Posters Presentations

False Positives Associated with Enterococcus Quantification Using EPA-Approved Methods for Industrial Discharge

Presenter: Emelie Andersson
University of North Carolina, Chapel Hill, Institute of Marine Sciences

Authors: Emelie Andersson, Angelia Blackwood, Kellen Lauer, Rachel Noble

Abstract

Industrial wastewater discharged into recreational waters is routinely monitored for Enterococcus sp., fecal coliforms, or *E. coli*. The most commonly used culture-based, U.S. Environmental Protection Agency- (EPA-) approved methods for quantification of Enterococcus sp. are EPA method 1600 and Enterolert*. For this study, discharge samples were collected twice per month for 1 year and quantified by EPA 1600 and Enterolert. Additionally, a suite of qPCR analyses were conducted on the samples, including total Enterococcus (EPA 1611), quantification of specific Enterococcus species, *E. faecalis*, *E. faecium*, and *E. casseliflavus*, and quantification of three human-associated Bacteroidales-based markers (Fecal Bacteroides, HF183, and BacHum). Published guidelines were closely followed to ensure quality quantitative data. Enterococcus sp. concentrations ranged from 109–2,070 (mean=819) CFU/100 ml and 86,207–5,246,223 (mean=719,679) CE/100 ml as determined by EPA 1600 and EPA 1611, respectively. There was no correlative relationship between EPA 1600 and 1611 (r=-0.10). Concentrations of *E. faecalis* and *E. faecium* were below detection limit for 10 of 24 samples (42%). None of the human-associated markers were quantifiable in any of the samples. Twenty isolates were selected from each EPA 1600 plate for confirmation using Vitek-2 (Biomerieux, Inc.), and the results indicated that the fecally associated Enterococcus sp. were not strongly present, while 42% of the isolates were identified as non-Enterococcus species. This finding was supported by the molecular analyses. Interestingly, plant and dairy-associated species (such as *Aerococcus viridans*) dominated the characterized species. The results indicate that for certain types of industrial discharge, Enterococcus sp. quantification is a questionable proxy for presence of fecal contamination.

Biosketch

Ms. Emelie Andersson graduated in 2013 with a bachelor of science degree in biology from Mount Olive College, North Carolina, where she also was a student athlete. Since March 2014, she has been working as a research technician in Dr. Rachel Noble's laboratory at the Institute of Marine Sciences of the University of North Carolina at Chapel Hill in Morehead City, North Carolina. Ms. Andersson has an interest in microbial source tracking and how it impacts public health.

Application of Hydrodynamic Modeling to Predict Viral Impacts from Wastewater Treatment Plant Discharges Adjacent to Shellfish Growing Areas

Presenter: Yaping Ao
U.S. Food and Drug Administration

Authors: Yaping Ao, Gregory Goblick

Abstract

Since the inception of the National Shellfish Certification Program in 1925—now the National Shellfish Sanitation Program—dilution analysis has been used as a means to minimize the presence of enteric pathogenic microorganisms in shellfish growing areas. Today, failures and bypasses at wastewater treatment plants (WWTPs) and combined sewer overflows (CSOs) that release untreated or partially treated sewage into shellfish receiving waters are of particular interest to shellfish control authorities and public health officials. To assess the risk posed by wastewater effluents, the U.S. Food and Drug Administration (FDA) has used several approaches, including hydrodynamic modeling, to predict the extent of sewage impacts on receiving waters. Models used by FDA were calibrated and validated by hydrographic dyes studies and microbiological analyses. The simulated results are extremely helpful to shellfish management authorities for determining appropriate classification and management for shellfish growing areas. Hydrodynamic modeling also has proven to be a valuable tool for a current joint United States/Canada quantitative norovirus risk assessment. FDA and the National Oceanic and Atmospheric Administration will be collaborating on efforts to develop ecoforecasting capabilities using modeling and weather (precipitation) forecasts to predict sewage impacts on shellfish growing areas that occur from WWTP bypasses and CSOs during storm-related events.

Biosketch

Ms. Yaping Ao is a visiting associate with the U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition in College Park, Maryland. She serves as a lead modeler in the application of computer fate and transport models to assess pollution source impacts to shellfish growing areas. She assisted with the development of dilution models to support a joint U.S.-Canada norovirus risk assessment and has provided training on and led specialized field and hydrographic studies to identify and assess pollution sources in the environment. Ms. Ao's expertise includes supporting the development of guidance for irrigation water use for produce safety. She received her master of science degree in civil engineering from Marquette University in Wisconsin and her bachelor of science degree in environmental engineering from the Chengdu University of Technology, China.

Beach Sand and the Potential for Infectious Disease Transmission: Observations and Recommendations

Presenter: João Brandão, PhD
National Institute of Health
Department of Environmental Health - Water and Soil Unit

Authors: Helena Solo-Gabriele, João Brandão

Abstract

Recent studies suggest that sand can serve as a vehicle for exposure of humans to pathogens at beach sites, resulting in increased health risks. Sampling for microorganisms in sand should, therefore, be considered for inclusion in regulatory programs aimed at protecting recreational beach users from infectious disease. We reviewed the literature on pathogen levels in beach sand and their potential for affecting human health. In an effort to provide specific recommendations for sand sampling programs, we outline published guidelines for beach monitoring programs, which are currently focused exclusively on measuring microbial levels in water. We also provide background on spatial distribution and temporal characteristics of microbes in sand, as these factors influence sampling programs. First steps toward establishing a sand sampling program include identifying appropriate beach sites and using initial sanitary assessments to refine site selection. A tiered approach is recommended for monitoring, which would include analyzing samples from many sites for fecal indicator organisms and other conventional analytes, while testing for specific pathogens and unconventional indicators would be reserved for high-risk sites and possible outbreaks. Given the diversity of microbes found in sand, studies are urgently needed to identify the most significant etiological agent of disease and to relate microbial measurements in sand to the risk to human health. (J Marine Biol Ass UK, 2015

Biosketch

Dr. João Brandão is an applied chemist-biotechnologist from the New University of Lisbon, Portugal, specializing in clinical mycology and environmental research. He has conducted research in those two areas of expertise since 2001 and lectured on them at Lusófona University in Lisbon. Mr. Brandão is the environmental microbiology research manager at the Department of Environmental Health of the National Institutes of Health, a national delegate of the Portuguese Association of Medical Mycology to the European Confederation of Medical Mycology, (council member) and national member of the European Microbiology Research Group for the European Commission (Bathing Water Directive). Of special interest to Dr. Brandão are microbial pathogen contaminants in sand, air, and water; nosocomial and endemic fungal infections of environmental origin; and prevention and early molecular detection. Other special interests include water contaminants and quantitative microbial risk assessment in water quality and microbial source tracking tools (for point-source pollution detection and resolution).

Risk Levels of Toxic Cyanobacteria in Portuguese Recreational Freshwaters

Presenter: João Brandão, PhD
National Institute of Health

Authors: Carina Menezes, Catarina Churro, João Brandão, Elsa Dias

Abstract

In this work, we present the results from monitoring cyanobacteria and associated toxins in several recreational freshwaters from central and southern Portugal. We identified two distinct scenarios: (a) reservoirs that never exhibited potentially toxic cyanobacteria; and (b) reservoirs that showed a persistent occurrence of cyanobacterial blooms, often associated with the presence of microcystins. Comparing our results with the guideline values for cyanobacterial cells in bathing waters and the corresponding expected microcystin levels, we determined that 30% of the reservoirs had high risk for public health. However, the cyanobacterial cell density criterion might overestimate the risk because it includes nontoxic strains of potentially toxic species. Also, this criterion is based on the worst scenario for toxin cell production quota, which might be higher than the real toxin cell content detected in cyanobacterial bloom samples. In fact, the risk level decreases considerably when we compare the real microcystin concentrations in water samples with the expected values. The applicability of these criteria in risk assessment of cyanobacteria and cyanotoxins in recreational freshwaters is discussed.

Biosketch

Dr. João Brandão is an applied chemist-biotechnologist from the New University of Lisbon, Portugal, specializing in clinical mycology and environmental research. He has conducted research in those two areas of expertise since 2001 and lectured on them at Lusófona University in Lisbon. Mr. Brandão is the environmental microbiology research manager at the Department of Environmental Health of the National Institutes of Health, a national delegate of the Portuguese Association of Medical Mycology to the European Confederation of Medical Mycology, (council member) and national member of the European Microbiology Research Group for the European Commission (Bathing Water Directive). Of special interest to Dr. Brandão are microbial pathogen contaminants in sand, air, and water; nosocomial and endemic fungal infections of environmental origin; and prevention and early molecular detection. Other special interests include water contaminants and quantitative microbial risk assessment in water quality and microbial source tracking tools (for point-source pollution detection and resolution).

Stretching Budgets with Composite Sampling

Presenter: Shannon Briggs, PhD
Michigan Department of Environmental Quality

Author: Shannon Briggs

Abstract

The advantage of using composite samples is that combining multiple samples provides an indication of the water quality at the cost of one sample. In general, results for composite samples are equivalent to taking the arithmetic mean of the analytical results of each of the samples that comprise the composite. Mathematically, an arithmetic mean is never less than and is generally greater than a geometric mean, although this is not always true in real-world sampling. A composite beach sample could potentially result in more beach closings and advisories than would a geometric mean for the same beach.

Arithmetic and geometric means, however, tend to be similar for samples from beaches that have homogeneous historical monitoring data. Homogeneity can be determined with the Kruskal-Wallis test, which is a nonparametric test equivalent to a one-way analysis of variance that determines whether there are between-site differences. Furthermore, an arithmetic or geometric mean is less likely to exceed criteria by selecting beaches that have homogeneity and consistently low bacteria levels.

Wymer et al. (2005), Kinzelman et al. (2006), Bertke (2007), Wymer and Wade 2007, Reicherts and Emerson (2010), and USEPA (2010) support the use of composite sampling to characterize the mean indicator density and encourage more sampling while minimizing costs. In Michigan, composite sampling is the cost-effective approach for implementing qPCR methods. Results from culture and qPCR methods on composite samples will be evaluated to establish correlations between the two methods.

Biosketch

Dr. Shannon Briggs is a toxicologist for the Water Resources Division of the Michigan Department of Environmental Quality (DEQ). She received her bachelor of science degree in animal science and her doctorate in pharmacology and toxicology from Michigan State University. She is a member of a planning team that will host the 2016 Great Lakes Beach Conference in Marquette, Michigan, October 5–7, 2016. Dr. Briggs assists local health departments with state and federal grants for monitoring beaches across the State of Michigan. She is leading a water quality initiative of the DEQ to provide rapid testing equipment and training for 10 new laboratories that will test beaches using the U.S. Environmental Protection Agency's draft Method C (i.e., qPCR method for *E. coli*). Dr. Briggs is an active member, past president, and cofounder of the Great Lakes Beach Association.

Pollution Source Identification, Tracking, and Sanitary Survey on Italian Beaches

Presenter: Annalaura Carducci
University of Pisa

Author: Annalaura Carducci, Marco Verani, Ileana Federigi, Renato Iannelli

Abstract

In Italy, sea-bathing tourism is a very important socioeconomic resource, but it is being impacted by the rapid urbanization of the coastal environment, which represents pollution pressure on water quality with point and diffuse sources of fecal contamination. This study was carried out at Versilia, a popular bathing destination northwest of Tuscany, where short-term pollution posed the problem of possible classification as "scarce" (according to the current European Bathing Water Directive) because of fecal contamination from drainage ditches. Our goal was to understand the impact of polluted streams on seawater contamination and the effect of meteorological conditions on freshwater and seawater bacterial indicator levels. The monitoring results from the 2012 to 2015 bathing seasons were analyzed and, only for 2015, we used cultural and biomolecular techniques to detect fecal-oral pathogens and viral indicators at ditch mouths. Our results demonstrate a relationship between bacterial indicator levels and rainfall amount; however, microbiological pollution also was highly variable in dry weather, suggesting the presence of undetected sources of fecal contamination. Collected data from seawater and river mouths show a time-dependent dilution effect of the sea that varied between the two halves of each bathing season. During 2015—the last year of monitoring—besides bacteria indicators, we found only the human adenovirus genome. This analytical survey of the pollution sources in Versilia recreational water could be used to create larger monitoring data sets for developing predictive models of microbial contamination in relation to climatic conditions and disinfection intervention

Biosketch

Ms. Carducci is a professor of general and applied hygiene in the Department of Biology at the University of Pisa, where she graduated with bachelor's and master's degrees in biology, specializing in hygiene and public health. Prof. Carducci supervises the Biology Department's Hygiene and Environmental Virology Laboratory and Health Communication Observatory. The focus of her teaching activity as well as her research includes microbiological monitoring of food and environmental matrices and biological risk analysis for life and working settings and for foods, health, and risk communication.

Expanding EPA Method 544: Addition of Seven Microcystin Congeners for Analysis of Lake Erie Beach Samples, a Comparative Study with ELISA

Presenter: Mark Citriglia
Northeast Ohio Regional Sewer District

Authors: Cheryl Soltis-Muth, Deborah Schordock

Abstract

Thirty-seven Lake Erie beach water samples from the summer of 2015 were analyzed for microcystins by U.S. Environmental Protection Agency (EPA) method 544 using solid phase extraction (SPE) and analysis on a Shimadzu LCMS-8050 Liquid Chromatograph Tandem Mass Spectrometer (LC/MS/MS). These same samples were lysed by a series of three freeze/thaw cycles and filtered. The resulting extracts were analyzed by ELISA and also by directly injecting them into the LC/MS/MS with the same operating conditions as with EPA method 544. In 19 cases, the ELISA result was greater than the sum of the individual microcystin results from EPA method 544 (SPE & LC/MS/MS). In all except one of the 19 instances, the ELISA also was greater than the sum of the individual microcystin results from the direct inject LC/MS/MS method. This indicates that there may be additional microcystin congeners in the samples that are not being detected by EPA method 544.

Seven additional microcystin congeners were added to the EPA method 544 analysis. The beach water extracts from 2015 will be rerun to determine if the difference between the ELISA and EPA method 544 results is due to microcystin congeners previously untested by the method.

Future work includes validating the SPE extraction procedure in EPA method 544 with the additional microcystin congeners and continuing the ELISA and LC/MS/MS method comparison study into 2016.

