qPCR (Elizaqu_vel et al., 2011). The preliminary culturable E.coli data (November 2013-January 2014) has shown that indicator bacteria in the influent was 2.84 x10^6 CFU/100 ml. The E. coli concentrations decreased to 3.63 x 10^4 CFU/100 ml after secondary treatment and 1.72 x 10^1 CFU/100 ml after UV disinfection. Overall, the preliminary data has shown that the log reduction of E. coli was 5.24 throughout the treatment process. Further analysis will include molecular detection of total E.coli and shiga-toxin producing E. coli O157:H7 by qPCR to compare the efficacy of the UV disinfection process for indicators versus pathogen removal. These results will also be compared to the upstream concentrations of the pathogens in the creek to address the non-point sources. Population trends as well as precipitation data will be included for assessing the microbial risks associated with human activities related to the Ogeechee River. References: American Public Health Association 2005. "Standard methods for the examination of water and wastewater", 21st ed. American Public Health Association, Washington, DC. Elizaqu_vel, P., Gabald_n, J.A., Aznar, R. 2011. Quantification of Salmonella spp., Listeria monocytogenes and Escherichia coli O157:H7 in non-spiked food products and evaluation of real-time PCR as a diagnostic tool in routine food analysis. Food Control, 22, 158-164. Georgia Department of Natural Resources Environmental Protection Division 2001. Ogeechee River Basin Management Plan 2000. Noble R.T., Blackwood, A.D., Griffith, J.F., McGee, C.D., Weisberg, S.B. 2010. Comparison of rapid quantitative PCR-based and conventional culture-based methods for enumeration of Enterococcus spp. and Escherichia coli in recreational waters. Applied and Environmental Microbiology, 76 (22), 7437-7443.

Non-anthropogenic factors have major influence on ecogenomics of microbial communities in well-managed canal networks of highly urbanized environments

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Growing demand for water in cities has led to development of extensive canal infrastructure in cities to capture storm water. While much is known about influences of anthropogenic pressures in contaminated water systems, influence of non-anthropogenic factors including both physical and chemical are highly understudied such environments. Therefore, there is an increasing need to understand how well-managed waterways can function as an ecological system in highly urbanized environments. Microbial communities in these waterways provide ecological services, whose understanding can be used to enhance self-cleaning capacities of such freshwater systems. This study was designed using a model, well-managed canal network in an urban watershed to understand the spatial ecology, influence of inputs from land-use patterns pulse disturbance due to rain on the structure and functional potential of microbial communities of the waterways. Fragmentation in the

land-use patterns of the watershed due to elevation topology or another land-use type, showed no influence on microbial communities in the waterways within a given land-use. Such non-fragmented microbial communities can thus be indicators of well-functioning waterways. As sediment microbial communities were more diverse than in water phase and their functional potential distinguished different land-use types, they seem closely linked to ecological services responding to environmental inputs and can be a better monitoring parameter than those in water phase. The response of sediment associated microbial communities to the pulse disturbance due to rain can be used to reset the system with further understanding on the extent and frequency of such natural manipulations. Regulated contaminants were below their allowable limits and did not affect microbial communities. Only two of 36 non-anthropogenic inputs, aluminum and copper, formed the key drivers for these communities and explained nearly 25% of variation in microbial communities. Ecological principles can thus be used to identify key practices for managing urban watersheds and canal networks.

Successional response of microbial taxa and functions of sediment microbiome remain conserved in different land use types

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Urban waterways in tropical areas are influenced by frequent perturbations of top-layer sediments, caused by rain due to high water discharge, velocity and turbulence. While many studies have focused on microbial ecology of natural fresh water systems, an understanding of microbial communities in engineered infrastructures, such as, urban waterways is still lacking. An understanding of sediment associated microbial communities, which provide ecological services, can be used to enhance the self-purification capabilities of these systems. To improve the water quality and appearance, dense network of canals provide high residence time to adopt such microbial ecology based soft approaches. With this motivation, this study was designed to understand the fate of sediment associated microbial communities' structure and function from different land use types after a storm event. To investigate this, a mesocosm experiment was conducted in 2m long flumes with sediment and water seeded from residential and industrial sites after a rain event. A third sediment type was created by mixing the two sediment types and termed as mixed. A linear flow regime was designed based on partial replacement and dilution principle. The experiment was conducted with complete random design of three replicates each of reactors for three sediment types for 30 days period with daily measurement of 12 environmental parameters and 10 time points for in situ physicochemical parameters such as oxygen and ORP levels, nutrient ions, metals, cell counts, T-RFLP and metatranscriptome. Non-metric Multidimensional Scaling (NMDS) and Analysis of Similarity (ANOSIM) showed that microbial communities shifted in a clear successional pattern. The changes were conserved in the three different land-use types. Microbial community changed to anaerobic phase on day 8 during the successional period with nitrate reduction to ammonium as their principle energy harvesting pathway. The microbial community profile kept changing between two broad groups during rest of the experiment indicating continuous successions which are interrupted by pulse disturbances due to rain. Relative abundance of active mRNA transcripts also showed similar trends with relatively higher resistance to change. Similar successional trend of microbial taxa irrespective of land-use types provide evidences for core assemblage and functional properties in microbial communities in these environments.

Behavior of Noroviruses in Coastal Environments and Implications for Seafood Cultivation and Human Health

