Final Report

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Fecal Indicator Bacteria and Potential Human Pathogens: Analysis of Sand, Sediment and Water in Maryland/Delaware Tidal Waters.

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Table of Contents

Introduction 1

Material and Methods 2

12-Month Study 5

Individual Sites Reports:

Assateague Island 5

Delaware Shore. 10

Granary Creek. 15

Sandy Point. 20

Combined Sites 12-Month Study Report. 26

Regrowth Study. 34

Individual Sites Reports:

Assateague Island 36

Delaware Shore. 42

Granary Creek. 47

Sandy Point. 56

Combined Sites Regrowth Study Report. 62

Conclusions 68

References 69

Acknowledgements 70

Appendix I. 71

Appendix II 72

INTRODUCTION

Enterococcus and *Escherichia coli* (*E. coli*) are fecal indicator organisms used to assess the human safety of freshwater beaches and recreational waters, while the enterococci level alone is used for marine coastal and estuarine recreational waters that are used for water contact activities (Federal Register 2004). The single sample maximum allowable densities at all areas are 33 Most Probable Number (MPN) per 100 ml and 126 MPN per 100 ml for enterococci and *E. coli*, respectively, for fresh water beaches and 35 MPN per 100 ml for enterococci for marine coastal recreational waters. When water monitoring finds that levels of these indicators have been exceeded, an advisory notice is issued, and the particular beach may be closed due to bacterial contamination.

It is assumed that the elevated densities result from pollution from sewage treatment plants or leaking on-site septic systems, agricultural runoff, input from contaminated waterways, or even bathers (Elmir et al. 2007). Recent studies, however, have shown that beach sands and sediments themselves can be sources of enterococci and *E. coli*. Investigators have found indicators organisms in beach sands and/or sediments of Lake Superior (Ishii et al. 2007), Lake Michigan (Beversdorf et al. 2006; Whitman and Nevers 2003; Alm et al. 2003), California (Ferguson et al. 2005), Florida (Anderson et al. 2005), and Southeastern Brazil (de Oliveira and Pinhata 2008).

Beversdorf and co-investigators (2006) suggest that regrowth and the moisture content of sand may be determinants of the persistence of *E. coli* in the sand environment in Lake Michigan beaches. In nonsterile, unseeded experiments, enterococci can grow in beach sand at rates equivalent to doubling times of 1.1 to 3.5 days after wetting by seawater (Yamahara et al. 2009). These findings suggest that water quality standards based on these two indicators may be problematic due to regrowth and their presence in sand. Anderson and others (2005) state that the presence of these bacterial indicators in beach sediments can falsely indicate recent water column pollution after rain or storm events, as well as when construction or recreational use occurs and bacteria are washed out into the water. Hartz and co-authors (2008) suggest that water quality managers consider moving the routine monitoring site further offshore to avoid increased densities due to bacteria washing out from the sand. Altogether, the evidence indicates that the sand/sediment contributions to recreational waters' bacterial levels may be significant and may need to be accounted for when conducting routine beach and recreational water monitoring.

The present investigation was conducted with two goals in mind. The first was to assess the levels of indictor bacteria in beach sand, beach sediment, and water at four (4) Mid-Atlantic marine coastal recreational waters, including three (3) sites in Maryland and one (1) in Delaware. The relationship, if any, among the bacterial levels in sand, sediments and water would also be determined. The second goal was to determine whether regrowth is occurring where elevated indicator levels are found and/or to discover the presence of bacterial pathogens that might suggest a pollution source.

INTRODUCTION

Enterococcus and *Escherichia coli* (*E. coli*) are fecal indicator organisms used to assess the human safety of freshwater beaches and recreational waters, while the enterococci level alone is used for marine coastal and estuarine recreational waters that are used for water contact activities (Federal Register 2004). The single sample maximum allowable densities at all areas are 33 Most Probable Number (MPN) per 100 ml and 126 MPN per 100 ml for enterococci and *E. coli*, respectively, for fresh water beaches and 35 MPN per 100 ml for enterococci for marine coastal recreational waters. When water monitoring finds that levels of these indicators have been exceeded, an advisory notice is issued, and the particular beach may be closed due to bacterial contamination.

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The present investigation was conducted with two goals in mind. The first was to assess the levels of indictor bacteria in beach sand, beach sediment, and water at four (4) Mid-Atlantic marine coastal recreational waters, including three (3) sites in Maryland and one (1) in Delaware. The relationship, if any, among the bacterial levels in sand, sediments and water would also be determined. The second goal was to determine whether regrowth is occurring where elevated indicator levels are found and/or to discover the presence of bacterial pathogens that might suggest a pollution source.

Materials and Methods

Site description

Four tidal beaches, one in Delaware and three in Maryland, were studied. The Delaware site was located in Sussex County, along the Atlantic coast (38° 38.552' N, 75° 10.077' W) (referred to as the Delaware Shore (DS) throughout the report). The three Maryland sites include: A bayside beach on Assateague Island (AI) National Seashore Federal Park located in Worcester County Maryland (38° 12.607' N, 75° 10.077' W); Granary Creek (GC), a tributary of the Wye River located in Queen Anne's County, Maryland (38° 53.042' N, 76° 08.091' W); and a beach on the west side of the Chesapeake Bay at Sandy Point (SP) State Park in Anne Arundel County, Maryland (39° N, 76° 23.928' W).

Sample collection

Monthly sampling was conducted during 2008 from January through April and from October through December, with bimonthly sampling from May through September. At each of the study sites, single cylindrical core sediment samples of approximately 2 cm x 25 cm were collected. Sediment collected in the swash zone between the high tide line and the water's edge was referred to as the "dry" sample, and sediment collected under the water at a distance from the shore line that was at knee depth was referred to as the "wet" sample. At the knee-depth location, 4 replicate water samples were also collected. Three (3) were used to determine indicator bacteria densities, and the 4th was used to screen for pathogens. All samples were stored on ice and delivered to the lab within 4 hours of collection.

Enumeration of fecal indicator organisms

Water

Triplicate water samples from each sampling site and date were tested by the Quanti-Tray® method of Idexx Laboratories, Inc. For the enumeration of *Escherichia coli* (*E. coli*) Colisure® was used for samples collected from January through July 8th and Colilert-18™ for samples collected from July 22nd through December 9th (midway through the project we determined that the Colilert-18™ system was preferable to Colisure® when testing marine waters). Enterolert® was used for the enumeration of enterococci. Results are read and recorded as Most Probable Number (MPN)/100 ml.

Sediment

Sediment was removed from each collection tube and placed into a sterile bottle for mixing. Twenty grams of sediment was added to 100 ml of extraction buffer (0.1% gelatin/0.1 M $(NH_4)_2HPO_4$) (Ishii et al. 2005) and mixed for 30 minutes using a wrist-action shaker. Sediment was allowed to settle for an additional 30 minutes. Various volumes of supernatant were passed through membrane filters and plated onto Difco™ mEnterococcus Agar for selective growth and enumeration of enterococci or Difco™ mTEC agar for the selective growth and enumeration of *E. coli*. A known weight of each sediment sample was also placed in a drying oven (60ºC) for 24–48

hours. Samples were then reweighted to determine the original moisture content of each sample. The number of *E. coli* and enterococci were expressed as CFU/100g dry weight.

Selection for potential pathogens

Various volumes of water and supernatant from sediment samples (as described above) were passed through membrane filters and placed onto different types of media for selective growth of potential pathogens. Selective agars used included: Acumedia® SS agar for growth of *Salmonella* and *Shigella*, BBL™ CIN agar for growth of *Yersinia*, BBL™ XLT4/Brilliant Green Agar for growth of *Salmonella enteritidis* and *Salmonella typhimurium*, Difco™ Campy CSM agar for growth of *Campylobacter*, Difco™ MS Agar (MacConkey's Sorbitol Agar) for growth of *E. coli* O157:H7, Rainbow® Agar O157 for growth of *E. coli* O157:H7, Rainbow® Agar Salmonella for growth of H_2S-producing *Salmonella*.

Identification of selected bacterial isolates

Isolates taken from selective media were considered potential pathogens and were analyzed for identification to genus and species. All isolates were processed by the BioLog MicroStation™, a phenotypic carbon utilization identification system. Several isolates were also analyzed by 16S rDNA sequence analysis as conducted by Midi Labs Inc., Newark, DE. A number of isolates were identified as "possible" *E. coli* O157:H7, but none of these isolates were confirmed as such by DNA or BioLog analysis. An Enzyme-Linked Immunosorbent Assay (ELISA) was conducted on these 15 isolates to determine if they could be confirmed as *E. coli* O157:H7 using serology.

Regrowth Studies

Selected water and sediment samples were analyzed for their ability to allow for growth of *E. coli* and/or enterococci. After initial counts of both fecal indicator organisms, water and/or sediment samples were placed at different temperatures (4 ºC, 21 ºC (room temp.), 24ºC, 30 ºC, and/or 37 ºC) for periods of time ranging from four to 13 days. The number of fecal indicator organisms in sediment and water, at the various time points, were determined as described above.

Statistical Analyses

Statistical analyses were performed using Minitab 15 Statistical Software (Minitab, Inc., State College, PA). *E. coli* and enterococci density data were $\log_e$ tranformed to meet assumptions of parametric analyses. Tests for normality used the Ryan-Joiner test for normality and a normal probability plot. Significance is set at $\alpha = 0.05$, with a trend toward statistical significance at $0.05 > p < 0.10$.

Pearson correlation analysis was performed to determine the strength of the linear association between Ln *E. coli* and Ln enterococci in sands, sediments, and water at each site. Percent moisture in sediments was also analyzed to determine the strength of the linear relationship with bacterial densities in the sediment samples. A one-way ANOVA was also performed to determine the presence of a difference in means of Ln *E. coli* and Ln enterococci in sands, sediments, and water. A *p*-value of ≤ 0.05 was used to designate statistical significance.

Regression analysis of the form $Y = b_o + b_1x$ was used to determine the direction, size, and statistical significance of the predictive linear relationship between the response and the predictor variable(s). The resulting R-Sq (adj) indicates the percentage variation in the response variable that can be explained by the predictor variables and regression equation. Again, an α-level of 0.05 was used to determine significance.

Each of the 4 sites was analyzed separately to determine any unique relationships between and among the variables. The composite data were then analyzed to determine any trends with regard to the indicator bacteria in the "dry" and "wet" sediments, and water.

12-Month Study of *E. coli* and Enterococci Densities and Potential Pathogens: Initial Analysis of Water and Sediment Samples

The single sample maximum density standards for fresh coastal recreational waters are 126 MPN/100 ml for *E.* coli and 33 MPN/100 ml for enterococci. For marine recreational waters, the enterococci single sample maximum density standard is 33 MPN/100 ml (U.S. Environmental Protection Agency (EPA) 2004). There are no standards or recommendations for bacteria levels in beach or submerged sand; however, as a way to compare the number of days that sediment densities were high with the number of exceedance days for water, we choose to set a "high" density value for sediment at the same level as for the standard in water, 126 CFU (per 100 g dry weight sediment) for *E. coli* and 33 CFU (per 100 g dry weight sediment) for enterococci.

Assateague Island 12-Month Study

The bacterial densities in water, in wet sediment, and in dry sediment collected at Assateague Island are shown in Tables 1 and 2 and displayed graphically by bar graphs and times plots in Figures 1-4. The descriptive statistics, along with their geometric means, are in Tables 3 and 4.

Only two of the *E. coli* densities in water exceeded 126 MPN/100 ml (Table 1). In contrast, 3 *E. coli* densities in wet sand exceeded that level and 4 in dry sediment, with both of the highest densities occurring in January.

TABLE 1. *E. coli* densities in water (MPN/100 ml), wet and dry sediments (CFU/100 g dry weight) at Assateague Island.

Date	Water	Wet Sed	Dry Sed
January 30th	5	1030	12000
February 19th	5	50	5
March 12th	5	50	5180
April 9th	5	5	5
May 7th	5	5	5
May 21st	5	5	290
June 10th	5	340	5
June 24th	5	61	5
July 8th	5	5	5
July 22nd	83	5	2500
August 5th	10	5	5
August 25th	2100	5	5
September 9th	845	241	5
September 23rd	12	5	5
October 14th	10	5	5
November 18th	14	5	5
December 9th	5	5	5

The enterococci densities in water exceeded the 33 MPN/100 ml level once (Table 2). Thirty-five percent (35%) of the densities in wet sediment were in excess of 33 MPN/100 g dry weight and 76% in dry sediment.

TABLE 2. Enterococci densities in water (MPN/ 100 ml), wet and dry sediments (CFU/100 g dry weight) at Assateague Island.

	Water	Wet Sed	Dry Sed
January 30th	20	50	880
February 19th	5	5	5
March 12th	5	190	5
April 9th	5	5	50
May 7th	17	5	5
May 21st	5	5	490
June 10th	5	170000	24000
June 24th	5	5	172
July 8th	5	150	50
July 22nd	5	120	230
August 5th	5	5	57
August 25th	109	5	61
September 9th	10	241	345
September 23rd	5	5	588
October 14th	5	5	57
November 18th	5	5	60
December 9th	5	5	5

TABLE 3. Descriptive Statistics: *E.coli* in water (MPN/100 ml), in wet and dry sediments (CFU/100 g dry weight) at Assateague Island.

Variable	**N**	**N***	**Mean**	**SE Mean**	**StDev**	**Median**	**Maximum**	**Geometric Mean**
E. coli Water	17	0	181.0	130.0	535.0	3.0	2100.0	12.4 MPN/100 ml
E. coli WetSed	17	0	104.2	62.4	257.2	0.0	1030.0	16.7 CFU/100 g
E. coli DrySed	17	0	1175.0	752.0	3099.0	0.0	12000.0	21.8 CFU/100 g

TABLE 4. Descriptive Statistics: Enterococci in water (MPN/100 ml), in wet and dry sediment (MPN/100 g dry weight) at Assateague Island.

Variable	**N**	**N***	**Mean**	**SE Mean**	**StDev**	**Median**	**Maximum**	**Geometric Mean**
Ent. Water	17	0	9.5	6.4	26.4	0.0	109.0	6.9 MPN/100 ml
Ent. WetSed	17	0	10044.0	9997.0	41220.0	0.0	170000.0	24.2 CFU/100 g
Ent. DrySed	17	0	1591.0	1402.0	5780.0	60.0	24000.0	89.6 CFU/100 g

FIG. 1. *E. coli* densities in water and sediments by collection date.

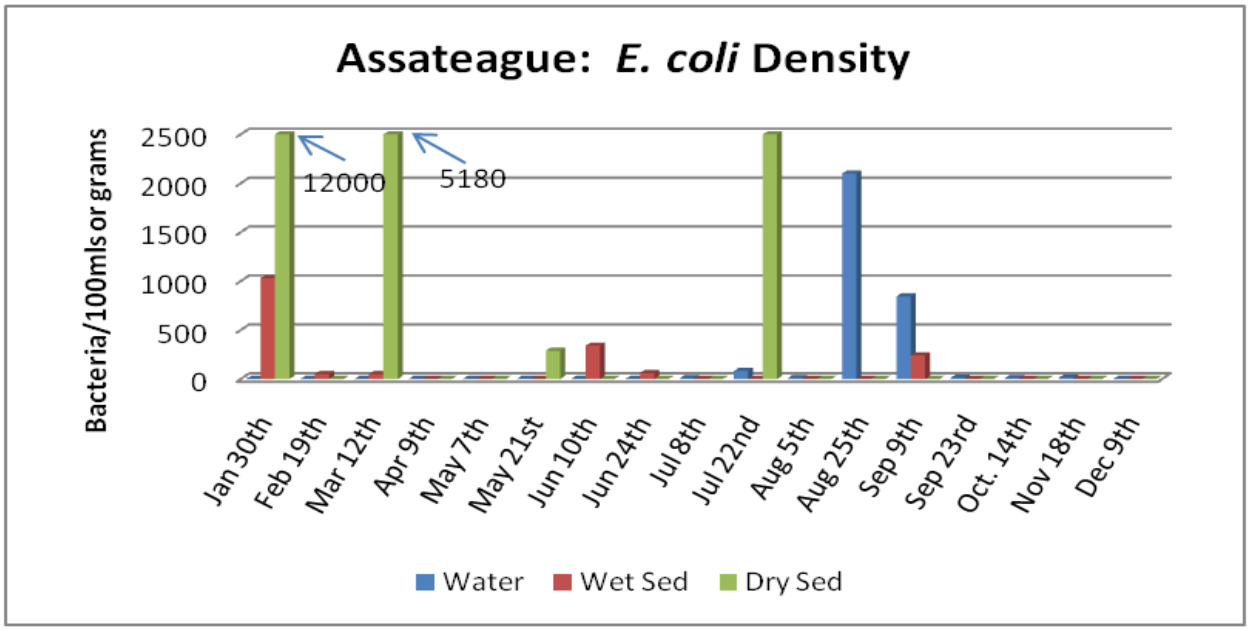


FIG. 2. Time plot of Ln *E. coli* densities collected samples of water, wet sediment, and dry sediment.

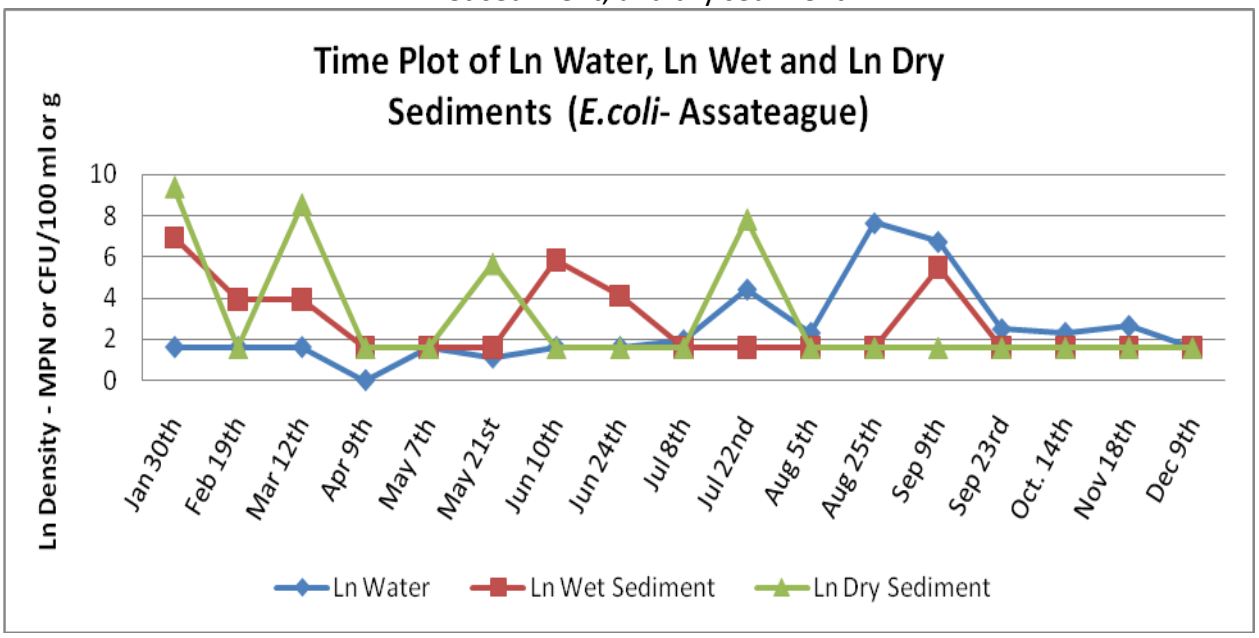


FIG. 3. Enterococci densities in water and sediments by collection date.

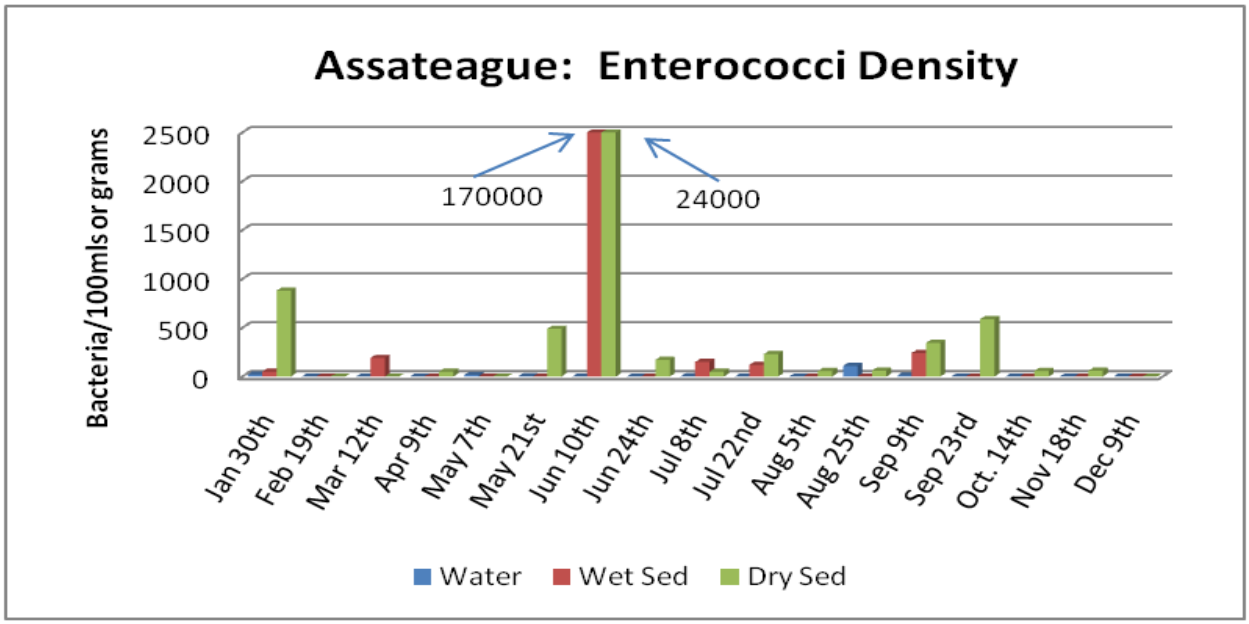
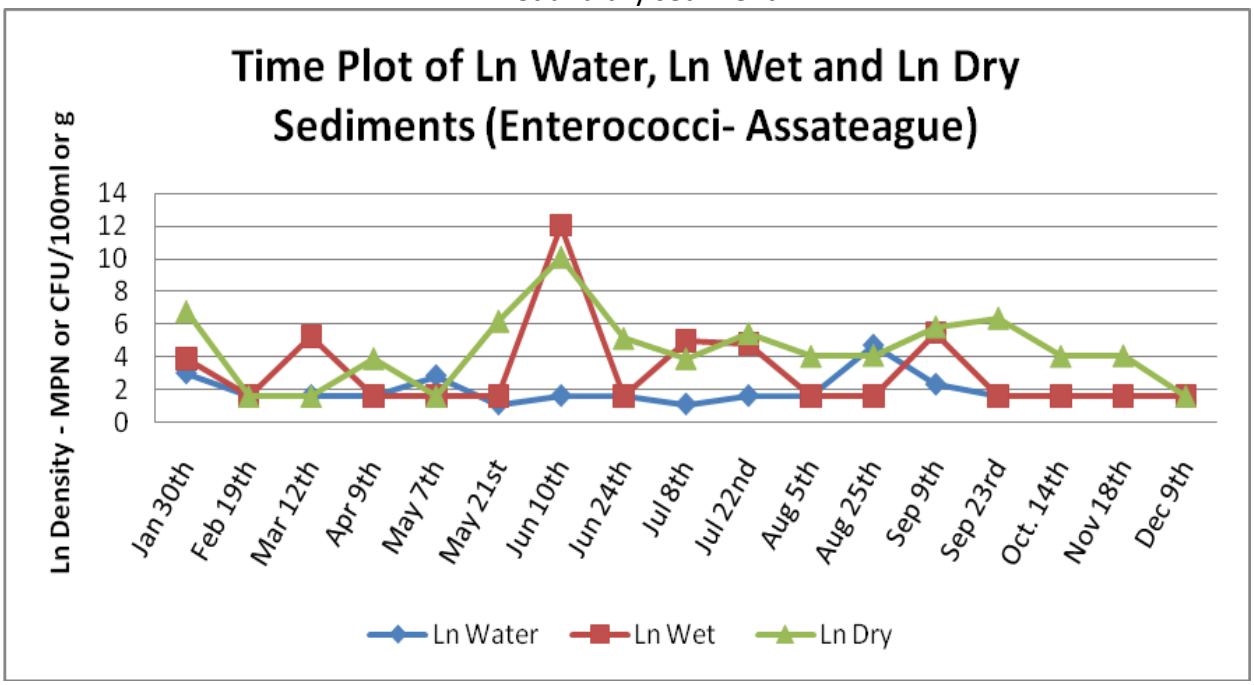


FIG. 4. Time plot of Ln enterococci densities in the samples of water, wet and dry sediment.