Biosketch

Mr. Mark Citriglia is currently the manager of analytical services for the Northeast Ohio Regional Sewer District (NEORSD). Mr. Citriglia has a bachelor of science degree in biology with a minor in chemistry from Cleveland State University and is a certified State of Ohio wastewater class III operator and a class IV certified wastewater analyst. He has worked for NEORSD for 27 years, beginning his career there as a wastewater analyst in the Analytical Services Department (laboratory), successfully gaining the skills and knowledge necessary to advance to managing the lab. As the laboratory manager for the past 12 years, Mr. Citrigilia has been committed to operating the lab in a safe, professional, and proficient manner, generating data of known and documented quality. He voluntarily adopted the quality policies and procedures outlined by the National Environmental Laboratory Accreditation Program, obtaining accreditation in 2007. Currently, he is expanding the lab's capabilities to include a research and development department along with additional accreditations. Mr. Citriglia is a member of the Water Environment Federation (WEF), NELAC Institute, and Lab Safety Institute. He was the recipient of the 2007–2008 Northeast Section Ohio Water Environment Association Lab Analyst Award, 2009 Ohio Water Environment Association Lab Analyst Award, and 2010 WEF Laboratory Excellence Award.

Make It "Tough:" qPCR Master Mix Comparison

Presenter: Mark Citriglia
Northeast Ohio Regional Sewer District

Author: Nichole Schafer

Abstract

Within the 2012 Recreational Water Quality Criteria guidelines, the U.S. Environmental Protection Agency (EPA) provides information for states that want to adopt water quality standards based on rapid methods that EPA has developed and validated using qPCR. Currently, EPA has validated methods 1609 and 1611 for the quantification of Enterococcus in environmental waters using qPCR. The Northeast Ohio Regional Sewer District laboratory collected samples from two northeast Ohio beaches and analyzed them using EPA method 1609 and a qPCR assay for *E. coli*. The objectives of this study were to identify a master mix that (1) could be used for both assays, (2) reduced inhibition, (3) is similar to the Environmental Master Mix (EMM) recommended by EPA, (4) uses an internal amplification control allowing for analysis on the same platform, and (5) contains a purified DNA polymerase that is validated to be free of extraneous *E. coli* DNA. A total of 241 samples were analyzed for both organisms using two different chemistries, EMM as stated in method 1609 and Tough Mix. The data for each organism and master mix combination were compared for inhibition, method QC, level of quantification, and difference between assay absolute and relative quantitation. Accuracy, sensitivity, and specificity were calculated as compared to the conventional method. In conclusion, we have determined that the Tough Mix yielded data comparable in accuracy and reproducibility to the EPA-recommended mix, but the level of inhibition and instance of positive QC data decreased with the Tough Mix.

Biosketch

Mr. Mark Citriglia is currently the manager of analytical services for the Northeast Ohio Regional Sewer District (NEORSD). Mr. Citriglia has a bachelor of science degree in biology with a minor in chemistry from Cleveland State University and is a certified State of Ohio wastewater class III operator and a class IV certified wastewater analyst. He has worked for NEORSD for 27 years, beginning his career there as a wastewater analyst in the Analytical Services Department (laboratory), successfully gaining the skills and knowledge necessary to advance to managing the lab. As the laboratory manager for the past 12 years, Mr. Citrigilia has been committed to operating the lab in a safe, professional, and proficient manner, generating data of known and documented quality. He voluntarily adopted the quality policies and procedures outlined by the National Environmental Laboratory Accreditation Program, obtaining accreditation in 2007. Currently, he is expanding the lab's capabilities to include a research and development department along with additional accreditations. Mr. Citriglia is a member of the Water Environment Federation (WEF), NELAC Institute, and Lab Safety Institute. He was the recipient of the 2007–2008 Northeast Section Ohio Water Environment Association Lab Analyst Award, 2009 Ohio Water Environment Association Lab Analyst Award, and 2010 WEF Laboratory Excellence Award.

qPCR: A Screening Tool for Harmful Algal Blooms

Presenter: Mark Citriglia
Northeast Ohio Regional Sewer District

Authors: Nichole Schafer, Mark Citriglia

Abstract

Lake Erie has seen an increase in the number and severity of harmful algal blooms (HAB) caused by cyanobacteria (blue-green algae). The cyanobacteria present in the HABs can potentially produce toxins capable of causing illness and/or death. Timely and accurate identification and reporting of these toxins is critical for issuing water quality advisories. The methods used to analyze toxin are very expensive, and selecting the correct one can be difficult. Another challenge is the necessity for a skilled analyst to be available to identify the microscopic algae. Furthermore, some cyanobacteria can produce multiple toxins (e.g., the genus Anabaena can produce anatoxin-a, saxitoxin, and microcystin; the genus Aphanizomenon can produce saxitoxin, anatoxin-a, and clylindrospermopsin). The Northeast Ohio Regional Sewer District (NEORSD) decided to experiment with a rapid method using qPCR to screen for a total cyanobacteria gene and specific toxin-producing genes (i.e., microcytins, saxitoxin, and cylindrospermopsin). The NEORSD laboratory experimented with the Phytoxigene, CyanoDTec qPCR assays to screen samples submitted for cyanotoxin analysis. A portion of the sample submitted for analysis was filtered, and the DNA was extracted and analyzed on multiple qPCR platforms. The results of our study indicate that this method has the potential to eliminate the need for microscopic examination and to simplify the process of selecting the appropriate method for toxin analysis.

Biosketch

Mr. Mark Citriglia is currently the manager of analytical services for the Northeast Ohio Regional Sewer District (NEORSD). Mr. Citriglia has a bachelor of science degree in biology with a minor in chemistry from Cleveland State University and is a certified State of Ohio wastewater class III operator and a class IV certified wastewater analyst. He has worked for NEORSD for 27 years, beginning his career there as a wastewater analyst in the Analytical Services Department (laboratory), successfully gaining the skills and knowledge necessary to advance to managing the lab. As the laboratory manager for the past 12 years, Mr. Citrigilia has been committed to operating the lab in a safe, professional, and proficient manner, generating data of known and documented quality. He voluntarily adopted the quality policies and procedures outlined by the National Environmental Laboratory Accreditation Program, obtaining accreditation in 2007. Currently, he is expanding the lab's capabilities to include a research and development department along with additional accreditations. Mr. Citriglia is a member of the Water Environment Federation (WEF), NELAC Institute, and Lab Safety Institute. He was the recipient of the 2007–2008 Northeast Section Ohio Water Environment Association Lab Analyst Award, 2009 Ohio Water Environment Association Lab Analyst Award, and 2010 WEF Laboratory Excellence Award.

Rapid and Efficient Extraction Method of Chlorophyll by Bead Beating

Presenter: Mark Citriglia
Northeast Ohio Regional Sewer District

Authors: Debmalya Bhattacharyya, Mark Citriglia

Abstract

Estimation of chlorophyll *a* has been routinely applied to determine algal and phytoplankton levels in marine and freshwater to assess the eutrophic status and, thereby, monitor water quality. The existing method extracts chlorophyll *a* from glass fiber filters by manual mastication in the presence of cold 90% acetone. The manual mastication of the filters is a prolonged and arduous process that allows processing of only two samples (in triplicates) in 20 minutes (i.e., about 3 minutes on average for processing each glass fiber filter). An alternative method of bead beating the glass fiber filters is using an Omni bead ruptor 24. The new method significantly reduced the time of processing of two samples analyzed in triplicates. Comparative analyses of the new and existing extraction methods were conducted keeping all other processing parameters constant and the chlorophyll *a* content was determined. Comparison of the results from the two methods was statistically analyzed by Passing and Bablok regression, which suggests that there were no significant systematic or proportional differences between the methods of extraction. It also was noted that the standard deviations among the data obtained by the bead beating method were less than by the existing method, which probably is due to less handling being required. We conclude that the application of bead beating of the glass fiber filters can be used with better efficiency to routinely process chlorophyll *a* samples.

Biosketch

Mr. Mark Citriglia is currently the manager of analytical services for the Northeast Ohio Regional Sewer District (NEORSD). Mr. Citriglia has a bachelor of science degree in biology with a minor in chemistry from Cleveland State University and is a certified State of Ohio wastewater class III operator and a class IV certified wastewater analyst. He has worked for NEORSD for 27 years, beginning his career there as a wastewater analyst in the Analytical Services Department (laboratory), successfully gaining the skills and knowledge necessary to advance to managing the lab. As the laboratory manager for the past 12 years, Mr. Citrigilia has been committed to operating the lab in a safe, professional, and proficient manner, generating data of known and documented quality. He voluntarily adopted the quality policies and procedures outlined by the National Environmental Laboratory Accreditation Program, obtaining accreditation in 2007. Currently, he is expanding the lab's capabilities to include a research and development department along with additional accreditations. Mr. Citriglia is a member of the Water Environment Federation (WEF), NELAC Institute, and Lab Safety Institute. He was the recipient of the 2007–2008 Northeast Section Ohio Water Environment Association Lab Analyst Award, 2009 Ohio Water Environment Association Lab Analyst Award, and 2010 WEF Laboratory Excellence Award.

Child Exposure to Water and Sand at the Beach: Findings from Studies of over 80,000 Subjects at 13 Beaches

Presenter: Stephanie DeFlorio-Barker, PhD
U.S. Environmental Protection Agency, Human Studies Facility

Authors: Stephanie DeFlorio-Barker, Benjamin F. Arnold, John M. Colford Jr., Steve Weinberg, Ken Schiff, Elizabeth Sams, Alfred Dufour, Timothy J. Wade

Abstract

Swimming and recreating in lakes, oceans, and rivers are common activities among adults and children; some studies have suggested that children could be at greater risk of illness than adults following such exposures. These effects could be caused by differences in immunity or differing behavioral factors such as poorer hygiene, as well as longer exposures to and greater ingestion of potentially contaminated water and sand. We pooled data from 13 prospective cohorts to examine exposures to potentially contaminated media such as beach water and sand among children compared to adults, and summarized time spent in the water among swimmers (n=48,573) by age category. Age categories were younger than 1 year, 1–3 years, 4–7 years, 8–12 years, 13–18 years, 19–34 years, and 35 years and older. Children aged 4–7 and 8–12 had the highest exposures at the beach of any of the age groups. Among children aged 8–12, 27% swallowed water, 63% dug in the sand, and 17% got sand in their mouth, compared to 6%, 22%, and 5% of adults 35 and older, respectively. Across all beach sites, children 8–12 spent 122 minutes in the water (SD= 85 minutes), while adults aged 35 and over spent 75 minutes in the water (SD=68 minutes). All exposures reported among children were statistically significantly greater than those reported among adults ($p < 0.001$). These findings and estimates might help inform quantitative microbial risk assessments to better estimate health risks associated with recreational water exposures.

Note: This abstract does not represent EPA policy.

Biosketch

Dr. Stephanie DeFlorio-Barker is a postdoctoral research fellow at the U.S. Environmental Protection Agency. She received her bachelor of science degree in molecular and cellular biology from the University of Illinois at Urbana-Champaign, and her master of public health degree in epidemiology and doctorate in environmental and occupational epidemiology from the School of Public Health at the University of Illinois at Chicago. Dr. DeFlorio-Barker's research interests include water quality, economic burden of environmental exposures, risk assessment, and communication of environmental health risks. For her doctoral dissertation, she conducted research on the severity and economic burden associated with surface water recreation in the United States.

Recreational Water Quality in Massachusetts: Historical Inventory of Freshwater and Marine Beach Surveillance from 2001–2015

Presenter: Irena Draksic
Massachusetts Department of Public Health, Bureau of Environmental Health

Authors: Michael Celona, Vanessa Curran, Irena Draksic, Michael Beattie, Margaret Round, Marc Nascarella

Abstract

Water quality data in Massachusetts represents a rich resource for understanding recreational water quality dynamics. With over 1,100 freshwater and marine beaches, Massachusetts has the greatest number of beaches in the northeastern United States. The Massachusetts Department of Public Health has been monitoring bacterial levels at these beaches since 2001, where they have collected a robust data set of over 200,000 individual water quality samples. This data set represents a robust historical inventory of laboratory results from individual water quality samples, coupled with field observations recorded by the sample collector. For example, in addition to data describing bacterial levels (bacterial levels in colony-forming units per 100 ml), this inventory includes parameters such as temperature, bather density, and rainfall. These samples were all collected during the meteorological summer, at a frequency of approximately once per week. Multiyear data analyses suggest that exceedances of water quality standards occur less frequently now, as compared to previous years. For example, of 14,874 samples collected at 1,086 beaches in 2014, approximately 3.5% of beaches exceeded the Enterobacteriaceae bacteria standard. Historically, about 5% of beaches exceed the respective marine or freshwater bacterial standard. Our field-collected data and field observation suggest that the majority of exceedances are directly related to rain events and likely the result of increased surface runoff. The data described here will be used in future efforts to understand the relationship between climate-related changes in rainfall and the health impacts of potential increased exposure to fecal bacteria.

Biosketch

Ms. Irena Draksic is a beach inspector at the Massachusetts Department of Public Health, Bureau of Environmental Health's Environmental Toxicology Program (ETP). She has been with the department for 11 years. Ms. Draksic manages beach data and closures during the bathing beach season, assists with sanitary surveys and freshwater algae bloom sampling, and provides training and technical support to contract laboratories and local communities. She has a bachelor of science degree in biology from Denison University and a master of arts degree in environmental science and policy from Clark University.

Preparing for New Water Quality Standards at NYC Beaches: Retrospective Evaluation of Water Quality Data and Updating Hydrodynamic Models

Presenter: Charles Dujardin
Great Lakes Environmental Center

Authors: Christopher Boyd, Anand Kumaraswamy, Charles Dujardin

Abstract

The New York City (NYC) Department of Health and Mental Hygiene (DOHMH) is responsible for monitoring and surveillance of all of the city's permitted beaches. The U.S. Environmental Protection Agency's (EPA's) 2012 Recreational Water Quality Criteria will impact beach notification in NYC because of its more conservative geometric mean, new statistical threshold value capturing long-term risk, and new beach action value alerting the public before water quality declines to unacceptable levels. DOHMH conducted a retrospective analysis of beach monitoring data to analyze the potential increase of advisory and closure days if the new 2012 criteria had been in effect. The study found on average a 31-day increase in the number of pollution advisory days per year and a 6-day increase in the number of closure days per year. These increases were not uniformly distributed across the city's beaches. The implications of the new criteria include the need for increased beach program staffing for more frequent sampling and risk communication and education outreach.

