# SURVIVAL AND REGROWTH OF FECAL ENTEROCOCCI IN DESICCATED AND REWETTED SEDIMENTS

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**Abstract**. Fecal enterococci are bacteria widely used as indicators of fecal contamination in marine and estuarine waters. One assumption is that these bacteria do not persist or regrow in the environment. Our continuing problems with high numbers of fecal enterococci in sediment suggested that these bacteria may persist and regrow. Therefore, we conducted experiments with fecal enterococci to determine their ability to survive desiccation and to regrow in marine and estuarine sediments from Georgia, New Hampshire, and Puerto Rico after 0, 2, 30, and 60 days. Although numbers of fecal enterococci generally decreased with increased length of drying, many fecal enterococci survived desiccation and regrew in rewetted sediment, violating the assumption that fecal bacteria not persist or regrow in the environment. Because there is not a better alternative to fecal enterococci as fecal indicator bacteria, these results suggest that care should be taken not to disturb the sediment when sampling water for fecal contamination, or if the sediment is already disturbed (e.g., on windy days or during runoff conditions), then the influence of sediment should be considered.

## INTRODUCTION

Fecal enterococci are bacteria widely used as indicators of fecal contamination in marine and estuarine waters. The State of Georgia began using fecal enterococci as indicator bacteria for marine and estuarine waters in April 2004. One assumption is that fecal enterococci, indeed all fecal indicator bacteria, do not persist or regrow in the environment (Clesceri et al., 1998).

Sediments have long been known as a reservoir for fecal bacteria (Stephenson and Rychert, 1982). For example, when cattle have access to streams, their fecal bacteria survive for long periods in the sediment (Howell et al., 1996). This discovery is unsurprising because clay particles and organic matter often protect the bacteria from adverse environmental conditions (Robert and Chenu, 1992).

We were concerned about the survival and potential regrowth of fecal enterococci in sediments during conditions of desiccation and rewetting because of our interest in bacterial source tracking. Bacterial source tracking is based on the principle that specific markers or strains of bacteria are associated with specific animal species. Therefore, it may be possible to match fecal contamination in environmental waters to specific animal species. However, if these bacteria survive and regrow in sediment, then this survival and regrowth may affect bacterial source tracking because the bacteria may represent long past sources of fecal contamination.

Few studies have been reported on the survival or regrowth of fecal bacteria in desiccated sediments. Fecal enterococci can regrow when sterile sediment is added to nonsterile sediment and the wetting and drying of the tidal cycle is simulated (Desmarais et al., 2002). Furthermore, soil bacteria regrow in rewetted soils as survivors dine on the deceased (Birch, 1958). Also, enterococci are known to survive desiccation for >11 weeks on surfaces associated with farm buildings (Bale et al., 1993).

Given the potential for fecal enterococci to survive and regrow in dried sediments, we conducted experiments to determine the extent to which fecal enterococci survive and regrow in marine and estuarine sediments from Georgia, New Hampshire, and Puerto Rico. These locations were selected because of their differences in latitude and differences (or lack of differences) in annual seasonal temperature. Also, clays may protect bacteria from environmental conditions (and presumably from the selective properties of culture medium). This protection may yield a large number of false positive isolates. For this reason, all fecal enterococci were confirmed according to the USEPA definition (2002).

## METHODS

Sediment samples were collected at low tide, once during winter/spring and once during summer, at three locations: Academy Creek, located near Brunswick, Georgia; Bunker Creek, located in the Great Bay of New Hampshire; and Chun-Chin Creek, located in the Jobos Bay National Estuarine Research Reserve in south central Puerto Rico.

Surface sediment samples (uppermost few millimeters) were collected with an ethanol-disinfected spoon and were placed into sterile polypropylene bottles. Bottles were placed on ice and the sediment was processed within 6 hours. Sediment samples were allowed to resettle for 1 h, after which the overlying water was removed aseptically. The sediment was analyzed for organic carbon and texture (sand, silt, and clay) by standard methods (Table 1).

For microbiological analysis, a 20-mL portion of sediment was placed in a 10-cm pre-weighed Petri dish and the weight recorded. The top of the Petri dish was removed and the sediment was allowed to air-dry at room temperature (20 to 22 °C) for 0, 2, 30, and 60 days. Each sampling was done in triplicate. In addition, triplicate 20-mL portions of sediment were placed in preweighed aluminum dishes and the dry weight determined gravimetrically after drying at 95 °C for 24 h.

After 0, 2, 30, and 60 days, triplicate samples of sediment in the Petri dishes were rewetted with sterile distilled water to their original weight. The dishes sat for 1 h before half the sediment was processed for fecal enterococci with the Enterolert System (IDEXX Laboratories, Westbrook, ME). The Most-Probable-Number (MPN) was determined as described by Hartel et al. (2004). The three sediment samples were then left covered at room temperature for 24 hours and the sampling was repeated with the remaining half of the sediment. In this manner, any regrowth could be recorded.