The mean *E. coli* density in dry sediment was over 6 times more than that in beach water, but densities in submerged wet sediment was less than that in water.

A significant predictive linear relationship (F statistic = 9.84; $p = 0.007$) was found between Ln *E. coli* in water and Ln enterococci in water that explained 36% of the variation in Ln *E. coli* in water. No statistically significant difference was found between the mean densities of Ln *E. coli* in water and Ln enterococci in water ($p = 0.264$).

No significant linear association was found between Ln *E. coli* in water and Ln *E. coli* density in either of the sediments. For Ln enterococci, the only other significant correlation was between the wet and dry sediments ($p = 0.11$).

Potential Pathogens.

There was one potential pathogen found in water collected from Assateague Island (*Escherichia*/*Shigella*), but when this isolate was tested against two DNA libraries and by ELISA, no exact match to a pathogen was confirmed. The list of identified organisms cultured from these samples is in Appendix I. The complete list of organisms cultured from Assateague Island samples and those from the other sites is in Appendix II.

Delaware Shore 12-Month Study

Water and Sediments.

The bacterial densities in water, in wet sediment, and in dry sediment collected at Delaware Shore are shown in Tables 5 and 6 and displayed graphically by bar graphs and time plots in Figures 5-8. The descriptive statistics, along with their geometric means, are in Tables 7 and 8.

Eighteen percent (18%) of *E. coli* densities in water were above the detection limit and none was more than 13 MPN/100 ml. Only 1 sample each of the wet and dry sediment samples was above the detection limit.

TABLE 5. *E. coli* densities in water (MPN/100 ml), wet and sediments (CFU/100 g dry weight) at Delaware Shore.

Date	Water	Wet Sediment	Dry Sediment
January 30th	5	5	5
February 19th	5	5	5
March 12th	5	5	5
April 9th	5	5	5
May 7th	5	5	5
May 21st	5	5	5
June 10th	5	5	5
June 24th	5	5	5
July 8th	7	5	5
July 22nd	5	1500	1200
August 5th	13	5	5
August 25th	5	5	5
September 9th	10	5	5
September 23rd	5	5	5
October 14th	5	5	5
November 18th	5	5	5
December 9th	10	5	5

Only 29% of enterococci densities in water samples was above the detection limit, and none was higher than 20 MPN/100 ml (Table 6). Twenty-four percent (24%) of wet sediment exceeded 33 CFU/100 g dry weight, while 18% of dry sediment samples exceeded that level.

Table 6. Enterococci densities in water (MPN/100 ml), wet and dry sediments (MPN/100 g dry weight) at Delaware Shore.

Date	Water	Wet Sediment	Dry Sediment
January 30th	5	5	5
February 19th	5	5	5
March 12th	7	50	5
April 9th	5	5	5
May 7th	7	5	5
May 21st	5	5	5
June 10th	5	60	5
June 24th	5	61	5
July 8th	10	100	5
July 22nd	5	5	5
August 5th	5	5	153
August 25th	5	5	465
September 9th	5	5	5
September 23rd	5	5	5
October 14th	10	5	227
November 18th	20	5	5
December 9th	5	5	5

Table 7. Descriptive Statistics: *E.coli* in water (MPN/100 ml), in wet and dry sediment (CFU/100 g dry weight) at Delaware Shore.

Variable	N	N*	Mean	SE Mean	StDev	Median	Maximum	Geometric Mean
E. coli Water	17	0	5.6	0.7	2.9	5.0	13.0	4.9 MPN/100 ml
E. coli WetSed	17	0	92.9	87.9	362.6	5.0	1500.0	7.0 CFU/100 g
E. coli DrySed	17	0	75.3	70.3	289.8	5.0	1200.0	6.9 CFU/100 g

Table 8. Descriptive Statistics: Enterococci in water (MPN/100 ml), in wet and dry sediment (CFU/100 g dry weight) at Delaware Shore.

Variable	N	N*	Mean	SE Mean	StDev	Median	Maximum	Geometric Mean
Ent. Water	17	0	6.1	1.0	4.3	5.0	20.0	5.1 MPN/100 ml
Ent. WetSed	17	0	19.8	7.1	29.1	5.0	100.0	9.2 CFU/100 g
Ent. DrySed	17	0	53.8	29.8	123.1	5.0	465.0	10.0 CFU/100 g

FIG. 5. *E. coli* densities in water and sediments by collection date.

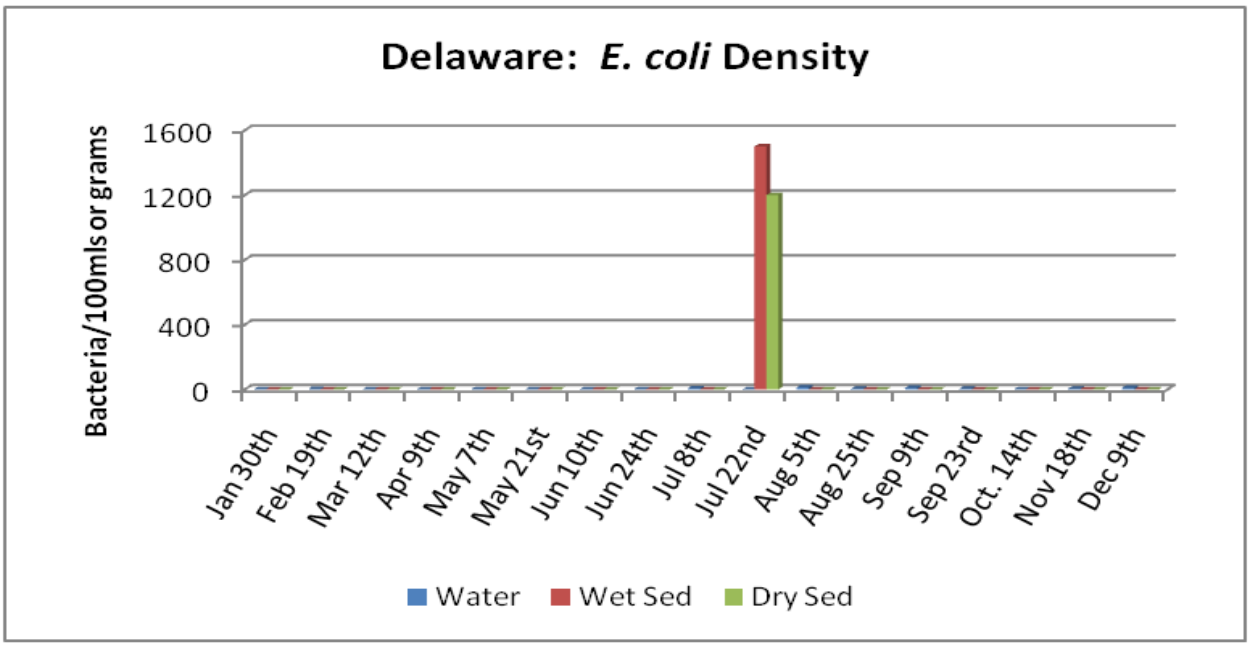


FIG. 6. Time plot of Ln *E. coli* densities in water, wet sediment, and dry sediment.

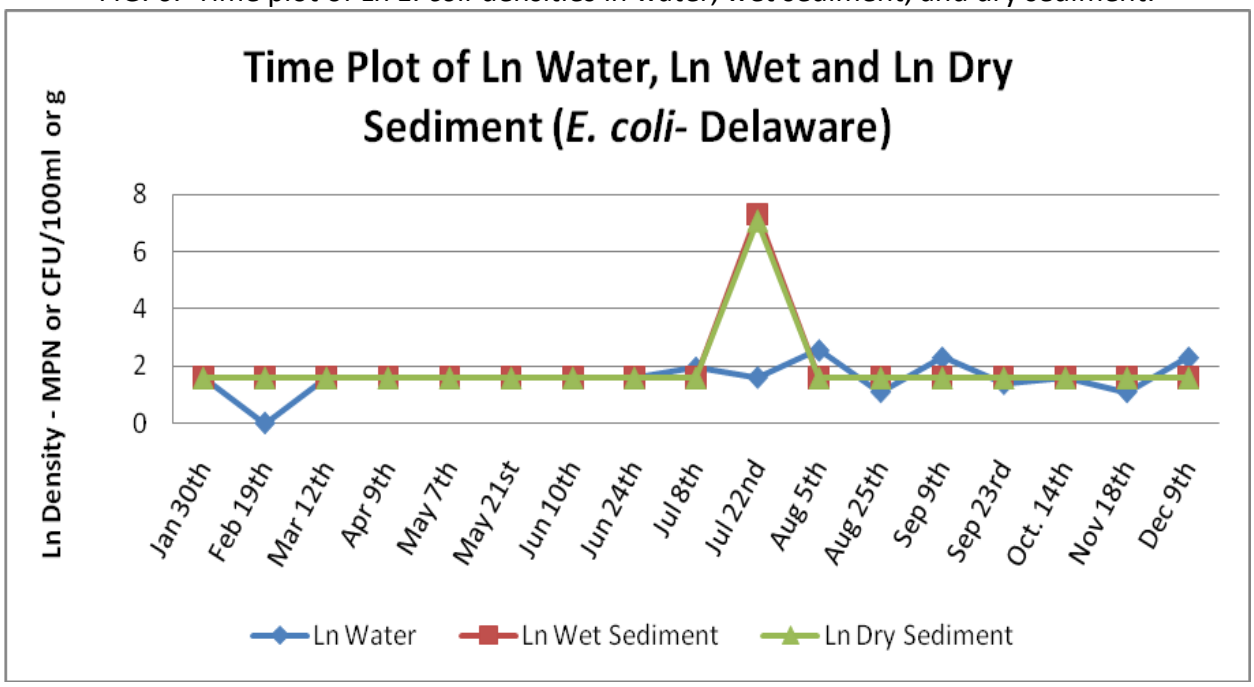


FIG. 7. Enterococci densities in water and sediments by collection date.

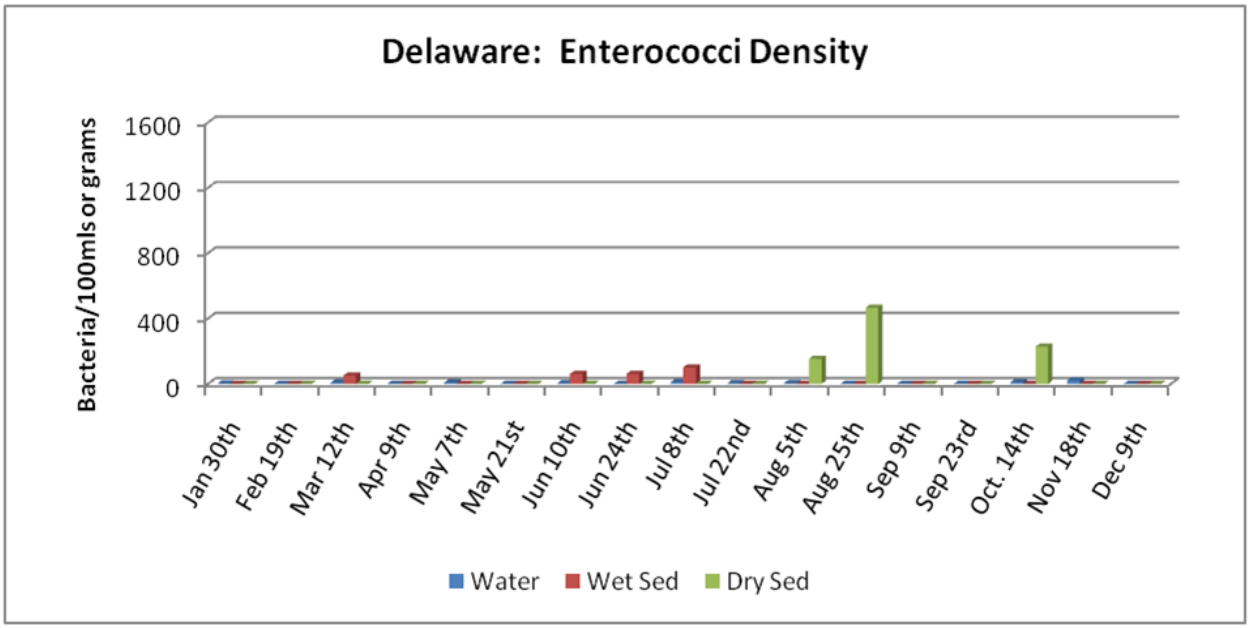
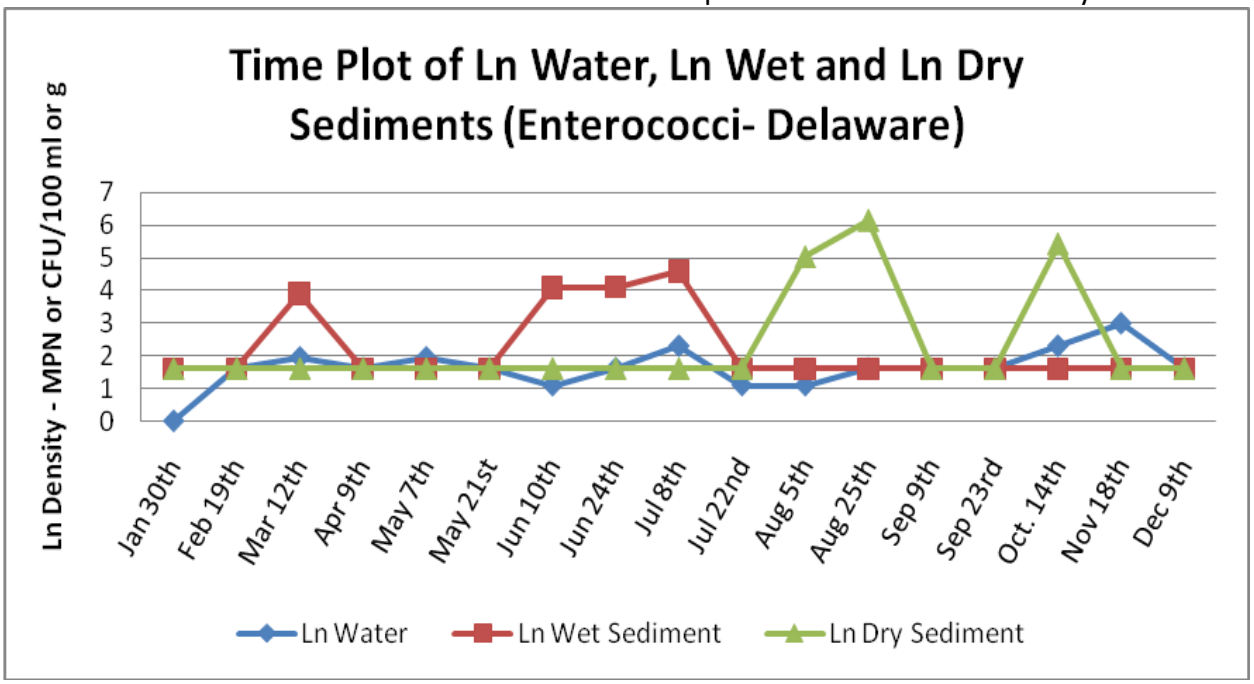


FIG. 8. Time of Ln enterococci densities in the samples of water and wet and dry sediment.



The mean *E. coli* density in submerged wet sediment was over 16 times more than that in beach water, and the mean density in dry sediment was 13 times that in water. The mean enterococci level in wet sediments was only about three (3) times than in water, while the mean dry sediment was about (9) times more than that in water.

No significant linear association was found between Ln *E. coli* in water and density in either wet or dry sediments, nor was there a significant linear association found among Ln enterococci and the sediments or between Ln *E. coli* and Ln enterococci.

No predictive relationship existed between Ln *E. coli* in water versus Ln enterococci in water or between with Ln *E. coli* in water and Ln enterococci in water versus their respective wet and dry sediments. There was no statistically significant difference in the mean densities of Ln *E. coli* in water and Ln enterococci in water ($p = 0.892$).

Potential Pathogens.

There was one potential pathogen found in a Delaware Shore water sample: *Shigella sonnei*. The identity of this organism was confirmed in a DNA match to sequences in GenBank[1], but was not confirmed when tested against sequences in the Midi Labs DNA certified library[2]. The list of identified organisms cultured from these samples is in Appendix I. The complete list of identified organisms in this study is in Appendix II.

[1]DNA sequences entered without verification
[2]DNA sequences derived from known bacterial cultures obtained from the American Type Culture Collection.

Granary Creek 12-Month Study

Water and Sediments.

The bacterial densities in water, in wet sediment, and in dry sediment collected at Granary Creek are shown in Tables 9 and 10 and displayed graphically by bar graphs and time plots in Figures 13-14. The descriptive statistics, along with their geometric means, are in Tables 11 and 12.

Four (4) of the *E. coli* densities in water exceeded 126 MPN/100 ml (Table 9). In contrast, all *E. coli* densities in wet sand exceeded that standard, while 41% of the *E. coli* densities in dry sand did.

TABLE 9. *E. coli* densities in water (MPN/100 ml), wet and dry sediments (CFU/100 g dry weight) at Granary Creek.

Date	Water	Wet Sediment	Dry Sediment
January 30th	34	1520	5140
February 19th	9	1330	223
March 12th	5	7130	120
April 9th$_1$	5	50	5
May 7th	17	125	5
May 21st	7	320	1180
June 10th	7	5	180
June 24th	10	1600	14000
July 8th	17	1200	300
July 22nd	234	192	57
August 5th	126	5	57
August 25th	147	5	5
September 9th	759	864	23000
September 23rd	5	5	5
October 14th	52	185	122
November 18th	33	5	5
December 9th	24	5	5

The enterococci densities in water exceeded the 33 MPN/100 ml level only twice (Table 10). Seventy-one percent (71%) the enterococci densities in wet sediment were above that standard level, while 88% of dry sediment did.

TABLE 10. Enterococci densities in water (MPN/100 ml), wet and dry sediments (CFU/100 g dry weight) at Granary Creek.

Date	Water	Wet SedIment	Dry Sediment
January 30th	23	300	340
February 19th	20	5840	223
March 12th	5	9290	170
April 9th	5	290	290
May 7th	7	310	310
May 21st	13	640	2130
June 10th	14	5	490
June 24th	5	1500	3100
July 8th	20	5	650
July 22nd	5	128	682
August 5th	17	5	431
August 25th	27	125	139
September 9th	144	247	2100
September 23rd	5	5	5
October 14th	5	5	5
November 18th	82	122	123
December 9th	23	123	3500

TABLE 11. Descriptive Statistics: *E.coli* means in water (MPN/100 ml), in wet and dry sediment (CFU/100 g dry weight), and Geometric Means at Granary Creek.

Variable	N	N*	Mean	SE Mean	StDev	Median	Maximum	Geometric Mean
E. coli Water	17	0	87.4	44.8	184.6	17.0	759.0	22.5 MPN/100 ml
E. coli WetSed	17	0	853.0	418.0	1723.0	185.0	7130.0	92.5 CFU/100 g
E. coli DrySed	17	0	2612.0	1530.0	6310.0	120.0	23000.0	109.7 CFU/100 g

TABLE 12. Descriptive Statistics: Enterococci in water (MPN/100 ml), in wet and dry sediment (CFU/100 g dry weight) at Granary Creek.

Variable	N	N*	Mean	SE Mean	StDev	Median	Maximum	Geometric Mean
Ent. Water	17	0	24.6	8.7	.9	14.0	144.0	13.4 MPN/100 ml
Ent. WetSed	17	0	1114.0	613.0	2529.0	128.0	9290.0	121.1 CFU/100 g
Ent. DrySed	17	0	864.0	270.0	1114.0	340.0	3500.0	309.8 CFU/100 g

FIG. 9. *E. coli* densities in water and sediments by collection date.

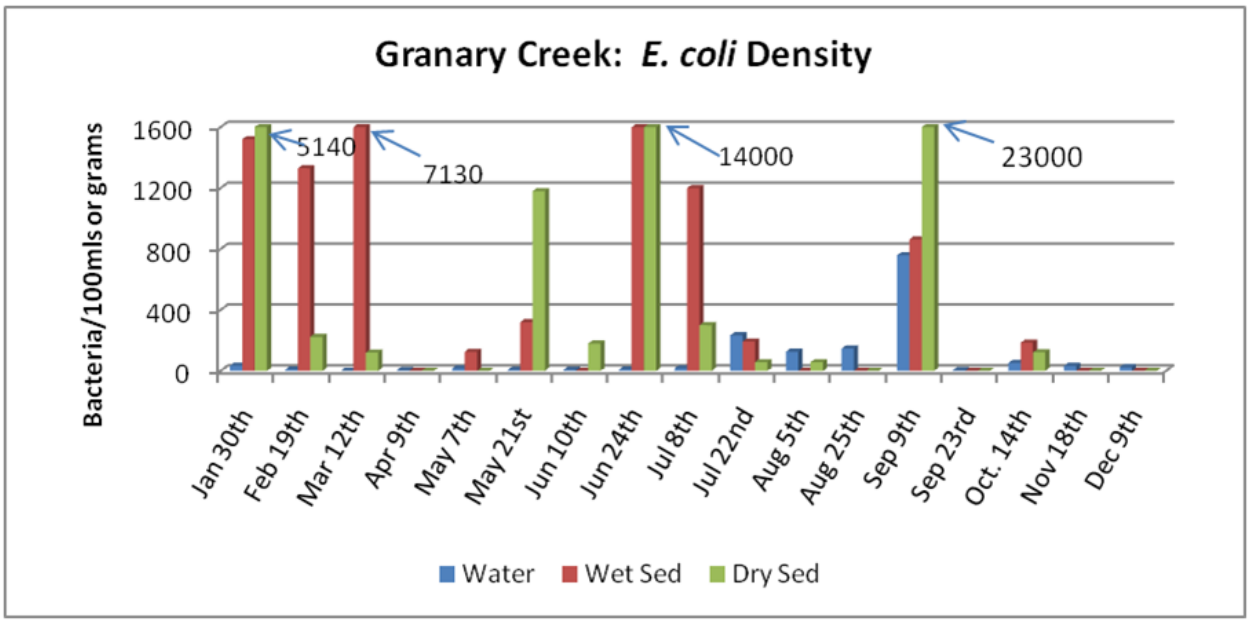


FIG. 10. Time plot of Ln *E. coli* densities in samples of water, wet and dry sediment.

