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

Aleksandar Dimkovski¹ and J. Michael Trapp²

¹Department of Marine Science, Coastal Carolina University, P.O. Box 261954, Conway, South Carolina 29528-6054
²Burroughs & Chapin Center for Marine and Wetland Studies, Coastal Carolina University, P.O. Box 261954, Conway, South Carolina 29528-6054

Abstract

Fecal indicator bacteria (FIB) are an important form of pollution in inland and coastal recreational waters and are of particular concern to human health. In this study, we conduct a comparison of regulatory FIB (E. coli) to host-specific qPCR assays on direct canine and sea bird fecal grab samples from the Grand Strand of South Carolina. Results suggest that inter-specimen variability makes interpretation of qPCR results difficult to attribute a percentage of the FIB load to a particular host. Temporal variability of the addition of waste to the system further complicates interpretation.

Introduction

Water quality impairments are commonly associated with elevated concentrations of fecal indicator bacteria (FIB). Microbial source tracking (MST) aims to identify the sources of FIB pollution so targeted remediation strategies can be used to improve water quality. Bacterial culture-based methods dependent on active substrate metabolism are typically used to quantify FIB and can offer some geographic source information. Results from these methods, however, do not provide information on the source of the pollution. Molecular techniques, such as polymerase chain reaction (PCR), offer a quick and sensitive approach for quantifying FIB concentrations and host specific quantification by targeting genetic markers in the bacteria unique to the host organism. In this study, dog and bird fecal grab samples were examined for regulatory E. coli (Colilert®-18), a general FIB qPCR assay (GenBac) and host specific qPCR assays for dog and avian sources (BacCan and Bird GFD) assays. In a second experiment dog and bird fecal matter was aged and sampled over time to compare how the ratio between E. coli and genetic markers changes temporally.

Methodology

- Fecal samples collected from selected canines were diluted to 10⁻³ mL. final dilution factor, while selected sea bird (Laridae) samples were combined and diluted to 10⁻³ mL. final dilution factor.
- Collected samples from an Italian Greyhound and bird mass-fecal sample were incubated (22°C for ~ 14 days) and dilution and filtering protocols were repeated.
- Bacterial DNA was extracted from filters by cell lysis, and quantitative PCR was used to quantify the presence of FIB with the GenBac Assay, the BacCan Assay and the Bird GFD Assay. Additionally cultivable E. coli was measured by Colilert®-18.

Results

- Concentrations of FIB and species specific genetic markers varied greatly between individuals. Surprisingly some individuals showed no E. coli in fresh feces but had relatively strong genetic marker results.
- A weak correlation existed across individually sampled canines between E. coli and BacCan (R² = 0.0166, p = 0.037), and E. coli and GenBac (R² = 0.036; p = 1.97 X 10⁻³) determined FIB concentrations.
- Genetic marker decay rates for aged canine fecal sample was rapid and logarithmic in slope. Corresponding E. coli concentrations progressively increased with time to maximum quantifiable level.
- Decay rate for aged sea bird fecal sample was very rapid while E. coli persisted longer with high concentrations.

Summary

- There is a great deal of variability in the concentration of FIBs and genetic markers between individuals.
- The source specific signal seems to disappear quickly while FIB concentrations appear to continue to rise after leaving the organism. Thus any detection of a source specific signal should be considered significant.
- This variability between bacterial concentrations in fresh samples limits interpretation of qPCR and Colilert results which complicates the assignment of FIB percent load to a particular host.
- These results suggest that a multiple tracer weight of evidence approach including traditional WQ methods and qPCR methods are necessary for meaningful data interpretation.

Bibliography