Because the presence of sediment might affect the accuracy of the Enterolert system, the content of each positive (fluorescing) Quanti-tray well was confirmed for the presence of fecal enterococci as described by Hartel et al. (2004). To be recorded as positive, at least one isolate from a positive Quanti-tray well had to conform to the USEPA (2002) definition of fecal enterococci: be able to hydrolyze esculin, be able to grow on brain heart infusion agar with 6.5% NaCl, and be catalase negative. Wells containing at least one isolate that conformed to this definition were counted towards the MPN. The results were expressed on a per gram dry weight basis.

**Table 1. Organic carbon and texture of sediments from Academy Creek, Georgia; Bunker Creek, New Hampshire; and Chun-Chin Creek, Puerto Rico.**

| Location | Organic C | Clay | Silt | Sand |
|---|---|---|---|---|
| | ----------%---------- | | | |
| Academy Creek | 6.2 | 10.6 | 80.3 | 9.1 |
| Bunker Creek | 3.5 | 32.4 | 60.5 | 7.2 |
| Chun-Chin Creek | 6.4 | 4.2 | 48.9 | 46.9 |

## RESULTS

All moist sediments had large numbers of fecal enterococci (Table 2). The corrected MPNs of fecal enterococci $g^{-1}$ dry weight of moist sediment varied from an average of 8,172 in Academy Creek (Georgia) to an average of 167 in Bunker Creek (New Hampshire) to an average of 166 in Chun-Chin Creek (Puerto Rico).

With a few exceptions, numbers of fecal enterococci decreased with increased length of drying. After 60 days, Georgia and New Hampshire sediments still contained between 63 and 1,200 fecal enterococci $g^{-1}$ dry weight of sediment, whereas Puerto Rico sediment had low numbers of fecal enterococci (between 2 and 17).

Fecal enterococci regrew in some rewetted sediments and not in others. For example, in the Academy Creek sediment from Georgia, fecal enterococci increased from 1,202 to 28,840 $g^{-1}$ dry weight after 60 days (December 2003). In contrast, in the Bunker Creek sediment from New Hampshire, fecal enterococci decreased from 302 to 53 $g^{-1}$ dry weight after 60 days (March 2004).

The number of false positive Enterolert wells (wells that fluoresced but contained no fecal enterococci) was highly variable and resulted in decreases between the original and corrected MPN ranging from 0 to >99.9%. This variability was observed in sediments from Georgia, New Hampshire, and Puerto Rico regardless of when the sediment was sampled or whether the sediment was moist, dried and rewetted for 1 h (survival), or dried and rewetted for 24 h (regrowth). The greatest decreases between original and corrected counts were observed in Puerto Rico sediments (average >99.6%).

**Table 2. Most probable number (MPN) of fecal enterococci (± 1 SD) obtained with the Enterolert system (IDEXX, Westbrook, ME) at two sampling times (summer, winter/spring) from the sediment of a) Academy Creek in Georgia b) Bunker Creek in New Hampshire, and c) Chun-Chin Creek in Puerto Rico. Each MPN was obtained from moist sediment and sediment dried for 2, 30, and 60 d before rewetting for 1 and 24 h at 25°C. The presence of fecal enterococci in each positive (fluorescing) Quanti-tray well (original MPN) was confirmed according to our protocol. The difference between the original MPN and the corrected MPN is given separately as a percent decrease.**