Additionally, to better anticipate poor water quality as a result of rain events, DOHMH and the NYC Department of Environmental Protection (DEP) revisited the advisory "triggers" using a hydrodynamic model developed by Stevens Institute and mathematical kinetics previously developed by DEP. Regression curves were developed between duration of hours the model exceeded 110 colony-forming units/100 milliliters and rainfall volume. From these regressions, advisory triggers and "durations" can be developed for each beach. For a given rainfall volume, expected time and duration of rainfall, and expected duration of exceedance, an assessment can be conducted to determine whether an advisory should be issued for a particular day.

Biosketch

Mr. Charles Dujardin received his bachelor's degree in chemical engineering and master's degree in environmental engineering from Manhattan College. Mr. Dujardin has extensive experience with water quality assessments, including total maximum daily load development for pathogens and dissolved oxygen, particularly in urban settings. He has been the project manager for many water quality modeling studies conducted for the City of New York, which have evaluated engineering alternatives for controlling combined sewer overflows and stormwater discharges. Also included has been the development of a state-of-the-art eutrophication model to assess the effectiveness of nutrient abatement schemes. For Region 2 of the U.S. Environmental Protection Agency, he was project manager for the development of wasteload allocations for toxic metals discharged to the New York-New Jersey Harbor. He has extensive experience with assimilative capacity studies, site feasibility studies, and seasonal treatment alternatives.

On Rapid Assessment Methods Using Statistical Modeling: Multiple Least Squares Regression vs. Logistic Regression

Presenter: Jay Fleisher, PhD
NOVA Southeastern University, College of Medicine

Author: Jay Fleisher

Abstract

Background: There is a need to develop rapid assessment of bacterial water quality. To this end many statistical models have been published mostly using environmental variables to predict concentrations of a particular FIO. The majority of these statistical models have used multiple least squares regression, in which the major indicator of the goodness of fit of these models have largely depended on the R2 value, which to date has been quite low. Since beach management decisions have to be dichotomous in nature (i.e., open/close beach), we explored the use of the multiple logistic model in relation to the multiple least squares approach.

Methods: 668 samples were used in this analysis. 10 major environmental variables and several FIOs were collected on each sample date. Both types of models were run on those data.

Results: Our best multiple least squares regression was computed with an R2 value of 0.26, while the multiple logistic regression model yielded a maximum sensitivity of 72.9 percent and a maximum specificity of 65.9 percent at a cut point = 0.1. A backward selection routine was used in both the models.

Conclusions: Since the logistic regression yields a much less nebulous goodness of fit statistic, coupled with the fact that the beach manager's decision is a dichotomous one, more attention should be paid to research using the multiple logistic model.

Biosketches

Dr. Jay Fleisher received his bachelor of science degree in environmental health science and master of science degree in environmental science from the City University of New York, his master of science degree in epidemiology from Columbia University's School of Public Health, and his doctorate in environmental epidemiology/biostatistics from the Institute of Environmental Medicine, New York University. Dr. Fleisher holds faculty positions at Florida's Nova Southeastern University and University of Miami. Dr. Fleisher's research interests are in the fields of chronic and infectious illnesses. He has focused his research efforts on the health effects of exposure to waters contaminated with domestic sewage, indicator organism variability, indicator organism-pathogen relationships, risk assessment, statistical water quality sampling protocols, assessing compliance, setting of microbial water quality standards, population health burden assessment, risk perception, and risk vs. current standards. Dr. Fleisher has advised numerous international committees, organizations, and government agencies on various aspects of these recreational water quality issues. In addition, he has authored over 70 peer-reviewed publications and six book chapters.

Wayne Creek: A Microbial Source Tracking Case Study

Presenter: Raul Gonzalez, PhD
Hampton Roads Sanitation District

Authors: Raul Gonzalez, Kyle Curtis, Danny Barker

Abstract

Microbial source tracking (MST) methods were used by Hampton Roads Sanitation District to characterize chronic bacterial contamination during dry and wet weather in the Lafayette River (Norfolk, Virginia). The objectives of this study were to 1) partition the sources of fecal contamination entering the Lafayette River, and 2) define the origin of contamination to work in a smaller, more manageable area. During this study, the City of Norfolk identified a leaking sewer pipe in the subwatershed. Therefore, an additional objective was to evaluate water quality after the repair. Sites were sampled on a downstream-to-upstream transect, high-priority stormwater segments were visited early in the sampling campaign, and subsequent samples were collected upstream in the stormwater network. Septic systems, which were initially suspected of causing the observed contamination, did not influence the headwaters at the sample locations monitored. To quantify the magnitude of human contamination relative to raw wastewater, MST data were calibrated using pump station raw influent. Human fecal contamination, measured by the HF183 marker, was confined to one "main" channel, which reduced the problem area to a manageable size. After the leaking force main was found and repaired, human contamination in the headwaters disappeared. Prior to this repair, water quality adjacent and downstream of the leak was similar to raw influent levels. Costs and obstacles encountered when starting an MST laboratory will be discussed.

Biosketch

Dr. Raul Gonzalez is an environmental scientist at Hampton Roads Sanitation District (HRSD), where he uses state-of-the-art source tracking techniques to identify potential sources of contamination in Hampton Roads. Since joining HRSD, he has been working on various water quality projects to quantify stormwater loads and bacteria runoff. Dr. Gonzalez is a native of California, where he graduated from University of California, Los Angeles with a degree in biology. After graduation, he worked at the Los Angeles County Sanitation Districts before returning to graduate school at University of North Carolina- (UNC-) Chapel Hill, where he earned his doctorate in environmental science and engineering. While at UNC, Dr. Gonzalez used molecular methods to pinpoint causes of high bacteria levels in surface waters. He now uses this microbial source tracking experience to assist regional stakeholders.

Heal the Bay's Beach Report Card: Communicating Complex Water Quality Issues and Improving Public Health

Presenter: Leslie Griffin
Heal the Bay

Authors: Leslie Griffin, James Alamillo

Abstract

Beach water quality often gains widespread attention only when there is a substantial sewage spill. Beachgoers typically do not concern themselves with the potential health effects associated with swimming in ocean waters impacted by more everyday sources such as polluted runoff, most likely due to a simple lack of awareness. Historically, it has been challenging for health agencies to effectively convey the health risks associated with swimming at these beaches. The focus of beach water quality risk management is typically on epidemiological research, risk assessment, and limitations with conventional sampling and measurement; yet few resources have been directed toward ensuring that the messaging effectively reaches the beachgoer. For 25 years, Heal the Bay has provided the public with an easy-to-use tool for deciding where to swim based on the most recent bacteriological data. The Beach Report Card® (BRC) provides the beachgoing public on the West Coast with easy-to-understand water quality information about their favorite beaches. The BRC assigns a letter grade based on sample results from local health agencies. Grades are calculated using an algorithm that takes into account frequency and magnitude of exceedances of single sample and geometric mean recreational water quality standards. The BRC has been an incredibly successful and effective tool for public notification. Today, the BRC provides health information for more than 600 beaches along the West Coast and has become part of the public and regulatory vernacular. This past summer, the BRC expanded its dissemination of beach water quality information by posting predictive model or "Nowcasting" results for three southern California beaches. This effort was the result of a Clean Beaches Initiative grant from the state and will expand to include 18–25 beach locations over the next 3 years.

Biosketch

Ms. Leslie Griffin is the beach water quality scientist at the Los Angeles-based environmental organization, Heal the Bay. Native to the East Coast, she relocated across country to receive her bachelor and master of science degrees in environmental science with an emphasis in water quality from Loyola Marymount University. She worked on passive sampling of PAHs for 2 years while obtaining her master's degree. While pursuing her education, Ms. Griffin interned at Heal the Bay as an aquarist and a watershed educator. In 2015, she began working full time with the organization as the data analyst for the Beach Report Card program. Currently, Ms. Griffin manages the Beach Report Card program—working to ensure accurate and timely dissemination of weekly beach water quality info for over 600 locations along the West Coast, as well as implementing a daily predictive modeling—or "nowcasting"—program for five beaches in Southern California.

The Next Generation PCR-Based Quantification Method for Ambient Waters: Digital PCR

Presenter: John Griffith, PhD
Southern California Coastal Water Research Project

Authors: Yiping Cao, John Griffith, Stephen Weisberg

Abstract

Real-time quantitative PCR (qPCR) is increasingly being used for ambient water monitoring, but development of digital polymerase chain reaction (digital PCR) has the potential to further advance use of molecular techniques in such applications. Digital PCR refines qPCR by partitioning the sample into thousands to millions of miniature reactions that are examined individually for binary end-point results, with DNA density calculated from the fraction of positives using Poisson statistics. This direct quantification removes the need for standard curves, eliminating the labor and materials associated with creating and running standards with each batch, and removing biases associated with standards variability and mismatching amplification efficiency between standards and samples. Confining reactions and binary end-point measurements to small partitions also leads to other performance advantages, including reduced susceptibility to inhibition, increased repeatability and reproducibility, and increased capacity to measure multiple targets in one analysis. As such, digital PCR is well suited to ambient water monitoring applications, and is particularly advantageous as molecular methods move towards autonomous field application.

Biosketch

Dr. John Griffith is principal scientist in the Department of Microbiology as well as coordinator of molecular technology at the Southern California Coastal Water Research Project. He received his doctorate in marine biology and biological oceanography from the University of Southern California. Dr. Griffith has been at the forefront of research to develop, evaluate, and implement methods for rapid assessment of beach water quality, microbial source identification, and detection of waterborne pathogens. He is the author of more than 60 articles on the use of Foraminifera as bioindicators, prokaryotic and viral diversity in marine plankton, microbial source tracking, rapid water quality measurement methods, and relationships between microbial water quality indicators and swimming-related illness.

Comparison of Agar- and Pectin-Based Methods for the Detection of Male-Specific Coliphage

Presenter: Joseph Guzman
Orange County Public Health Laboratory

Authors: J.A. Guzman, T.T. Chiem, R.C. Alexander

Abstract

Water quality is an important public health concern affecting millions of lives. Many waterborne illnesses are caused by enteric virus contamination in the water. Current test methods involve the detection of bacterial indicators, which is inadequate in predicting the pathogenic levels of enteric virus. Due to similarities to enteric virus in structure, composition, and resistance to environmental extremes, coliphages have been considered as a surrogate for determining enteric virus levels in water quality assessment. This study focused on the detection of male-specific (F+) coliphage using agar-based U.S. Environmental Protection Agency (EPA) method 1602—single agar layer (SAL)—in comparison to commercially available pectin-based Easyphage. Each method involved combining surface water, sewage, or spiked samples with log-phase *Escherichia coli* on the associated media. After appropriate incubation, detection of coliphage was identified by plaque formation in a lawn of *E. coli* host growth. In spiking experiments, samples with a predetermined concentration of F+ coliphage corresponding to 80 plaque-forming units (PFU) per 100 milliliters (mL) were tested by both methods to determine recovery rates. In spiked samples, the highest recovery rate for SAL was 25% (20/80) and 98% (78/80) for Easyphage with a mean recovery rate of 7% and 69%, respectively. In surface water samples, Easyphage consistently showed more recovery of coliphage than the SAL method with a mean recovery of 123.6 PFU/100ml for Easyphage and 53.8 PFU/100ml for SAL. In one sample, Easyphage recovered 25 times more coliphage than SAL. The pectin-based Easyphage method is less labor-intensive and more effective in the recovery of F+ coliphage in comparison to the agar-based SAL method. Incorporating the Easyphage method with traditional bacterial indicators would provide a more complete assessment of water quality.

Biosketch

Mr. Joseph Guzman is the supervising public health microbiologist overseeing the Orange County Public Health Water Quality Laboratory located in Newport Beach, California. He received his bachelor of science degree in medical microbiology from California State University, Long Beach, and, for the last 25 years, has been with the Orange County Public Health Laboratory working in all areas of public health microbiology. Mr. Guzman first started to focus on water quality testing in 1998, becoming involved in the bacterial monitoring of Orange County's beaches, harbors, and estuaries. The laboratory continues to do routine beach monitoring while also participating in research projects in the development of rapid indicator methods, development of alternative fecal indicators, predictive modeling, and determining sources of bacterial pollution in receiving waters.

Beachgoer Behavior During a Retrospectively Detected Algal Bloom at a Great Lakes Beach

Presenter: Elizabeth D. Hilborn, PhD
U.S. Environmental Protection Agency, Environmental Public Health Division

Authors: Elizabeth D. Hilborn, Whitney S. Krueger, Blake A. Schaeffer, Richard P. Stumpf, Elizabeth A. Sams, Timothy J. Wade

Abstract

Algal blooms occur among nutrient rich, warm surface waters and can adversely impact recreational beaches. During July–September 2003, a prospective study of beachgoers was conducted on weekends at a public beach on one of the Great Lakes in the United States. We measured each beachgoer's activity at the start and end of their beach visit and the environmental factors—water and air temperature, wind speed, and wave height—at the study site each day. At the time, there was no notification of algal blooms; however, we retrospectively evaluated the presence of algal blooms using MERIS data from the Envisat-1 satellite.

A total of 2,840 people participated in the study over 16 study days. The majority (55%) were female, and 751 (26%) were < 18 years of age. An algal bloom was detected retrospectively by remotely sensed satellite imagery during August 16–24. This peak bloom period (PB) included 4 study days. During PB study days, more study participants 226/742 (31%) reported body contact with the water compared to contact 531/2098 (25%) on nonpeak days. During the 4 PB days, of the environmental factors, only mean water temperature was significantly different—25 degrees Centigrade (°C) vs. 23 °C ($p < 0.05$)—from other days.

These results suggest that beachgoer body contact with water was not deterred by the presence of an algal bloom and that interventions to actively discourage water contact during a bloom are needed to reduce exposure to blooms.

Note: This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

Biosketch

Dr. Elizabeth D. Hilborn is a senior health scientist (epidemiologist) in the Environmental Public Health Division in the Office of Research and Development of the U.S. Environmental Protection Agency (EPA). Dr. Hilborn's expertise is in the human health effects of waterborne contaminants including toxic cyanobacteria. She currently serves on the Interagency Working Group for the Harmful Algal Blooms, Hypoxia, Research and Control Act; on the Scientific Committee for the 10th International Conference on Toxic Cyanobacteria; and on multiple cyanobacteria-focused EPA committees and workgroups. Dr. Hilborn earned her bachelor of science degree in biology from the University of North Carolina (UNC) at Chapel Hill, a doctor of veterinary medicine degree at North Carolina State University College of Veterinary Medicine, and a master of public health degree at the UNC at Chapel Hill. Dr. Hilborn served in the Centers for Disease Control and Prevention's Epidemic Intelligence Service and is board-certified with the American College of Veterinary Preventive Medicine.

