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Connecticut Department of Environmental Protection

Final Report

FOR THE PROJECT

Impact of Summer Ambient Temperatures on Elevated Levels, Persistence and Regrowth of the *Enterococcus* Indicator Bacteria at the Silver Sands State Park Beach in Milford, CT in the Long Island Sound Coastal Area.

Prepared by

INTERSTATE ENVIRONMENTAL COMMISSION

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I. Abstract

In the summer of 2007, the Interstate Environmental Commission (IEC) conducted a study funded by the Connecticut Department of Environmental Protection (CT DEP) Long Island Sound Fund program. The purpose of the project was to analyze localized conditions contributing to high concentrations of indicator bacteria that have caused and may cause additional beach closures in the Long Island Sound coastal area. The study area, Silver Sands State Park in Milford, Connecticut, includes two creeks, Great Creek and Fletcher Creek, both emptying into the Long Island Sound. This project was crucial to better predicting and understanding elevated bacteria levels in the study area and similar Long Island Sound coastal areas and, subsequently, improving overall water quality and promoting safe recreational use of Long Island Sound bathing beaches.

The focus of this study was to examine the specific impact of summer temperatures - as well as pH, total suspended solids (TSS), turbidity and salinity - on the concentration, persistence and potential regrowth of indicator bacteria in sediments and the water column during summer months, and also, to determine if creek sediments serve as a source of *Enterococci* to overlying waters through resuspension and remobilization. Samples were analyzed for DNA markers to indicate sources of fecal pollution within the watershed. In addition, DNA fingerprinting was used to determine if indicator bacteria are re-growing or concentrating in the environment.

The study found that there was no significant correlation between either sediment or water temperatures and *Enterococcus* levels. However, it was found that birds are a major contributor of fecal pollution in the study area. DNA fingerprinting yielded a highly diverse population of *Enterococci* in the sediment, which suggests that upstream creek sediments may serve as a sink and act as a concentrating environment for indicator bacteria, although they are not proliferating within the sediment. Therefore, at Silver Sands State Park, sediments may have a certain, but most likely, limited, contribution to bacterial pollution in overlying creek and downstream waters.

II. Background

Previous CT DEP sampling of indicator bacteria in the tidal creek draining into Silver Sands State Park Beach in Milford, CT, performed on a weekly basis, had revealed that *Enterococci* concentrations at the beach periodically exceeded bathing water criteria. Since CT DEP's surveys indicated that significant sources of human sewage might not be present, additional data were needed in order to enhance the understanding of estuarine processes and localized conditions that may contribute to elevated levels of indicator bacteria that are detrimental to the sanitary quality of bathing beaches and overall water quality of the Long Island Sound.

Therefore, the focus of this study was to determine the specific impact of summer temperatures - as well as pH, Total Suspended Solids (TSS), turbidity and salinity - on the concentration of indicator bacteria in both the water column and sediments at the Silver Sands State Park Beach during summer months.

III. Description of Funding Source – Long Island Sound Fund

The Long Island Sound Fund supports four categories of activities: public access, public education and outreach, habitat restoration and research. More specifically, the Long Island Sound Fund supports:

1. The development of public outreach and education programs to increase the public's awareness of the need to preserve and protect Long Island Sound and its resources with special attention to developing in our young people a sense of the value of the Sound to our quality of life.
2. The increase of public access to Long Island Sound through the development of boardwalks, walkways, benches, fishing piers and signage and the acquisition of appropriate sites.
3. The protection and restoration of habitat essential to the Long Island Sound ecosystem including tidal wetlands, mudflats, beaches and dunes, riverine

migratory corridors, and coves and embayments to ensure the future survival of important plant and animal species and their habitats.

4. The support of scientific research of Long Island Sound that provides clear direction for management decisions to enhance our understanding and management of the Sound's natural resources.

IV. Findings

1. The results of this study show no significant correlation between either water or sediment temperature and *Enterococcus* levels.