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Noroviruses (NoVs) are the most common cause of viral gastroenteritis worldwide, responsible for two thirds of human illnesses caused by food-borne pathogens in the United States. There is emerging evidence that viruses are widespread in land-based runoff, posing a health risk to swimmers in coastal waters and through consumption of raw shellfish. Association of pathogens with aquatic aggregates could significantly impact their transmission dynamics to susceptible hosts including humans, but to date the association of fecal pathogens including viruses with these ubiquitous particles has been largely unexplored. The work had two main objectives: (i) to test whether waterborne pathogens including viruses are associated with aquatic macroaggregates and whether pathogen aggregation is increased in saline waters; and (ii) to evaluate the distribution of human NoVs in coastal California surface waters and shellfish by determining the prevalence, fate and transport of NoVs in freshwater, estuarine and coastal waters. The study was divided into three parts. In the first part, a laboratory study was conducted to assess the association of viruses and zoonotic pathogen with macroaggregates. Fresh, estuarine and marine water samples were spiked with PP7 virus particles, Cryptosporidium parvum, Giardia lamblia, and Salmonella enterica serovar Typhimurium and rolled for 24 h to enhance the production of macroaggregates. Two fractions, the top 1.1 L, operationally defined as 'aggregate-poor' water and the lower visible aggregates containing 'aggregate-rich' fraction, were collected and analyzed for the spiked microorganisms. The bacteriophage PP7 was quantified using real-time PCR. Protozoa were enumerated using immunomagnetic separation followed by direct fluorescent antibody staining and microscopy (IMS-DFA). Salmonella cells were quantified by membrane filtration and aerobic culture on Luria Bertani agar, followed by colony enumeration under UV illumination. The second part focused on the detection of NoVs in freshwater and seawater samples. The prevalence of known human NoVs was analyzed during dry and wet seasons at eight freshwater sites, where human fecal markers have previously been detected, and two seawater sites near shellfish harvesting waters. Collected sea water samples were concentrated via hollow fiber ultrafiltration (HFF) and analyzed for human NoV genogroups I and II (GI and GII) using quantitative PCR. Total coliforms, fecal coliforms, E. coli and Enterococcus were measured using standardized microbiological methods. The third part assessed NoV prevalence in aggregates and mussels. At each of four coastal locations, we collected a seawater sample and 30 mussel samples. The collected water samples were placed in horizontal cylinders and after settling for 30 minutes, separated into the top aggregate poor and the bottom aggregate rich water fraction. Hemolymph from pools of five mussels each, aggregate-rich fractions, and aggregate-poor fractions that had been concentrated using HFF were analyzed for human NoV GI and GII using RT-qPCR. In the laboratory aggregation study, the viral surrogate PP7 and tested pathogens showed increased association with macroaggregates in estuarine and marine waters, as compared to natural freshwater and an ultrapure water control. Enrichment factor estimations demonstrated that pathogens were 2-4 orders of magnitude more concentrated in aggregates than in the estuarine and marine water surrounding the aggregates. NoV GI was detected at five of ten sampling locations, with the highest signature (21 genomes/mL) found at the mouth of Carmel River with brackish salinity. NoV GI was also detected in all aggregate samples analyzed to date, ranging from 33 genomes/mL to 2,361 genomes/mL, with concentrations consistently 1-2 orders of magnitude higher in aggregate-rich fractions compared with aggregate-poor seawater. Furthermore, NoV GI has been detected in 75% of the mussel

hemolymph samples analyzed to date, ranging in concentration from 233 genomes/mL to 2,343 genomes/mL. The study provides evidence of human NoV contamination in freshwater discharges to the ocean along the central California coast. Detection of NoV in seawater fractions with and without marine aggregates as well as in mussel hemolymph provides novel insight on the transport and fate of NoV in coastal ecosystems. Furthermore, PP7 virus particles and the pathogens Giardia, Salmonella, and Cryptosporidium were all found to enrich in marine and estuarine aggregates, highlighting the potential significance of aquatic aggregates in mediating pathogen waterborne transport and bioavailability to susceptible hosts. These findings are relevant for estimating public health risk and food safety concerns since invertebrates, including those consumed by humans as seafood, are known to ingest and retain microscopic particles associated with aggregates more readily than free suspended particles.

Adaptation of a microbial source-tracking tool developed in the water microbiology field to ascertain risk factors in the fresh produce environment

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Over the last few decades, consumption of fresh and minimally processed produce has been associated with an increasing proportion of U.S. foodborne illnesses. The pathogens causing these outbreaks are primarily fecally derived. Little is known about the relative importance of various risk factors in introducing fecal contamination to fresh produce, although increasing attention is being paid to the role of waters used for irrigation. Microbial source tracking (MST) is an emerging tool developed in the water microbiology field for identifying and quantifying the dominant source(s) of fecal contamination in surface waters. The purpose of this project was to ascertain risk factors for fecal contamination of fresh produce by applying general and source-specific 16S rDNA Bacteroidales MST assays to samples of irrigation water, produce rinses, and hand rinses collected over the course of one year from 9 farms (growing tomatoes, jalape_o peppers, and cantaloupe) in Northern Mexico. Of 174 samples, 39% were positive for a universal Bacteroidales marker (AllBac), with a geometric mean concentration of 2.1 log10 GEC/100 ml. By sample type, 47% of produce rinses, 34% of hand rinses, and 28% of irrigation water samples were positive for AllBac. A positive correlation was found between levels of AllBac on both produce and hands and between produce and irrigation water. Thirty-one samples (18% of total) were positive for human fecal contamination, with a geometric mean concentration of 2.4 log10 GEC/100 ml. By sample type, 18% of produce rinses, 20% of hand rinses, and 15% of irrigation water samples were positive for a human-specific marker. For the human marker, a positive correlation was detected between produce and hands but not between produce and irrigation water. These results suggest that both irrigation water and human handling may serve as risk factors for fecal contamination of produce, although the contamination source (e.g., human vs. animal) appears to be different for the two. Multiple statistical analyses were performed to compare the presence and concentration of Bacteroidales markers with E. coli in samples, including Pearson Chi-Square analyses, logistic regressions, and Spearman Rank correlations. Consistent with others working in the water quality area, no statistically significant relationship was detected between E. coli and the general or human-specific Bacteroidales markers across all samples using any statistical test ($p > 0.05$). However, when individual produce types were examined separately, AllBac presence was positively correlated with E. coli concentration in samples from cantaloupe farms ($X2 = 6.29$; $p < 0.01$), suggesting a similar contamination source. This research provides for an increased understanding of the relative importance of irrigation water and human handling in introducing contamination to fresh produce. Nevertheless, our sample sizes were

small, so a more comprehensive effort involving additional produce farms and commodities, as well as additional source-specific markers, would be required to further refine interpretations regarding sources of Bacteroidales contamination in the produce production environment. This information is essential for the development of effective measures to reduce the risk of produce contamination, thereby reducing risks to human health.