Washington County, Minnesota, Lake Surveillance for *Naegleria fowleri* from 2011–2014

Presenter: Vincent Hill, PhD
Centers for Disease Control and Prevention

Authors: Bonnie Mull, Jessica L. Collin-Pilarski, Amy Kahler, Erik Anderson, Matt Downing, Fred Anderson, Vincent Hill

Abstract

The presence of the thermophilic free-living ameba, *Naegleria fowleri*, was investigated in lakes in Washington County, Minnesota, including one lake associated with the deaths of two children in 2010 and 2012 from primary amebic meningoencephalitis (PAM). Samples of lake water and sediment were collected from 10 lakes in the summer months annually from 2011 to 2014. In addition to culture and PCR analyses for *N. fowleri*, samples were analyzed for a suite of physical, chemical, and biological parameters potentially related to its presence and growth. Environmental parameters such as rainfall and air temperature also were investigated as potential factors related to detecting the pathogen. *N. fowleri* was detected in five of the 10 lakes in 2011, in one lake in 2012, and in none of the lakes in 2013 or 2014. It was detected in 2011 and 2012 in the lake associated with the two cases of PAM. Average air temperatures prior to sample collection were higher in 2011 and 2012 than in 2013 and 2014, suggesting that the hot weather during those summer periods might have been associated with the growth and detection of *N. fowleri*. The amount of precipitation within 13 days of sample collection was positively associated with detection of *N. fowleri*. The results of this study provide environmental and water quality data that will help increase understanding of *N. fowleri* ecology and potential environmental and water quality factors that could be associated with the detection of this pathogen in recreational water bodies.

Biosketch

Dr. Vincent Hill is a research environmental engineer in the Waterborne Disease Prevention Branch at the Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases. Dr. Hill's work focuses on the development and application of environmental sampling methods, microbial detection methods, and treatment technologies for water and wastewater systems. He is an author of over 90 peer-reviewed journal articles and scientific reports focused on environmental microbiology and engineering. In addition to directing the Waterborne Disease Prevention Branch's Environmental Microbiology Laboratory, Dr. Hill also is team lead for the Water, Sanitation, and Hygiene Laboratory Team.

An Economic Evaluation of the Demand for Beach Safety Information

Presenter: Abigail Kaminski
University of Maine, School of Economics

Authors: Abigail Kaminski, Kathleen Bell, Caroline Noblet, Keith Evans

Abstract

Coastal beaches and the recreation opportunities they provide are important economic assets. It is vital that the health and safety of users be considered in determining how these resources are managed. The health and safety of coastal waters is impacted by a diverse and changing set of problems; large-scale nutrient runoff, land use change, heavy precipitation events, and failing or aging infrastructure can all have negative impacts on coastal water quality. State and local organizations use a variety of means to communicate this information to beach users, but little is known about the diversity of beach users and how they seek out information on beach safety.

In this study, we analyze beach safety information-seeking behavior using data from a survey of Maine and New Hampshire beach users. We estimate a series of discrete regression models to explain search behaviors. Our sample seek out surf condition information at a higher rate than water quality information, and results to date show differences in the factors that influence demand for these two types of information, suggesting that users might regard the risks associated with each differently. These findings provide valuable insight for coastal resource managers and public health officials as they develop effective plans to reach diverse beach users.

Biosketch

Ms. Abigail Kaminski is a graduate student in the School of Economics at the University of Maine, pursuing a master of science degree in resource economics and policy. She received her bachelor of arts degree in economics and geography from Clark University in Worcester, Massachusetts. After graduating from Clark, Ms. Kaminski spent 2 years teaching middle school math with Teach for America in Miami, Florida, and 1 year as an assistant director of admissions at Clark University. She is currently working as a research assistant on an interdisciplinary research team focused on strengthening decision-making around the management of coupled coastal systems. Ms. Kaminski's thesis research focuses on the decision-making behavior of coastal beach users, and she has a broad interest in the ways diverse individuals interact with and value natural resources.

Prolonged Survival of Viable Fecal *Bacteroidales* in Marine Sediments as Assessed by Propidium Monoazide Real-time PCR

Presenter: Minji Kim, PhD
Department of Civil and Environmental Engineering, University of California—Davis

Authors: Minji Kim and Stefan Wuertz

Abstract

Members of the order *Bacteroidales* are the most widely used fecal identifiers in microbial source tracking (MST). While their decay in natural waters has been investigated at length and rate constants have been determined under various conditions, fate and transport studies in sediments are few and far between. Yet this knowledge is critical when using MST to evaluate recreational water quality.

To estimate the decay of fecal *Bacteroidales* in oxygen-free sediments, we constructed anaerobic sediment microcosms in which human, cow, and dog feces were completely mixed with sediments and incubated them at 6 degrees Centigrade (°C) and 20 °C. In addition, intact sediment core microcosms in which fecal slurry was spiked in the overlying water were established to investigate the rate of transport of *Bacteroidales* to the sediment surface. Microcosms were sacrificed in triplicate on each sampling date up to 42 d followed by qPCR analysis with or without addition of the DNA-modifying dye propidium monoazide to differentiate genetic markers from viable cells and total intracellular as well as extracellular marker DNA.

In anaerobic microcosms, the survival and persistence of host-associated *Bacteroidales* cells and DNA were considerably extended, especially at the lower temperature of 6ºC; cells and DNA showed two-log reduction times (T_{99}) of 109 d or higher at 6°C and 14-25 d (cells) as well as 27-47 d (DNA) at 20°C. In core microcosms, *Bacteroidales* cells in sediments decayed up to ten times more slowly than cells in the overlying water column. In both microcosms, various host markers decayed at similar rates under each condition tested. Cells decayed significantly faster than total DNA in marine sediments at 20°C while their decay was comparable at 6°C, suggesting seasonal variation should be considered in application of MST in marine sediments.

The prolonged persistence of fecal *Bacteroidales* in sediments indicated in the study implies that sediments can act as nonpoint sources of fecal markers, causing false positive results when monitoring beach water quality because the bacteria do not reflect recent fecal pollution events. This study demonstrates the need to consider the contribution of MST markers in sediments in field monitoring approaches.

Biosketch

Dr. Minji Kim received her doctorate from the University of California, Davis (UCD) and has conducted postdoctoral research in Stefan Wuertz's laboratory at UCD. In her graduate studies, she investigated the decay of host-associated microbial source tracking (MST) genetic markers in marine and freshwater sediments. In addition, she performed two MST water quality monitoring projects to identify the sources of fecal contamination in a northern California coastal area. As a postdoctoral scholar, Dr. Kim continues her interest in integrating engineering with microbiology to address public health concerns. She has worked to identify pathogenic bacteria and viruses in aerosols and to optimize a propidium monoazide quantitative polymerase chain reaction (qPCR) method for analysis of viable parasite oocysts on produce.

How EPA's Source ID Technology and Digital PCR Enhance Watershed Management

Presenter: Mauricio Larenas
Source Molecular Corporation

Author: Mauricio Larenas

Abstract

Identifying sources of fecal pollution is important for effective watershed management. The need to more accurately identify where the contamination is coming from and how much bacteria is present has spurred the development of the U.S. Environmental Protection Agency's (EPA's) source ID and Digital Polymerase Chain Reaction (PCR) technology.

EPA's patented genetic testing methods developed specifically to detect human, cattle, chicken, and dog fecal pollution have undergone rigorous review and been proven to be more sensitive and more accurate. The upcoming EPA standard for microbial source tracking (MST) will enable watershed managers to use genetic-based methods for broader applications. It also will increase the credibility of MST as a useful source of information in assessing potential sources of fecal contamination and possible public health risks.

Digital PCR is an advanced technology that provides absolute quantification of the target DNA, allowing water managers to know the source of fecal pollution as well as exactly how much fecal bacteria is in the water. It also improves sensitivity, allowing watershed managers to find the fecal source even if it has been some time since the pollution event, and creates the possibility of direct pathogen detection.

The presentation will provide case studies and practical insight on how DNA-based test methods have enabled water quality managers to more effectively craft remediation plans.

Biosketch

Mr. Mauricio Larenas is a project manager as well as the chief operating officer at Source Molecular Corporation. In the past 10 years, he has provided advice and assistance to hundreds of water managers across the country on source identification projects. He has helped many more to understand the importance of identifying the sources of fecal pollution and the cost-saving that comes with applying microbial source tracking technology. Mr. Larenas leads the company's development of breakthrough technologies (e.g., Digital PCR) as well as its participation in California's Source Identification Protocol Project. He has worked with the U.S. Environmental Protection Agency in obtaining a license for Source Molecular's laboratory to use the regulator's newly developed and patented genetic testing markers. Mr. Larenas is currently overseeing the development of Source Molecular's analytical data management system, which will be in full compliance with the laboratory's ISO/IEC 17025:2005 accreditation requirements. He holds a bachelor of science degree in biology from Florida International University in Miami, Florida.

A Customized DNA Microarray for Microbial Source Tracking in Environmental Systems

Presenter: Xiang Li, PhD
U.S. Environmental Protection Agency, Office of Research and Development

Authors: Xiang Li, Valerie Harwood, Bina Nayak, Christopher Staley, Michael Sadowsky, Jennifer Weidhaas

Abstract

It is estimated that more than 160,000 miles of rivers and streams in the United States are impaired due to the presence of waterborne pathogens. These pathogens typically originate from human and other animal fecal pollution sources; therefore, a rapid microbial source tracking (MST) method is needed to facilitate water quality assessment and impaired water remediation. We report a novel qualitative deoxyribonucleic acid (DNA) microarray technology consisting of 453 probes that can detect general fecal and host-associated bacteria, viruses, antibiotic resistance, and other environmentally relevant genetic indicators. A novel data normalization and reduction approach also is presented to help alleviate false positives often associated with high-density microarray applications. To evaluate the performance of the approach, DNA and complementary DNA (cDNA) were isolated from swine, cattle, duck, goose, and gull fecal reference samples, as well as soiled poultry liter and raw municipal sewage. Based on nonmetric multidimensional scaling analysis of the results, findings suggest that the new microarray approach might be useful for detecting pathogens and identifying fecal contamination in recreational waters. The ability to simultaneously detect a large collection of environmentally important genetic indicators in a single test has the potential to provide water quality managers with a wide range of information in a short period of time. Future research is warranted to measure microarray performance in different geographic regions and water types.

Biosketch

Dr. Xiang Li is an Oak Ridge Institute for Science and Education (ORISE) postdoctoral fellow at the U.S. Environmental Protection Agency in Cincinnati, Ohio. He received his bachelor and master of science degrees from Liaoning Normal University in China, and his doctorate in environmental engineering from West Virginia University. Prior to that, Dr. Xiang Li worked at the Center of Disease Control and Prevention in Dandong, China. His main research interests are microbial source tracking in environmental waters, microbiological water quality modeling, and molecular microorganism detection methods assessment.

Citizen Science on the Bronx River: An Analysis of Water Quality Data for an Urban Recreational Resource

Presenter: Diane Mas, PhD
Fuss & O'Neill, Inc.

Authors: Diane Mas, Kathalene Lamboy

Abstract

Citizen science not only offers the opportunity to leverage scarce resources for collecting data, but also encourages a tangible connection between the citizen scientists and the waters they monitor. Adapting monitoring programs based on insight gained from the evaluation of data collected and changing priorities in a watershed is a critical step in the continued success and relevance of a volunteer monitoring program.

Since 1990, over 1,000 water quality samples have been collected by Bronx River stewards at 19 locations along the 23-mile Bronx River in New York. The number of samples collected at each location varied from 10 to 200, and 15 water quality parameters were included in the data set. Analysis of this large data set was undertaken to summarize the data, identify water quality trends and data gaps, and make recommendations for the water quality monitoring program to improve the quality, usefulness, and educational value of future monitoring data for the Bronx River. The analysis consisted of a graphical and tabular statistical summary to provide a comprehensive characterization of the data collected, analysis of correlation with other environmental parameters, and spatial and temporal trend analysis. The review and analysis of the water quality data resulted in several recommendations for ongoing and future citizen scientist monitoring efforts for this important urban recreational resource, including adding visual monitoring for algal blooms and establishing sites for long-term data collection.

Biosketch

Dr. Diane Mas is an associate in the Water Environment and Natural Resources group at Fuss & O'Neill, Inc. She received her bachelor of arts degree in geology from Amherst College, her master of science in engineering degree from the Water Resources Program at Princeton University, and her doctorate in civil engineering from the University of Massachusetts Amherst. Dr. Mas was formerly a hydrologist for the National Weather Service and has held teaching appointments at Smith College and Hampshire College. She has spent nearly 20 years working in the areas of water quality modeling, watershed management, and environmental impact assessment. Dr. Mas's current areas of practice focus on climate change resiliency and adaptation for water resources, water quality assessment and modeling and watershed management, harmful algal bloom impacts to drinking and recreational waters, and the relationship between water quality and public health.

Microbial Source Tracking Toolbox Approach in Florida's Surface Waters

Presenter: Daisys Matthews
Florida Department of Environmental Protection

Authors: Daisys Matthews, David Whiting, Puja Jasrotia, Loretta Wolfe

Abstract

Microbial source tracking is a set of techniques used to investigate and identify potential sources of elevated levels of fecal indicator bacteria in a water body. Indicator bacteria such as fecal coliforms, *E. coli*, and enterococci are commonly found in the feces of humans and other warm-blooded animals, but they also can grow as free-living, non-enteric environmental strains that are not indicative of increased pathogen risk. Standard microbiological culture-based methods cannot distinguish enteric bacteria (i.e., from the gut of a host animal) from non-enteric environmental bacteria. Listing a water body as impaired when no increased risk to human health exists can create significant economic burdens. To focus time and money on eliminating or reducing fecal pollution more quickly, the Florida Department of Environmental Protection has devised a multipronged approach that uses molecular fecal source markers and chemical tracers of human fecal wastewater. Commonly used human wastewater tracers include artificial sweeteners (sucralose), drugs (carbamazepine and primadone), pain relievers (acetaminophen), and fragrances (tonalide). In addition, the laboratory recently implemented a method to distinguish between DNA from live bacteria and DNA from dead bacteria in a water sample using the dye propidium monoazide when performing qPCR analyses.