2. The study showed that while upstream creek sediments may serve as a sink and act as a concentrating environment for indicator bacteria, there was a low regrowth of bacteria in the sediment, demonstrated by the high diversity of the samples. Therefore, at Silver Sands State Park Beach, sediments may have a certain, but most likely a limited, contribution to bacterial pollution in overlying creek and downstream waters.

3. The results indicated that birds are significant contributors to bacterial pollution of beach water. There is limited indication of humans as a possible source, and no indication of dogs or deer as sources of pollution.

4. The results from the July 10, 2007, sampling at the beach sampling location exceeded Connecticut Beach Closure Criteria. This would have led to the beach being resampled and potentially being closed.

V. Summary of Actions

IEC successfully conducted five sampling events between June and August 2007 (See Table 1). Three sampling events had no rain in the 48 hours prior to the sampling, and the other two events had less than 0.07 inches of rain in the prior 48 hours. This amount of rain is considered to be negligible and should have little to no effect on the results of the study. Sampling was performed as described in the Quality Assurance Project Plan (QAPP) with a total of 28 samples collected and analyzed on each run. This consisted of four grab samples

collected at each of the seven sampling points (See Table 2 and Appendix 1 for sampling locations).

Table 1: Sampling Dates and Parameters

Sampling Dates	Parameters Sampled					
	Fecal coliform	*Enterococcus*	pH	Temperature	Salinity	TSS
June 27, 2007	X	X	X	X	X	X
July 10, 2007	X	X	X	X	X	X
August 7, 2007	X	X	X	X	X	X
August 14, 2007	X	X	X	X	X	X
August 29, 2007	X	X	X	X	X	X

Table 2: Sampling Locations

Station	Type	Location
U1	Water	Upstream Creek 1
U2	Water	Upstream Creek 2
D1	Water	Downstream Creek 1
D2	Water	Downstream Creek 2
B	Water	Beach
U1S	Sediment	Upstream Creek 1
U2S	Seiment	Upstream Creek 2

Field measurements were taken for temperature, salinity, and pH and laboratory analyses were performed at the IEC laboratory for *Enterococci*, fecal coliform, TSS and turbidity. Temperature data was supplemented with data used from the in-situ HOBO continuous temperature data recorders set to record at hourly intervals from May to November 2007.

Samples were analyzed for *Enterococci* and fecal coliform using the MPN 3-tube, 4-dilution method as specified in Standard Methods. Also, depth measurements were taken at both downstream creek locations in conjunction with sample collection during each event in order to assess tidal variability within both creeks; the flow direction was also taken.

As described in the QAPP, one water sample from each of the two downstream water sampling locations was collected during four sampling events. Additionally, as an extension of the sampling plan, one upstream water sample was also collected. All of these samples were sent to Dr. Troy Scott of Biological Consulting Services[1] for further molecular characterization using Host Specific PCR analyses testing the presence or absence of specific DNA sequences associated with the human or particular animal source of bacterial pollution. During three of the sampling events, a small subset of *Enterococci* isolated from sediment samples from both upstream locations was collected and sent to Dr. Scott where it was analyzed for sediment regrowth by ribotyping DNA fingerprinting.

VI. Analytical Methods

1. Interstate Environmental Commission Methods

Table 2 (below) outlines the procedures used for analyses by IEC. Temperature, salinity, turbidity, and pH are field measurements; microbiological and TSS samples were not analyzed in the field. The Project Action Levels for pathogens and pH were all taken from effluent limitations within the IEC District. Project Action Levels all are within laboratory reporting limits.

[1] BCS Laboratories, Inc.-Miami
4842 SW 74th Court
Miami, FL 33155
Telephone: (352) 317-5713, www.microbioservices.com