Household drinking-water in developing countries: variability of contamination between collection and storage by water supply type

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There has been significant debate for over 20 years on the relative importance of water quality improvements at the source and in the household. We conducted a systematic review of studies published between 1990 and August 2013 that measured water quality at two points along the pathway from water source to consumption: the point of collection and in water stored in the household. Studies were identified from online databases, including PubMed and Web of Science, and from grey literature. Studies were included which assessed drinking-water for the presence of E. coli or thermotolerant coliforms and which associated results with particular water supply types. The results of the 39 studies which fit the inclusion criteria were analyzed using meta-analysis and meta-regression techniques to provide estimates of contamination between water at the point of collection and in household storage containers. The majority (52%) of water supplies studied were piped. Water for 13 water supplies was found to be more contaminated at the point of collection while water from 29 water supplies was found more contaminated in household stored water. Random effects models were used to account for significant heterogeneity (I_ = 95.8%) between studies when pooling risk ratios. Water sampled from household storage containers overall was at 28% higher risk of contamination than water from the point of collection. Water from piped supplies had a 94% higher risk of contamination at in stored water compared to water at the point of collection. A country's income level was the covariate found to explain the most heterogeneity (12.2%) among studies. Water supply type, urban versus rural geography, Millennium Development Goals region did not explain a significant amount of between study heterogeneity. Overall, water that is cleanest at the point of collection was found to have the proportionately highest contamination in when sampled from household water storage containers. As more and more households have access to piped water systems, this review highlights the need to disaggregate the piped water category in study reporting. We suggest tap location (in-house, in-yard or off-premises), levels of water reliability, and residual chlorine as important data that will allow for further differentiation of the quality and safety of piped supplies.

Assessing hog lagoon waste contamination in the Cape Fear Watershed using Bacteroides 16S rRNA gene pyrosequencing

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Pig-specific bacterial source tracking methods that target Bacteroides 16S rRNA genes may be biased towards the detection of fecal matter only. In North Carolina, the majority of pig related waste in the Cape Fear Watershed is from hog waste lagoon runoff and not direct fecal contamination. In this study,

we pyrosequenced 31,459 Bacteroides 16S rDNA amplicons from water samples from 4 river sites during 5 different months in the Cape Fear Watershed, and waste samples from 6 hog lagoons within the Cape Fear watershed. Using a strict 99% similarity OTU cutoff, specific Bacteroides markers for pig feces, hog lagoon wastewater, animal wastewater, and cosmopolitan wastewater were identified in the river and hog lagoon samples. In the hog lagoon samples pig fecal markers were relatively low (1.0 to 13.9%) and were instead dominated by 3 hog lagoon wastewater markers (28.1% to 68.9%), suggesting that Bacteroides communities undergo a shift from feces to hog lagoon storage and relying solely on fecal indicators is not sufficient for determining hog lagoon waste contamination in watersheds. By employing pyrosequencing and strict host-specific clustering, 20% of the river samples contained hog lagoon markers, but only one water sample collected in March 2010 in the Black River showed significant hog lagoon waste contamination. In addition, Bacteroides communities in the contaminated water also showed similar β diversity to hog lagoon communities. Thus, we report a new and discriminatory method for assessing hog lagoon waste contamination in watersheds hosting industrial swine production facilities.

Microbial coagulation using chitosan and influences of water parameters on its performance

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Introduction Microbially contaminated drinking water causes diarrheal and other infectious diseases especially in children age under 5. Providing safe drinking water for underserved populations remains a challenging task. Chitosans, biopolymers obtained from crustacean shells, have coagulant properties and have shown the ability to remove microbes from water. This study examined impact of water quality parameters on the efficacy of a candidate chitosan as an alternative coagulant for bacteria and virus removal from water. _ Methods Jar test method was used to measure the efficacy of a candidate chitosan at different doses for removal of the test microbes from test water. Test water was artificially created as recommended by the US Environmental Protection Agency for evaluation of point-of-use water treatment technologies. This water was modified to test effects of water parameters on microbial removal, with pH of 6, 7.5 or 9; salinity of 0.1, 0.3 and 1 ppt; and turbidity of 5, 10 and 30 NTU. Jar testing was performed with rapid mixing at 100 rpm for 1 minute, slow mixing at 25 rpm for 15 minutes then settling for 30 minutes. Supernatant was recovered for bacterial and viral analysis. Chitosan polymer with molecular weight 100,000 daltons which showed high kaolinite and bentonite removal (our previous studies) was used for this study. Water pH was measured both before and after testing. Chitosan doses were 1, 3, 10 and 30mg/L. E. coli and bacteriophage MS2 were seeded into test water and mixed for 30 minutes, then dosed with chitosans. E. coli was analyzed by membrane filtration and double agar layer plaque assay was used for MS2 on host E. coli Famp. Microbial removal as log10 reductions were compared among the various test conditions of chitosan coagulation. Results Effects of water pH and coagulant dose on microbial removal by chitosan Bacterial removal was not significantly affected by water pH levels of 6, 7.5 or 9 ($p>0.05$). However, chitosan exhibited highest E. coli removal at dose 10mg/L for all 3 water pH tested. Chitosan at dose 10mg/L exhibited high bacterial removals; 4.76, 4.08 and 4.29log10 at pH 6, 7.5 and pH 9, respectively; that were not significantly different ($p>0.05$). At dose 3mg/L, bacterial removal was significantly lower at pH 9 (0.64log10) than pH 6 (3.65log10) ($p<0.05$) and pH 7.5 (3.91log10) ($p<0.01$). Virus removal was significantly affected by different pH levels ($p<0.0001$). At all pH tested, virus removals were lowest at the lowest chitosan dose of 1mg/L (1.64, 0.26 and 0.03log10 at pH 6, 7.5 and 9) and higher at higher coagulant doses (4.86, 3.47 and 2.91log10 at pH 6, 7.5 and 9 with a 10mg/L dose, respectively). Virus removal at pH 6 was significantly better than virus

removal at pH 9 at every dose ($p<0.01$) but virus removal at pH 6 was better than removal at pH 7.5 only at 1 and 10mg/L doses ($p<0.05$). _ Effects of water salinity and coagulant dose on microbial removal by chitosans Based on log10 E. coli removals, varying water salinity at pH 7.5 had no significant effects on bacteria removal by chitosan coagulants. All three salinity levels showed similar trends that bacteria removals were much lower (<1.2log10) at 1mg/L dose and higher (>3log10) at higher chitosan doses of 3, 10 and 30mg/L. Water salinity at pH 7.5 affected virus removal somewhat. At chitosan dose 10mg/L, virus removal was significantly better at salinity 1 ppt (3.96log10) than salinity at 0.1 ppt (3.19log10) ($p<0.01$), although the removals were considerable and > 3log10 at both salinities tested. Virus removals were not statistically different in waters with 0.3 and 1 ppt salinities ($p>0.05$ value). Effects of water turbidity on microbial removal by chitosans Water turbidity did not affect the extent of bacterial removal by chitosan coagulation at each chitosan dose tested ($p> 0.05$). However, E. coli reductions were consistently low (<1.5log10) at 1mg/L chitosan dose and consistently high (> 3log10) at the higher chitosan doses of 3, 10 and 30mg/L. Water turbidity also generally did not affect the extent of virus removal at each chitosan dose tested. Although virus removal was significantly different ($p < 0.001$) among water turbidities at dose 3mg/L, all virus reductions were extensive and >3.5log10; 3.56, 4.89 and 4.71log10 at turbidity 5, 10 and 30 NTU, respectively. There were no statistically significant differences in virus reductions at 10 and 30 NTU turbidities at each chitosan dose tested, with all reductions >3.5log10. Conclusion Overall, turbidity, salinity, and pH influenced chitosan performance for virus removal but not bacteria removal. Overall, low doses of 3-10mg/L chitosan can achieve between 3 and 5log10 removal of E. coli bacteria and indicator virus MS2 at varying levels of turbidity, salinity, and pH. Efficacy across a range of water qualities and at a wide range of chitosan doses from 3 to 30mg/L makes chitosan a promising potential coagulant to use for household water treatment.