Biosketch

Ms. Daisys Matthews is the environmental manager of the molecular biology laboratory at the Florida Department of Environmental Protection (DEP). She received her bachelor of science degree in biological sciences from Florida International University in Miami, Florida. For the past 15 years, Ms. Matthews has been a part of the DEP's biology laboratory. Her expertise is in microbiology and molecular biology, and she currently is focused on microbial source tracking.

Deadend Hollow-Fiber Ultrafiltration for the Concentration of Coliphage from Freshwater

Presenter: Brian McMinn
U.S. Environmental Protection Agency

Authors: Brian McMinn, Asja Korajkic, Emma Huff, Eric Rhodes

Abstract

To more accurately signify the presence of pathogenic viruses in ambient waters, the U.S. Environmental Protection Agency is investigating the potential use of coliphage for assimilation into new recreational water quality guidelines. Culturable coliphage are typically present in lower densities than fecal indicator bacteria, requiring more sensitive and robust concentration methods for their detection. Here, we evaluated deadend hollow-fiber ultrafiltration (D-HFUF) in conjunction with single agar overlay (SAL) to concentrate and detect F+ and somatic coliphage from ambient freshwater. Two volumes (1 liter [L] and 10L) of river and lake water were evaluated to determine if either impacted coliphage recovery. For 1L samples, recoveries ranged from 62.2% to 78.6%, while higher volumes resulted in lowered recoveries (36.4–51.2%) of both coliphage types. Sample volume significantly impacted recovery in river water ($P < 0.001$), but not in lake water, while there was no significant difference in recoveries between phage types irrespective of the water type or volume. Method sensitivity was assessed by serially diluting sewage in river water. Both coliphage types were reliably and consistently detected in highly diluted sewage (10,000-fold). The ability of the D-HFUF-SAL method to perform in highly turbid waters (> 100 NTU) also was assessed and there was generally no significant difference in recoveries at turbidities ranging from 38.4 to 118.7 NTU. Our study indicates that the D-HFUF-SAL method is a robust and sensitive method for recovery of coliphage from difficult environmental matrices and its simple design makes it a good candidate for routine water quality monitoring.

Biosketch

Mr. Brian McMinn is a microbiologist in the U.S. Environmental Protection Agency's Office of Research and Development, National Exposure Research Laboratory in Cincinnati, Ohio. He received his bachelor of science degree in environmental health from Missouri Southern State University and his master of science degree in environmental microbiology from Missouri State University. Mr. McMinn's research interests include development and optimization of methods to concentrate adenoviruses from drinking and ambient waters. More recently, the focus of his research has been on investigating the utility of different bacteriophage as indicators of human fecal pollution as well as developing improved methods for their concentration and detection.

Microbial Source Tracking at Two Beaches

Presenter: Heather Merritt
Maryland Department of the Environment

Author: Mark Frana

Abstract

The Maryland Department of the Environment and Delaware Department of Natural Resources and Environmental Control are working with the University of Maryland Salisbury to identify sources of enteric bacteria at Maryland and Delaware beaches. They are using microbial source tracking (MST), a subspecialty of microbiology that incorporates various techniques into identifying sources of fecal contamination in water samples. Recent studies using genetic markers for pollution source tracking in coastal waters have proven successful in identifying nonpoint sources of pollution. Over the past 15 years, a variety of MST methods have been developed, each with its own advantages and limitations. One of the most promising techniques includes the use of quantitative PCR (qPCR) using DNA probes specific for fecal bacteria (e.g., Bacteroides spp.) originating from a specific source. This project involves collecting water samples at one site in Maryland and one site in Delaware where previous monitoring studies have shown high levels of fecal indicator organisms, particularly after a rain event. At each location, an intensive survey was conducted to determine the likely sources of fecal indicator bacteria. The samples are analyzed using qPCR and four genetic markers (i.e., human, gull, poultry, and ruminants) specific for bacteria associated with those sources of contamination. The results of this project will help guide remediation efforts designed to reduce the levels of fecal contamination in the study areas.

Biosketch

Ms. Heather Merritt is the beaches coordinator in the Science Services Administration of the Maryland Department of the Environment (MDE), a position she has held for nearly 10 years. Ms. Merritt has a bachelor of science degree in wildlife science from Virginia Tech in Blacksburg, Virginia, and master of science degree in environmental science from Marshall University in Huntington, West Virginia.

Hydraulic Connectivity Between Wastewater Injection Wells and Submarine Springs (Seeps) in Lahaina, Maui

Presenter: Scott Murakawa
Hawaii Department of Health, Clean Water Branch

Authors: Scott Murakawa, Wataru Kumagai

Abstract

The Lahaina Groundwater Tracer Study identified a definite hydraulic connection between the injection wells at the Lahaina Wastewater Reclamation Facility (WWRF) and the nearshore coastal waters off of Lahaina, Maui (Glenn, Whittier, Dailer, et al., 2013). Beginning in October 2011, the Hawaii State Department of Health-Clean Water Branch (DOH-CWB) began characterizing the submarine spring (seep) water entering nearshore surface waters to determine the potential impacts of injection well effluent on coastal waters.

This presentation will focus on the hydraulic connections identified in the Lahaina Groundwater Tracer Study, the methods used to sample seep water, and the fecal indicator bacteria and nutrient results from the ongoing nearshore seep coastal monitoring conducted in Lahaina, Maui. The data suggest that there is an underground microbial community in underground aquifers that can affect bacteria and nutrient levels of the effluent from the Lahaina WWRF. Chlorination of treated effluent injected into the injection wells between January 2012 and July 2014 resulted in an increase in total nitrogen at nearshore seep groups, yet showed no effects on fecal indicator bacteria levels. This suggests that chlorination has resulted in the die-off of the denitrifying bacteria population residing in the complex groundwater networks of West Maui.

Biosketch

Mr. Scott Murakawa and Mr. Wataru Kumagai are environmental health specialists with the State of Hawaii Department of Health, Clean Water Branch. Mr. Murakawa has been with the Clean Water Branch for 11 years, and Mr. Kumagai has been with the Clean Water Branch for 2 years. Both Mr. Murakawa and Mr. Kumagai have worked on various water quality projects throughout the State of Hawaii during their time with the Department of Health, including Hawaii's recreational beach monitoring, Lahaina seep monitoring, and national aquatic resource surveys.

Utilizing Virtual Beach for Bacteria Forecast Modeling in Sarasota, FL

Presenter: Matthew Neet, PhD
University of South Carolina

Authors: Matthew Neet, Dan Ramage, Dwayne Porter, Heath Kelsey

Abstract

Bacteria level forecasts were developed for beach waters around Sarasota, Florida. Specifically, daily forecast procedures were automated for 12 beach areas. The Virtual Beach (VB) software package, developed by the U.S. Environmental Protection Agency (EPA), provided a set of statistical tools to develop the robust models created in the study. VB was utilized because it requires limited statistical or programming skill to understand and operate. As data records were incomplete for all variables of interest, multiple model runs were made and various techniques were tested to increase the number of records available for modeling and ultimately adjusted r-square values. Once predictive (statistical) bacterial models were created and analyzed, a website application was developed to show each beach location and its associated forecast. The website application was utilized to provide daily recommendations about swimming in beach waters at each of the 12 study sites. Model results and daily Web application recommendations will be presented to local stakeholders for buy-in and critique.

Biosketch

Dr. Matthew Neet is a temporary research professor in the Belle W. Baruch Institute for Marine and Coastal Sciences at the University of South Carolina. He received his bachelor of science degree in biological sciences from Clemson University and his master of earth and environmental resource management degree, master of public health degree in environmental health sciences, and doctorate in environmental health sciences from the University of South Carolina. Dr. Neet has more than 15 years of experience in the geographic information system (GIS), global positioning system, remote sensing, and image processing fields. He also has geospatial experience at a variety of institutions, including higher learning, state and county government, consulting, defense contracting, and commercial business institutions. Having recently completed his doctorate, Dr. Neet currently is studying bacterial modeling along multiple beach habitats in the southeast. His research interests include environmental justice, GIS, remote sensing, bacterial forecasting, and ecological modeling.

Validation of Host-Associated Bacteroidales Gene Marker Assays for Microbial Source Tracking in an Urban Tropical Environment of Singapore

Presenter: Jean Pierre Nshimyimana
Singapore-MIT Alliance for Research and Technology – CENSAM
Singapore Centre for Environmental Life Sciences Engineering

Authors: Jean Pierre Nshimyimana, Mercedes Cecilia Cruz, Janelle Thompson, Stefan Wuertz

Abstract

Microbial source tracking (MST) is now a widely applied tool in many temperate regions of Europe and North America as well as Australia, New Zealand, and Japan, but it has not seen much use yet in tropical urban environments. This study applied qPCR to validate the use of host-associated *Bacteroidales* marker assays based on the 16S rRNA gene to identify human fecal pollution in the urban tropical environment of Singapore. We collected a total of 295 animal and human stool and sewage samples. Animals tested included cats, dogs, rabbits, chickens, birds, monkeys, and wild boars. Following DNA extraction, samples were analyzed by qPCR using seven assays targeting human-associated *Bacteroidales*—HF183-SYBR, HF183, BacHum, BacH and *B. thetaiotaomicron* (B. theta), dog-associated *Bacteroidales* (BacCan), and total *Bacteroidales* (BacUni). The qPCR results were used to compute and compare sensitivity and specificity among assays.

The five human-associated assays (HF183-SYBR, HF183, BacHum, BacH, and B. theta) and the total *Bacteroidales* assay BacUni had 100% sensitivity for sewage. The overall sensitivity for human stool samples ranged from 50 to 70% and B. theta and BacHum displayed the highest human stool sensitivity of 68.6% and 65.7%, respectively. In addition, these two assays had the highest specificity (98.6% for B. theta and 91.4% for BacHum). The HF183 assay recently recommended for MST in California, USA, had a specificity of 90% and sensitivity of 60% and 100%, respectively, to human stool and sewage samples. BacCan assay sensitivity and specificity to dog fecal samples were 80% and 97.3%, respectively. We recommend that B.theta, BacHum, and BacCan be used for MST studies in Singapore.

Biosketch

Mr. Jean Pierre Nshimyimana is currently pursuing doctoral studies in civil and environmental engineering jointly at Nanyang Technological University in Singapore and Massachusetts Institute of Technology (MIT). He will complete his studies later this year. Mr. Nshimyimana received his master of science degree in civil and environmental engineering from MIT and an undergraduate degree in environmental health sciences from the School of Medicine and Health Sciences at the University of Rwanda. In his research at the Singapore-MIT Alliance for Research and Technology (SMART) and Singapore Centre for Environmental Life Sciences Engineering (SCELSE), Mr. Nshimyimana is applying molecular biology tools (e.g., qPCR, digital PCR, and next generation sequencing) to address (1) how bacterial communities and subgroups of sewage-associated taxa and pathogen-like bacteria vary as a function of land use, sites, and water quality in tropical urban catchments; (2) whether 16S rRNA host-associated Bacteroidales assays can be used to effectively identify and distinguish nonpoint sources of fecal microbial pollution in urban tropical environments; and (3) what the effect of predation is on persistence and decay of human Bacteroidales-associated genetic markers in a tropical sediments-rich environment.

Rapid Concentration to Support Improved Detection of Indicator Bacteria in Recreational Waters

Presenter: Andy Page
InnovaPrep, LLC

Authors: Andy Page, Michael Hornback, David Alburty

Abstract

Rapid detection of fecal indicator bacteria in recreational waters is needed to ensure public safety. Molecular and other rapid microbiological detection technologies have progressed significantly in the last several decades and provide the greatest potential for overcoming these challenges. However, their development has significantly outpaced development of sample concentration techniques, which are necessary for rapid detection of low concentrations of bacteria.

InnovaPrep has developed a suite of systems for concentration of bacteria and other biological particles from liquid samples. Volumes of water from a few milliliters to tens of liters of water are processed through flat membrane filters or hollow-fiber membrane filters to capture any biological particles that are present. The biological particles are then efficiently recovered from the membrane surface with a tangential flush using a carbonated "wet foam." The wet foam is expanded up to six times the original liquid volume and becomes highly viscous, allowing it to act as the membrane surface and recover the particles into volumes significantly smaller than can be attained with traditional liquid elutions. The process is scalable, efficient, and typically results in concentration factors of approximately 1000X per concentration stage.

The InnovaPrep concentration and wet foam elution processes will be presented along with developmental and commercially available InnovaPrep concentration systems. Data from use of the Concentrating Pipette system for concentration of indicator bacteria from recreational waters and detection by quantitative polymerase chain reaction will be presented, including concentration efficiencies, concentration factors, and detection limits.

Biosketch

Mr. Andy Page, president and CTO of InnovaPrep LLC, is an expert in the field of collection and concentration of microorganisms from air, surfaces, and liquids. He holds multiple patents for biological collection and concentration, including patents for membrane concentration processes using wet foam elution. Mr. Page's patents underlie the InnovaPrep Concentrating Pipette instrument, Large Volume Concentrator, and other fractionation and concentration technologies developed by InnovaPrep for enabling improved rapid detection of microorganisms in liquid samples, including pathogens in environmental waters.

Moving from the Shore to the Water: Advancements in Local Health Department Beach Water Quality Monitoring within the City of Milwaukee

Presenter: Lindsey Page
City of Milwaukee Health Department

Authors: Paul Biedrzycki, Lindsey Page, Todd Miller, Nicholas Tomaro

Abstract

Recreational water monitoring by local health departments (LHDs) at public beaches represents a core environmental health competency. In the City of Milwaukee, three public beaches are monitored by the Milwaukee Health Department (MHD) on a seasonal basis for levels of *Escherichia coli*, the indicator organism for pathogens of human health concern. Recently, water sampling and sanitary survey data collection has been conducted in partnership with the University of Wisconsin-Milwaukee (UWM) Zilber School of Public Health (ZSPH). This collaboration has resulted in leveraging analytical capabilities for developing beach water quality predictive models and investing in student workforce development.

For the 2016 beach season, MHD and faculty and students from ZSPH and the UWM-School of Freshwater Sciences will develop and deploy offshore buoys configured with sensors to monitor real-time water quality conditions. These advancements in LHD monitoring infrastructure will ultimately enhance the quality, type, and amount of data collected at city beaches to improve the accuracy of daily water quality advisories issued by MHD.