TABLE 3: IEC's Analytical Methods

Analytes	***Sample Matrix***	***Project Action Level***	***Analytical Method***	***Method Detection Limit***	***Laboratory Reporting Limit***
Fecal and Total Coliform	Non-Potable Water	Fecal Coliform 2400 /100 ml (single sample) Total Coliform 5000 / 100 ml (80 % of samples)	Standard Methods for the Examination of Water and Wastewater, 14th Edition: Method 9221 A, B, C & D	MPN ≤3 and ≥24,000 or MPN ≤30 and ≥240,000	MPN ≤3 and ≥24,000 or MPN ≤30 and ≥240,000
Enterococci	Non-Potable Water	35/ 100 ml (geo mean)	Standard Methods for the Examination of Water and Wastewater, 14th Edition: 9230 A & B	MPN ≤3 and ≥24,000 or MPN ≤30 and ≥240,000	MPN ≤3 and ≥24,000 or MPN ≤30 and ≥240,000
Temperature	Non-Potable Water	N/A	US EPA Method # : 150.1	-5 to 65 Degrees C	-5 to 65 Degrees C
Salinity	Non-Potable Water	N/A	US EPA Method # : 120.1	0 to 80 ppt	0 to 80 ppt
TSS	Non-Potable Water	N/A	Standard Methods for the Examination of Water and Wastewater, 20th Edition: 2540 D	.1mg/L-20,000mg/L	.1mg/L-20,000mg/L
PH	Non-Potable Water	<6.0 to >9.0 SU	US EPA Method # : 150.1	0 to 14 SU	0 to 14 SU
Turbidity	Non-Potable Water	N/A	US EPA Method # : 180.1 Revision 2.0	0.1 NTU-40 NTU	0.1 NTU-40 NTU

1. Biological Consulting Services Laboratories Methods

a) Host specific *Enterococcus* PCR analysis. For each sample, 100 ml of water was filtered through a 0.45-micron membrane filter. The filter was placed on m*Enterococcus* media supplemented with indoxyl substrate and the plate was incubated for 24 hours according to the protocol outlined in EPA Method 1600. Colonies exhibiting a blue halo were enumerated as *Enterococci*. Host specific PCR was carried out for respective targets using a modified version of the method described by Scott, T.M., et al., (2005, 2007). DNA extraction was prepared using the Qiagen DNA extraction kit, as per manufacturer's instructions. Five micro-liter aliquots of purified DNA extract were used directly as template for subsequent PCR reactions. Amplification of PCR primers were carried out using HotStarTaq polymerase (Qiagen, Inc.) and master mix, which contained a final concentration of 1.5 mM MgCl2, 150 mM dNTP, and 0.3 mM of each primer. An Eppendorf Gradient Thermocycler was used with the following cycling parameters: 95ºC for 15 minutes (to lyse cells and activate polymerase), followed by 35 cycles of 94ºC for 1 minute, 55ºC for 1 minute, and 72ºC for 1 minute and a final extension at 72ºC for 5 minutes. PCR products were electrophoresed on 2% agarose gels, stained with GelStar nucleic acid stain (Cambrex, Inc.) and visualized under UV light.

b) Specific Bacteroides spp. PCR analysis (used for dog analyses only) For each sample, 100 ml of water was filtered through a 0.45-micron membrane filter. DNA was directly extracted from the membrane using the Qiagen DNAextraction kit, as per manufacturer's instructions. Five microliter aliquots of purified DNA extraction product were used directly as template for subsequent PCR reactions. Amplification of Bacteroides target sequence was carried out using HotStarTaq polymerase (Qiagen, Inc.), specific primers, and reaction master mix. The Master mix contained a final concentration of 1.5 mM MgCl2, 150 mM dNTP, and 0.3 mM of each primer. An Eppendorf Gradient Thermocycler was used with the following cycling parameters: 95ºC for 15 minutes (to activate polymerase), followed by 35 cycles of 94ºC for 1 minute, 55ºC for 1 minute, and 72ºC for 1 minute, and a final extension at 72ºC for

5 minutes. PCR products were electrophoresed on 2% agarose gels, stained with GelStar nucleic acid stain (Cambrex, Inc.) and visualized under UV light.

c) DNA Fingerprinting. Ribotyping of *Enterococcus* isolates was accomplished by the method described in Scott, T.M., et al., (2004) and Scott, T.M., et al., (2003). The ribotyping was performed on a subset of three samples per event collected during three sampling events. Chromosomal DNA was extracted from *Enterococci* isolates and digested with Hind/III. Fragments were separated by agarose electrophoresis. The DNA was then transferred and fixed to a Zeta-probe membrane. A cDNA probe complementary to the *Enterococcus* 16S and 23S rDNA was labeled with digoxigenin-dUTP and was used to probe the membranes. The resulting genetic fingerprint was then analyzed using Bionumerics software and compared with fingerprints from other isolates for similarity to assess clonality.