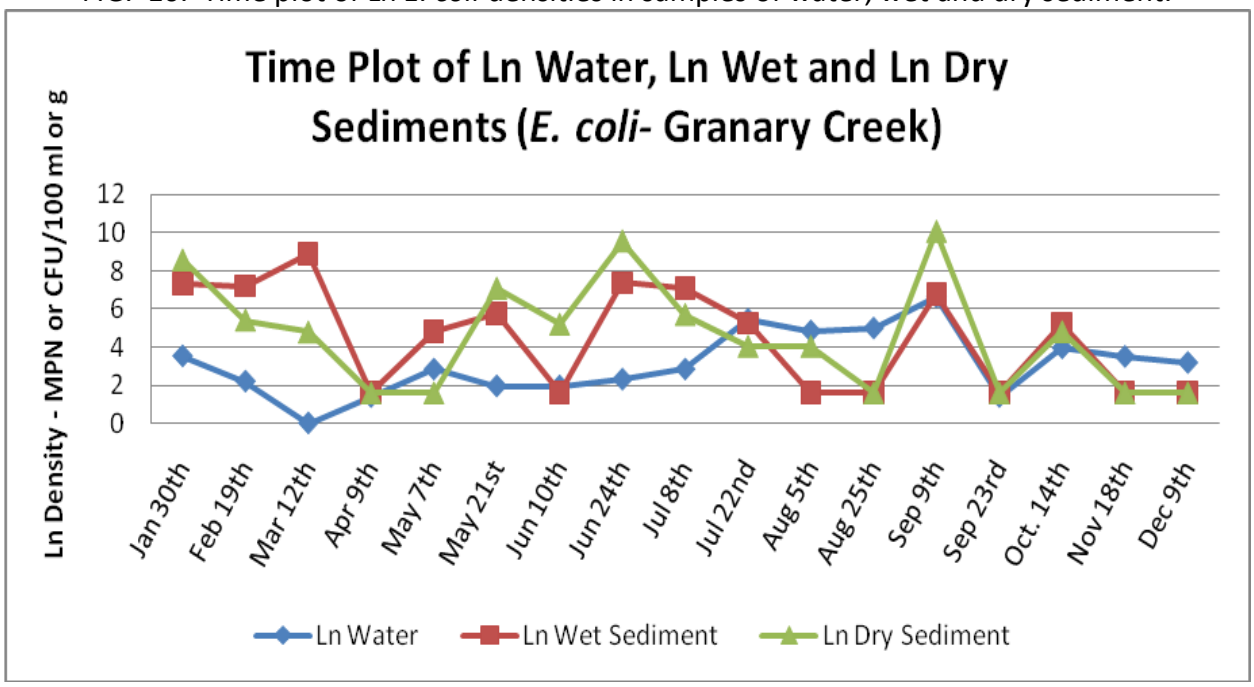


FIG. 11. Enterococci densities in water and sediments by collection date.

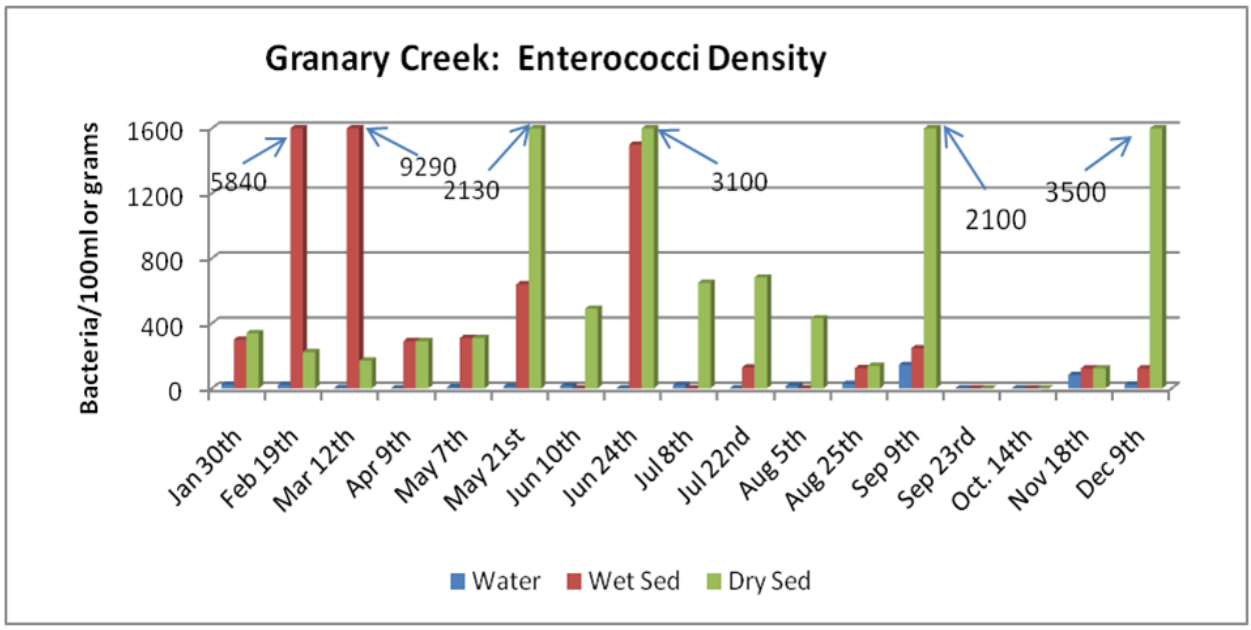
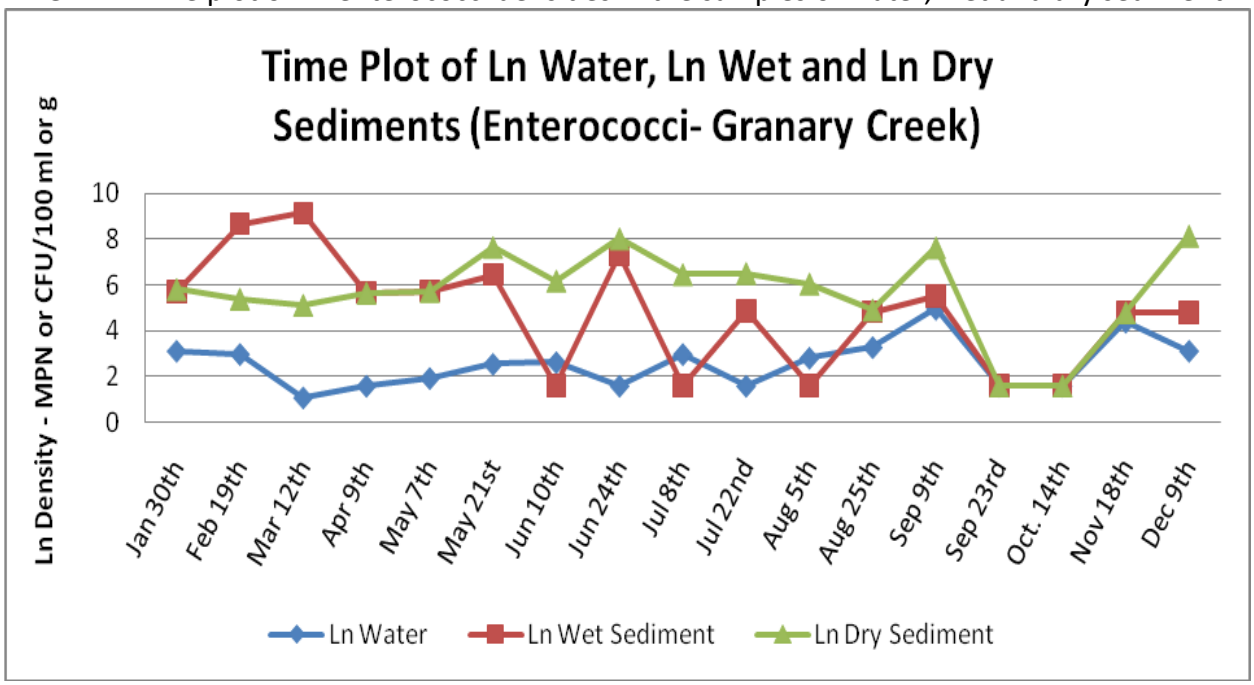


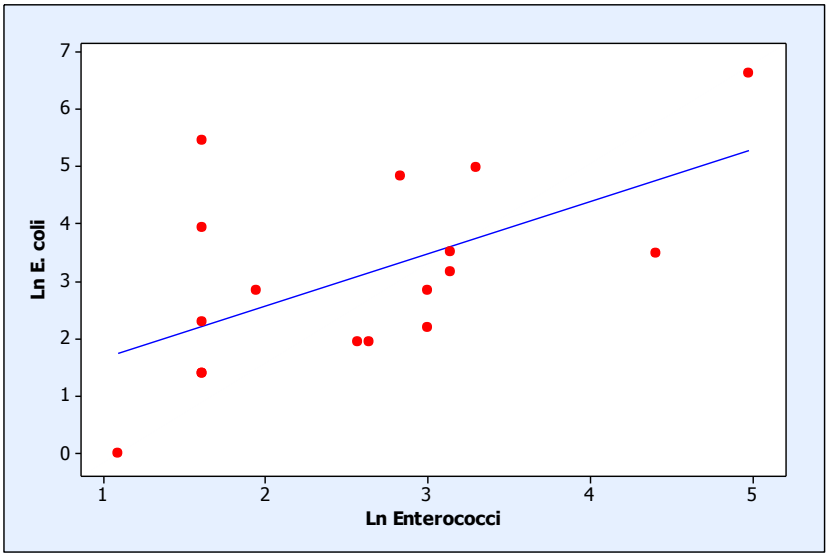
FIG. 12. Time plot of Ln enterococci densities in the samples of water, wet and dry sediment.



There was a highly significant correlation between Ln *E. coli* in dry sediment and Ln *E. coli* in wet sediment (p = 0.001).

A significant predictive relationship (F statistic = 7.48; *p* = 0.015) was found between Ln *E. coli* in water versus Ln enterococci in water, which explained 29% of the variation in Ln *E. coli* densities in water (Figure 13). There was no statistically significant difference in the mean densities of Ln *E. coli* in water and Ln enterococci in water (*p* = 0.290).

FIG. 13. Scatter plot and regression line for Ln *E. coli* in water versus Ln enterococci in water.



Potential Pathogens.

Four (4) potential pathogens were found in water or dry sediments collected from Granary Creek, including *E. coli* (2), *Shigella* and *Yersinia*, but none were confirmed matches when tested against a certified library (Midi Labs, Newark, DE). The *E. coli* culture was also tested for *E. coli* 0157:H7 by ELISA analysis in the BST Laboratory and found negative for that strain. The list of these potential pathogens is in Appendix I. The complete list of identified organisms cultured from water and sediment samples at Granary Creek and the other sites is in Appendix II.

Sandy Point 12-Month Study

Water and Sediments.

The bacterial densities in water, in wet sediment, and in dry sediment collected at Sandy Point are shown in Tables 16 and 17 and displayed graphically by scatter plots, bar graphs, and time plots in Figures 19-22. The descriptive statistics, along with their geometric means, are in Tables 18 and 19.

Two (2) of the *E. coli* densities in water exceeded 126 MPN/100 ml in late July and late August (Table 16). Twenty-nine percent (29%) of the wet sediment samples exceeded that level, while 47% of dry sediment exceeded it.

TABLE 16. *E. coli* densities in water (MPN/100 ml), wet (CFU/ 100 g dry weight) and dry sediments (CFU/100 g dry weight) at Sandy Point.

Date	Water	Wet Sediment	Dry Sediment
January 30th	10	5	5440
February 19th	5	100	5
March 12th	43	780	860
April 9th	5	5	5
May 7th	94	110	5770
May 21st	5	170	120
June 10th	38	270	2490
June 24th	5	380	160
July 8th	7	5	500
July 22nd	422	298	118
August 5th	24	116	119
August 25th	160	115	176
September 9th	74	5	116
September 23rd	5	5	5
October 14th	5	5	5
November 18th	20	115	176
December 9th	5	5	5

The enterococci densities in water exceeded the 33 MPN/100 ml level 24% of the time (Table 17). All of the samples of wet sediment and dry sediment with densities above the detection level exceeded 33 CFU/100 g level.

TABLE 17. Enterococci densities in water (MPN/100 ml), wet and dry sediments (CFU/100 g dry weight) at Sandy Point.

Date	Water	Wet Sediment	Dry Sediment
January 30th	17	5	5
February 19th	5	5	5
March 12th	10	5	5
April 9th	10	5	5
May 7th	5	5	110000
May 21st	5	170	300
June 10th	7	5	280
June 24th	10	590	430
July 8th	183	100	350
July 22nd	244	5	588
August 5th	7	5	179
August 25th	303	977	941
September 9th	10	115	291
September 23rd	5	5	5
October 14th	5	5	119
November 18th	41	5	5
December 9th	*5*	*5*	*5*

The mean *E. coli* density in dry sediment was over 17 times more than that in beach water, and densities in submerged wet sediment was over twice that in water (Table 18). The enterococci mean in sediments was 134 times more in dry sediment than in water, but just over twice times that in wet sediment Table 19). These means indicate a differentially high accumulation of E. *coli* and enterococci in sand than in water.

TABLE 18. Descriptive Statistics: *E.coli* means in water (MPN/100 ml), in wet and dry sediment, (CFU/100 g dry weight), and geometric means at Sandy Point.

Variable	N	N*	Mean	SE Mean	StDev	Median	Maximum	Geometric Mean
E. coli Water	17	0	53.0	25.3	104.5	10.0	422.0	16.2 MPN/100 ml
E. coli WetSed	17	0	144.4	48.9	201.7	110.0	780.0	42.8 CFU/100 g
E. coli DrySed	17	0	944.0	450.0	1855.0	120.0	5770.0	114.7 CFU/100 g

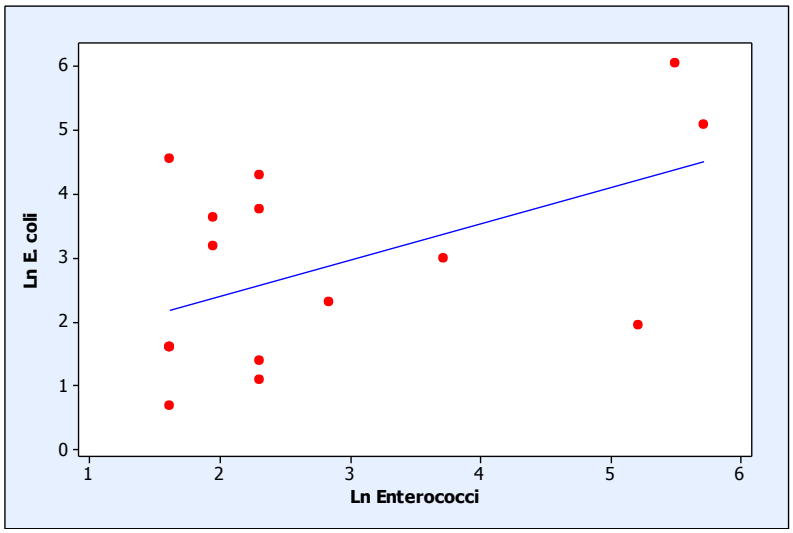
TABLE 19. Enterococci means in Water (MPN/100 ml), in wet sediment and dry sediment (CFU/100 g dry weight), and geometric means at Sandy Point.

Variable	N	N*	Mean	SE Mean	StDev	Median	Maximum	Geometric Mean
Ent. Water	17	0	49.5	23.1	95.4	10.0	303.0	14.7 MPN/100 ml
Ent. WetSed	17	0	114.8	64.5	265.8	0.0	977.0	15.9 cfu/100 g
Ent. DrySed	17	0	6675.0	6458.0	26627.0	179.0	110000.0	82.4 cfu/100 g

A significant linear association was found between Ln *E. coli* in dry sediment and Ln *E. coli* in water ($p = 0.037$). There was also a significant relationship between Ln *E. coli* with Ln enterococci in water ($p = 0.030$). There was an additional statistically significant linear relationship between Ln *E. coli* in water and Ln enterococci in dry sediment ($p = 0.033$).

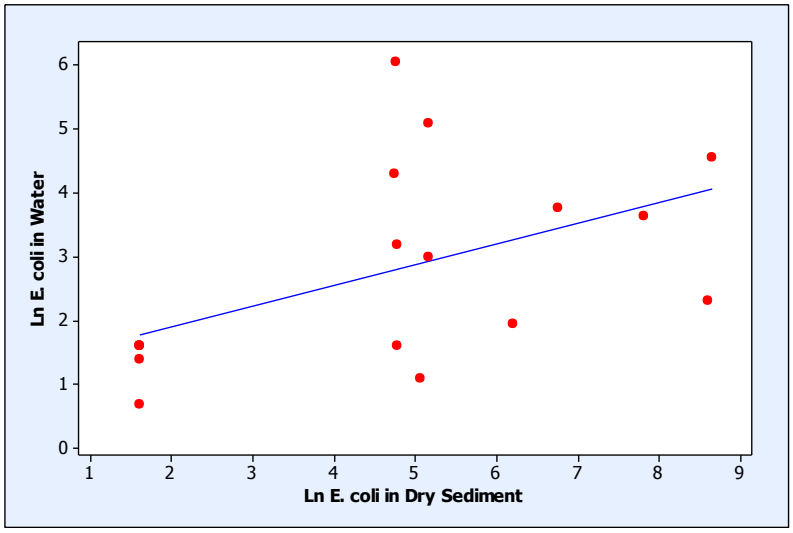
A significant predictive relationship ($F = 5.73$; $p = 0.030$) was found between the Ln *E. coli* in water and Ln enterococci in dry sediment. This relationship that explained 23% of the variation in Ln *E. coli* densities in water (Figure 19).

FIG. 19. Scatter plot and regression line for Ln *E. coli* in water versus Ln enterococci in water.



A significant predictive relationship ($F = 5.23$; $p = 0.037$) was also found between Ln *E. coli* in water and in dry sediment. This relationship explained 21% of the variation in Ln *E. coli* densities in water (Figure 20).

FIG. 20. Scatter plot and regression line for Ln *E. coli* in water versus Ln E. coli in dry sediment.



An additional significant (F = 5.34; p = 0.019) predictive relationship was found between Ln *E. coli* versus Ln enterococci and Ln *E. coli* in dry sediment, which explained 35% of the Ln *E. coli* variation in water. A significant (F=3.94; p = 0.033) predictive relationship was found between Ln enterococci in water versus Ln *E. coli*, Ln enterococci in wet sediment and Ln enterococci in dry sediment that explained 36% of the variation of Ln enterococci in water.

FIG. 21. *E. coli* densities in water and sediments by collection date.

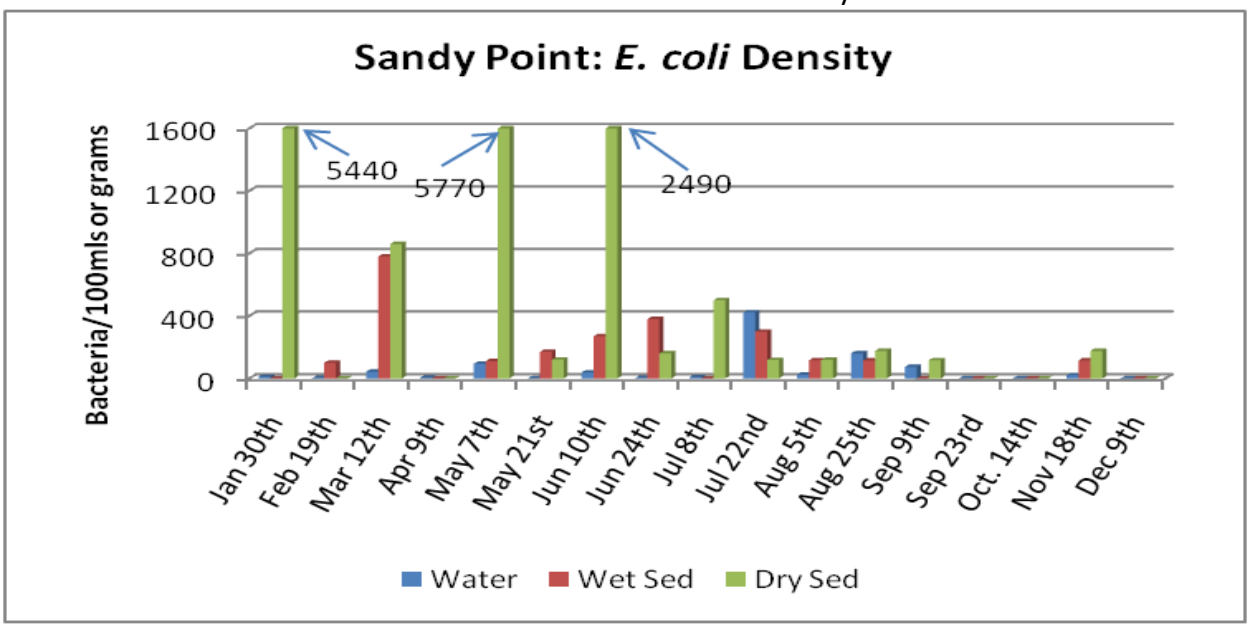


FIG. 22. Time plot of Ln *E. coli* densities in samples of water and wet and dry sediments.

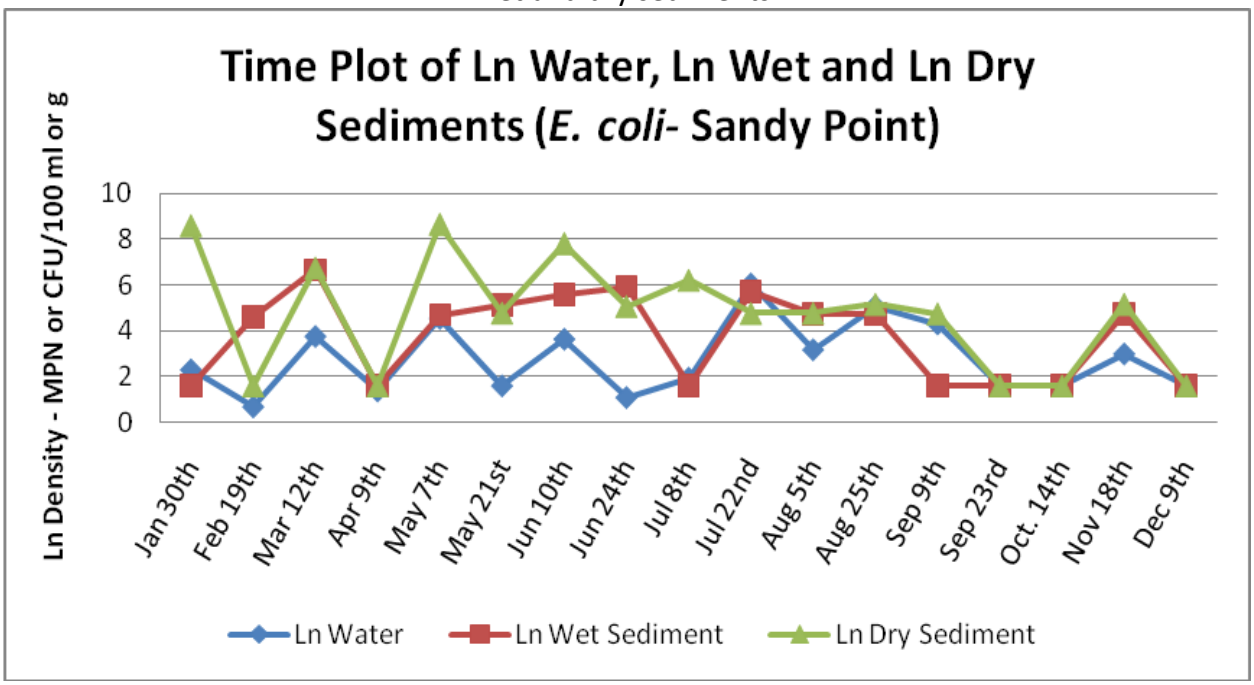


FIG. 23. Enterococci densities in water and sediments by collection date.