Relations between DNA- and RNA-based molecular methods for cyanobacteria and concentrations of microcystin at Great Lakes and inland lake beaches in Ohio

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Toxic cyanobacterial blooms, or harmful algal blooms (HABs), are of concern in many parts of the world because of their effects on drinking water, water-based recreation, and watershed ecology. The Great Lakes have seen an increase in the number and the size of HABs over the last several years. At this time, there are more than 40 freshwater species of cyanobacteria that are known to produce toxins that can affect the skin, liver, and the nervous system. However, the presence of cyanobacteria does not mean that toxins are being produced, since toxin production is strain-specific and dependent upon environmental factors. Molecular methods are being used to better identify and understand the potential for toxin production in cyanobacterial populations in environmental waters. The U.S. Geological Survey (USGS), in cooperation with the University of Toledo, the Ohio Lake Erie Commission, and Ohio Water Development Authority evaluated relations between results from DNA- and RNA-based molecular methods and microcystin concentrations. Water samples were collected from Maumee Bay State Park Lakeside Beach, Oregon, Ohio, during the 2012 recreational season and from seven additional Lake Erie and inland lake sites during 2013. Samples were analyzed for cyanobacteria gene sequences by DNA-based quantitative polymerase chain reaction (qPCR) and RNA-based quantitative reverse-transcription polymerase chain reaction (qRT-PCR). Results from five DNA assays (quantifying total cyanobacteria, total Microcystis, and Microcystis, Planktothrix, and Anabaena strains that contain the toxin gene mcyE) and three RNA assays (quantifying Microcystis, Planktothrix, and Anabaena strains that are expressing the toxin gene mcyE) were compared to microcystin concentration results determined by

an enzyme-linked immunosorbent assay (ELISA). Using Spearman's correlation analysis, three of the DNA-based assays and one of the RNA-based assays were correlated to microcystin concentrations by ELISA. Quality control samples for the molecular methods provided insights into analytical variability, with the DNA-based total cyanobacteria assay having the least analytical variability and the DNA-based Planktothrix toxin mcyE assay having the most. Additionally, a sample holding-time comparison was done for the RNA-based Microcystis toxin mcyE assay because RNA is known to have a shorter half-life than DNA. Filtering approximately 2 hours after sampling yielded higher RNA concentrations than filtering after 24 hours. The results from this study indicate that DNA- and RNA-based molecular methods can be useful for studying the potential for and the expression of cyanobacterial toxins. These molecular assays may be useful in large-scale research projects to help better understand the factors that cause cyanobacterial blooms.

Effects of Microalgae on the Survival of Waterborne Viruses-Using Microcystis and Somatic Coliphage as example

Chenxi Sun, National University of Singapore

Waterborne viruses have drawn wide attention across the world because of the threat they posed on public health and relatively less information on their survival. Algal bloom at the same time has also grown to be an important international environmental issue. This study focused on the interactions between these two microorganisms, mainly the effects of microalgae on the survival of waterborne viruses. In the study, microcystis was used as model microalgae and somatic coliphage phiX174 was used as model virus. Both adsorption and inactivation of coliphage phiX174 was studied in the presence of microcystis. For adsorption study, after coliphage phiX174 was mixed with microcystis in buffer solution and slowly shaken for 30 minutes, no obvious adsorption of coliphage phiX174 onto microcystis was observed. For inactivation study, the effects of microcystis and UVA/visible light were studied. When coliphage phiX174 was added into buffer solution containing microcystis at a density of 2x10^5 cell/ml under UVA/visible light, the inactivation rate constant for coliphage phiX174 was increased. This value increased more significantly for coliphage phiX174 samples mixed with inactivated algae cells than with live algae cells. When the experiment was conducted in dark, no significant inactivation of coliphage phiX174 was observed when mixed with live and inactivated algae cells during the experiment. The increased inactivation of coliphage phiX174 was believed to be due to an increase in the exogenous oxidation process and positively correlated to the production of OH?. The effect of microcystis at same density under different irradiation intensities and effect of different microcystis density under fixed irradiation intensity were also evaluated. The results showed it was possible the increased inactivation of coliphage phiX174 was linked to chlorophyll activity. Also, as the density of microcystis increases, the reduction on solar irradiation became more dominant than the increase of the exogenous oxidation process and it started to prolong coliphage phiX174 survival in water environment.

A Quantitative Microbial Risk Assessment for Lettuce Irrigated with Wastewater Treated by On-Farm Bank Filtration Systems in Bolivia