VII Sampling Results

The detailed results from all five runs are included in the Appendix 2.

1. Exceedences of Single Sample Maximum Criteria

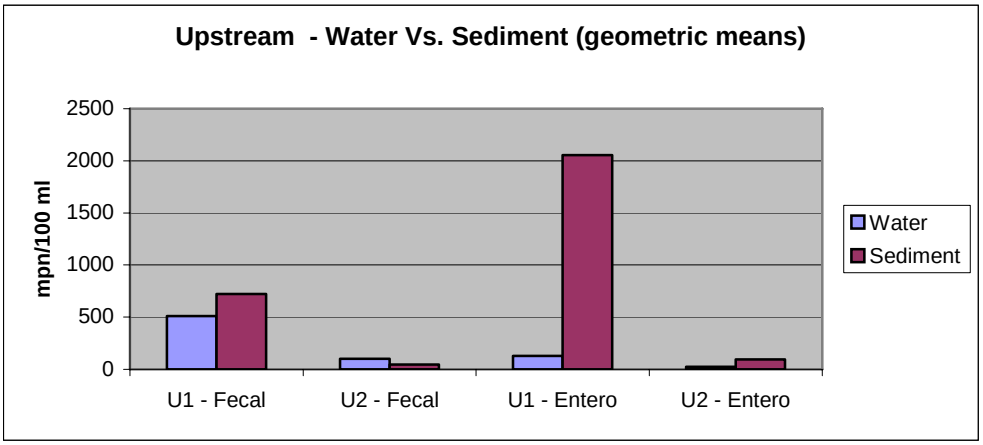
Of the 20 samples taken from the beach, a total of four samples exceeded the Connecticut Beach Closure Criteria single sample maximum of 104 MPN[2] per 100 ml[3]. Three of the four exceedences occurred on July 10, 2007. The other exceedence occurred on August 7, 2007, though in that case the geometric mean of the four beach samples collected that day was 14 MPN per 100 ml which is below the acceptable geometric mean limit 35 MPN per 100 ml[4].

[2] Most Probable Number, used for enumeration of target bacterial indicator.
[3] Connecticut Beach Closure Criteria for Single Sample Maximum requires resampling if *Enterococcus* >104 MPN/ml and if second result > 104 MPN/ 100 ml, then the beach is closed.
[4] Connecticut uses 35 MPN per 100 ml as the limitation that the geometric mean of five samples taken over a 30-day period should not be exceeded.

2. Water vs. Sediment Samples

When comparing the bacterial indicator results for the upstream (Station U1 and U2) water versus corresponding sediment samples, only the U1-*Enterococcus* results showed a great disparity between the two types of samples with the sediment sample being over one magnitude greater than the corresponding water results.

Figure 1.