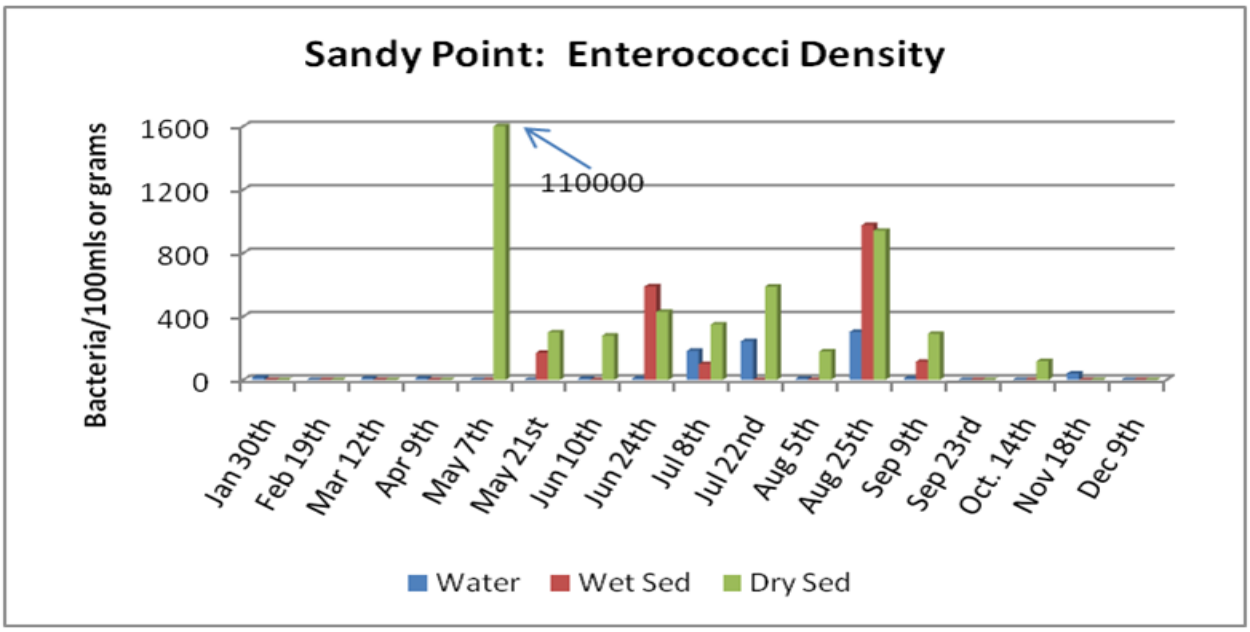
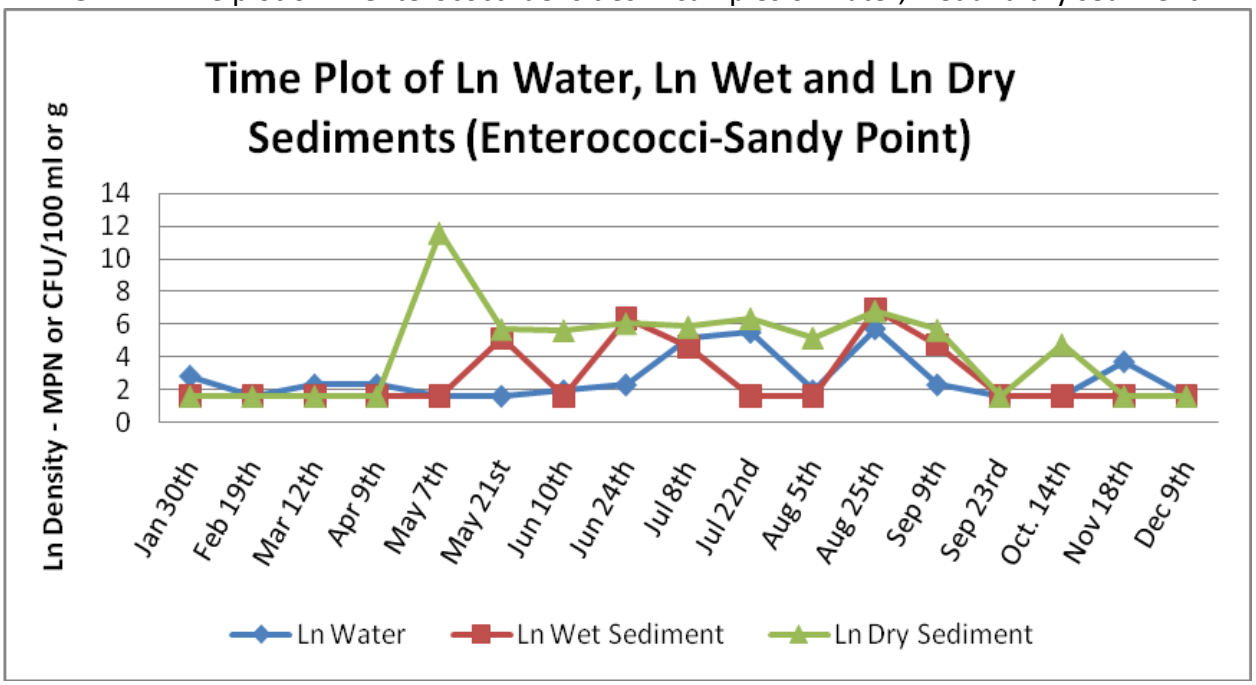


FIG. 24. Time plot of Ln enterococci densities in samples of water, wet and dry sediment.



Potential Pathogens.

Seven (7) potential pathogens were found in water, wet sediments, or dry sediments collected from Sandy Point, including *Escherichia* spp., *E. coli, E. coli (0157:H7), E. coli/Shigella, E. coli/Shigella dysenteria,, Vibrio furnissii,* and *Shigella flexneri.* Only one of these, *Vibrio furnissii* was a confirmed match when tested against a certified library (Midi Labs, Newark, DE) The *E. coli* cultures were also tested for *E. coli* 0157:H7 by ELISA analysis in the BST Laboratory and found negative for that strain (Appendix I). The complete list of identified organisms cultured from water and sediment samples at Sandy Point and the other sites is in Appendix II.

Combined 12-Month Study Sites Summary

Water and Sediments.

The descriptive statistics, along with geometric means, for *E. coli* and enterococci densities in water, in wet sediment, and in dry sediment are shown by study site in Tables 20 and 21 and displayed graphically in time plots in Figures 25-30. The means of bacterial densities for the combined sample results are shown in Tables 22 and 23.

The single sample maximum density standards for fresh coastal recreational waters were 126 MPN/100 ml for *E. coli* and 33 MPN/100 ml for enterococci. For marine recreational waters, the enterococci single sample maximum density standard is 33 MPN/100 ml (U.S. Environmental Protection Agency (EPA) 2004). There are no standards or recommendations for bacteria levels in beach or submerged sand; however, as a way to compare the number exceedance days with sediment densities, we chose to set a "high" density value for sediment at the same level as for the standard in water, 126 CFU (per 100 g dry weight sediment) for *E. coli* and 33 CFU (per 100 g dry weight sediment) for enterococci.

Mean E. coli Densities by Site.

Assateague Island had the highest mean *E. coli* density in water, and it was the only site with a mean that exceeded the recreational standard for *E. coli* in water (Table 20) (Figure 25). Delaware Shore had the lowest mean *E. coli* density in water and also the lowest in wet and dry sediments of any of the 4 sites. Granary Creek samples yielded the highest *E. coli* densities of the 4 locations for both wet and dry sediments (Figures 26-27). The mean densities of *E. coli* at Granary Creek and Sandy Point in wet sediments also exceeded 126 CFU/100 g dry weight, while all locations except Delaware Shore had means above that level in dry sediments.

TABLE 20. Descriptive statistics: Means of *E. coli* densities in water, in wet and dry sediments, and geometric means of samples by study site.

Variable	Site	N	N*	Mean	SE Mean	StDev	Median	Maximum	Geometric Mean
E. coli Water	AI	17	0	184.0	129.0	534.0	5.0	2100.0	12.4
	DS	17	0	5.7	0.7	2.9	5.0	13.0	4.9
	GC	17	0	87.4	44.8	184.6	17.0	759.0	22.5
	SP	17	0	54.2	25.3	103.9	10.0	422.0	16.2
E. coli WetSed	AI	17	0	107.5	62.0	255.8	5.0	1030.0	16.7
	DS	17	0	92.9	87.9	362.6	5.0	1500.0	7.0
	GC	17	0	853.0	418.0	1723.0	185.0	7130.0	92.5
	SP	17	0	146.4	48.6	200.2	110.0	780.0	42.8
E. coli DrySed	AI	17	0	1179.0	751.0	3098.0	5.0	12000.0	21.8
	DS	17	0	75.3	70.3	289.8	5.0	1200.0	6.9
	GC	17	0	2612.0	1530.0	6310.0	120.0	23000.0	103.3
	SP	17	0	945.0	450.0	1855.0	120.0	5770.0	114.7

FIG. 25. Time plot of Ln *E. coli* in water by study site.

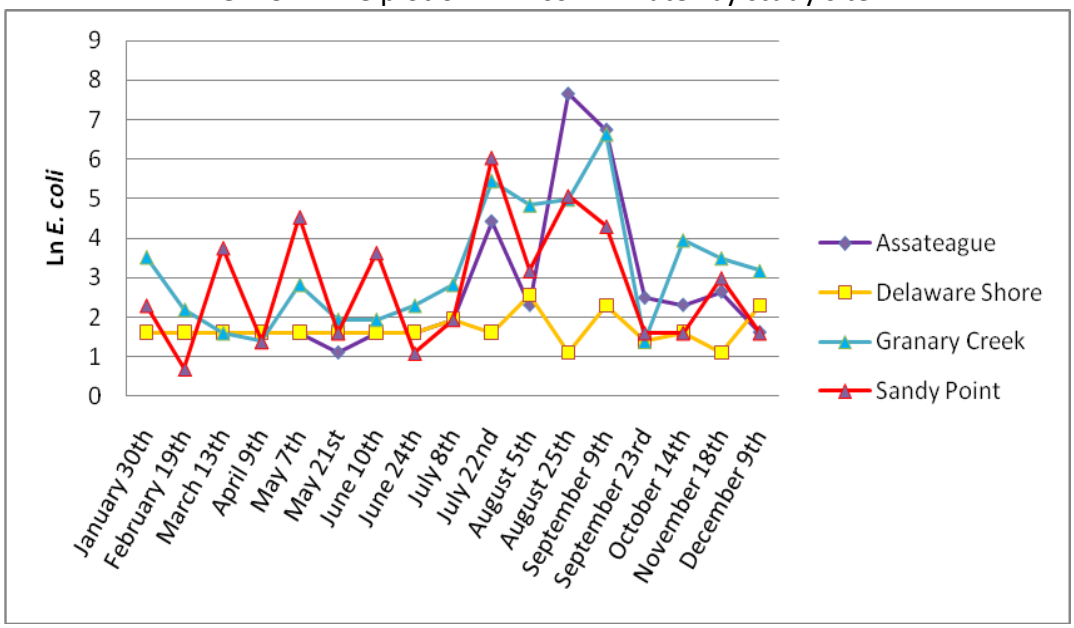


FIG. 26. Time plot of Ln *E. coli* in wet sediment by study site.

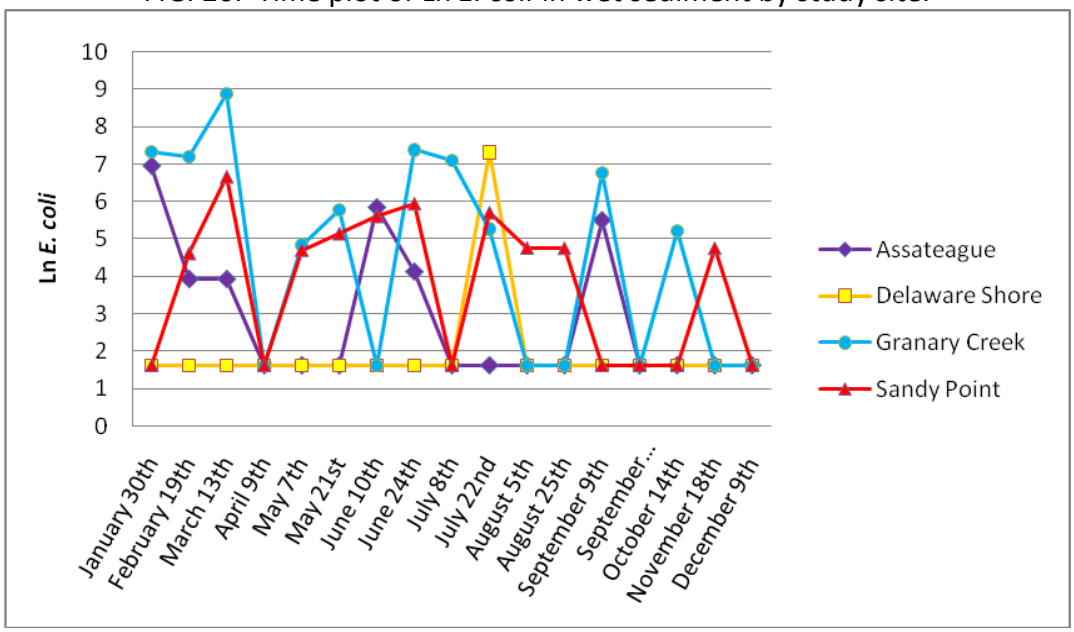
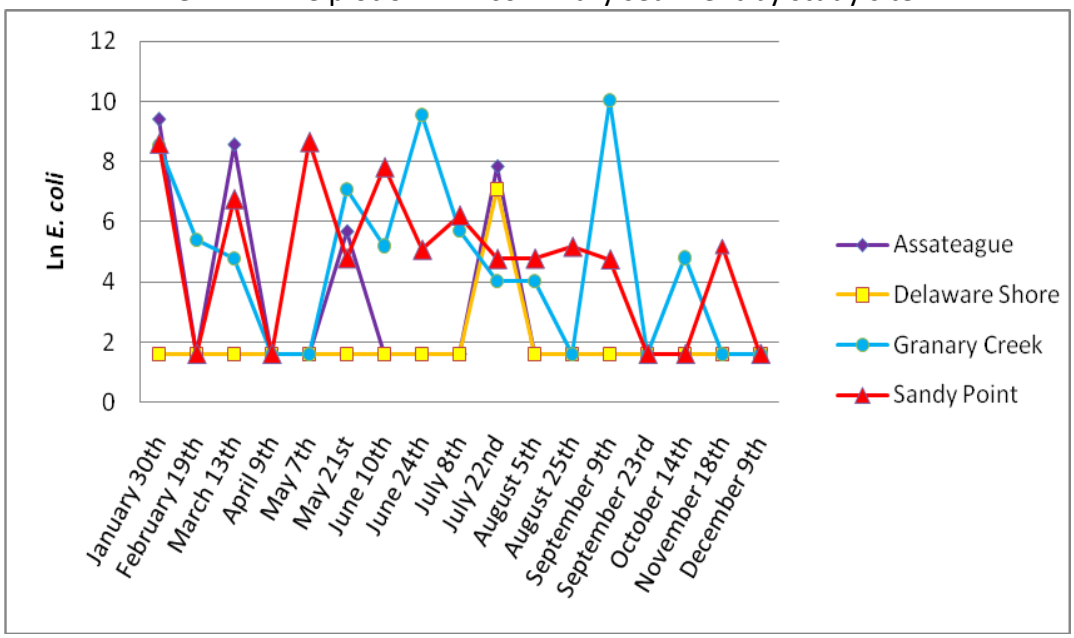


FIG. 27. Time plot of Ln *E. coli* in dry sediment by study site.



Mean Enterococci Densities by Site.

The Delaware Shore had the lowest mean densities for both enterococci and *E. coli* in water and in both wet and dry sediments (Table 20) (Figures 25-27). In contrast to the *E. coli* mean densities, Sandy Point, not Assateague Island, had the highest mean concentration of enterococci in water (Table 21). This density was the only mean concentration that exceeded the recreational standard of 33 MPN/100ml for enterococci in water. Assateague Island had the highest mean enterococci density in wet sediment. Except for Delaware Shore, all locations had in excess of 33 CFU/100 g dry weight in wet sediment, and all 4 locations exceeded that level in dry sediment.

TABLE 21. Descriptive Statistics: Means of study site enterococci densities in water(MPN/100 ml) and sediments (CFU/100 g dry weight) and geometric means of samples collected at the 4 study sites.

Variable	Site	N	N*	Mean	SE Mean	StDev	Median	Maximum	Geometric Mean
ENT Water	AI	17	0	1.9	0.2	0.9	1.6	4.7	6.9
	DS	17	0	6.1	1.0	4.3	5.0	20.0	5.1
	GC	17	0	24.6	8.7	35.9	14.0	144.0	13.4
	SP	17	0	51.3	22.9	94.4	10.0	303.0	14.7
ENT WetSed	AI	17	0	10047.0	9997.0	41219.0	5.0	170000.0	24.2
	DS	17	0	19.8	7.1	29.1	5.0	100.0	9.2
	GC	17	0	1114.0	613.0	2529.0	128.0	9290.0	121.1
	SP	17	0	118.4	64.1	264.2	5.0	977.0	15.9
ENT DrySed	AI	17	0	1592.0	1402.0	6780.0	60.0	24000.0	9.0
	DS	17	0	53.8	29.8	123.1	5.0	465.0	10.0
	GC	17	0	864.0	270.0	1114.0	340.0	3500.0	309.8
	SP	17	0	6677.0	6458.0	26627.0	179.0	110000.0	82.4

FIG. 28 Time plot of Ln enterococci in water by study site.

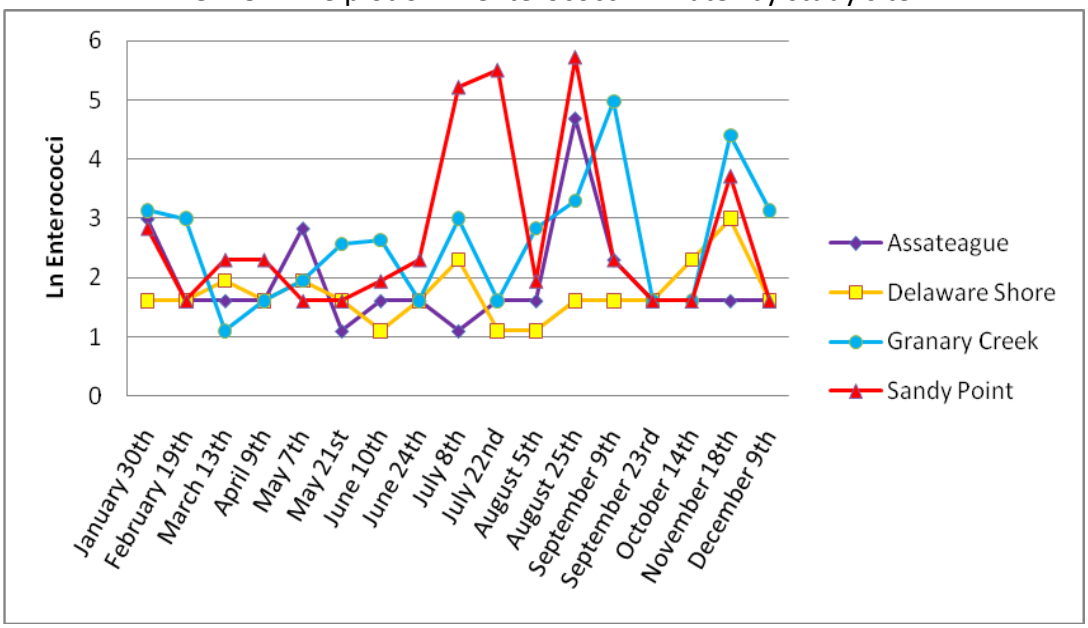


FIG. 29. Time plot of Ln enterococci in wet sediment by study site.