Matthew Verbyla, University of South Florida

Rivers in the Cochabamba Valley of Bolivia are highly-contaminated with untreated wastewater, especially during the dry season, when irrigated farming is practiced the most. Some farmers have constructed bank-filtration systems to improve the quality of irrigation water, instead of using water from the river. The goal of this project was to characterize risks of viral infection resulting from the

consumption of irrigated lettuce. Water samples from the river and from three bank filtration system extraction wells were analyzed for human rotavirus (group A) using quantitative polymerase chain reaction (qPCR) with reverse transcription (RT). An average concentration of 2,505 rotavirus target copies/ml were detected in river water samples (n=5; SD=757), with an average extraction efficiency of 40% (SD=10%). Rotavirus was not detected in any of the 25 water samples from the three bank filtration system extraction wells. The limit of quantitation for the assay was 0.3 copies/ml. Using a short convenience-sample survey and participant observation, data was gathered from market-goers and lettuce vendors to address the quantity of lettuce sold, purchased, and the local practices for lettuce consumption and treatment. Systematic observation of restaurant meals produced near the market was performed to gather samples of average lettuce weights from meals served at local restaurants, and observation of market vendors was also completed. Results indicated that market-goers purchase enough lettuce for each member of their household to eat 19 grams/day on average (SD=25). Some interviewees reported rinsing lettuce at home with water; others reported using lime juice, iodine or chlorine. A quantitative microbial risk assessment (QMRA) model was developed, using a Bayesian approach with Markov Chain Monte Carlo, to assess the potential exposure to rotavirus given the results from the market surveys and the water quality analyses. The results indicate that consuming lettuce irrigated with river water in Cochabamba would correspond to an average daily risk of infection of 89% (SD=2%). The estimated average daily risk associated with consuming lettuce irrigated with water from the bank filtration systems may be very low, but could be as high as 23%, due to the uncertainty associated with some of the model parameters. One limitation for quantifying risk in this context is the limit of quantification for the virus assay.

A prospective cohort study of risks associated with beaches in Brazil

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INTRODUCTION The association between exposure to contaminated recreational water and disease has been studied since the 1950s. Epidemiologic studies in the US and UK have demonstrated higher rates of gastrointestinal, respiratory, and skin illnesses in swimmers compared to non-swimmers at recreational waters. These studies focused primarily on beaches in developed areas dominated by point source pollution and has led to the creation of worldwide standards for risk criteria (WHO 2003; USEPA 2012). Additional studies conducted in New Zealand, Egypt, Hong Kong, Australia, Israel, France, Canada, and The Netherlands yielded similar findings (Pruss 1998). The developing world, including South America, is underrepresented in the scope of global recreational water quality epidemiological studies. These areas rely on water quality criteria developed on other continents with relatively little information known about their local applicability. In this manuscript we describe a large cohort study performed at five urban beaches near Sao Paulo, Brazil. The aim of this project was to compare rates of highly credible gastrointestinal illness (HCGI) among swimmers and non-swimmers and to evaluate whether there was a dose-response relationship among swimmers based on water quality measures. Furthermore, we investigate whether sensitive populations (children and elderly) are at higher risk. These analyses lay the foundation for localized QMRA. METHODS At baseline of the prospective cohort study, 23235 participants were interviewed at five Brazilian beaches. Triplicate beach water samples were collected once daily at each beach on days when initial interviews were conducted and assayed for fecal coliforms, E. coli, and enterococci using defined substrate technology. Follow-up telephone interviews were conducted 10 days later to determine the onset of specific symptoms with 16637 individuals (72% response rate). Cases reporting vomiting or diarrhea or stomach ache plus nausea were classified as

HCGI cases. Cumulative incidence (per 1000 individuals) and Odds Ratios (OR) of health outcomes (HCGI, diarrhea, nausea, vomiting, and fever) were calculated for three age groups (0-10, 11-54, and 55+) at the five beaches. Dose-response models were created for each beach using the maximum likelihood estimation to fit the data to a Beta-Poisson distribution ($P=1-[1+C/N50(21/\alpha-1]-\alpha$). Where N50 is the number of organisms required to infect 50% of the population, C is the dose, and α represents pathogen survival probabilities (derived from the model). RESULTS In total, 150 water samples were collected over 10 days (five weekends) at five beaches. Enterococci ranged from 0.69 to 6.55 (ln MPN/100 ml) with a geometric mean of 3.15. E. coli ranged from 0.69 to 8.87 (ln MPN/100 ml) with a geometric mean of 4.20. Of the 23235 enrolled individuals, 16637 successfully completed the follow-up interview which included 14010 individuals entering the water that day (9456 of which usually submerge their heads and 5844 tend to swallow water). Other initial survey data collected included sand contact at the beach (n=11943), eating food on the beach (n=11909), and exposure to pool water in previous week (n=6212). Pooled beach data found the odds of HCGI by age group after entering the water to be OR=1.34 (0-10), OR=1.22 (11-54), and OR=0.80 (55+). Overall HCGI OR for all beaches when exposure included only entering the water was 1.59 (95% CI 0.97-1.57) but increased to 2.57 (95% CI 2.05-3.23) when sand contact was included as an exposure. Among individuals that entered the water and usually swallow water, a dose-response relationship was observed for HCGI, where illness was positively related to enterococci concentrations ($\alpha=0.10$; N50=1893). DISCUSSION This study demonstrated a Beta-Poisson distributed dose-response relationship between enterococci and HCGI in swimmers and non-swimmers. However, we also found water exposure to be one of many factors influencing illness risk at beaches. Sand contact had higher HCGI OR compared to entering the water alone; indicating that exposure to sand may increase the risk of reporting HCGI. Furthermore, this study demonstrated that HCGI risk is not equal across the defined age groups, with the risk of HCGI highest in the youngest age group. Other factors potentially influencing HCGI risk at Brazil beaches include food consumption and time spent at the beach. Our results, distinct from the USEPA and UK data, should be used when developing regulations in Brazil. The results of this study indicate that a universal recreational water quality standard is not adequate for all geographical locations and a local risk assessment is required to inform local regulations. Both developing and developed countries will benefit from local QMRA and beach criteria development.

Characterizing E. coli in the nearshore reservoir at four Great Lakes beaches

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Recent studies have shown that foreshore sand at non-tidal beaches, such as those on the Great Lakes, can act as a reservoir for FIB, with microbial concentrations often much higher than in adjacent waters. Not only does the sand/sediment provide a direct route of exposure, it can also release any associated microbes into the surface waters by resuspension or through interstitial porewater flow and groundwater discharge. To enhance understanding of how E. coli concentrations in the nearshore reservoir effect surrounding shallow surface waters, an interdisciplinary study focusing on the hydrogeological, biogeochemical, and microbial processes was conducted on four distinctly different Great Lakes beaches (two urban and two rural beaches ranging from degraded to pristine). E. coli concentrations were measured in ankle and waist deep surface water, foreshore porewater, and sand/sediment at 3-4 transects along each of the four beaches. Samples were taken bi-weekly from May to September 2013 with several of those trips including biogeochemical and extensive hydrogeological measurements. Results show that E. coli concentrations were significantly higher in the sand, sediment, porewater, and surface water at the more degraded urban beach. The concentrations in the foreshore sand and porewater were much higher than in the surface water at both urban beaches, suggesting that

this reservoir may act as a non-point source of E. coli. The ratio of E. coli associated with foreshore saturated sand to those in the foreshore pore water was significantly different at each beach signifying that methods for characterizing the nearshore reservoir may have to take into account specific physical parameters unique to each beach.