Beach water quality monitoring programs at many LHDs have been compromised by funding shortfalls in the past decade, impacting capacity and capabilities necessary to adequately meet the public health mission. LHD collaboration with academic and community partners through the development of cost-effective and scientifically valid beach monitoring outcomes is foundational to future sustainability of this important function. The MHD program is an example of how cross-sectoral and coordinated partnerships can meaningfully benefit community health, meet educational outreach objectives and satisfy relevant research interests.

Biosketch

Ms. Lindsey Page is the emergency preparedness coordinator in the Division of Disease Control and Environmental Health at the City of Milwaukee Health Department. She received her master of public health degree from Northern Illinois University and, prior to working for the City of Milwaukee, she was an infectious disease epidemiologist for the North Dakota Department of Health. Ms. Page currently is the cochair of the Wisconsin Coastal Beaches Workgroup and manages the beach monitoring program for three beaches in the City of Milwaukee.

Beyond Swimming: Playing in the Cold Waters of Alaska

Presenter: Gretchen Pikul
State of Alaska, Department of Environmental Conservation

Author: Gretchen Pikul

Abstract

The Kenai River is located on the Kenai Peninsula in south central Alaska, and is considered the most popular sport fishing destination in Alaska. Due to a nearby bird rookery and poor fish waste management by anglers, bacteria levels exceed water quality standards during the salmon fisheries in July and might pose human health risks. The purpose of the multi-year monitoring study was to determine bacteria levels and sources, and develop data trends and actions needed to improve the effectiveness of public outreach and notifications. Water conditions in Alaska are different from typical beaches used for swimming. Recreational use of Alaskan beaches is highly seasonal, with low temperature waters even during summer. Recreational water use periods are limited, the fisheries are short (usually 3 weeks), and migratory birds are plentiful. Swimming is not the primary activity on Alaska beaches, but fishing, wading, dog walking, kayaking, and boating are. Alaska needs a secondary recreation criteria to properly address our specific bacteria concerns.

We are searching for better, more quantitative risk assessment tools and more progressive ways to improve beach sanitation resulting in long-term solutions, beyond issuing beach notices. Alaska is also searching for more transparent ways to connect the BEACH Act with other Clean Water Act programs. How do bacteria exceedances lead to 303(d) listings, Total Maximum Daily Loads, or other community planning and state regulations? The study results and implementation challenges will be discussed during this presentation.

Biosketch

Ms. Gretchen Pikul is an environmental program specialist at the Alaska Department of Environmental Conservation (ADEC), where she works in the Non-point Source section of the Division of Water. She received her bachelor of science degree in geological sciences from Salem State College in Massachusetts and worked in the environmental private sector before joining ADEC, where she has worked in the Contaminated Sites DoD Oversight and Water Quality sections. Ms. Pikul's main areas of interests are total maximum daily loads, restoration of impaired waters, and green infrastructure to manage urban stormwater.

Measuring the Impact of Harmful Algal Blooms: The One Health Harmful Algal Bloom System (OHHABS)

Presenter: Virginia Roberts
Centers for Disease Control and Prevention
National Center for Emerging and Zoonotic Infectious Diseases

Authors: Virginia Roberts, Joana Yu, Irina Pyrkh, Lorraine Backer, Kathleen Fullerton, Jonathan Yoder, Michael Beach

Abstract

Harmful cyanobacteria and algae blooms (harmful algal blooms, or HABs) are an emerging public health issue in both coastal and inland waters. Waterborne and foodborne disease outbreaks associated with HAB events or algal toxin exposures are reported to the U.S. Centers for Disease Control and Prevention (CDC) by state health departments via the Web-based National Outbreak Reporting System (NORS). However, these outbreak reports do not include the individual-level data on harmful algal bloom-related illnesses or exposures needed to improve case definitions or prioritize other HAB-related activities related to human or animal health. In 2014, CDC established a working group of state and federal partners with expertise in HABs and illness surveillance to design the One Health Harmful Algal Bloom System (OHHABS), a Web-based reporting system able to receive data about HAB-related human illnesses, animal illnesses, and HAB events using the same technological platform as NORS. During 2014 and 2015, the OHHABS working group drafted reporting forms; developed initial reporting definitions for human illnesses, animal illnesses, and HAB events; and launched the OHHABS pilot system. Following the full OHHABS launch in 2016, maintenance of the system will require ongoing efforts to support state activities and demonstrate the utility of the system through use of the data. Continued and expanded collaboration with human health, animal health, and environmental health communities will be essential to meeting One Health goals of the system, both in the implementation of OHHABS and the optimization of data collected in the system.

Biosketch

Ms. Virginia Roberts, MSPH is an epidemiologist in the Waterborne Disease Prevention Branch of the Centers for Disease Control and Prevention (CDC). Ms. Roberts manages the waterborne disease outbreak component of the National Outbreak Reporting System (NORS) and coordinates a CDC Great Lakes Restoration Initiative project building waterborne disease prevention capacity in Great Lakes states.

Importance of Staying Informed

Presenter: Gerald Ruiz
ATC Associates

Author: Gerald Ruiz

Abstract

As more than five cities in Texas each has a population of almost 240,000 people (including Houston, one of the largest cities in the country), programs such as Texas Beach Watch are indispensable to the way of life that many enjoy on the gulf coast. With the influx of recreational activities such as fishing, surfing, and boating, it has become essential for the public to be fully aware of any hazards that could potentially endanger such pastimes. Texas Beach Watch and similar programs are designed to survey and determine the quality of water in these regions and to discover the dangers they could pose to the public. However, more compelling and accessible communication with the public environment is necessary if those programs are to effectively accomplish their goal of helping the communities they serve. Possible methods of effectively informing the public include (1) holding seminars in proximity to the water-surveying locations, (2) promoting the programs through popular social media platforms, and (3) reaching out to local businesses around the area that would have an interest in the quality of the water from which some of their products might originate. To sum up, communication is key in helping promote environmental programs such as Texas Beach Watch to better serve the public.

Biosketch

Mr. Gerald Ruiz is an environmental technician for ATC Group Services LLC, an environmental engineering consulting firm located in Houston, Texas. Mr. Riuz is a graduate of the University of Houston, where he graduated with a bachelor of science degree in environmental science. Prior to working with ATC, Mr. Riuz interned at Joyce, McFarland, and McFarland LLP, where he assisted with trial work. He also is a member of the American Meteorological Society and has volunteered at the organization's environmental awareness events, such as Earth Day festivals and weather balloon launches aimed at informing young adults and children about the environment they live in. Currently, Mr. Ruiz is in charge of the Texas Beach Watch, a program aimed at informing the public of Texas Gulf coasts beaches by acquiring samples and determining the level of bacteria that is sometimes present and can be harmful to people.

Washington State Marine Beach Bacteria Trends, 2003–2014

Presenter: Debby Sargeant
Washington State Department of Ecology and Health

Authors: Deborah Sargeant, Julianne Ruffner

Abstract

The Beach Environmental Assessment, Communication, and Health (BEACH) Program is an ongoing monitoring and notification program implemented throughout Puget Sound and along the coast of Washington State. Washington marine beaches are tested for enterococci , a fecal bacteria, to determine possible health risks to the public from water contact recreation. The program began with a pilot project in 2003.

The BEACH program consistently monitors 51 core beaches, which were selected as the highest use and highest risk beaches in the state. Data from the core beaches were analyzed to identify any long-term trends in marine water quality based on summer bacteria data from 2003 through 2014.

Results indicate that the core beaches overall show no significant bacteria trends in water quality for the period tested. Individual core beaches were tested for bacteria trends over time and showed mixed results.

Increasing trends in bacterial levels were detected at the following beaches:

- Bayview State Park Beach in Skagit County
- Dash Point Metro Park Beach in Pierce County
- Freeland County Park Beach in Island County

Decreasing trends in bacteria levels were detected at Richey Viewpoint Beach in King County and Howarth Park Beach in Snohomish County.

Biosketch

Ms. Debby Sargeant is the program manager of Washington State's Beach Environmental Assessment, Communication, and Health (BEACH) Program, which is administered by the Washington departments of Ecology and Health. Ms. Sargeant received her bachelor of science degree in environmental studies from The Evergreen State College, home of the Geoducks. She has 25 years of experience working on bacteria-related issues in Washington, including designing bacterial water quality studies to track sources of pollution, bacterial total maximum daily load studies, tracking sources of bacteria to marine areas for shellfish bed and beach water quality restoration, and microbial source tracking.

How to Achieve a Major Reduction in Shore Pollution Using a Business Quality Approach

Presenter: Phillip Scanlan
Past AT&T Corporation Quality, Vice President
Past Ocean Conservancy Board Member

Author: Phillip Scanlan

Abstract

In 1988, I learned that half of the North Atlantic bottlenose dolphins had been killed due to pollution. I offered my help to the New Jersey Department of Environmental Protection to apply AT&T business quality methods to help reduce the pollution problem.

The AT&T network of fiber and switches is similar to the New Jersey network of sewage pipes and plants. However, the New Jersey network is managed by over 100 different government municipalities and counties. We used AT&T quality methods to set goals, establish clear responsibilities for processes, share best practices, and recognize improvement.

In 1988, New Jersey had 803 beach block days of closings due to pollution, the worst in the United States. In 1991, that number had been reduced to 10 and, in 1995, to 4. In the latest National Resources Defense Council report for 2014, New Jersey is still one of the top U.S. states in quality of beach water.

This presentation reviews the business quality approach that was used to help the New Jersey team achieve the greatest improvement in water quality in the United States. The full story is in my book *The Dolphins are Back*.

Biosketch

Mr. Phil Scanlan holds bachelor and master of science degrees in engineering from Northeastern University. He has spent his 33-year business career at AT&T, starting as an engineer and advancing to become the company's quality vice president. Phil also has been working on environmental projects for more than 30 years, with the goal of making the "quality of life" better wherever he lives by using a quality approach to get results. As a volunteer, he has helped apply that approach to achieving major environmental improvements. He spent 10 years supporting a team effort to clean up the New Jersey shore after 2,500 North Atlantic bottlenose dolphins died from pollution-related diseases, helping to turn the Jersey shore from the worst in the nation to the best in the nation. Phil was recognized for his leadership efforts by the New Jersey Department of Environmnetal Protection and the New Jersey Secretary of Commerce. He also wrote a book titled *The Dolphins are Back* to share how other states could improve the health of their shores. Because of his work on the Jersey shore, Phil was invited to join the national board of the Ocean Conservancy—the largest nonprofit organization focused on protecting our ocean wildlife—where he served for 10 years.

Bacterial Source Tracking of Fecal Contamination Using Digital PCR

Presenter: Samendra Sherchan, PhD
Tulane University

Author: Samendra Sherchan

Abstract

Identifying sources of fecal contamination—a serious problem in coastal and fresh waters—is important in preventing disease and ensuring water quality. Using molecular techniques to indicate the presence of human fecal pollution and to determine water quality has become increasingly popular. Detecting Enterococci and *Escherichia coli* is a traditional method of indicating fecal pollution. Both are naturally found in the intestinal tracts and feces of different types of animals and have a high survivability rate in the environment. The U. S. Environmental Protection Agency (EPA) water quality criterion using membrane filtration for Enterococci is 33 colony-forming units (CFU)/100 milliliters (ml) and for *E. coli* is 126 CFU/100 ml in freshwater systems. However, determination of the amount of Enterococci and *E. coli* in a water system indicates only the presence of fecal contamination, is time-consuming, and will take days to be concluded. Several molecular methods have been developed to distinguish whether the contamination is human or nonhuman. Bifidobacteria and Bacteriodes are two examples of bacterial indicators for host-specific identification of human fecal pollution. Bifidobacteria are gram-positive rods widely found in the intestinal microflora of humans and some species like Bifidobacteria adolescentis can be used as a genetic marker in the Quantitative Polymerase Chain Reaction (qPCR) technique, allowing source tracking of human fecal contamination. This review poster presentation will discuss a recent advanced molecular method, Digital PCR, a microbial source tracking technique that can help to determine whether the source of fecal contamination in estuaries and rivers is human or nonhuman.

Biosketch

Dr. Samend Sherchan is an assistant professor in the Department of Global Environmental Health Sciences at Tulane University. His areas of expertise include environmental bioinformatics, environmental microbiology, water quality, and water reuse. He received his doctorate from the University of Arizona in 2013. Dr. Sherchan's research interests include environmental monitoring, health-related water microbiology, water quality, food safety, microbial source tracking, environmental metagenomics, environmental microbiology, emerging contaminants, water reuse, water treatment, waterborne diseases, and public health.

Evaluation of *Enterococcus* and *E. coli* Measurements at Recreational Beaches in Door County by Quantitative Polymerase Chain Reaction and Define Substrate Culture Analysis

Presenter: Nilay Sheth
University of Wisconsin Oshkosh, ERIC Lab

Author: Nilay Sheth

Abstract

Recently the U.S. Environmental Protection Agency (EPA) has approved the use of the qPCR monitoring method 1611 for surface water regulatory decisions. This method allows for results in as little as 4 hours, rather than the 24+ hours necessary for culture-based tests. Method 1611 is an enterococci-based test method, while all Great Lake states have adopted *E. coli* as the indicator organism of choice for recreational water monitoring. While EPA qPCR method C is an *E. coli* specific method, it has not been approved as a method for regulatory decisions. The change in the basis for the test (molecular versus culture-based), and the heterogeneous nature of Great Lake beach communities presents significant challenges to implementation of the rapid method. The objective of this study was to determine the feasibility of using both qPCR method 1611 and C for beach water monitoring and management in northern Lake Michigan communities. Currently, EPA has utilized 1,000 CCE/100 mL as a target value for enterococci-based qPCR method. However, no such target value has been determined for *E. coli* qPCR method C. The study will also aim to find a target value for qPCR *E. coli* method C for northern Lake Michigan beaches. This presentation will present results from multiple years with samples collected from northern Lake Michigan beaches (Door County, Wisconsin), comparing both qPCR EPA method 1611 and C with culture based enterococci and *E. coli* method.