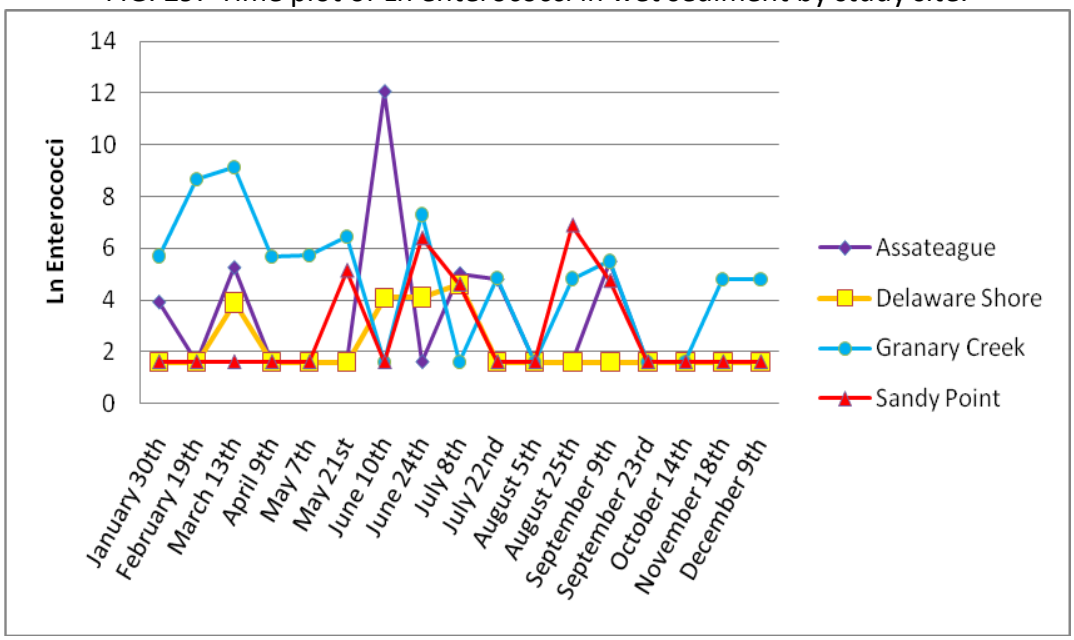
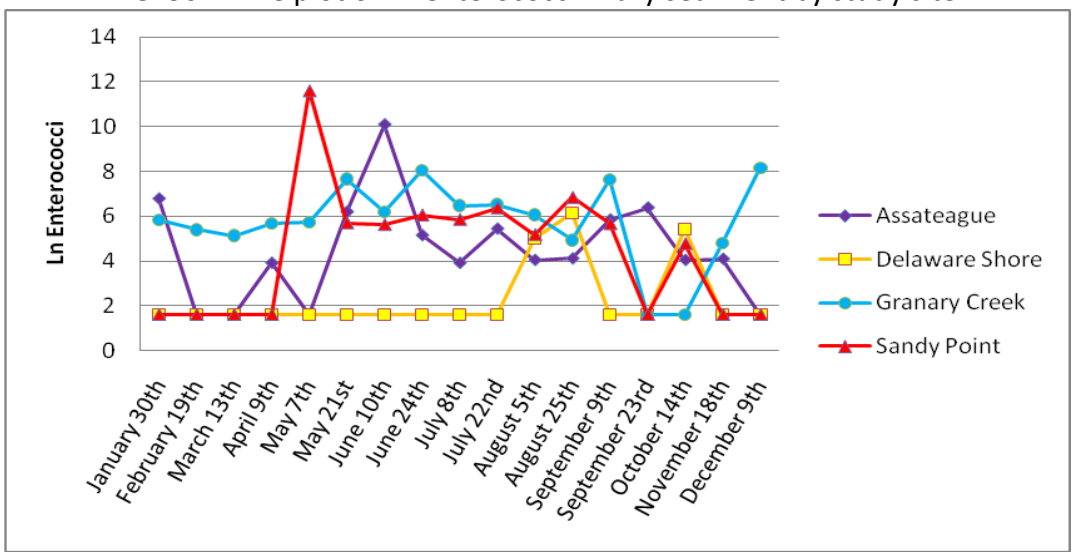


FIG. 30. Time plot of Ln enterococci in dry sediment by study site.



Combined E. coli Densities.

When results from all locations were combined (Table 22), *E. coli* concentrations in water were lower than in either wet or dry sediments. Wet sediment yielded a mean density that was over 3.5 times that in water. Dry sediment mean density was a much larger 14.5 times *E. coli* in water.

Table 22. Descriptive statistics for the combined E. coli densities in water and sediments, and and geometric means for all 4 study sites.

Variable	N	N*	Mean	SE Mean	StDev	Median	Maximum	Geometric Mean
E. coli Water	68	0	82.7	34.9	288.2	7.0	2100.0	12.2
E.coli WetSed	68	0	300.0	113.0	932.0	5.0	7130.0	26.1
E. coli DrySed	68	0	1203.0	445.0	3672.0	5.0	23000.0	36.5

Combined Enterococci Densities.

Combined enterococci concentrations in water, like *E. coli* mean densities, were lower than in either wet or dry sediments (Table 23); however, the differences between water and sediments were much greater than for *E. coli*. Mean enterococci density in wet sediment was 119 times that in water (2,825 CFU/g dry weight) and 97 times than enterococci in water (2,297 CFU/g dry weight).

Table 23. Descriptive statistics: Combined enterococci densities in water and sediments, and geometric means, for all 4 study sites.

Variable	N	N*	Mean	SE Mean	StDev	Median	Maximum	Geometric Mean
ENT Water	68	0	23.7	6.5	53.8	5.0	303.0	9.1
ENT WetSed	68	0	2825.0	2500.0	20618.0	5.0	170000.0	25.6
ENT DrySed	68	0	2297.0	1647.0	13579.0	121.0	110000.0	69.1

Potential Pathogens.

Seven (7) different potential pathogens were found in 11 samples of water, wet sediments, or dry sediments collected from the 4 study sites, including *Escherichia/Shigella; E. coli (0157:H7)* (3 samples)*; E. coli/Shigella and E. coli/Shigella dysenteria*; *Shigella* spp*; Shigella flexneri;* and *Vibrio furnissi*. None were confirmed matches when tested against a certified library (Midi Labs, Newark, DE). All *E. coli* cultures were also tested for *E. coli* 0157:H7 by ELISA analysis in the BST Laboratory and found negative for that strain. The *Pathogen Summary* and the full list of identified organisms cultured from water and sediment samples all study sites are included in Appendix I and Appendix II, respectively.

Regrowth Study

From collection dates from late June through mid October during the 12-month beach water and sediment study, samples were analyzed for initial indicator organism density and then a subset of samples were set aside for the Regrowth Study. In that study, several experiments were conducted in order to determine the ability of indicator organisms (*E. coli* and enterococci) to grow in water and sediments and the length of survival time, if any. The Regrowth Study examined the survivability and/or regrowth of the two indicator organisms. Here, the term "survivability" is defined as any level of maintenance of bacterial density after the initial density measured on the day of collection. "Regrowth" is defined here as any increase in bacteria density over a 1 day period, recognizing that small increases may be due to procedural error. Each sample was held at various temperatures and reanalyzed for bacterial indicator density on a daily basis (see Materials and Methods). Some details of the protocol were changed between experiments as the study progressed.

Sample incubation conditions included 5 temperatures: 4°C, 21°C, 24°C, 30°C, and 37°C, but not all regrowth/survivability experiments were conducted on samples from all collection sites or at all temperatures. The temperatures of 21°C and 24°C were derived from the average soil temperatures at time of collection. Also, the total incubation time for different samples varied according to bacterial survival. Generally, the measurement of the indicator density of a given sample was recorded daily and was continued until indicator organism counts were below the detection level (5 CFU/100 ml or 5 MPN/100 g dry weight) or until all of the sample had been used up by the analysis. The longest regrowth/survivability experiment extended for 13 days (day = 24 hr $\pm$ 2 hrs). A summary of the regrowth experiments is shown in Table 24.

The statistical analysis described in the report below was performed only on data from the samples included in the Regrowth Study. Initial water density values represent the average of measurements on 3 replicate water samples; all other values are from a single measurement.

TABLE 24. Summary of the regrowth experiments conducted during the study period.

Date	Indicator	water sediment	Study Site*	Temp	Analysis Time (up to:)
24-Jun-08	E. *coli*	water	All	37°C	72 hrs (3 days)
		sediment	All	37°C	72 hrs (3 days)
	Enterococci	water	All	37°C	72 hrs (3 days)
		sediment	All	37°C	72 hrs (3 days)
8-Jul-08	E. *coli*	water	All	37°C	48 hrs (2 days)
		sediment	All	37°C	144 hrs (6 days)
	Enterococci	water	All	37°C	48 hrs (2 days)
		sediment	All	37°C	144 hrs (6 days)
22-Jul-08	E. *coli*	water	GC & SP	4°C & 37°C	72 hrs (3 days)
		sediment	GC & SP	4°C & 37°C	144 hrs (6 days)
	Enterococci	water	GC & SP	4°C & 37°C	72 hrs (3 days)
		sediment	GC & SP	4°C & 37°C	144 hrs (6 days)
5-Aug-08	E. *coli*	water	All	4°C & 30°C	72 hrs (3 days)
		sediment	All	4°C & 30°C	48 hrs (2 days)
	Enterococci	water	All	4°C & 30°C	72 hrs (3 days)
		sediment	All	4°C & 30°C	48 hrs (2 days)
9-Sep-08	E. *coli*	water	All	4°C, 21°C & 37°C	312 hrs (13 days)
		sediment	All	4°C, 21°C & 37°C	312 hrs (13 days)
	Enterococci	water	All	4°C, 21°C & 37°C	312 hrs (13 days)
		sediment	All	4°C, 21°C & 37°C	312 hrs (13 days)
23-Sep-08	E. *coli*	water	All	4°C, 24°C & 37°C	72 hrs (3 days)
		sediment	All	4°C, 24°C & 37°C	48 hrs (2 days)
	Enterococci	water	All	4°C, 24°C & 37°C	72 hrs (3 days)
		sediment	All	4°C, 24°C & 37°C	48 hrs (2 days)
14-Oct-08	E. *coli*	water	AI & GC	4°C, 24°C & 37°C	72 hrs (3 days)
		sediment	GC	4°C, 24°C & 37°C	48 hrs (2 days)
	Enterococci				
		sediment	DS	4°C, 24°C & 37°C	48 hrs (2 days)

*AI = Assateague Island, DS = Delaware Shore, GC = Granary Creek, SP = Sandy Point

Assateague Island: Statistical Analysis of Regrowth/Survivability Studies from Water and Sediment Samples Collected at Assateague Island.

Water.

E. coli Density in Water.

The mean density of the initial *E. coli* in water samples in the regrowth study was 181 MPN/100 ml, which was 29 times that of the mean initial enterococci in those same water samples (6 MPN/100 ml). As would be expected, this difference was statistically significant (F = 9.82; p = 0.004).

A very highly significant linear relationship was found between Ln *E. coli* in water and Ln *E. coli* in wet sediment (p = 0.000), although there was none with Ln E. coli in dry sediment (p = 0.396). The relationship between Ln *E. coli* in water and the percent moisture in the wet sediment was also statistically significant (p = 0.020), as was the association between percent moisture in wet and dry sediments (p = 0.042). In addition, there was a highly significant relationship between Ln *E. coli* in water and Ln enterococci in water (p = 0.003).

The initial Ln *E. coli* concentrations in wet sediment and in dry sediment were very highly significant predictors of the Ln *E. coli* density in water (F = 19.27; p < 0.000). The resulting equation was able to explain 72.3 % of the variation in Ln *E. coli* densities in water. When temperature was added to the equation, the relationship was highly significant, but reduced in predictive power. This second result could explain 71.2% of the variation in Ln E. *coli* densities in water.

Ln *E. coli* density in water versus Ln *E. coli* in wet and dry sediment and the percent moisture in them was very highly significant (F = 344.60; p < 0.000), with an R-Sq (adj) and could explain virtually all (99.3%) variation in Ln *E. coli* density in water.

A significant predictive relationship existed between Ln *E. coli* in water and Ln Enterococci in water (F = 13.66; p = 0.003) and could explain 47.5% of Ln *E. coli* variation.

E. coli in Water Regrowth.

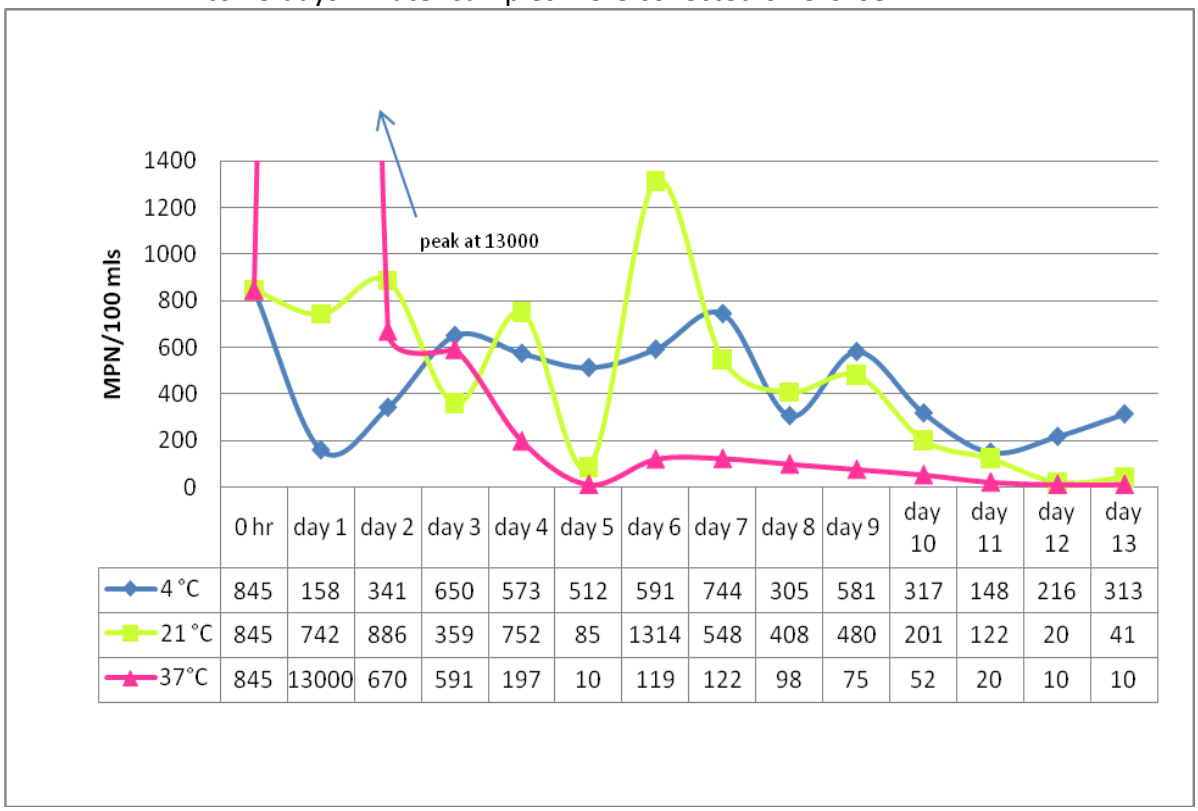
No regrowth of *E. coli* in water occurred in experiments conducted from June through August 2008 (Table 25). During the September 9 experiments, increased or continued growth was seen after day 1, continuing through day 13 at all incubation temperatures. For the rest of the study period (through October 14), an increase or maintenance of growth was seen through day 3 incubation only.

TABLE 25. *E. coli* densities found in water samples during 10 regrowth experiments.

	0 hr	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10	day 11	day 12	day 13
Date: Temp														
6-24: 37°C	5	5	5	5										
7-8: 37°C	5	5	5											
8-5: 4°C	10	10	10	10										
8-5: 30°C	10	5												
9-9: 4°C	845	158	341	650	573	512	591	744	305	581	317	148	216	313
9-9: 21°C	845	742	886	359	752	85	1314	548	408	480	201	122	20	41
9-9: 37°C	845	13000	670	591	197	10	119	122	98	75	52	20	10	10
9-23: 4°C	12	62	97	109										
9-23: 24°C	12	181	119	31										
9-23: 37°C	12	41	31	52										

An example of regrowth/survivability from water samples collected at Assateague Island is shown below in Figure 1.

FIG. 31. *E. coli* densities in water samples maintained at different temperatures for up to 13 days. Water samples were collected on 9-9-08.



Enterococci Density in Water.

As stated in the *E. coli Density in Water* section above, the mean initial density of enterococci in water was only 6 MPN/100 ml. A very highly significant linear relationship existed between Ln enterococci concentration in water and Ln enterococci density in wet sediment ($p < 0.000$). There was no significant correlation between Ln enterococci density in water and in dry sediment ($p = 0.591$).

The predictive relationship between Ln enterococci density in water versus Ln enterococci in wet sediment and Ln enterococci in dry sediment was highly significant (F = 10.77; p = 0.002) and could explain 58% of the variation in Ln enterococci's density in water. The association of Ln enterococci in water versus Ln enterococci in wet sediment was very highly significant (F = 23.08; $p < 0.000$), with 61% of the variation explained.

Ln enterococci in water versus Ln *E. coli* in water, Ln enterococci in wet sediment, and % moisture in wet and in dry sediments was significant (F = 4.52; $p = 0.024$) and explained half of the variation in Ln enterococci in water.

Enterococci in Water Regrowth.

All of the initial enterococci densities in water samples were at detection level or at 10 MPN/100 ml (Table 26). An increase of enterococci density in water was seen only once, during the September 23 experiment.

TABLE 26. Enterococci densities found in water samples from 10 Regrowth experiments.

	0 hr	**day 1**	**day 2**	**day 3**	**day 4**	**day 5**	**day 6**	**day 7**	**day 8**	**day 9**	**day 10**	**day 11**	**day 12**	**day 13**
Date: Temp														
6-24: 37°C	5	5	5	5										
7-8: 37°C	10	5	5											
8-5: 4°C	5	5												
8-5: 30°C	5	5												
9-9: 4°C	10	5												
9-9: 21°C	10	5												
9-9: 37°C	10	5												
9-23: 4°C	5	10	5											
9-23: 24°C	5	5												
9-23: 37°C	5	5												

Sediment.

E. coli in Sediment.

The mean initial *E. coli* density in wet sediment was 56 CFU/100 g dry weight. The maximum initial *E. coli* density was 241 CFU/100 g dry weight in the September 9 sample (Table 27). Except for the June sample all of the remaining initial densities were at the minimum detectable concentration.

A very highly significant predictive relationship was found between Ln *E. coli* in wet sediment and Ln *E. coli* in dry sediment (F = 19.27; $p = < 0.000$), which could explain 72% of Ln *E. coli* variability in wet sediment.

The mean initial *E. coli* density in dry sediment was 338 CFU/100 g dry weight. The maximum initial *E. coli* density was 2,500 CFU/100 g dry weight in late June. The initial densities for experiments conducted on the remaining dates were at the minimum detectable density.

No significant difference was found in the mean densities of Ln *E. coli* in wet sediment versus Ln *E. coli* in dry sediment ($p = 0.874$).

TABLE 27. *E. coli* densities found in wet and dry sediment samples during 16 regrowth experiments.

		0 hr	**day 1**	**day 2**	**day 3**	**day 4**	**day 5**	**day 6**	**day 7**	**day 8**	**day 9**	**day 10**	**day 11**	**day 12**	**day 13**
	Date: Temp														
Wet Sediment	**6-24: 37°C**	61	5	5	5										
	8-5: 30°C	5													
	9-9: 4°C	241	5	5											
	9-9: 21°C	241	5	5											
	9-9: 37°C	241	5	5											
	9-23: 4°C	5	5												
	9-23: 24°C	5	5												
	9-23: 37°C	5	5												
Dry Sediment	**6-24: 37°C**	5	5	5	5										
	8-5: 30°C	5													
	9-9: 4°C	5	5	5											
	9-9: 21°C	5	5	5											
	9-9: 37°C	5	5	5											
	9-23: 4°C	5	5												
	9-23: 24°C	5	5												
	9-23: 37°C	5	5												

E. coli in Sediment Regrowth

No regrowth of *E. coli* density was seen in wet sediment samples analyzed from Assateague Island.

Enterococci in Sediment.

The mean initial enterococci density in wet sediment was 77 CFU/100 g dry weight, and the maximum initial enterococci density was 241 CFU/100 g dry weight in early September (Table 28). The mean initial enterococci density in dry sediment was 251 CFU/100 g dry weight, and the maximum density was 588 CFU/100 g dry weight in late September.

TABLE 28. Enterococci densities found in wet and dry sediment samples during 9 regrowth experiments.

		0 hr	**day 1**	**day 2**	**day 3**	**day 4**	**day 5**	**day 6**	**day 7**	**day 8**	**day 9**	**day 10**	**day 11**	**day 12**	**day 13**
	Date: Temp														
Wet Sediment	**6-24: 37°C**	5	5	5	5										
	7-8: 37°C	50	457	5											
	8-5: 30°C	5	5												
	9-9: 4°C	41	5	5											
	9-9: 21°C	41	5	5											
	9-9: 37°C	41	5	5											
	9-23: 4°C	5	5												
	9-23: 24°C	5	5												
	9-23: 37°C	5	5												
Dry Sediment	**6-24: 37°C**	172	5	5	5										
	7-8: 37°C	5	5	5											
	8-5: 30°C	57	5												
	9-9: 4°C	34	5	5											
	9-9: 21°C	34	5	287											
	9-9: 37°C	34	5	5											
	9-23: 4°C	588	147	882											
	9-23: 24°C	588	147	441											
	9-23: 37°C	588	5												

There was a very highly significant linear relationship ($p < 0.000$) existed between Ln enterococci in dry sediment and the percent moisture in dry sediment; however, the relationship between Ln enterococci in wet sediment and the percent moisture in wet sediment was not significant ($p = 0.505$).

A very highly significant predictive relationship existed between Ln enterococci in dry sediment versus the percent moisture in dry sediment ($F = 25.47$; $p < 0.000$). Sixty-eight percent (68%) of the variation in Ln enterococci in dry sediment was explained.

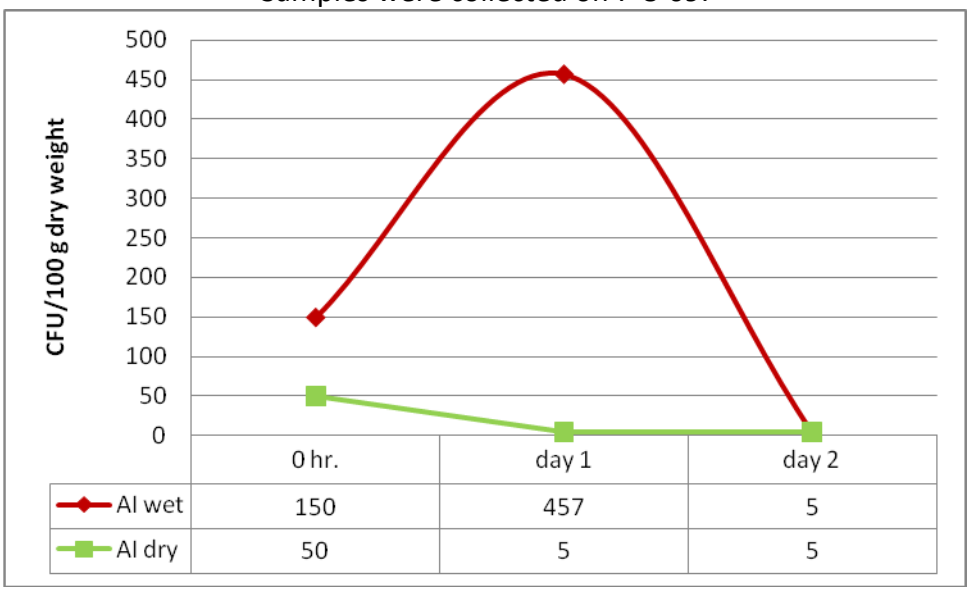
A highly significant difference ($p = 0.001$) was found between the mean densities of Ln enterococci density in wet sediment versus in dry sediment. In contrast, there was no significant difference in the means of Ln *E. coli* density in wet sediment versus Ln enterococci density in wet sediment ($p = 0.452$).

Enterococci in Sediment Regrowth.

Regrowth of enterococci density was seen in wet sediment in early July through day 1 ((Table 28). Regrowth was also seen in dry sediment during the early and late September experiments through day 2.

Examples of regrowth/survivability of enterococci from water samples collected at Assateague Island are shown below in Figure 31.

FIG. 31. Enterococci densities (CFU/100 g dry weight) in wet and dry sediments held at 37°C. Counts fell to detection levels after day 2. Samples were collected on 7-8-09.



Regrowth Study

Delaware Shore: Statistical Analysis of Regrowth/Survivability Studies from Water and Sediment Samples Collected at Delaware Shore

Water.

E. coli Density in Water.

The mean density of the initial *E. coli* in water for the regrowth study was 6 MPN/100 ml, which was the same density as that of the mean initial enterococci in water.