The Impact of Background Loadings: An Assessment of Contributions Using Edge-of-Field Studies and Watershed Scale Bacterial Source Tracking in Texas

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Additional Authors: Terry Gentry; George Di Giovanni; Lucas Gregory; Emily Martin; Daren Harmel; Elizabeth Casarez

The number one cause of water quality impairment in Texas is bacteria. According to the 2012 Texas Integrated Report for Clean Water Act Sections 305(b) and 303(d), recreation and oyster harvest in 272 waterbody segments in the State are impaired due to excessive levels of E. coli, Enterococcus, and fecal coliforms. In 2003, Texas initiated work to develop a bacterial source tracking (BST) library to help better assess bacterial sources. Since that time, the library has been populated with 1,669 E. coli isolates obtained from 1,455 different domestic sewage, wildlife, livestock and pet fecal samples collected from a dozen watersheds. Utilizing the statewide BST library, comprehensive BST is being conducted or has been completed to identify the source of bacterial impairments in 16 watersheds across the state. Throughout these studies, wildlife contributions have been found to be the predominant source of bacteria. Similarly, recent evaluations of edge-of-field runoff from grazed and ungrazed pastures have found background loading - loadings from wildlife and naturalized soilborne E. coli - to be significant. Background concentrations in runoff from sites evaluated ranged from 5,000 to 6,000 cfu/100 mL with median values ranging from 3,500 to 5,500 cfu/100 mL. When compared to the concentration allowed by the Texas Water Quality Standards (126 cfu/100 mL), these background concentrations are significant. Annual E. coli loading from ungrazed sites generally ranged from 4E 10 to 8E 10 cfu/ha. This background loading is not currently considered in water quality models and/or is considered 0. This presentation will summarize the findings from watershed scale BST and edge-of-field and small watershed runoff studies completed to date and discuss the implications of these findings on regulatory and non-regulatory water quality programs.

Metagenomic analysis of viral communities associated with irrigation water and fresh produce

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Additional Authors: Tiong Aw; Joan Rose

Introduction Foodborne outbreaks associated with fresh produce have become increasingly relevant in the United States. This indicates the vulnerability of our food systems to contamination and public health risk. Despite the increased importance of fresh produce as a vehicle for human pathogens, there is currently limited knowledge about where in the supply chain contamination occurs. Potential pre-harvest sources of contamination include irrigation water, soil, manure and human handling. The difficulty in identifying emerging new strains and tracking the contamination source is accentuated by the lack of methods to understand the change in microbial populations associated with fresh produce. Viruses are small biological nanoparticles and the host specificity of viruses suggests that they could be a promising library-independent tool to determine the major microbial sources in food systems, including

irrigation water. The rapid development of culture- and sequence-independent metagenomic technologies presents an opportunity for generating an improved understanding of the virus communities (virome) associated with fresh produce and irrigation water. Metagenomic approaches applied to study viral communities in environmental samples including sewage have demonstrated useful when comparing genomes and determining general presence and diversity. The objective of this study was to generate a comprehensive view of the virome in irrigation water and lettuce using an established method of virus recovery and metagenomics approach. Methods Irrigation water and lettuce samples for viral metagenomics were collected during the winter season in 2013. Viruses were concentrated from 100L of irrigation water using a low cost disposable hollow fiber ultrafiltration system. In addition, Colilert and Enterolert tests were performed to detect the presence of indicator bacteria Escherichia coli (E.coli) and enterococci accordingly. Romaine and head (iceberg) lettuce from small and large field operations were sampled both pre- and postharvest. Pre-harvest samples were cut with a sterile knife at ground level with the removal of outer leaflets and placed into whirl-pack bags. Post-harvest samples are hand cut by crew members and placed on a packaging machine for bagging. Virus recovery from lettuce included an elution step with Tris-glycine buffer at a pH of 9.5 and constant shaking followed by concentration of the virus particles by polyethylene glycol (PEG) and centrifugation. Samples were then passed through 0.22-_m filters and treated with DNase prior to viral nucleic acid extraction. Sequencing will be performed using Illumina HiSeq technology, allowing us to increase overall sequencing depth for larger sample sizes. Results and Discussion Seeded virus recovery experiments with Salmonella bacteriophage P22 and coliphage MS2 had an average recovery between 42 and 73 percent. Water samples were collected at 6 different locations along irrigation water supply canals. The concentrations of E.coli and enterococci ranged from 2.0 to 4.1 and 3.1 to 8.4 MPN/100 ml with a geometric mean of 3.3 and 4.9 MPN/100ml, respectively. A total of 42 romaine and head lettuce samples were collected. Six romaine and head lettuce samples were collected from a small field operation, half of which were pre and postharvest. A total of 15 romaine and head lettuce samples were taken from large field operations at different stages of harvesting including pre-harvest, postharvest (after worker break), and pre- and post-chopping. Irrigation water and lettuce samples are currently being processed for nucleic acid extraction and sequencing results will be presented.

Microbiological and chemical quality of packaged drinking water in Freetown, Sierra Leone

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Consumption of packaged drinking water (PW) sold in bottles and "sachets" (sealed plastic bags) has grown explosively in recent years, particularly in developing countries. While many perceive PW to be safer than other drinking water options, the quality of PW products is not effectively monitored in many developing countries, including Sierra Leone. The microbiological and chemical quality of PW in Freetown, Sierra Leone was assessed by collecting and analyzing a representative sample of PW products for Escherichia coli (E. coli), total coliforms (TC), and selected chemical parameters using standard methods. Samples of raw water, finished PW products at manufacturing facilities, and PW products at points of sale (POS) were collected. At manufacturing facilities, 47% of raw water samples (n=49) contained detectable E. coli (≥1 CFU/100 mL). By contrast, 18% of finished PW products sampled at these facilities contained detectable E. coli (≥1 CFU/100 mL). Of 36 manufacturers using municipal piped water from the main utility serving Freetown as their raw water source, finished PW samples had significantly lower E. coli concentrations than raw water samples ($p<0.05$). At POS, 35% of samples contained detectable E. coli (n=50). Samples from street vendors contained significantly higher E. coli

and TC concentrations than samples from shops ($p<0.05$), and the exteriors of samples from street vendors were also significantly more contaminated ($p<0.01$). PW products met national and international (WHO) standards for all chemical parameters studied except manganese and pH, however neither parameter poses major health risks at the levels measured in these samples. These results suggest that, while improvements are needed in the manufacturing, distribution, and regulation of PW in Freetown, PW may be safer than some municipal supplies. It is therefore recommended that household drinking water sources be sampled and analyzed as well, to better understand the water quality of PW products in context.