| Condition | Original MPN | Corrected MPN | Decrease | Original MPN | Corrected MPN | Decrease |
|---|---|---|---|---|---|---|
| | ---$\log_{10}$ MPN $g^{-1}$ sediment---- | | % | ---$\log_{10}$ MPN $g^{-1}$ sediment---- | | % |
| *A) Academy Creek, GA* | December, 2003 | | | August, 2004 | | |
| Moist sediment | 3.63±0.09 | 3.50±0.10 | 25.9 | 4.90±0.17 | 4.12±0.30 | 83.4 |
| Dried 2 days and rewet | 4.45±0.30 | 4.23±0.19 | 39.8 | 4.31±0.00 | 2.72±0.08 | 97.4 |
| 24 hours after rewet | 4.57±0.59 | 4.37±0.40 | 37.0 | 4.31±0.00 | 3.26±0.67 | 91.1 |
| Dried 30 days and rewet | 2.73±0.07 | 2.71±0.10 | 4.5 | 4.38±0.00 | 3.03±0.18 | 95.5 |
| 24 hours after rewet | 4.55±0.26 | 4.23±0.27 | 52.1 | 4.38±0.00 | 3.79±0.16 | 74.3 |
| Dried 60 days and rewet | 3.17±0.17 | 3.08±0.25 | 18.7 | 3.53±0.01 | 2.60±0.13 | 88.3 |
| 24 hours after rewet | 5.21±0.00 | 4.46±0.45 | 82.2 | 3.61±0.28 | 2.85±0.34 | 82.6 |
| *B) Bunker Creek, NH* | March, 2004 | | | July, 2004 | | |
| Moist sediment | 2.38±0.32 | 2.36±0.28 | 3.8 | 2.06±0.36 | 2.02±0.36 | 9.0 |
| Dried 2 days and rewet | 2.60±0.23 | 2.19±0.02 | 61.5 | 2.39±0.48 | 2.34±0.50 | 10.7 |
| 24 hours after rewet | 2.81±0.46 | 2.76±0.42 | 10.1 | 3.37±0.39 | 3.33±0.34 | 8.0 |
| Dried 30 days and rewet | 1.61±0.31 | 1.61±0.31 | 0 | 1.60±0.77 | 1.38±0.61 | 39.7 |
| 24 hours after rewet | 3.31±0.22 | 3.31±0.22 | 0 | 2.33±0.68 | 2.30±0.63 | 7.0 |
| Dried 60 days and rewet | 2.68±0.29 | 2.48±0.16 | 36.4 | 3.48±0.01 | 1.80±0.20 | 97.9 |
| 24 hours after rewet | 2.06±0.67 | 1.73±0.62 | 63.9 | 3.35±0.22 | 2.17±0.48 | 93.4 |
| *C) Chun-Chin Creek, PR* | March, 2004 | | | June, 2004 | | |
| Moist sediment | 4.00±0.01 | 2.20±0.01 | 98.4 | >4.38±0.01 | 2.24±0.01 | >99.3 |
| Dried 2 days and rewet | >4.38±0.01 | 1.98±0.01 | >99.6 | 3.93±0.01 | 1.46±0.01 | 99.7 |
| 24 hours after rewet | >4.38±0.01 | 2.11±0.01 | >99.5 | 3.71±0.01 | 1.30±0.01 | 99.6 |
| Dried 30 days and rewet | >4.38±0.01 | 1.81±0.01 | >99.7 | 3.68±0.01 | 0.60±0.01 | 99.9 |
| 24 hours after rewet | >4.38±0.01 | 1.27±0.01 | >99.9 | 3.87±0.01 | 0.81±0.01 | 99.9 |
| Dried 60 days and rewet | >4.38±0.01 | 1.22±0.01 | >99.9 | 3.53±0.01 | 0.20±0.01 | 99.9 |
| 24 hours after rewet | >4.38±0.01 | 1.68±0.01 | >99.8 | 3.82±0.01 | 0.77±0.01 | 99.9 |

## DISCUSSION

There were large numbers of fecal enterococci in moist sediments. These results are similar to many other studies that suggest sediments are reservoirs of large numbers of fecal bacteria (e.g., Howell et al., 1996). Disturbing this sediment may affect fecal counts. Therefore, care should be taken not to disturb the sediment when sampling, or if the sediment is already disturbed (e.g., on windy days or during runoff conditions), then the influence of sediment should be considered.

Fecal enterococci survived desiccation and sometimes regrew in sediment after rewetting. The most reasonable explanation for this survival and regrowth is the ability of the fecal enterococci to tolerate the high salt concentrations in the sediment. Fecal enterococci can tolerate 6.5% NaCl (USEPA, 2002).

Fecal enterococcal survival was poorest in Puerto Rican sediment, likely because of soil texture. Puerto Rican sediment contained a higher percentage of sand (46.9%) than sediments from New Hampshire (7.2%) or Georgia (9.1%). Soils with a high percentage of sand dry faster and have poorer bacterial survival than soils with a high percentage of clay (Hartel and Alexander, 1986).

According to the definition in *Standard Methods for the Examination of Water and Wastewater* (Clesceri et al., 1998), fecal indicator bacteria should not persist in the environment. Survival and regrowth of fecal enterococci violate this criterion. Furthermore, survival and regrowth affect bacterial source tracking results because the bacteria may represent a source of long past fecal contamination. These results reaffirm that an ideal fecal indicator bacterium does not exist, and care should be taken in interpreting fecal enterococcal data.

There was a serious methodological problem with the Enterolert system because false positive wells affected the MPN results. The greatest problem was observed in Puerto Rico sediment. Why there was so much variability among the sediments is unclear. Nevertheless, these results suggest that the Enterolert system should be used with caution in waters containing high amounts of sediment.

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## LITERATURE REVIEW

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