The relationship between Ln *E. coli* density in water versus Ln *E. coli* in wet and in dry sediments was not significant (F = 0.086; $p = 0.0449$). No significant difference was found in the mean densities of Ln *E. coli* in water and Ln enterococci in water ($p = 0.990$).

E. coli in Water Regrowth.

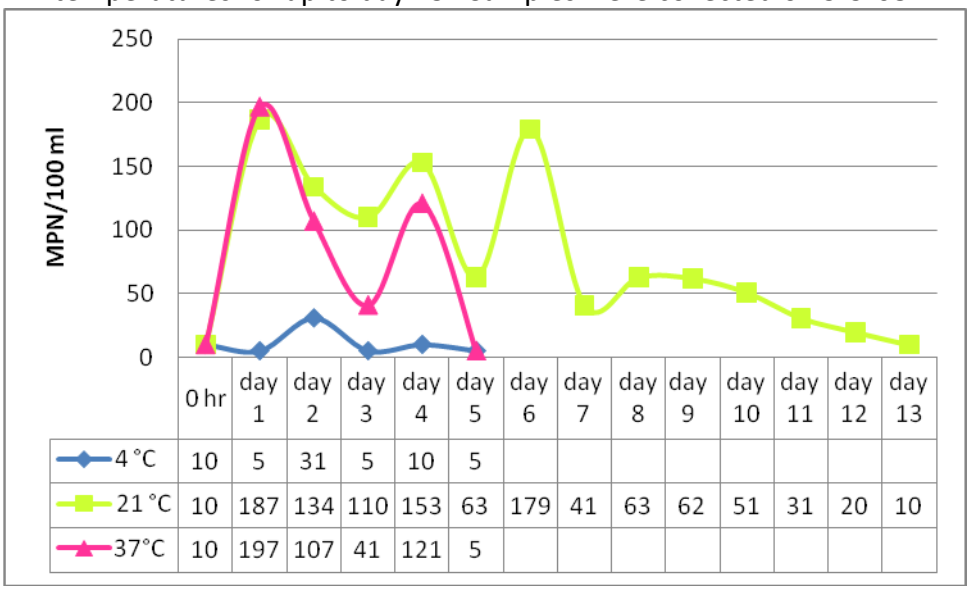
No regrowth of *E. coli* in water occurred in experiments conducted in June and July (Table 29). During August through early September, 33% of the samples demonstrated regrowth, with 3 samples continuing regrowth or maintenance of growth through day 5. One of these samples continued regrowth and maintenance of growth until day 13. For the rest of the study period (through October 14, 2008), only maintenance of growth, if any, was seen.

TABLE 29. *E. coli* densities found in water samples during10 regrowth experiments.

	0 hr	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10	day 11	day 12	day 13
Date: Temp														
6-24: 37°C	5	5	5	5										
7-8: 37°C	5	5	5											
8-5: 4°C	10	5												
8-5: 30°C	10	61	5											
9-9: 4°C	10	5	31	5	10	5								
9-9: 21°C	10	187	134	110	153	63	179	41	63	62	51	31	20	10
9-9: 37°C	10	197	107	41	121	5								
9-23: 4°C	5	5												
9-23: 24°C	5	5												
9-23: 37°C	5	5												

An example of regrowth/survivability from water samples collected at the Delaware Shore is shown below in Figure 32.

FIG. 32. *E. coli* densities in water samples maintained at different temperatures for up to day 13. Samples were collected on 9-9-08.



	0 hr	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10	day 11	day 12	day 13
4 °C	10	5	31	5	10	5								
21 °C	10	187	134	110	153	63	179	41	63	62	51	31	20	10
37°C	10	197	107	41	121	5								

Enterococci Density in Water.

As stated in the <u>*E. coli*</u> *Density in Water* section above, the mean initial density of enterococci in water was 6 MPN/100 ml (Table 30). A highly significant linear relationship existed between Ln enterococci concentration in water and Ln enterococci density in dry sediment ($p < 0.026$). There was no significant correlation between Ln enterococci density in water and in wet sediment ($p = 0.377$).

A highly significant predictive relationship was found between Ln enterococci in water versus Ln E. *coli* in water, Ln enterococci in wet sediment, and % moisture in wet and in dry sediments was highly significant (p = 0.030). This predictive relationship explained 95.5% of the variation of Ln enterococci in water. The relationship between Ln enterococci in water versus both Ln enterococci in wet and in dry sediments was not significant ($F = 17.47$; $p = 0.055$).

No significant difference was found in the mean densities of Ln *E. coli* in water versus that of Ln enterococci in water ($p = 0.990$).

TABLE 30. Enterococci densities found in water samples from 10 regrowth experiments.

	0 hr	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10	day 11	day 12	day 13
Date: Temp														
6-24: 37°C	5	20	20	5										
7-8: 37°C	10	5	5											
8-5: 4°C	5	5												
8-5: 30°C	5	5												
9-9: 4°C	5	10	5											
9-9: 21°C	5	5												
9-9: 37°C	5	5												
9-23: 4°C	5	5												
9-23: 24°C	5	5												
9-23: 37°C	5	5												

Enterococci in Water Regrowth.

All of the initial enterococci densities in water were at the detection level or at 10 MPN/100 ml. In June and on September 9, possible regrowth of enterococci density in water was seen. One (1) experiment, in June, showed maintenance of detectable enterococci density through day 3.

Sediment.

E. coli Density in Sediment.

The mean initial *E. coli* density in wet sediment was 204 CFU/100 g dry weight. The maximum initial *E. coli* density was 1,200 CFU/100 g dry weight in late July. Aside from initial densities found during both experiments on July 22, all of the remaining initial densities were at the minimum detectable concentration.

The mean initial *E. coli* density in dry sediment was 164 CFU/100 g dry weight. The maximum initial *E. coli* density was also 1,200 CFU/100 g dry weight in late July. The initial densities for experiments conducted on the remaining dates were at the minimum detectable concentration.

A perfect linear relationship was found between Ln *E. coli* in wet sediment and in dry sediment (R = 1.000). As expected, the predictive linear relationship between Ln *E. coli* in wet sediment versus Ln *E. coli* in dry sediment was very highly significant ($p = 0.000$) and could explain 100.0 % of the Ln *E. coli* density variation in wet sediment.

The means of Ln *E. coli* density in wet sediment and in dry sediment were not significantly different ($p = 0.116$).

E. coli Density in Sediment Regrowth

No regrowth of *E. coli* was seen in wet sediment or dry sediments, although the minimum detectable level of growth was maintained in both through day 3 incubation in June (Table 31).

TABLE 31. *E. coli* densities in wet and dry sediment samples during 8 regrowth experiments in wet and dry sediments.

		0 hr	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10	day 11	day 12	day 13
	Date: Temp														
Wet Sediment	**6-24: 37°C**	5	5	5	5										
	8-5: 30°C	5	5												
	9-9: 4°C	5													
	9-9: 21°C	5	5												
	9-9: 37°C	5	5												
	9-23: 4°C	5	5												
	9-23: 24°C	5	5												
	9-23: 37°C	5	5												
Dry Sediment	**6-24: 37°C**	5	5	5	5										
	8-5: 30°C	5	5												
	9-9: 4°C	5													
	9-9: 21°C	5	5												
	9-9: 37°C	5	5												
	9-23: 4°C	5	5												
	9-23: 24°C	5	5												
	9-23: 37°C	5	5												

Enterococci in Sediment.

The mean initial enterococci density in wet sediment was 15 CFU/100 g dry weight (Table 32). The maximum initial enterococci density was 100 CFU/100 g dry weight in early July. Initial densities for experiments conducted on the remaining dates were at the minimum detectable concentration.

The mean initial enterococci density in dry sediment was 251 CFU/100 g dry weight. The maximum initial enterococci density was 227 CFU/100 g dry weight in October. The initial densities for experiments conducted on remaining dates were at the minimum detectable level.

A significant linear relationship existed between Ln enterococci in water and in dry sediment (p = 0.026). The predictive relationship between the two was highly significant (p = 0.026) and could explain over one-quarter of the variation (27.7%) in Ln enterococci in water.

A highly significant (F = 43.26; $p < 0.000$) predictive relationship was found between Ln enterococci in water versus Ln enterococci in wet and dry sediments and the % moisture in those sediments. Almost all (92.4%) of the variation in Ln enterococci in water could be explained by these variables.

At Delaware Shore, a highly significant predictive relationship was found between Ln enterococci in dry sediment versus the percent moisture in dry sediment (F = 8.86; p < 0.010). Thirty-eight percent (38%) of the variation in Ln enterococci in dry sediment was explained by this relationship.

No significant difference ($p = 0.116$) was found between the mean densities of of Ln enterococci in wet sediment and in dry sediment.

Enterococci in Sediment Regrowth.

Regrowth of enterococci was not seen in wet or in dry sediments, although maintenance of Day 1 growth was maintained in both in wet and dry sediments through day 3 in June.

TABLE 32. Enterococci densities found in 9 wet sediment samples and 9 dry sediment samples during regrowth experiments.

		0 hr	**day 1**	**day 2**	**day 3**	**day 4**	**day 5**	**day 6**	**day 7**	**day 8**	**day 9**	**day 10**	**day 11**	**day 12**	**day 13**
	Date: Temp														
Wet Sediment	**6-24: 37°C**	61	5	5	5										
	7-8: 37°C	100	5												
	8-5: 30°C	5	5												
	9-9: 4°C	5	5	5											
	9-9: 21°C	5	5												
	9-9: 37°C	5	5												
	9-23: 4°C	5	5												
	9-23: 24°C	5	5												
	9-23: 37°C	5	5												
Dry Sediment	**6-24: 37°C**	5	5	5	5										
	7-8: 37°C	5													
	8-5: 30°C	153	5												
	9-9: 4°C	5	5	5											
	9-9: 21°C	5	5												
	9-9: 37°C	5	5												
	9-23: 4°C	5	5												
	9-23: 24°C	5	5												
	9-23: 37°C	5	5												

Regrowth Study

Granary Creek: Statistical Analysis of Regrowth/Survivability Studies from Water and Sediment Samples Collected at Granary Creek

Water.

E. coli Density in Water.

The mean initial density of *E. coli* in water for the regrowth study was 206 MPN/100 ml, which was almost six (6) times that of the mean initial enterococci in water density of 35 MPN/100 ml. The difference in means of the two initial densities was significant (F = 5.79; $p = 0.023$).

A significant linear relationship between Ln *E. coli* in water and Ln *E. coli* in wet sediment was found ($p = 0.020$), although there was none between Ln *E. coli* in water and Ln *E. coli* in dry sediment ($p = 0.182$). The correlation between Ln *E. coli* in water and Ln enterococci in water was highly significant ($p = 0.002$).

The predictive relationship between Ln *E. coli* in water versus Ln *E. coli* in wet and in dry sediments and the % moisture in those sediments was highly significant (F = 71.94; $p = 0.014$). This relationship with Ln *E. coli* densities in sediments and their percent moisture content explains virtually all (97.9%) of the variation in Ln *E. coli* in water.

The relationship between Ln *E. coli* versus Ln enterococci alone was highly significant (F = 15.73; $p = 0.002$). This predictive relationship explained just over half (51%) of the variation in Ln *E. coli* in water.

E. coli Density in Water Regrowth.

Regrowth of *E. coli* density in water occurred in 73% of the samples during the experiments. The amount of regrowth ranged from twice the previous density to the over 1,000 times the original density that was seen in August (Table 33). During the early September experiments, maintenance of growth and/or regrowth occurred through day 11 at 21 ^{o}C incubation temperature and through day 13 at 4 ^{o}C.

TABLE 33. *E. coli* densities found in water samples during 15 regrowth experiments.

	0 hr	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10	day 11	day 12	day 13
Date: Temp														
6-24: 37°C	5	5	41	5										
7-8: 37°C	10	41	5											
7-22: 4°C	183	175	98	109										
7-22: 37°C	183	6000	5											
8-5: 4°C	132	20	10	10										
8-5: 30°C	132	20000	426	5										
9-9: 4°C	759	504	432	496	591	317	556	420	450	496	218	216	211	84
9-9: 21°C	759	605	262	145	85	41	31	20	52	31	10	5		
9-9: 37°C	759	14000	836	63	<1									
9-23: 4°C	5	52	41	41										
9-23: 24°C	5	432	241	41										
9-23: 37°C	5	5400	187	20										
10-14: 4°C	227	5												
10-14: 24°C	227	5	5											
10-14: 37°C	227	5												

Enterococci Density in Water.

The mean initial density of enterococci in water at Granary Creek was 6 MPN/100 ml. A significant linear relationship between Ln enterococci in water and Ln enterococci in dry sediment ($p = 0.013$) and also with Ln E. coli in water ($p = 0.002$). In addition, a highly significant linear relationship existed between Ln enterococci in wet sediment and in dry sediment ($p = 0.001$).

A significant predictive relationship ($F = 3.92$; $p = 0.049$) was found between Ln enterococci in water versus Ln enterococci in wet sediment and Ln enterococci in dry sediment, which explained 29.4% of the variation in Ln enterococci in water. Unlike the finding for Ln *E. coli* in water, no significant relationship ($F = 0.23$; $p = 0.899$) existed between Ln enterococci in water versus Ln enterococci in wet and dry sediments and the percent moisture in the sediments.

The predictive linear relationship between Ln enterococci in water and Ln enterococci in dry sediment was significant ($F = 8.37$; $p = 0.013$), and explained 35% of the Ln enterococci in water variation.
In contrast, no significant relationship ($F = 3.21$; $p = 0.096$) exisited between Ln enterococci in water versus Ln enterococci in wet sediment, although it trended toward statistical significance.

The mean densities of Ln enterococci in wet sediment and Ln enterococci in dry sediment were not significantly different ($p = 0.082$), although the difference did trend to statistical significance.

Enterococci Density in Water Regrowth.

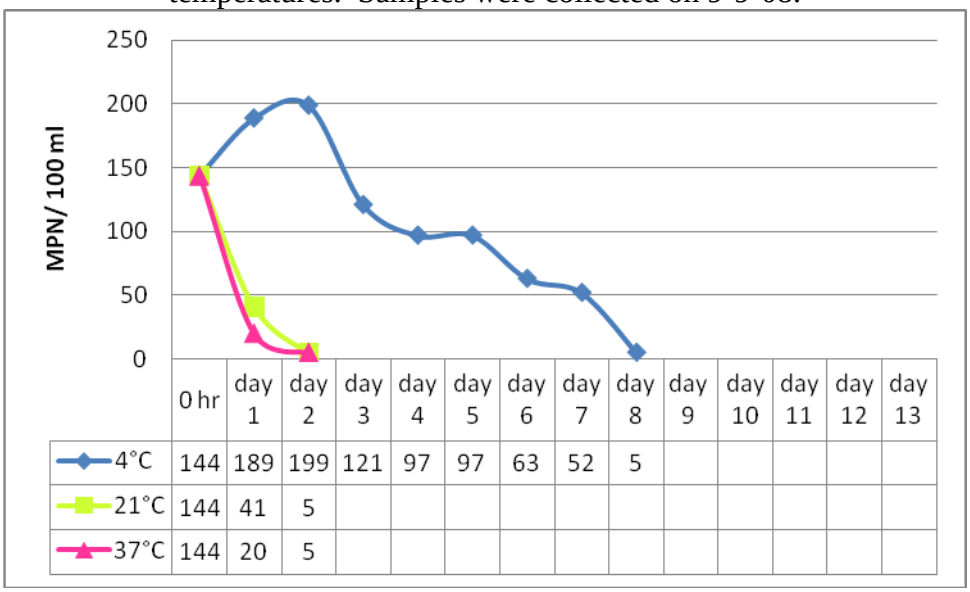
Enterococci regrowth in water was not nearly as striking as was that found with *E. coli* in water. Enterococci regrowth occurred during only 33% of the experiments. The early September experiment at 4°C saw maintenance of growth or regrowth through day 8 (Table 34).

TABLE 34. Enterococci densities found in water samples during 12 regrowth experiments.

	0 hr	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10	day 11	day 12	day 13
Date: Temp														
6-24: 37°C	5	41	10	5										
7-8: 37°C	10	20	5											
7-22: 4°C	5	5												
7-22: 37°C	5	10	10	5										
8-5: 4°C	20	5												
8-5: 30°C	20	5												
9-9: 4°C	144	189	199	121	97	97	63	52	5					
9-9: 21°C	144	41	5											
9-9: 37°C	144	20	5											
9-23: 4°C	5	20	10	5										
9-23: 24°C	5	5												
9-23: 37°C	5	5												

An example of Enterococci regrowth/survivability in water samples collected at Granary Creek is shown below in Figure 33.

FIG. 33. Enterococci densities in water maintained at three (3) different temperatures. Samples were collected on 9-9-08.



Sediment.

E. coli Density in Sediment

The mean initial *E. coli* density in wet sediment was 383 CFU/100 g dry weight, which was over twice the enterococci density (170 CFU/100 g dry weight) found in wet sediment. The maximum initial *E. coli* density in wet sediment was 1,600 CFU/100 g dry weight in June. One-third of the initial *E. coli* densities in wet sediment were at the minimum detectable density.

The predictive linear relationship between Ln E. *coli* in wet sediment and E. *coli* in dry sediment ($p = 0.001$) was highly significant ($F = 20.19$; $p = 0.001$) and explained 57.8% of the Ln E. coli variation in wet sediment.

The difference between the mean densities of Ln E. *coli* in wet and in dry sediments was not significant ($p = 0.384$), nor were the means of Ln E. *coli* in wet sediment and Ln enterococci in wet sediment ($p = 0.109$). Also, there was no significant difference in the means of E. *coli* in dry sediment and Ln enterococci in dry sediment ($p = 0.602$).

E. coli in Sediment Regrowth

E. *coli* density regrowth was seen in 78% of the wet sediment experiments (Table 35). The density increase ranged from 2.5 times the initial density to 158 times the previous E. coli density. The experiment in early July maintained growth through day 6, while the 4 ^{o}C experiment in early September showed regrowth and maintenance of growth through day 13.

Density regrowth was seen in 36% of the the dry sediment experiments (Table 35). The density increase ranged from 1.2 times the initial density to 29 times the previous E. *coli* density in dry sediment. The 37°C experiment in early September maintained growth through day 11, while the 4°C and 21°C experiments showed regrowth and maintenance of growth through day 13.

TABLE 35. *E. coli* densities found in wet and in sediment samples during 14 regrowth experiments each.

		0 hr	**day 1**	**day 2**	**day 3**	**day 4**	**day 5**	**day 6**	**day 7**	**day 8**	**day 9**	**day 10**	**day 11**	**day 12**	**day 13**
	Date: Temp														
Wet Sediment	**6-24: 37°C**	1600	16000	9000	456										
	7-8: 37°C	1200	19000	12000	219	63	63	5							
	7-22: 4°C	192	1900	801	1800										
	7-22: 37°C	192	481	320	5										
	8-5: 30°C	5	5												
	9-9: 4°C	864	1100	1200	1100	1200	1500	926	1500	463	617	1200	772	463	1900
	9-9: 21°C	864	1200	5											
	9-9: 37°C	864	1200	2200	5										
	9-23: 4°C	5	158	5											
	9-23: 24°C	5	5												
	9-23: 37°C	5	158	5											
	10-14: 4°C	185	463	231											
	10-14: 24°C	185	5												
	10-14: 37°C	185	463	5											
Dry Sediment															
	6-24: 37°C	14000	7500	2300	3400										
	7-8: 37°C	300	5												
	8-5: 30°C	57	5												
	9-9: 4°C	23000	13000	15000	26000	18000	22000	20000	17000	14000	17000	13000	17000	18000	16000
	9-9: 21°C	23000	2200	6400	6800	7000	7800	4500	2700	4800	2800	3900	5700	2600	2500
	9-9: 37°C	23000	20000	14000	6100	7400	4800	3200	3000	1200	872	727	5		
	9-23: 4°C	5	5												
	9-23: 24°C	5	5												
	9-23: 37°C	5	147	147											
	10-14: 4°C	122	5												
	10-14: 24°C	122	152	5											
	10-14: 37°C	122	5												

Examples of E. *coli* regrowth/survivability in wet and dry sediment samples collected at Granary Creek are shown below in Figure 34-36.

FIG. 34. *E. coli* densities in sediment samples maintained at 37°C. Samples were collected on 7-8-08.

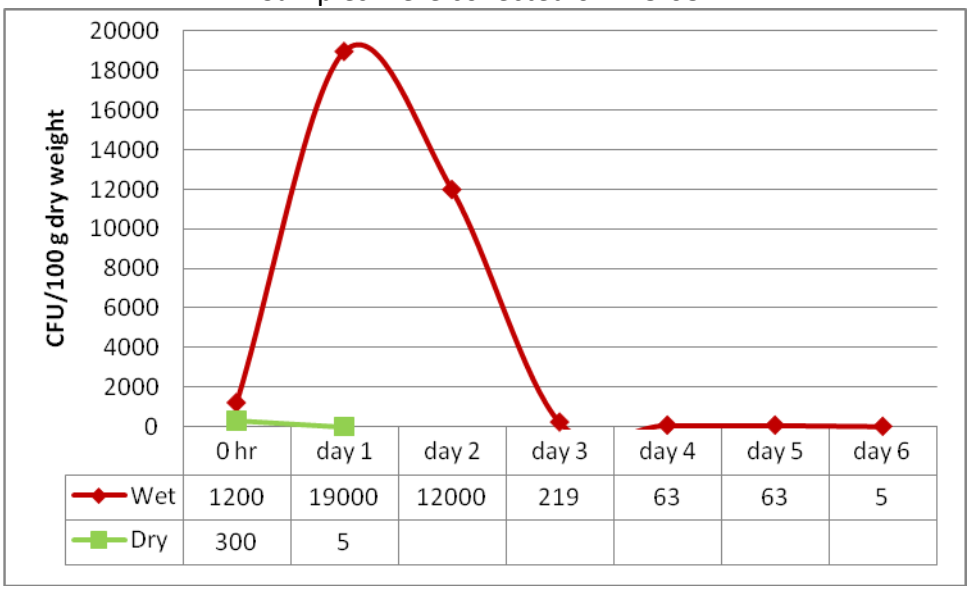


FIG. 35. *E. coli* densities in wet sediment maintained at three (3) different temperatures . Samples were collected on 9-9-08.