Effect of sunlight on the divergence of community structure of fecal bacteria in cowpats collected from three different farms

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Fecal pollution of environmental waters is a major concern for the general public because exposure to fecal-associated pathogens can have severe impacts on human health. In the last few years, numerous metagenomic studies applied next generation sequencing to understand the shift in microbial communities due to wastewater and sewage sludge treatment, antibiotic intake and nanoparticles exposure. However, the influence of sunlight, moisture and oxygen level on fecal bacterial diversity has rarely been investigated using metagenomic methods even though these stressors are some of the most important factors influencing the survival of bacteria in natural environments. In this study, we monitored bacterial community changes in cattle feces for 57 days after excretion (day 0, 2, 4 8, 15, 22, 29, 43, 57) by sequencing the 16s variable region 4, using Illumnia MiSeq. A total of 8 cattle feces from 2 different commercial farms (4 from each farm) were studied; half of the samples were directly exposed to sunlight (unshaded) and half were shaded. Results indicated that the relative abundance (RA) profiles at the class level in both shaded and unshaded samples at day 0 were similar, with Clostridia (~50%), Sphingobacteria (~10%), and Bacteroidia(~22%) being the most abundant bacteria. In the shaded samples, Gammaproteobacteria, Betaproteobacteria, Alphaproteobacteria, and Actinobacteria became the most dominant bacteria by day 57 and the diversity profiles between all shaded samples were very similar. However, for unshaded samples, the diversity profiles of samples collected from one farm diverge differently than the other, and by day 57 two farms had a significantly different community profile especially the RA of Bacilli, Actinobacteria and Alphaproteobacteria. Since the moisture content was relatively similar in all unshaded samples, and each sample was exposed to the same intensity of UV radiation, we speculate that the main reason causing different community profiles in the unshaded cowpats after 57 days was the breakdown of fecal materials to different organic compositions by UV radiation. Future studies should investigate the effect of UV radiation on nutrient and organic matter (OM) compositions in fecal material from different origins and identify the relationship between profiles of nutrient/OM and bacterial community. Overall, this study indicated that freshly excreted feces from different locations can have similar community profile but their profiles can become distinctly different after UV radiation and aging.

Recreational Beaches in Abu Dhabi City, United Arab Emirates.

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Additional Authors: James Cunningham; Glenn Whaley

This presentation will discuss a recent microbiological study of recreational marine waters in Abu Dhabi, United Arab Emirates. The study found that for recreational waters surrounding Abu Dhabi City, 99% of water samples were below action levels, defined as a level of enterococci >280 MPN per 100 mL sample. The study was under taken by the Environment Agency--Abu Dhabi (EAD), which is the entity charged with protecting water quality and public health. In keeping with international best practice for regular monitoring of recreational waters, EAD initiated a 12-week Pilot Marine Water Quality Monitoring Program (MWQMP) from April to June of 2012, to determine the status of potentially harmful pathogens (originating from domestic wastewater) in marine water at recreational beaches in Abu Dhabi, in order to better inform the Agency's planning for a long-term MWQMP. Water samples were collected on a weekly basis from 50 sites along 26 beaches located in Abu Dhabi City. At three of these beaches, water samples were collected from three sites each (for a total of nine sampling sites) three times per day, one day per week, in order to capture spatial and temporal impacts on water quality (variation assessment). In situ water measurements were taken at the time of collection and laboratory analysis of samples entailed quantification of the bacterial indicator enterococci for all samples, as well as total and fecal coliform bacteria for the samples from the variation assessment. In order to protect public health during the Pilot Program, an exceedance protocol was established that provided for follow up sampling in response to enterococci results >280 MPN per 100 mL sample at beaches with recreational users. Results from the laboratory analyses for enterococci showed eight exceedance events (>280 MPN per 100 mL sample) at four sites over the course of the 12-week Pilot Program. Out of the 899 samples analyzed, there were eight exceedances and therefore 99% of the samples were below action levels. Only two of the four exceedance sites were recreational use beaches, and follow-up testing the next day at one site showed bacterial counts dropped to below exceedance levels, whereas follow-up testing at the other site showed repeated exceedances. Concurrent testing of two drainage outfalls near this site showed the levels of enterococci in the drainage waters were high. The variation assessment component revealed one instance of a temporal variation over the course of one day that involved a moderately unhealthy water quality result (>140 and <280 MPN per 100 mL). The level of enterococci in the water during this sampling day varied from 86 to 161 to <10 MPN per 100 mL of sample during morning, noon, and afternoon collection times, respectively. The other two indicators (total and fecal coliforms) did not demonstrate a similar variation pattern. The results from microbial monitoring performed during this study have been used to evaluate risks and guide decisions regarding routine beach monitoring requirements and actions to protect public health at recreational beaches in Abu Dhabi City.

Biofilm formation by Escherichia coli strains persistent in tropic soil environments: evidence for niche adaptation?

Tao Yan, University of Hawaii

Additional Authors: Qian Zhang; Xingru Zhang; Yi Zuo

High levels of E. coli were frequently detected in tropical soils in Hawaii, which present important environmental sources of E. coli to waterbodies. In previous studies, we have isolated E. coli strains from

soil samples in the Manoa watershed on the Island of Oahu, Hawaii, and rep-PCR genotyping indicated several strains were persistent in the soil. A previous study also showed that these soil E. coli strains exhibited higher desiccation resistance than the lab K-12 strains and strains isolated from municipal sewage. In this study, we test the hypothesis that these soil E. coli strains are more capable of establishing biofilm, which would have contributed to their persistence in tropic soil environments. Cell surface energy measurement found the soil E. coli strains to be significantly more hydrophobic than lab K-12 strains, environmental E. coli strains isolated from water, and wastewater E. coli strains. Biofilm formation capability of these E. coli strains are currently being determined, and the presence/absence of genes involved in biofilm formation will also be tested.