Biosketch

Mr. Nilay Sheth is a research scientist at the Environmental Research and Innovation Center at the University of Wisconsin- (UW-) Oshkosh. He received his bachelor of science degree in cell biology from UW-Whitewater and his master of science degree in microbiology from UW-Oshkosh. Mr. Sheth currently supervises the Door County, Wisconsin, laboratory, where they adhere to regulatory body methods to detect coliform and *E. coli* in surface water using defined substrate methods and rapid molecular assay (qPCR) to quantify Enterococci and *E. coli* in surface water. For the past 5 years, Mr. Sheth has worked as a research scientist at UW-Oshkosh, where he manages various projects in the fields of drinking water and surface water.

Assessing Potential Sources and Influential Parameters of Fecal Contamination at F.W. Kent Park Lake, Oxford, Iowa

Presenter: Reid Simmer
University of Iowa, Department of Geographic and Sustainability Sciences

Authors: Reid Simmer, Mary Skopec, David Bennett

Abstract

Fecal contamination of recreational water bodies in Iowa poses a threat to water quality as well as human health. Concern for the health effects of waterborne pathogens resulted in 149 beach advisories across 39 state-owned beaches during the 2015 beach season. While the presence of pollution is often clear, its cause and source are difficult to identify. Furthermore, the current practice in Iowa of sampling once per week allows for high error and does little to prevent swimmer exposure. The objectives of this study were to identify the environmental factors causing spikes in fecal contamination as well as the sources of pollution at F.W. Kent Park Lake in Oxford, Iowa. Water samples were collected twice per week at the swimming beach and throughout the contributing watershed from May to October 2015. All samples were analyzed for *Escherichia coli* using the IDEXX Colilert enumeration method. The resulting data as well as numerous environmental parameters are being analyzed using Virtual Beach. The two expected sources of fecal contamination are agricultural runoff and the wild geese that frequent the beach. We will consider the spatiotemporal pattern of rainfall and *E. coli* concentrations in our analysis to determine the relative impact the two alternative sources have on water quality. The ultimate goal of this research is to develop a predictive model of water conditions to better inform beachgoers of the potential threat of exposure to fecal contamination.

Biosketch

Mr. Reid Simmer is a graduate student in geography at the University of Iowa, where he received his bachelor of science degree in environmental science. Mr. Simmer has worked as a summer field and lab technician for the Iowa Department of Natural Resources Beach Monitoring Program for the past 4 years. His main research interests include water quality monitoring and modeling as well as pollution source tracking.

Evaluation of Raw Sewage, Highly Treated Reclaimed Water, and Sewage-Impacted Surface Waters for Indicator and Pathogenic Microorganisms: Implications for Recreational, Agricultural, and Drinking Water Use

Presenter: Mark Sobsey, PhD
University of North Carolina, Chapel Hill

Authors: Emily Bailey, Mark Sobsey

Abstract

The microbial quality of reclaimed wastewaters and wastewater-impacted surface waters is a health concern for beneficial uses in recreation or agriculture, or as drinking water sources. In this study, indicator and pathogenic bacteria, viruses, and parasites were quantified in periodic samples of (1) raw sewage, (2) tertiary-treated, dual-disinfected (free chlorine and UV radiation) reclaimed water, and (3) wastewater-impacted surface waters used for recreation or as water supply sources collected from representative facilities in the North Carolina (NC) Research Triangle region. Specified treatment processes for high-quality reclaimed water under NC regulations effectively reduced indicator bacteria, virus, and protozoan parasite surrogate concentrations (to less than or equal to 3 *E. coli*/100 milliliter (mL), less than or equal to coliphages/100 mL, and less than or equal to Clostridium perfringens/100 mL). However, human enteric viruses (adenoviruses and noroviruses) and protozoan parasites (Cryptosporidium and Giardia) were still detectable in 10-liter samples of reclaimed water by nucleic acid (RT-PCR/PCR) and immunofluorescent microscopy methods, respectively. Indicators met NC state log10 reduction targets for high-quality reclaimed water, but log10 pathogen reductions were lower and highly variable, and did not meet log10 reduction performance targets. Pathogen concentration data, however, are not based on infectivity or culturability and may not predict human health risks. Microbial analysis to date of sewage-impacted surface water samples used recreationally and near drinking water supply intakes shows that they do not meet bacteriological quality requirements for recreational use and that dual-disinfected reclaimed water is of higher quality based on indicator microorganism concentrations. Developing better microbial water quality criteria for wastewater, recreation, and reuse requires further investigation.

Biosketch

Dr. Mark Sobsey is a Kenan Distinguished Professor of Environmental Sciences and Engineering specializing in environmental health microbiology and water, sanitation, and hygiene in the Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, University of North Carolina at Chapel Hill (UNC-CH). He received his bachelor of science degree in biology (1965) and master of science degree in hygiene (1967) from the University of Pittsburgh. Dr. Sobsey received his doctorate in environmental health sciences from the School of Public Health, University of California, Berkeley (1971). He held a postdoctoral position (1971), instructorship (1972), and assistant professorship (1973) in the Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas, and joined the UNC-CH faculty in 1974 as an assistant professor. Dr. Sobsey's research, teaching, and service encompass the detection, characterization, occurrence, environmental survival/transport/fate, treatment, human health effects characterization, and risk assessment of viruses, bacteria, and parasites of public health concern in water, wastewater, biosolids, soil, air, and food for the prevention and control of

water-, food-, and excreta-borne disease. His most recent research focuses on household water treatment for improved water quality and health; new, improved, and rapid microbial detection technologies for water and wastewater; wastewater reclamation and reuse; virus survival in fecal wastes and sewage; and antimicrobial resistance of bacteria associated with fecal waste sources in the environment.

Pathogenic Viruses and Bacteria in Stormwater Discharging to Beaches with Year-Round Surfer Populations in San Diego, California

Presenter: Joshua Steele, PhD
Southern California Coastal Water Research Project

Authors: Joshua Steele, Kenneth Schiff, A. Denene Blackwood, Laila Othman, Rachel Noble, John Griffith

Abstract

Microbial water quality, usually measured by fecal indicator bacteria (FIB), at California beaches near storm drains worsens following storms, leading to well-known 72-hour rain advisories. Until recently, however, the pathogenic bacteria and viruses in stormwater that likely cause illness could not be reliably measured, primarily because of the difficulty in quantifying dilute pathogens in complex water matrices. Digital PCR assays now have the sensitivity to enable direct quantification of pathogenic viruses and bacteria. Using digital PCR assays, we measured microbial water quality in stormwater discharges that drained into two beaches with large, year-round surfer populations during wet-weather seasons in San Diego, California. Tourmaline Creek drained a small urban watershed and the San Diego River drained a large mixed urban/undeveloped watershed. Stormwater was collected during six events with precipitation ranging from 0.19 to 2.5" from January to March 2014 and December 2014 to March 2015. Microbial water quality was determined by quantifying FIB, Campylobacter, Salmonella, human Norovirus, adenovirus, and enterovirus. We found high FIB and pathogen concentrations in stormwater from both the large and small watersheds during and in the 3 days following rainfall. Norovirus type GII and Campylobacter spp. were detected most frequently, while Salmonella and adenovirus were rarely detected, and enterovirus was not detected at all. We found no relationship between FIB and pathogen concentration. The ability to quantify pathogens in stormwater provides the ability to more precisely determine the microbial contamination. This direct quantification also will enable better risk estimates and improve management at beaches affected by nonpoint source pollution.

Biosketch

Dr. Joshua Steele is a scientist at the Southern California Coastal Water Research Project who specializes in environmental microbiology and microbial ecology, including cultivation—independent tracking of pathogens and natural microorganisms in the coastal ocean and watersheds. He received his bachelor of science degree in molecular biology from the University of California, San Diego and his doctorate in biological oceanography from the University of Southern California. Dr. Steele's current research focuses on developing and applying molecular techniques to detect and track pathogenic bacteria and viruses, determine their viability and infectivity, and link them to beachgoer risk using statistical models; detecting microbes of emerging concern in coastal water; employing next generation sequencing to connect the environmental microbiome to water quality and ecosystem health; and applying bioinformatics tools in environmental assessment.

Using Canine Scent Tracking To Determine Bacteria Source Contamination: A Case Study from the Grand Traverse Bay Watershed, Michigan

Presenter: Sarah U'Ren
The Watershed Center Grand Traverse Bay

Author: Sarah U'Ren

Abstract

The Watershed Center Grand Traverse Bay (TWC) is a nonprofit organization based in Traverse City, Michigan, whose mission is to advocate for clean water in Grand Traverse Bay and protect and preserve the bay's watershed. During the past 8 years, we have implemented our Healthy Beaches Program, which includes bacteria monitoring, source tracking work at local beaches, education via advertising and social media, and large-scale best management practices at beaches to reduce bacterial contamination. One of the first things TWC did in reaction to high bacteria counts at local beaches was to begin a source tracking monitoring program to determine if the bacteria was from human or animal sources, which would in turn dictate our management approach. In addition to partnerships with research agencies like Michigan State University and the U.S. Geological Survey, our source tracking program included the use of a canine unit trained to detect the presence of human sewage in water. Using the canine team from Environmental Canine Services proved to be a cost-effective and timely way to pinpoint and eliminate areas in which human sources may or may not have been contributing to bacteria counts. This poster summarizes TWC's source tracking efforts with the canine team to help protect local beaches from bacterial contamination and explains how those results were used to implement management efforts.

Biosketch

Ms. Sarah U'Ren has served as the program director for The Watershed Center Grand Traverse Bay (TWC) for the past 14 years. She is responsible for overseeing and coordinating all watershed projects and grant activities at TWC and has 16 years of experience in watershed project management, research, and fieldwork. Ms. U'Ren authored the *Grand Traverse Bay Watershed Protection Plan*, specializes in beach and stormwater management and stormwater-related restoration activities, and has overseen more than 60 grant-funded projects in her tenure at TWC. She earned her bachelor's degree in biology from Alma College and her master's degree in environmental science from the University of Maryland.

Utilizing Green Infrastructure to Protect Public Health at Beaches: A Case Study in Suttons Bay, MI

Presenter: Sarah U'Ren
The Watershed Center Grand Traverse Bay

Author: Sarah U'Ren

Abstract

The Watershed Center Grand Traverse Bay (TWC) is a nonprofit organization based in Traverse City, Michigan, whose mission is to advocate for clean water in Grand Traverse Bay and protect and preserve the bay's watershed. In 2013, TWC and the Village of Suttons Bay installed 18 rain gardens and 3,600 feet of underground infiltration trenches throughout the village in an effort to use green infrastructure to protect public health and reduce harmful stormwater runoff from reaching local beaches. Funded by the U.S. Environmental Protection Agency's Great Lakes Restoration Initiative, the rain gardens and trenches allow infiltration of most of the stormwater originating in the village and prevent it from reaching the bay. Additionally, the village formed a Friends of the Rain Gardens group in 2015 that consists of volunteers who will take care of the rain gardens as they become established over the next few years. This poster will display the types and locations of installed green infrastructure, lessons learned, and a look at the status of the project 2 years after implementation.

Biosketch

Ms. Sarah U'Ren has served as the program director for The Watershed Center Grand Traverse Bay (TWC) for the past 14 years. She is responsible for overseeing and coordinating all watershed projects and grant activities at TWC and has 16 years of experience in watershed project management, research, and fieldwork. Ms. U'Ren authored the *Grand Traverse Bay Watershed Protection Plan*, specializes in beach and stormwater management and stormwater-related restoration activities, and has overseen more than 60 grant-funded projects in her tenure at TWC. She earned her bachelor's degree in biology from Alma College and her master's degree in environmental science from the University of Maryland.

Methods to Sample *E. coli* in Foreshore Sand and Pore Water

Presenter: Laura Vogel
University of Western Ontario

Author: Laura Vogel

Abstract

In recent years, a number of studies have shown that microbial contaminants, including fecal indicator bacteria (FIB) such as *E. coli*, accumulate in sand and pore water close to the shoreline at beaches. This reservoir of bacteria can act as a nonpoint source, resulting in elevated microbial levels in adjacent surface waters. While health units following recommended sampling guidelines routinely monitor surface waters at many recreational beaches, there is no widely accepted method for collecting sand/sediment or pore water samples for FIB enumeration. The efficiency with which FIB attaches to different types of sand grains and the different moisture conditions under which FIB attachment can occur add to the uncertainty of characterizing the abundance of *E. coli* in the foreshore reservoir. Some studies have quantified the abundance of *E.coli* in the foreshore reservoir by collecting unsaturated surface sand samples, while other studies test the pore water and disturbed saturated sand samples. The utility of the different sampling strategies in providing quantifiable information related to the abundance of FIB in the foreshore reservoir and associated risk is not well understood. Field sampling was conducted to evaluate techniques for characterizing the abundance of *E. coli* in the foreshore reservoir. Sampling was performed at a range of beaches with different sand types including fine grain (0.125<d50<0.250 mm), medium grain (0.251<d50<0.500 mm), and coarse grain sand (0.501<d50<1.00 mm). Techniques used to sample the foreshore reservoir include collecting pore water samples (drive point, shovel, and careful excavation) and saturated sand samples (sediment core, shovel, and careful excavation). This data will help determine the optimal sampling strategy for characterizing FIB abundance. This understanding is critical to developing a standard recommended method for quantifying the abundance of FIB in the foreshore reservoir as required to ultimately evaluate the associated water quality impairment and public health risk.

Biosketch

Ms. Laura Vogel received both her bachelor's degree in environmental engineering and her master's degree in civil engineering from the University of Miami. She is currently a doctoral candidate at the University of Western Ontario in the Civil and Environmental Engineering Department, where she is focusing on microbial water quality at recreational beaches.

Differential Decay of Bacterial and Viral Fecal Indicators in Common Human Pollution Sources

Presenter: Pauline Wanjugi, PhD
U.S. Environmental Protection Agency

Author: Pauline Wanjugi

Abstract

Understanding the decomposition of microorganisms associated with different human fecal pollution sources is necessary for proper implementation of many water quality management practices, as well as predicting associated public health risks. Here, the decay of select cultivated and molecular indicators of fecal pollution originating from fresh human feces, septage, and untreated sewage in a subtropical marine environment was assessed over a 6-day period with an emphasis on the influence of ambient sunlight and indigenous microbiota. Ambient water mixed with each fecal source (1:10 dilution) was placed in dialysis bags and incubated in situ in a submersible aquatic mesocosm. Genetic and cultivated fecal indicators, including fecal indicator bacteria (enterococci, *E. coli*, and Bacteroidales), coliphage (somatic and F+), and human-associated genetic indicators (HF183/BacR287 and HumM2), were measured in each sample. Simple linear regression assessing treatment trends in each fecal type over time showed significant decay trends ($p \leq 0.05$) in most treatments of feces and sewage sources (27/28 and 32/40, respectively), compared to the septage source (6/26). A two-way analysis of variance comparison of log10 reduction values for sewage and fecal experiments indicated that treatment effects differentially impact survival of cultivated bacteria, cultivated coliphage, and genetic indicators. Findings suggest that sunlight is critical for coliphage decay, while indigenous microbiota play a greater role in decay of genetic indicators in sewage than in fecal source. This study offers new insights into the decay of common human fecal pollution sources in subtropical marine waters with important implications for future water quality management applications.