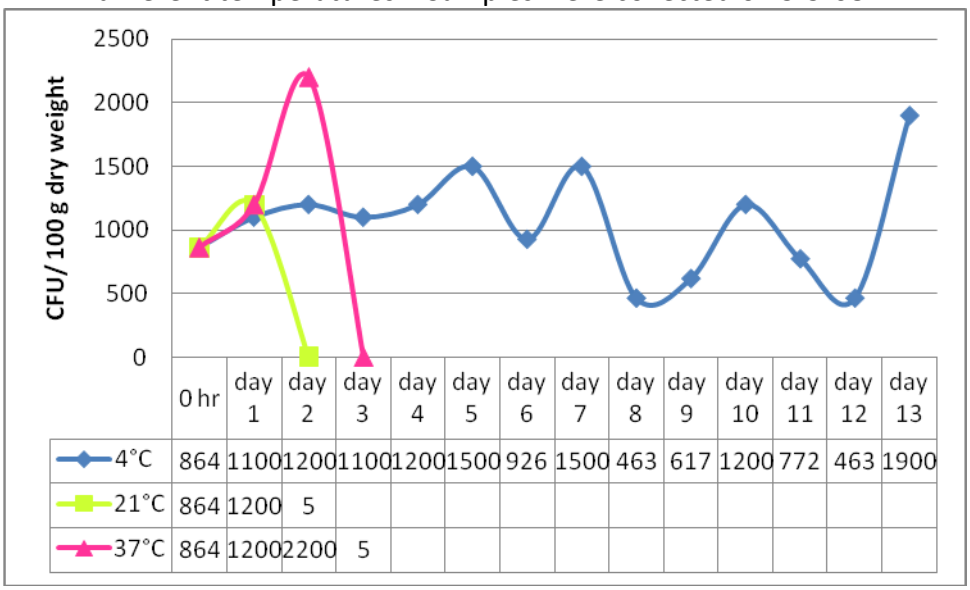
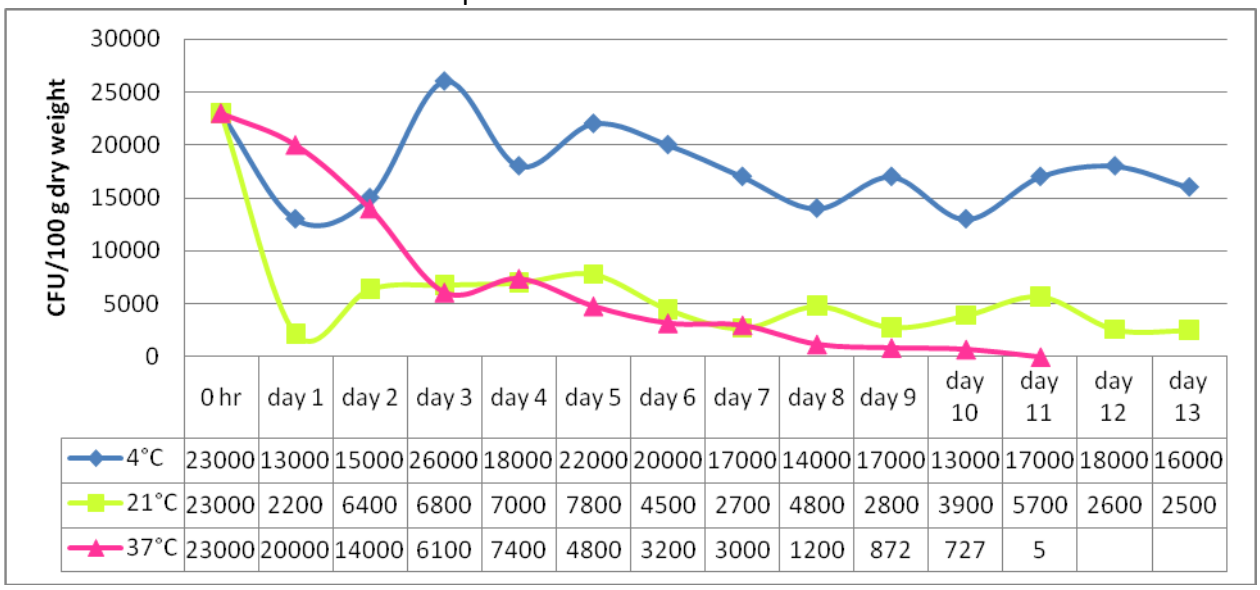


FIG. 36. *E. coli* densities in dry sediment maintained at three (3) different temperatures. Samples were collected on 9-9-08.



	0 hr	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10	day 11	day 12	day 13
4°C	23000	13000	15000	26000	18000	22000	20000	17000	14000	17000	13000	17000	18000	16000
21°C	23000	2200	6400	6800	7000	7800	4500	2700	4800	2800	3900	5700	2600	2500
37°C	23000	20000	14000	6100	7400	4800	3200	3000	1200	872	727	5		

Enterococci Density in Sediment.

The mean initial enterococci density in wet sediment was 170 CFU/100 g dry weight (as discussed in the *E. coli Density in Sediment* section) (Table 36). The maximum initial enterococci density was 1,500 CFU/100 g in June.

The mean initial enterococci density in dry sediment (820 CFU/100 g dry weight) was almost 5 times that in wet sediment. The maximum initial enterococci density was also seen in June (3,100 CFU/100 g dry weight). Forty percent (40%) of the experiments had initial enterococci densities in dry sediments at the minimum detectable density.

A statistically significant predictive linear relationship ($F = 18.90$; $p = 0.001$) was found between Ln enterococci in wet sediment and in dry sediment, which explained 56.1% of the variation of Ln enterococci in wet sediment.

No significant difference was found in the mean densities of Ln enterococci in wet and in dry sediments ($p = 0.082$), although it did trend toward statistical significance.

Enterococci Density in Sediment Regrowth.

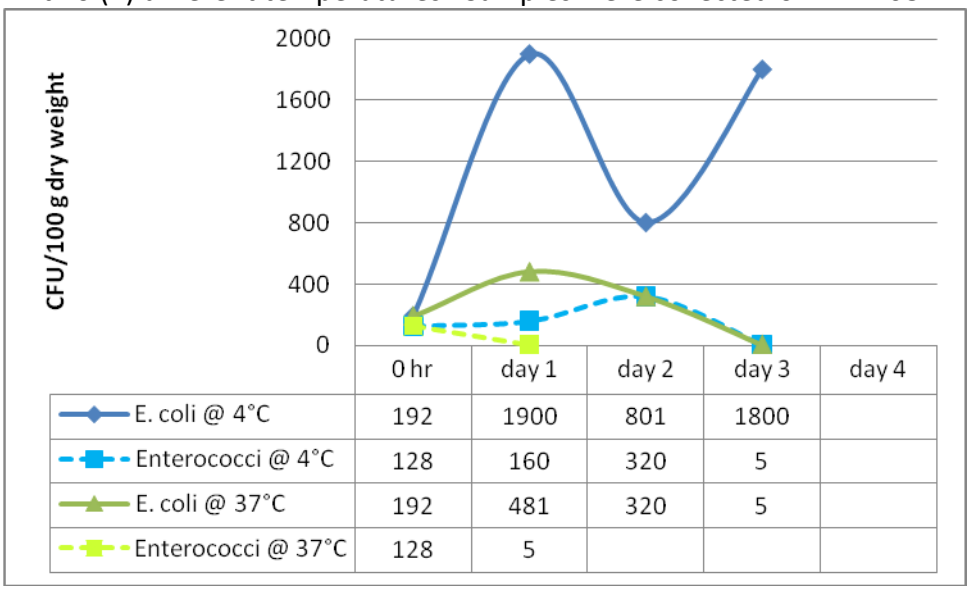
Regrowth in enterococci density was seen in 33% of the experiments (Table 36). The increases ranged from twice that of the previous day's density to 156 times the initial density. During the 4°C early September experiment, increases in density or maintenance of growth was seen through day 4.

TABLE 36. Enterococci densities found in sediment samples during 11 regrowth experiments.

		0 hr	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10	day 11	day 12	day 13
	Date: Temp														
Wet Sediment	**6-24: 37°C**	1500	5	456	152										
	7-8: 37°C	5	781	63	5										
	7-22: 4°C	128	160	320	5										
	7-22: 37°C	128	5												
	8-5: 30°C	5	156	5											
	9-9: 4°C	247	5	154	5	5									
	9-9: 21°C	247	5	5											
	9-9: 37°C	247	154	5	5										
	9-23: 4°C	5	5												
	9-23: 24°C	5	5												
	9-23: 37°C	5	5												
Dry Sediment															
	6-24: 37°C	3100	5	5	5										
	7-8: 37°C	650	149	5											
	8-5: 30°C	431	5												
	9-9: 4°C	2100	5	727	1200	1500	436	2300	1900	872	727	1100	1900	1900	
	9-9: 21°C	2100	1200	436	291	872	436	436	436	291	145	5			
	9-9: 37°C	2100	727	291	5	5									
	9-23: 4°C	5	5												
	9-23: 24°C	5	147												
	9-23: 37°C	5	294												

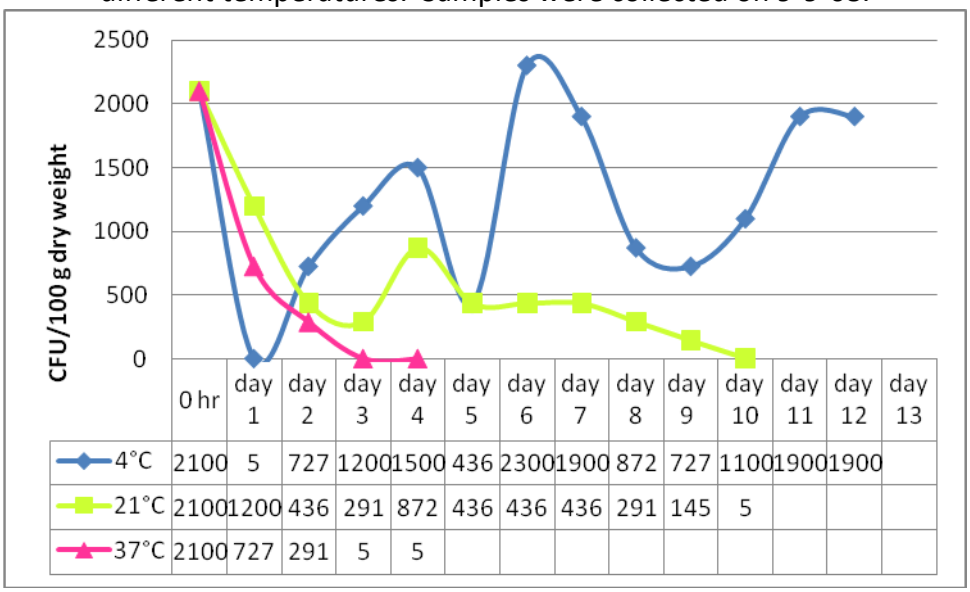
Examples of E. *coli* and/or enterococci regrowth/survivability of sediment samples collected at Granary Creek are shown below in Figures 37-38.

FIG. 37. Enterococci and *E. coli* densities in wet sediment maintained at two (2) different temperatures. Samples were collected on 7-22-08.



	0 hr	day 1	day 2	day 3	day 4
E. coli @ 4°C	192	1900	801	1800	
Enterococci @ 4°C	128	160	320	5	
E. coli @ 37°C	192	481	320	5	
Enterococci @ 37°C	128	5			

FIG. 38. Enterococci densities in dry sediment maintained at three (3) different temperatures. Samples were collected on 9-9-08.



	0 hr	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10	day 11	day 12	day 13
4°C	2100	5	727	1200	1500	436	2300	1900	872	727	1100	1900	1900	
21°C	2100	1200	436	291	872	436	436	436	291	145	5			
37°C	2100	727	291	5	5									

Regrowth Study

Sandy Point: Statistical Analysis of Regrowth/Survivability Studies from Water and Sediment Samples Collected at Sandy Point

Water.

E. coli Density in Water.

The mean initial *E. coli* density in water for Sandy Point (82 MPN/100 ml) was over twice the mean initial enterococci density in water. This difference in means was significant (F=6.55; p = 0.024). The maximum initial *E. coli* density was 441 CFU/100 g dry weight (Table 1).

A very highly significant (F = 134,707.19; $p < 0.000$) predictive relationship existed between Ln *E. coli* in water versus Ln *E. coli* in wet and dry sediments and the percent moisture in those sediments. One hundred percent (100 %) of the variation of Ln *E. coli* in water could be completely explained by these variables.

A very highly significant predictive linear relationship was found between Ln *E. coli* in water versus 2 variables: Ln *E. coli* in dry sediment and the percent moisture in dry sediment (F = 17.93; p = 0.000). This relationship indicated that almost three-quarters (71%) of the variation of Ln *E. coli* in water could be explained by the two sediment variables.

A highly significant (p = 0.005) correlation was found between Ln *E. coli* in water and Ln *E. coli* in dry sediment. No significant correlation was found between Ln *E. coli* in water and Ln *E. coli* in wet sediment.

The predictive linear relationship between Ln *E. coli* in water and Ln *E. coli* in wet and in dry sediments was highly significant (F= 7.79; p = 0.007) and explained 49% of the variation in Ln *E. coli* in water.

The predictive relationship between Ln *E. coli* in water and Ln enterococci in water was highly significant (F = 10.23; p = 0.007), and explained 40% of Ln *E. coli* variation in water.

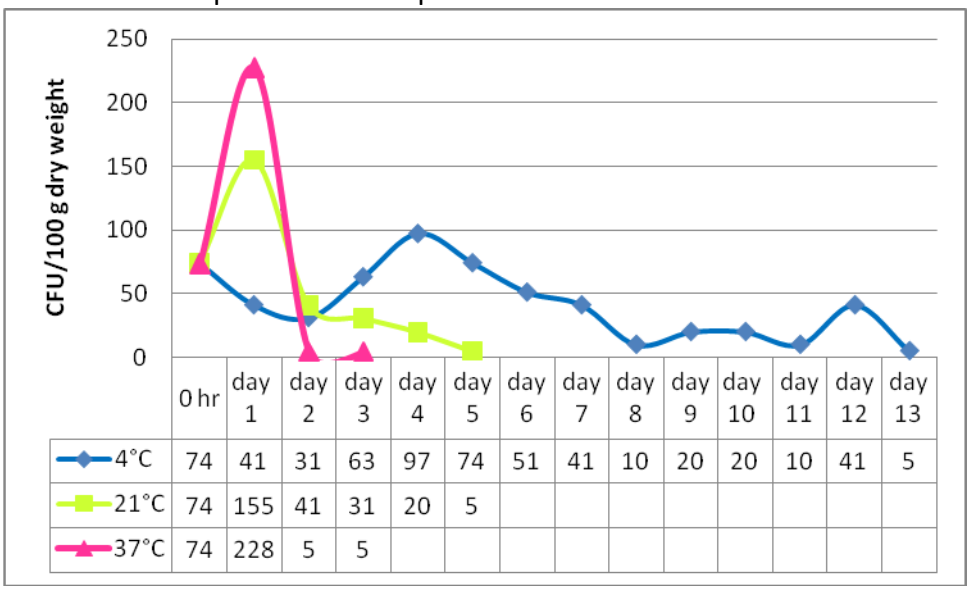
E. coli Density in Water Regrowth.

Regrowth was seen during 73% of the 15 experiments (Table 37). The increase in density ranged from a doubling of the initial density to > 100 times initial density. In the early September 4°C experiment, regrowth or maintenance of growth was seen through day 13 (Figure 39).

TABLE 37. *E. coli* densities found in water samples during 12 regrowth experiments.

	0 hr	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10	day 11	day 12	day 13
Date: Temp														
6-24: 37°C	10	5	20	5										
7-8: 37°C	10	20	5											
7-22: 4°C	441	441	496	362										
7-22: 37°C	441	275	120	5										
8-5: 4°C	41	10	30	5										
8-5: 30°C	41	1400	185											
9-9: 4°C	74	41	31	63	97	74	51	41	10	20	20	10	41	5
9-9: 21°C	74	155	41	31	20	5								
9-9: 37°C	74	228	5	5										
9-23: 4°C	5	146	95	52										
9-23: 24°C	5	41	41	10										
9-23: 37°C	5	504	20	5										

FIG. 39. *E. coli* densities in water samples maintained at 3 different temperatures. Samples were collected on 9-9-08.



Enterococci Density in Water.

The mean initial enterococci density in water (34 MPN/100 ml) was significantly less than that of E. *coli* density in water (as stated in the *E. coli Density in Water* section above). The maximum enterococci density was 165 MPN/100 ml.

The predictive relationship between Ln enterococci in water versus Ln enterococci in wet sediment and in dry sediment and the % moisture in these sediments was highly significant (F = 6.3; *p* = 0.008 and explained 60.8% of the Ln enterococci variation in water.

A significant predictive relationship was found between Ln enterococci in water and Ln enterococci in dry sediment (F = 5.78; p = 0.032), which explained 25% of Ln enterococci variation in water. No such relationship existed between Ln enterococci in water and Ln enterococci in wet sediment.

Again, as stated in the *E. coli Density in Water* section, a highly significant predictive relationship was found between Ln enterococci in water and Ln *E. coli* in water (F= 10.23; *p* = 0.007) and explained 40% of the variation of Ln enterococci in water.

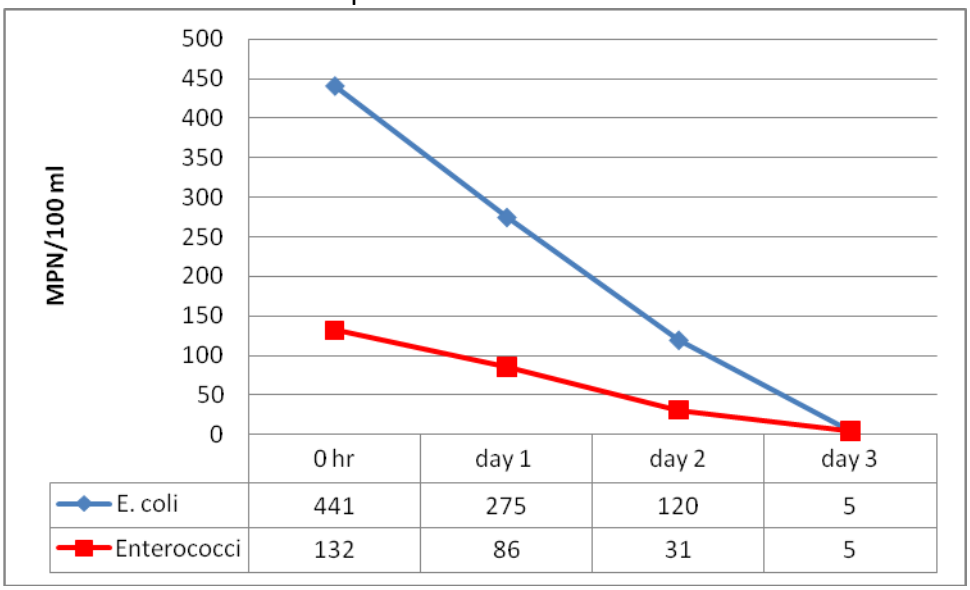
Enterococci Density in Water Regrowth.

Regrowth was seen in 20% of the water samples collected at Sandy Point (Table 38). The increase in enterococci density ranged from an 8-fold increase in density during the 4°C experiment in late September to a 4-fold increase during the June experiment and the 24 ^{o}C experiment in late September. Four (4) of the experiments maintained and/or increased growth through day 3 (Figure 40).

TABLE 38. Enterococci densities found in water samples during 12 regrowth experiments.

	0 hr	**day 1**	**day 2**	**day 3**	**day 4**	**day 5**	**day 6**	**day 7**	**day 8**	**day 9**	**day 10**	**day 11**	**day 12**	**day 13**
Date: Temp														
6-24: 37°C	5	20	5	5										
7-8: 37°C	165	5	5											
7-22: 4°C	5	5												
7-22: 37°C	132	86	31	5										
8-5: 4°C	10	10	5											
8-5: 30°C	10	5												
9-9: 4°C	10	5												
9-9: 21°C	10	5												
9-9: 37°C	10	5												
9-23: 4°C	5	41	10	10										
9-23: 24°C	5	20	5											
9-23: 37°C	5	5												

FIG. 40. *E. coli* and Enterococci densities in water samples maintained at 37°C. Samples were collected on 7-22-08.



Sediment.

<u>E. coli</u> Density in Sediment

The mean initial *E. coli* density in wet sediment was 91 CFU/100 g dry weight. The *E. coli* maximum density was 376 CFU/100 g dry weight in June. Sixty percent (60%) of the 15 experiments had an initial density at the minimum detectable density.

The mean initial *E. coli* density in dry sediment was 101 CFU/100 g dry weight. The maximum density was 500 CFU/100 g dry weight in June. Forty percent (40%) of the initial E. *coli* concentrations had an initial density at the minimum detectable density.

The predictive relationship of Ln *E. coli* in wet sediment versus Ln *E. coli* in dry sediment was significant ($F = 6.55$; $p = 0.024$), and explained 28% of Ln *E. coli* variation in wet sediment.

The mean densities of Ln *E. coli* in wet and in dry sediments were not significantly different ($F = 0.69$; $p = 0.413$), nor were the mean densities of Ln *E. coli* in wet sediment and Ln enterococci in wet sediment ($F = 0.23$; $p = 0.635$).

<u>E. coli</u> Density in Sediment Regrowth

Regrowth was seen in 20% of the *E. coli* density in wet sediment experiments (Table 39). The magnitude of increase ranged from 1.5 times the initial density in July and in early September to 29 times the initial density in early September. The longest period of maintenance or increase in *E. coli* was seen in early September and was through day 5.

TABLE 39. *E. coli* densities found in wet and dry sediment samples during 10 regrowth experiments each.

		0 hr	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10	day 11	day 12	day 13
	Date: Temp														
Wet Sediment	**6-24: 37°C**	376	134	5	5										
	7-22: 4°C	298	446	5	149										
	7-22: 37°C	298	5												
	8-5: 30°C	116	5												
	9-9: 4°C	5	5	144	287	431	5								
	9-9: 21°C	5	144	5											
	9-9: 37°C	5	5	5											
	9-23: 4°C	5	5												
	9-23: 24°C	5	5												
	9-23: 37°C	5	5												
Dry Sediment															
	6-24: 37°C	160	5	5	5										
	7-8: 37°C	500	284	142	28	5									
	8-5: 30°C	119	5												
	9-9: 4°C	116	145	5											
	9-9: 21°C	116	145	5											
	9-9: 37°C	116	5	5											
	9-23: 4°C	5	5												
	9-23: 24°C	5	5												
	9-23: 37°C	5	158	5											

Enterococci Density in Sediment.

The mean initial Ln enterococci density in wet sediment was 72 CFU/100 g dry weight, while the mean initial enterococci density in dry sediment was (2.5 times that in the wet sediment) (235 CFU/100 g dry weight). That difference was highly significant ($p = 0.004$). The maximum initial enterococci density in wet sediment was 591 CFU/100 g dry weight and was virtually the same (588 CFU/100 g dry weight) in dry sediment (Table 40).

Sixty-seven percent (67%) of the experiments with wet sediments had initial enterococci densities of the minimum detectable density, but only 20% had initial densities in dry sediments were as low as that minimum density.

The linear relationship between Ln enterococci in wet and in dry sediments was not significant (p = 0.092), but did trend toward statistical significance. A highly significant predictive relationship

was found between Ln enterococci and the percent moisture in wet sediment (F = 7.10; p = 0.019), indicating that 30% of the variation in Ln enterococci in wet sediment was explained by that variable.

Enterococci Density in Sediment Regrowth.

Regrowth of enterococci in wet sediment occurred during 20% of the experiments. The range of increase of enterococci ranged from 1.25 times the initial density to 30 times the initial density in July and in September, respectively. The June experiment continued maintenance of growth through day 3.

Regrowth in dry sediment occurred during 27% of the experiments (Table 40). The increase of enterococci in dry sediment ranged from < 1% in June to 29 times the previous density during early September experiments. One (1) experiment increased and/or maintained growth in dry sediment through day 5.

TABLE 40. Enterococci densities found in wet and dry sediment samples during 11 regrowth experiments.