Impact of Indigenous Sand Microbiota on the Decay of Fecal Indicator Bacteria and Actual Bacterial Pathogens

Tao Yan, University of Hawaii

Additional Authors: Quian Zhang

Fecal contamination of recreational water can adversely impact the public health and economic functions of many coastal communities. The current recreational water management practices focus primarily on water itself, while recent studies have found that other system components can also affect water quality. The objectives of this study were to determine (1) whether beach sand microbiota can enhance the decay of exogenous fecal indicator bacteria (FIBs), and (2) whether FIBs and actual bacterial pathogens follow similar decay patterns between beach sand and seawater. The decay patterns of exogenous Enterococcus faecalis cells in laboratory beach microcosms for three beaches in Hawaii were determined, and beach sand indigenous microbiota was identified to the major factor correlating to the bacterial decay. Subsequent experiments observed that higher indigenous microbiota corresponded to faster bacterial decay. Comparison between the two major beach system components (beach sand and seawater) indicated that the indigenous microbiota in beach sand played a significant role in bacterial decay. Manipulating two important beach characteristics (sand-to-water ration and sand particle size) also resulted in different bacterial decay rates. Actual municipal wastewater was also spiked into beach microcosms containing only sand, only seawater, or both sand and seawater. The FIBs (entercocci, E. coli, and C. perfringens) and actual human pathogens (Salmonella sp. and Vibrio sp.) exhibited different decay patterns in beach sand and seawater. The dynamics of microbial communities in the wastewater-contaminated beach sand and seawater are being determined by using 16S rRNA gene Illumina sequencing.

Effects of water stratification on microbial abundance and activity, bacterial community structure and ecosystem function

Zheng Yu, Institute of Urban Environment, Chinese Academy of Sciences

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Effects of water stratification on microbial abundance and activity, bacterial community structure and ecosystem function Zheng Yu 1,2, Jun Yang 1*, Lemian Liu 1 1Aquatic EcoHealth Group, Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, P. R. China; 2University of the Chinese Academy of Sciences, Beijing 100049, P. R. China. Microorganisms are well known to serve as the principal producers, consumers and decomposers, and

they play pivotal roles in maintain freshwater ecosystem health. However, little is known about how these functional groups coexist with each other in aquatic environment, particularly in stratified reservoirs. In this study, we describe the nature of microbial communities in a subtropical deep reservoir (Dongzhen Reservoir, southeast China). Clone library, 454 pyrosequencing and quantitative real-time PCR were used together to facilitate an in-depth investigation of the community structure of bacteria, phytoplankton, zooplankton, fungi and nitrogen-cycle related bacteria. Our results showed that thermal and oxygen stratification shaped the composition of the microorganisms in the reservoir. Stratification was evident among ecological functional groups: producers (phytoplankton) and consumers (zooplankton) were overwhelmingly dominant in the epilimnion, while decomposers (fungi) were inclined to inhabit the hypolimnion. The RNA: DNA ratios of 16S rRNA gene were significantly lower in epilimnion and metalimnion (8.64_1.75 and 3.01_0.64, respectively) and rapidly increased in hypolimnion (23.17_3.28, $P < 0.05$), suggesting that bacterial communities were more active in the low temperature, low dissolved oxygen and high TN/TP ratio water zones. For the denitrifier communities, α-, β- and γ- Proteobacteria were the overwhelmingly dominant phyla in the denitrifier communities, each functional gene had its own dominant groups which were different at the genus level. For the diazotrophic bacterial communities, Cyanobacteria, affiliated to the toxic bloom-forming Cylindrospermopsis raciborskii, was the dominant diazotrophic cluster in the surface waters, whereas diazotrophic Alphaproteobacteria were dominant in the bottom waters. These results contribute to our understanding of the relationship of ecosystem functional groups in the man-made aquatic systems and have important practical implications for reservoir management. Results suggest that the strategies for the control of eutrophication and harmful algal bloom prevention should focus on a fuller understanding of the consequences of both thermal stratification and vertical distribution of microorganisms.
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Development of a Rapid Enrichment and Multiplex qPCR F+ Coliphage Detection Method for Water Quality Monitoring

Yvonne Yuen, UNC Chapel Hill

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Introduction: Bacterial fecal indicators are commonly used for recreational water quality monitoring. However, quantitative microbial risk assessment demonstrated that human enteric viruses were most likely the pathogens that caused the majority of the swimming related illnesses observed in epidemiology studies conducted in the U.S. Furthermore, some epidemiology studies have shown fecal indicator viruses are predictive of adverse health effects from primary contact exposures. Therefore, testing for fecal indicator viruses can enhance recreational water quality monitoring efforts. Another limitation for current recreational water quality monitoring practice is that most methods take 18 hours or more for results. Microbial water quality can change quickly and monitoring results from samples obtained a day ago can potentially expose many to unnecessary risk or in contrast, unneeded beach closures may be made that result in economic loss. Rapid methods are needed for timely responses and coliphages are ideal candidates. This is because the presence of coliphages is shown to be associated to adverse health outcomes for swimmers and can produce more than 10,000 PFU/mL of progeny phages within a few hours, which then can be detected by molecular assays. The development of a rapid coliphage method is the basis of this research. Methods: Three different growth media: Tryptic Soy Broth (TSB), Lauria Broth (LB) and minimal media M9 with 20% glucose are evaluated for an optimized

rapid liquid enrichment procedure to propagate the few F+ coliphages in a water sample to >10,000 PFU/L progeny phages after 3 hours. Then, coliphage presence is confirmed by multiplex quantitative polymerase chain reaction (qPCR). F+ RNA coliphages are reverse transcribed into cDNA before a multiplex qPCR assay that simultaneously detects: F+DNA coliphage, F+RNA coliphages of genogroup (GG) I, II, III and IV and III and an internal amplification control. Freshwater, seawater and spiked water samples are analyzed. Results: F+ coliphage rapid enrichment was possible within 3 hours, with TSB and M9-glucose the best medium for MS2 (GG I), but TSB and LB the better media for GA (GG II) and QB (GG III), and all 3 media achieving about the same concentrations for SP (GG IV). FDNA growth assessment and water sample analyses by qPCR are ongoing but qPCR detection has been achieved in ~2.5 hours. Conclusions: Liquid culture enrichment can produce enough F+ coliphages in 3 hours to be detectable by qPCR in approximately 2.5 hours, and the whole procedure takes around 6 hours to complete. This new F+ coliphage detection method can potentially provide accurate and timely assessment for recreational water quality monitoring, if performance is further confirmed by ongoing studies from which results will be reported.