Biosketch

Dr. Pauline Wanjugi is a National Research Council postdoctoral research fellow working at the U.S. Environmental Protection Agency (EPA) Office of Research and Development in Cincinnati, Ohio. Dr. Wanjugi received her undergraduate degree from the University of Idaho, her master's degree from Pennsylvania State University, and her doctorate from the University of South Florida. Her current research pertains to the identification and quantification of sources of fecal pollution in recreational waters using traditional and molecular microbiology approaches. Prior to her current tenure at EPA, Dr. Wanjugi worked in a similar capacity at Texas A&M University, where she conducted research on assessment of sources of fecal pollution in Texas watersheds and expansion of the Texas bacterial source tracking library.

Trash Free Beaches via Trash Free Waters

Presenter: Kelsey Watts-FitzGerald
U.S. Environmental Protection Agency, Office of Water

Authors: Margaret Murphy, Laura Johnson, Robert Benson, Noemi Mercado, Rahul Madhusudanan

Abstract

Aquatic trash pollution is a global problem. Approximately 80% of trash in the world's oceans comes from inland sources. The U.S. Environmental Protection Agency's (EPA's) Trash Free Waters (TFW) Program pursues innovative strategies to prevent and reduce the amount of trash entering U.S. rivers, lakes, and coastal waters with the aim of moving towards a zero loading of trash (particularly plastic trash) entering our watersheds and ultimately the ocean. The four major objectives of the program are to 1) support stakeholder-driven initiatives and projects in cities and states; 2) support research on the ecological, economic, and potential human health impacts of trash in aquatic environments; 3) support U.S. Government engagement on marine trash/debris issues internationally; and 4) foster public/private partnerships to generate innovative ideas for technology, litter prevention, and material reuse. With these as the tenets of TFW, our program's progress includes having developed regional strategies with projects in the Mid-Atlantic, California and Pacific Islands, Gulf of Mexico, New York/New Jersey, and Puerto Rico; an assessment of the state of research pertaining to aquatic plastic impacts in order to explore the issue and inform EPA and non-EPA research; Our Oceans commitments to follow on the success of our Puerto Rico strategy by expanding the TFW approach into the wider Caribbean, as well as a partnership with China to share information and best practices on reducing aquatic trash; and partnerships with industry stakeholders by convening an executive dialogue with our Administrator to explore breakthrough ideas to achieve trash-free waters in the U.S.

Biosketch

Ms. Kelsey Watts-FitzGerald is an Oak Ridge Institute for Science and Education participant in the U.S. Environmental Protection Agency's Office of Water. She received her bachelor of arts degree in biology and environmental studies from Oberlin College, and her master of public health degree in environmental science and policy from Columbia University. Ms. Watts-FitzGerald's fellowship placement is in the Marine Pollution Control Branch, which is focused on programs related to trash-free waters; ocean acidification; corals; ocean dumping; and vessels, marinas, and ports.

Vancomycin-resistant Enterococci in a Domestic Sewage Spill in South Florida

Co-Presenters: Suzanne Young and Valerie J. Harwood, PhD
Department of Intergrative Biology, University of South Florida

Authors: Suzanne Young, Valerie J. Harwood

Abstract

Culturable *Enterococcus faecium* harboring the *vanA* gene were detected in water and sediment after a sewage spill in south Florida. The site was sampled for 7 weeks after over 1 million gallons of domestic sewage was released through a broken pipe. This is only the second instance in which vancomycin-resistant enterococci (VRE) harboring *vanA* genes that confer high-level resistance have been reported outside of clinical settings in the United States. Putative VRE were isolated on mEI agar amended with 32 μg·ml-1 vancomycin and could be cultured from water and sediment as much as 5 days after the event. Fecal indicator bacteria also were monitored at the site and in receiving waters and sediment. Culturable enterococci levels in water at the site exceeded recreational water quality guidelines for 4 weeks following the spill. Analysis of bacterial species via 16S rRNA gene sequencing showed changes in community structure through time following the sewage spill in both sediment and water. Communities in sediment were distinct from those in water, with different dominant families. Enteric pathogen families *Enterococcaceae* and *Enterobacteriaceae* were present at low levels in both water and sediment over the course of the study. Six sewage-associated families identified by sequencing results represented a small proportion of total reads, but declined over time and on a similar timescale to culturable enterococci and VRE in both water and sediment.

Biosketch

Ms. Suzanne Young is a fourth-year PhD candidate in Dr. Valerie (Jody) Harwood's water quality microbiology laboratory at the University of South Florida in Tampa, Florida. She completed her master's degree at Queens College/City University of New York in New York City, studying sewage contamination in the Hudson River in collaboration with the Riverkeeper water quality monitoring program. Her publication topics include antibiotic resistance in the environment, alternative habitats of Vibrio vulnificus, microbial source tracking, and disease ecology. Ms. Young received the 2013 EPA STAR Graduate Research Fellowship to study the ecology of antibiotic resistant bacteria in aquatic environments. The focus of her doctoral dissertation is antibiotic resistance gene transfer and the survival of vancomycin-resistant enterococci under varying environmental condition.

Dr. Valerie J. (Jody) Harwood is a professor and chair of the Department of Integrative Biology at the University of South Florida, where she has worked since 1998. She is an environmental microbiologist whose research focuses on microbial ecology, water quality, and the use of molecular biology tools for assessing the extent and sources of fecal contamination in water. She is a major contributor to the U.S. Environmental Protection Agency (EPA) guide document on microbial source tracking (MST) and is a coeditor of the book, *Microbial Source Tracking: Methods, Applications & Case Studies* (Springer 2011). Dr. Harwood also has

conducted extensive research on the persistence and ecology of enteric organisms in secondary habitats such as water and sediments, and is at the forefront of efforts to implement quantitative PCR methods as a tool in environmental microbiology research. She is the author of more than 80 peer-reviewed publications on subjects including MST, environmental persistence and fate of fecal indicators and waterborne pathogens, and Vibrio vulnificus ecology. Dr. Harwood collaborates with agencies ranging from local to international (e.g., EPA, WHO, UNESCO) on water quality issues. She is a member of the Council Policy Committee (executive board), a distinguished lecturer for the American Society for Microbiology, and a Fulbright Specialist Award recipient for work in Queensland, Australia, with the Commonwealth Scientific and Industrial Research Organisation.

Predictive Modeling of Microbial Indicators for Timely Beach Notifications and Advisories at Marine Beaches

Presenter: Richard Zepp, PhD
U.S. Environmental Protection Agency, National Exposure Research Laboratory/Office of Research and Development, Ecosystems Research Division

Authors: Richard Zepp, Mike Cyterski, Marirosa Molina, Chris Fitzgerald, Gene Whelan, Rajbir Parmar Kurt Wolfe, Mike Galvin

Abstract

Marine beaches are occasionally contaminated by levels of fecal indicator bacteria (FIB) that exceed U.S. Environmental Protection Agency water quality criteria. Here, we describe application of a recent version of the software package Virtual Beach tool, VB 3.0.5, to build and evaluate multiple linear regression (MLR) and generalized boosted models to predict microbial water quality for selected marine beaches in the eastern United States and Puerto Rico. For comparison, we used the same modeling technique to evaluate selected freshwater beaches of the Great Lakes. Culturable and qPCR methods were used to measure enterococci concentrations. Environmental variables for the beach sites such as rainfall, wind and current speed/direction, and solar radiation were obtained concurrently. VB 3.0.5 can be used to develop site-specific relationships —"models"—that link the densities of FIB to the environmental variables. VB was used to evaluate the best models for these beaches from an extensive list of choices. Comparisons of MLR modeling results for predicted FIB densities at the marine beaches and results obtained at selected Great Lakes beaches indicate that, on the basis of adjusted R2 values for predicted versus observed levels of FIB, model performance was better for the freshwater Great Lakes beaches than for the marine beaches (freshwater average adjusted R2 = 0.5, marine average adjusted R2 = 0.39). Modeling results for the culturable FIB data at the marine beaches also were somewhat better than for qPCR data (culturable average adjusted R2 = 0.46, qPCR average adjusted R2 = 0.42). Lower values for marine beaches likely reflect the interplay of several factors discussed in the presentation.

Biosketch

Dr. Richard G. Zepp is a Senior Research Scientist at the U.S. Environmental Protection Agency National Exposure Research Laboratory in Athens, Georgia. He received his bachelor of science degree Furman University and his doctorate from Florida State University. Dr. Zepp's research interests include predictive modeling and fate and transport of pathogens and fecal indicators in aquatic environments. He is a member of the United Nations Environment Programme Environmental Effects Assessment Panel and is an adjunct professor at the Rosenstiel School of Marine and Atmospheric Sciences at the University of Miami, Florida, and the Department of Chemistry at the State University of New York in Syracuse, New York. He is also a member of AGU, ACS, SETAC, ISES, ASM, ASLO, Sigma Xi, and AAAS.

Process Relationships for Evaluating the Role of Light-induced Inactivation of Coliphages at Selected Beaches and Nearby Tributaries of the Great Lakes

Presenter: Richard Zepp, PhD
U.S. Environmental Protection Agency, National Exposure Research Laboratory/Office of Research and Development, Ecosystems Research Division

Authors: Richard Zepp, Marirosa Molina, Kelvin Wong, Mike Cyterski, Gene Whelan, Rajbir Parmar, Brad Acrey, Rania Georgacopoulos

Abstract

One approach to predictive modeling of biological contamination of recreational waters and drinking water sources involves applying process-based models that consider microbial sources, hydrodynamic transport, and microbial fate. Coliphages are possible indicators of fecal contamination of recreational waters. Past studies have indicated that sunlight plays an important role in altering densities of coliphages, other indicator microorganisms, and pathogens in aquatic environments. Here, we report on laboratory studies of light-induced inactivation of two species of coliphage, an F-specific RNA coliphage and a somatic coliphage, under various conditions in phosphate-buffered solution and natural waters. Inactivation rates of the coliphages were determined in a series of irradiations that used simulated solar radiation passed through light filters that blocked different parts of the ultraviolet and visible spectral region. Inactivation rates and spectral irradiance were then analyzed by the Rundel technique to develop biological weighting functions (BWFs) for the light-induced inactivations. Direct exposure to solar radiation at midday and shallow depths in all waters resulted in rapid inactivation, with half-lives of hours or less. In the dark, inactivation half-lives were much longer—on the order of several days. BWFs were combined with data for underwater solar spectral irradiance to model light-induced inactivation of phages in selected recreational waters. We report on use of this approach to help evaluate potential effects of sunlight-induced inactivation on phage densities at Great Lakes sites located near Milwaukee, Wisconsin; Michigan City, Indiana; Cleveland, Ohio; Duluth, Minnesota; and the Manitowoc River basin.

Biosketch

Dr. Richard G. Zepp is a Senior Research Scientist at the U.S. Environmental Protection Agency National Exposure Research Laboratory in Athens, Georgia. He received his bachelor of science degree Furman University and his doctorate from Florida State University. Dr. Zepp's research interests include predictive modeling and fate and transport of pathogens and fecal indicators in aquatic environments. He is a member of the United Nations Environment Programme Environmental Effects Assessment Panel and is an adjunct professor at the Rosenstiel School of Marine and Atmospheric Sciences at the University of Miami, Florida, and the Department of Chemistry at the State University of New York in Syracuse, New York. He is also a member of AGU, ACS, SETAC, ISES, ASM, ASLO, Sigma Xi, and AAAS.

Development of a Human-Specific *B. thetaiotaomicron* IMS/ATP Assay for Measuring Viable Human Contamination in Surface Waters in Baja California, Mexico

Presenter: Amity Zimmer-Faust, PhD
U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory

Authors: Amity Zimmer-Faust, Vanessa Thulsiraj, Leopoldo Espinosa-Mendoza, Jennifer Jay

Abstract

Immunomagnetic separation/ adenosine triphosphate (IMS/ATP) assays utilize paramagnetic beads and target-specific antibodies to isolate target organisms. Following isolation, adenosine tri-phosphate (ATP) is extracted from the target population and quantified. An inversely coupled (Inv-IMS/ATP) assay for detection of *Bacteroides thetaiotaomicron* was developed and applied for rapid detection of human-associated fecal contamination in surface waters in Baja California. Specificity of the assay was tested against challenge solutions of varying concentration of dog, gull, horse, and chicken feces, and a field validation survey of assay measurements in coastal and wastewater treatment plant effluent water quality in Rosarito and Ensenada, Baja California, was conducted. Inv-IMS/ATP measurements were shown to be specific and sensitive to human fecal contamination. At test concentrations of challenge feces less than 1000 MPN ENT/100 mL, sensitivity and specificity of the assay both exceeded 80%. Moreover, the Inv-MS/ATP assay yielded measurements of viable *B. thetaiotaomicron* that were comparable to the HF183 human marker in complex surface waters impacted with both wastewater and stormwater runoff, and the Inv-IMS/ATP assay was able to effectively differentiate between surface waters impacted with adequately and inadequately treated wastewater. The Inv-IMS/ATP assay shows promise for rapid evaluation of recreational water quality in areas where access to more expensive methods is limited and in areas where water quality is unpredictable.

Biosketch

Dr. Amy Zimmer-Faust is a postdoctoral researcher for the U.S. Environmental Protection Agency at the National Health and Environmental Exposure Research Lab, Pacific Coastal Ecology Branch located in Newport, Oregon. She received her bachelor of science degree in aquatic biology from the University of California at Santa Barbara and her doctorate in environmental science and engineering from the University of California at Los Angeles. Dr. Zimmer-Faust's PhD focused on the application of rapid methods (MST markers and IMS/ATP assays) for evaluation of coastal water quality. Her main research interests include evaluation of the sources and fate of fecal pollution in coastal systems and the environmental parameters that control fecal inputs.