Wet Sediment		**0 hr**	**day 1**	**day 2**	**day 3**	**day 4**	**day 5**	**day 6**	**day 7**	**day 8**	**day 9**	**day 10**	**day 11**	**day 12**	**day 13**
	Date: Temp														
	6-24: 37°C	591	5	5	5										
	7-8: 37°C	100	5												
	7-22: 4°C	5	149	5											
	7-22: 37°C	5	5												
	8-5: 30°C	5	5												
	9-9: 4°C	115	5	5											
	9-9: 21°C	115	144	5											
	9-9: 37°C	115	5	5											
	9-23: 4°C	5	147												
	9-23: 24°C	5	5												
	9-23: 37°C	5	5												
Dry Sediment															
	6-24: 37°C	426	266	270	5										
	7-8: 37°C	350	284	142	2000	5									
	8-5: 30°C	179	5												
	9-9: 4°C	291	5	145											
	9-9: 21°C	291	5	145	5										
	9-9: 37°C	291	5	5											
	9-23: 4°C	5	5												
	9-23: 24°C	5	5												
	9-23: 37°C	5	5												

Combined Regrowth Study Sites Summary

Descriptive Statistics: Combined Data from All Locations

Initial Bacterial Density in Water

Initial E. coli Density in Water

- The initial mean *E. coli* density in water from the combined locations was almost six (6)) times more than the mean enterococci density from the combined locations (Table 1).

- From most to least, initial *E.coli* mean densities in water were highest at Granary Creek (206 MPN/100 ml), Assateague Island (181 MPN/100 ml), Sandy Point (82 MPN/100 ml), and Delaware Shore (6 MPN/100 ml) (Table 41).

TABLE 41. Descriptive Statistics: Initial *E. coli* density in dater for combined sites and by study site.

Variable	Location	N	N*	Mean	SEMean	StDev	Median	Maximum
E. coli Water	ALL	60	0	20.5	5.4	41.6	5.0	845.0
E. coli Water	AI	15	0	180.9	88.8	343.9	12.0	845.0
E. coli Water	DS	15	0	6.5	0.7	2.6	5.0	10.0
E. coli Water	GC	15	0	206.0	75.7	293.0	52.0	759.0
E. coli Water	SP	15	0	82.4	38.3	148.1	10.0	441.0

Initial Enterococci Density in Water

- The initial mean enterococci density in water from the combined locations fewer than the *E. coli* density in water at the combined locations, except for the Delaware Shore (Table 42).

- From most to least, initial enterococci mean densities in water were highest at Granary Creek (35 MPN/100 ml), Sandy Point (34 MPN/100 ml), Delaware Shore (6 MPN/100 ml), and Assateague Island (6 MPN/100 ml) (Table 42).

TABLE 42. Descriptive Statistics: Initial enterococci density in dater for combined sites and by study site.

Variable	Location	N	N*	Mean	SE Mean	StDev	Median	Maximum
ENT Water	ALL	60	0	20.5	5.4	41.6	5.0	165.0
ENT Water	AI	15	0	6.3	0.6	2.3	5.0	10.0
ENT Water	DS	15	0	6.3	0.6	2.3	5.0	10.0
ENT Water	GC	15	0	35.1	14.6	56.6	5.0	144.0
ENT Water	SP	15	0	34.3	14.7	56.8	10.0	165.0

Initial Bacterial Density in Sediment

E. coli Density in Sediment

- The initial mean *E. coli* density from the combined locations is highest in Dry Sediment (1,549 CFU/100 g), followed by Wet Sediment (184 CFU/100 g), then water (119 MPN/100 ml) (Table 43).

- The initial *E. coli* mean densities in Dry Sediments were highest at Granary Creek (5,594 CFU/100 g), followed by Assateague Island (338 CFU/100 g), Delaware Shore (164 CFU/100 g), and Sandy Point (101 CFU/100 g) (Table 44).

- The initial *E. coli* mean densities in Wet Sediments were highest at Granary Creek (383 CFU/100 g), followed by Delaware Shore 204 CFU/100 g, Sandy Point (91 CFU/100 g), then Assateague Island (56 CFU/100 g).

TABLE 43. Descriptive statistics: Initial *E. coli* density in wet sediment for combined sites and by study site.

Variable	Location	N	N*	Mean	SEMean	StDev	Median	Maximum
E. coli Wet	ALL	60	0					1600
E. coli Wet	AI	15	0	55.9	25.0	96.8	5.0	241.0
E. coli Wet	DS	15	0	204.0	136.0	526.0	5.0	1500.0
E. coli Wet	GC	15	0	383.0	130.0	502.0	185.0	1600.0
E. coli Wet	SP	15	0	90.0	33.5	129.6	5.0	376.0

TABLE 44. Descriptive statistics: Initial enterococci density in wet sediment for combined sites and by study site.

Variable	Location	N	N*	Mean	SE Mean	StDev	Median	Maximum
E. coli Dry	ALL	60						
E. coli Dry	AI	15	0	338.0	227.0	878.0	5.0	2500.0
E. coli Dry	DS	15	0	164.0	109.0	420.0	5.0	1200.0
E. coli Dry	GC	15	0	5594.0	2501.0	9687.0	122.0	23000.0
E. coli Dry	SP	15	0	100.8	32.4	125.3	116.0	500.0

Enterococci in Sediment

- The initial mean enterococci density from the combined locations was highest in Dry Sediment (344 CFU/100 g), followed by Wet Sediment (84 CFU/100 g), then water (21MPN/100 ml) (Table 45).

- The initial enterococci mean densities in Dry Sediments were highest at Granary Creek (820 CFU/100 g), followed by Assateague Island (251 CFU/100 g), Sandy Point (235 CFU/100 g), Delaware Shore (69 CFU/100 g) (Table 46).

- The initial mean enterococci density in Wet Sediments were highest at Granary Creek (170 CFU/100 g), followed by Assateague Island (77 CFU/100 g), Sandy Point (72 CFU/100 g), and Delaware Shore (15 CFU/100 g).

- The initial mean enterococci densities in both Wet and Dry Sediments were fewer than those of the initial mean *E. coli* in Sediments, except for Dry Sediment at Sandy Point and Wet Sediment at Assateague Island.

TABLE 45. Descriptive statistics: Initial enterococci in wet sediment for combined sites and by study site.

Variable	Location	N	N*	Mean	SE Mean	StDev	Median	Maximum
ENT Wet	ALL	60	0					1500
ENT Wet	AI	15	0	77.2	25.5	98.7	5.0	241.0
ENT Wet	DS	15	0	15.1	7.1	27.6	5.0	100.0
ENT Wet	GC	15	0	169.5	98.4	381.3	5.0	1500.0
ENT Wet	SP	15	0	72.4	39.1	151.3	5.0	591.0

TABLE 46. Descriptive statistics: Initial enterococci in dry sediment for combined sites and by study site.

Variable	Location	N	N*	Mean	SE Mean	StDev	Median	Maximum
ENT Dry	ALL	60	0					3100.0
ENT Dry	AI	15	0	251.0	53.6	207.4	230.0	588.0
ENT Dry	DS	15	0	69.1	24.9	96.3	5.0	227.0
ENT Dry	GC	15	0	820.0	262.0	1017.0	431.0	3100.0
ENT Dry	SP	15	0	235.0	49.8	192.9	179.0	588.0

Trends Seen at the 4 Study Sites

Initial Bacterial Density in Water

E. coli Density in Water

- At the 2 Maryland recreational areas in the study, very highly or highly significant predictive relationships existed between Ln *E. coli* in water versus Ln *E. coli* density in wet and dry sediments. These relationships explained 72% of the Ln *E. coli* in water variation at Assateague Island and 49% of the variation at Sandy Point.

- At the 3 Maryland study sites, the predictive relationship between Ln *E. coli* in water versus Ln *E. coli* in wet and dry sediments and the percent moisture in them was very highly significant. These relationships explained 82% of the variation in Ln *E. coli* in water at Assateague Island, 99% of the variation at Granary Creek, and 100% of the variation at Sandy Point.

- At the 3 Maryland study sites, the predictive relationship between Ln *E. coli* in water and Ln enterococci in water were highly or very highly significant and explained approximately one-half of the variation in Ln *E. coli* in water.

- At the 3 Maryland study sties, the initial mean densities of Ln *E. coli* in water were significantly more than that of Ln enterococci in water.

- At Assateague Island and Granary Creek, the predictive relationship between Ln enterococci in water versus Ln enterococci in wet and in dry sediments were highly significant or significant. The relationships explain 58% at Assateague Island and 29% at Granary Creek.

- The predictive relationships between Ln enterococci in water versus Ln enterococci in dry sediment at Delaware Shore, Granary Creek, and Sandy Point were significant. This relationship explained 92% of the variation in Ln enterococci in water at Delaware Shore, 35% at Granary Creek, and 25% at Sandy Point.

- The predictive relationship between Ln Enterococci in water versus Ln enterococci in wet sediment and in dry sediment and the percent moisture in wet and in dry sediments was highly significant at Delaware Shore and Sandy Point and significant at Assateague Island. The variation explained by this relationship was 92% at Delaware Shore, 61% at Sandy Point, and 50% at Assateague Island.

Initial Bacterial Density in Sediment

E. coli Density in Sediment

- The predictive relationship between Ln *E. coli* in wet sediment versus in dry sediment was very highly significant or highly significant at Delaware Shore, Granary Creek, and Sandy Point. The Ln *E. coli* in wet sediment variation explained was 100% at Delaware Shore, 58% at Granary Creek, and 28% at Sandy Point.

Enterococci in Sediment

- At Sandy Point, a highly significant predictive relationship was found between Ln enterococci and the percent moisture in Wet Sediment and explained 30% of the variation in Ln Enterococci in wet sediment.

- At Assateague Island and Delaware Shore, a very highly significant or significant relationship existed between Ln enterococci in dry sediment versus the percent moisture in dry sediment. The variation in Ln enterococci in dry sediment was 68% at Assateague Island and 38% at Delaware Shore.

- At Granary Creek, the predictive relationship between Ln enterococci in wet versus in dry sediments was highly significant and explained 56% of the variation in Ln enterococci in wet sediment.

- At Assateague Island and Sandy Point, Ln enterococci in dry sediment was highly significantly more than in wet sediment.

Regrowth of Bacteria Density in Water

E. coli Density In Water Regrowth/Maintenance of Growth

- The percentage of experiments that saw regrowth of *E. coli* in water was 47% at Assateague Island, 27% at Delaware Shore, 73% at Granary Creek, and 67% at Sandy Point.

Enterococci in Water Regrowth/Maintenance of Growth

- Assateague Island and Delaware Shore each had one experiment that showed regrowth, while 33% of the Granary Creek experiments and 27% of the Sandy Point experiments showed regrowth of enterococci in water.

Regrowth of Bacteria Density in Sediments

E. coli Density in Sediment Regrowth/Maintenance of Growth

E. coli Density in Wet Sediment

- Sixty-seven percent (67%) of the experiments at Granary Creek and 20% of the experiments at Sandy Point showed regrowth of *E. coli* in wet sediment.

E. coli Density in Dry Sediment

- Thirty-three percent (33%) of the Granary Creek experiments and 20% of Sandy Point experiments showed regrowth of *E. coli* in dry sediment.

Enterococci Density in Sediment Regrowth/Maintenance of Growth

Enterococci Density in Wet Sediment

- Assateague Island had one experiment that indicated regrowth of enterococci in wet sediment, while 33% of the experiments at Granary Creek and 20% of Sandy Point experiments showed regrowth of enterococci in wet sediment.

Enterococci Density in Dry Sediment

- Twenty-seven percent (27%) of the Granary Creek experiments and 20% of the experiments at both Assateague Island and Sandy Point showed enterococci in dry sediment.

Conclusions: 12-Month and Regrowth Studies

This study was conducted to address two major questions. The first question addresses the levels of indicator bacteria in beach sand, beach sediments and water at selected sites in Maryland and Delaware. The *12-month Water, Sediment, and Pathogen Study* provides the following answer.

- Detectable levels of indicator bacteria (either *E. coli* or Enterococci) were initially found in 47% of 408 assays.

- The study found significant to very highly significant relationships between bacteria levels in water versus in wet and/or in dry sediments at one to all study sites.

The second question dealt with whether regrowth was occurring where elevated bacterial levels were found and whether bacterial pathogens were present, suggesting a pollution source. The study found the following answers.

- Regrowth of both indicator bacteria occurred in sand, sediment, and water samples at all 4 locations. The increase varied from less than a doubling to over 1,000 times the previous day's density.

- Other than the indicator bacteria, 99 bacterial isolates cultured from sand, sediment, and water samples were identified.

- Only 1 of the 99 bacterial isolates was definitively identified as a potential intestinal illness pathogen, *Vibrio furnisii*.

Because this was a pilot study and necessarily had limitations, additional questions arise that might be answered by the following:

- Conduct a focused study of 1 or, at most, 2 beaches where 3 replicates would be collected and analyzed for wet and dry sediments (in the current study, only 1 sediment sample was collected on each sampling day). A focused study would confirm and/or expand findings of the study.

- Consider expanding the number and kind of samples collected at the study locations, e.g., water samples taken closer to shore or sediment samples collected above high tide line.

- Determine the minimum bacterial density increase that constitutes regrowth.

- Screen for potential pathogens using PCR methodology in addition to the culturing technique used in the current study in order to increase detection sensitivity.

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APPENDIX I.

EPA Project
Pathogen Summary

WaterShed (O157:H7)[3]	Midi Labs[1]	GenBank[2]	ELISA
Assateague	-*Escherichia/Shigella* No match	-*Escherichia sp.* (99%)	Negative
Delaware Shore	-*Shigella* No match	-*Shigella sonnei* [4] (100%)	
Granary Creek	-*E. coli* (O157:H7) No match	-*Shigella boydii* [4] (100%)	Negative
	-*Shigella* No match	-*Shigella sonnei* [4] (99%)	
	-*Rahnella*	-*Yersinia* sp.[5] (100%)	
	-*E. coli* (O157:H7) No match	-*E. coli* (100%)	Negative
Sandy Point	-*Escherichia/Shigella* No match	-*Escherichia sp.* (99%)	Negative
	-*E. coli* (O157:H7) No match	-*E. coli* (100%)	Negative
	-*E. coli* No match	-*Shigella sonnei* [4] (99%)	Negative
	-*E.coli/Shigella dysenteria* No match	-*Esherichia sp.* (99%)	Negative
	-*Vibrio furnissi*[4]		
	-*Shigella flexneri* No Match	-*Shigella sonnei*[4] (99%)	

[1] A validated library was used for identification
[2] GenBank is not a validated library
[3] Tested against O157:H7 antisera
[4] BioSafety Level 2 (moderate potential for mild disease)
[5] Unable to rate pathogenicity potential without species identification.

APPENDIX II Identification of Bacterial Isolates by 16S DNA Sequencing

(Sorted by Sampling Site)

Collection Date	Sample*	Midi Labs ID[1]	GenBank ID[1]
1-30.02	AI dry	*Pseudomonas-sp*	*99% Pseudomonas sp.*
5-21.14	AI dry	no match (Paenibacillus?)	*99% Paenibacillus favisporus*
7-8.09	AI dry	*Shewanalla-algae*	
12-9.04	AI water	No match (Enterobacter pyrinius?)	*99% Enterobacter sakazakii*
4-9.01	AI water	*Pseudomonas sp.*	*100% Pseudomonas nitroreducens*
4-9.02	AI water	no match (Chryseomonas/Pseudomonas?)	*97% Pseudomonas sp.*
4-9.03	AI water	*Pseudomonas sp.*	*98% Pseudomonas pseudoalcaligenes*
4-9.04	AI water	no match (E.coli/Shigella?)	*99% Escherichia sp.*
4-9.06	AI water	*Citrobacter sp.*	
5-7.07	AI water	*Acinetobacter calcoaceticus*	*99% Acinetobacter calcoaceticus*
9-9.05	AI water	No match (Chryseomonus?)	*98% Pseudomonus sp.*
5-21.20	DS dry	*Pseudomonas fulva*	
7-22.10	DS dry	*Citrobacter braakii/freundii*	
11-18.04	DS water	No match (Shigella?)	*100% Shigella sonnei*
12-9.05	DS water	No match (Klebsiella pneumoniae?)	*99% Klebsiella pneumoniae*
5-21.02	DS water	*Bacillus circulans*	*99% Bacillus sp.*
9-9.04	DS water	*Pseudomonas pseudoalcaligenes*	*100% Pseudomonus putida*
4-9.20	DS wet	*Planococcus kocurii*	*99% Planococus sp.*
1-30.24	GC dry	*Pseudomonas fluorescens-G*	*99% Pseudomonas putida*
8-5.06	GC dry	*Pseudomonas reinovorans*	*98% Pseudomonas sp.*
9-9.07	GC dry	*Proteus vulgaris*	
9-9.08	GC dry	no match (*E.coli O157:H7?)*[3]	*100% E.coli*
11-18.02	GC water	*Pseudomonas sp.*	*100% Pseudomonas nitroreducens*
12-9.01	GC water	No match (Shigella dysenteriae?)	*99% Escherichia sp.*
12-9.02	GC water	No match (Escherichia/Shigella)	*99% Escherichia sp.*
12-9.03	GC water	No match (E.coli 0157:H7?)	*100% E. coli*
1-30.12	GC water	*Enterobacter cancerogenus*	*99% Enterobacter cloacae*
1-30.17	GC water	*Citrobacter sp.*	
1-30.18	GC water	*Citrobacter sp.*	
2-19.01	GC water	*Aeromonas-popoffii*	*99% Aeromonas bivalvium*
2-19.04	GC water	*Hafnia-alvei*	*99% Hafnia alvei AB435606*
4-9.11	GC water	*Pseudomonas-lundensis/taetrolens*	
4-9.12	GC water	no match (Rahnella?)	*100% Yersinia sp.*
4-9.13	GC water	*Pseudomonas fluorescens*	
5-21.05	GC water	*Citrobacter freundii*	
5-21.06	GC water	*Citrobacter freundii/braakii*	
5-21.08	GC water	*Citrobacter sp.*	
5-21.09	GC water	*Citrobacter braakii*	
5-21.10	GC water	*Citrobacter sp.*	

Collection Date	Sample*	Midi Labs ID[1]	GenBank ID[1]
5-7.11	GC water	*Citrobacter sp.*	
7-22.07	GC water	*Citrobacter sp.*	
7-8.02A	Gc water	*Aeromonus caviae/enteropelogenes*	
7-8.02B	GC water	*Citrobacter sp.*	
9-9.01	GC water	*Proteus vulgaris*	
2-19.05	GC wet	*Citrobacter sp.*	
2-19.09	GC wet	*Bacillus sphaericus*	*100% Lysinibacillus sphaericus*
2-19.10	GC wet	*Lysinibacillus sphaericus*	*100 % Lysinibacillus sphaericus*
4-9.22	GC wet	*Bacillus sphaericus*	*98% Bacillus sp.*
4-9.24	GC wet	*Sporosarcina-globispora*	*97% Sporosarcina koreensis*
5-7.32	GC wet	*Aeromonas caviae/enteropelogenes*	
5-7.33	GC wet	no match (Serratia ficaria?)	*100% Rheinheimera sp.*
7-22.13	GC wet	*Aeromonas caviae/enteropelogenes*	
7-8.14	GC wet	*Shewanella algae*	
9-9.11	GC wet	*Serratia marcescens*	
5-21.23	GC wet	*Acinetobacter calcoaceticus*	*99% Acinetobacter calcoaceticus*
5-21.16	GC-dry	*Pseudomonas pseudoalcaligenes*	
5-21.21	GC-dry	no match *(Paenibacillus lautus?)*	*Paenibacillus favisporus*
5-7.34	GC-dry	*Aeromonas popoffii*	
5-7.36	GC-dry	*Pseudomonas spp.*	*100% Pseudomonas putida*
6-24.11	GC-dry	*Citrobacter freundii*	
8-5.11	GC-dry	*Aeromonas caviae/enteropelogenes*	
3-12.11	SP dry	no match (Escherischia/Shigella?)	*99% Escherichia sp*
5-7.42	SP dry	no match (Enterobacter aerogenes?)	*99% Klebsiella sp.*
5-7.43	SP dry	*Enterococcus faecalis*	
5-7.44	SP dry	*Acinetobacter genomospecies 3*	
9-9.10	SP dry	*Proteus vulgaris*	
11-18.05	SP water	No match (Shigella/Escherichia?))	*99% Escherichia sp.*
1-30.27	SP water	*Serratia marcescens*	
1-30.28	SP water	*Aeromonas sp.*	*99% Aeromonas salmonicida*
3-12.01	SP water	no match (E.Coli/Shigella?)	*99% Escherichia sp.*
3-12.03	SP water	*Pseudomonas sp.*	*100% Pseudomonas nitroreducens*
3-12.04	SP water	*Klebsiella pneumoniae-ozaenae*	
3-12.05	SP water	*Bacillus sp.*	*99% Lysinibacillus fusiformis*
3-12.05A	SP water	*Citrobacter sp.*	
3-12.06	SP water	*Citrobacter sp.*	
3-12.07	SP water	*Kurthia gibsonii*	*100% Bacillus sp.*
3-12.09	SP water	no match (Citrobacter?)	*Erwinia aphidicola*
3-12.10	SP water	no match (Hafnia?)	*100% Hafnia alvei*
4-9.16	SP water	*Bacillus badius*	*98% Bacillus sp.*
5-21.04	SP water	*Staphylococcus-equorum*	

Collection Date	Sample	Midi Labs ID	GenBank ID
5-21.07	SP water	*Citrobacter sp.*	
5-21.11	SP water	no match (Hafnia?)	*99% Hafnia alvei*
5-21.13	SP water	*Acinetobacter genomospecies 3*	
6-10.01	SP water	*Citrobacter youngii*	
7-22.01	SP water	no match *(E. coli O157:H7?)*	*100% E. coli*
7-22.02	SP water	no match *(E. coli/Shigella dysenteriae?))*	*99% Escherichia sp.*
8-5.01	SP water	to species *(E.coli O157:H7?)*	
8-5.05	SP water	No match (Aeromonas?)	*98% Shewanella amazonensis*
9-9.02	SP water	*Proteus vulgaris*	
12-9.09	SP wet	No match (Shigella flexneri?)	*99% Shigella sonnei*
5-7.39	SP wet	no match (E. coli 0157:H7?)	*100% E. coli*
5-7.40	SP wet	no match (E. coli Sigma W311?)	*99% Shigella sonnei*
7-22.08	SP wet	no match (E. coli 0157:H7?)	*99% E. coli*
7-22.11	SP wet	*Citrobacter braakii/freundii*	
7-22.12	SP wet	*Citrobacter braakii/freundii*	
7-8.10	SP wet	*Pseudomonas aeruginosa*	
8-5.07	SP wet	*(to genus) Pseudomonas sp.*	*95% Pseudomonas aeruginosa*
8-5.12	SP wet	*Vibrio furnissii*	

*AI – Assateague Island, DS – Delaware Shore, GC – Granary Creek, SP – Sandy Point

[1]The Midi Labs 16S DNA library was constructed using validated bacterial isolates obtained from the American Type Culture Collection

[2]Bacterial isolates that could not be identified with high confidence using the Midi Lab library were then analyzed using the GenBank DNA library (an non-validated library).

[3]Isolates that were potentially identified as *E. coli* O157:H7 were tested against O157:H7 antisera using the ELISA methodology. All such isolates were negative for this assay.