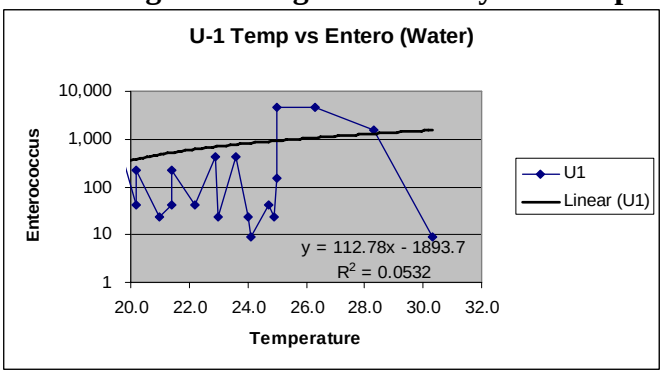


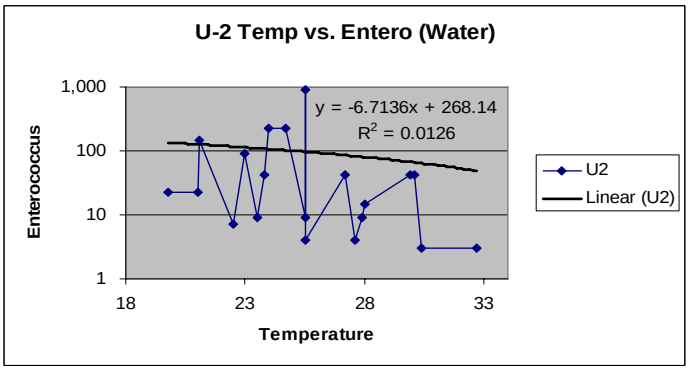
3. Temperature versus *Enterococcus*

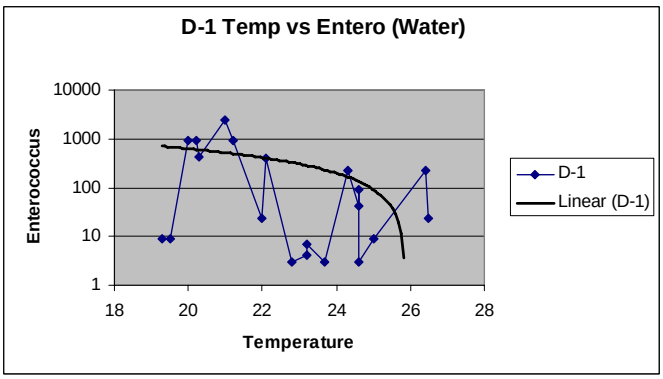
IEC conducted regression analyses (temperature versus *Enterococcus*) to determine a correlation between the two. Based on analyses for the seven sampling locations (twenty data points each), there was no significant correlation between temperature and *Enterococcus* at any of the seven locations (See Figure 2, below).

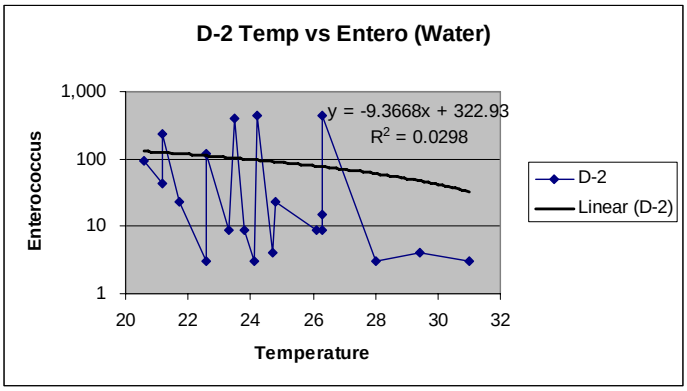
In addition to correlation analyses, daily plots of time vs. temperature and *Enterococcus* were examined (Appendix 3). Daily temperature fluctuations did not show any significant increase in *Enterococcus* results corresponding to an increase in temperature.

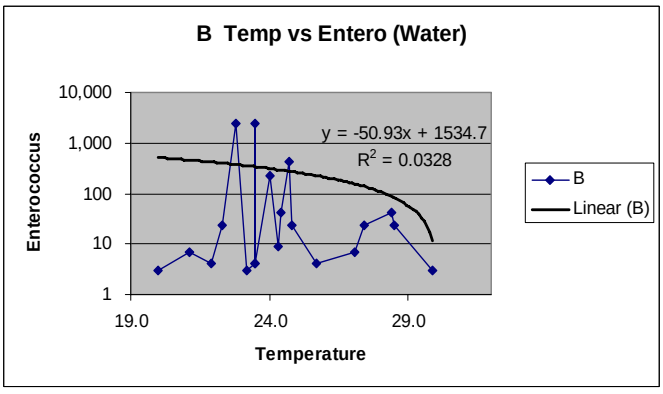
Figure 2 Regression Analyses: Temperature vs. Enterococcus

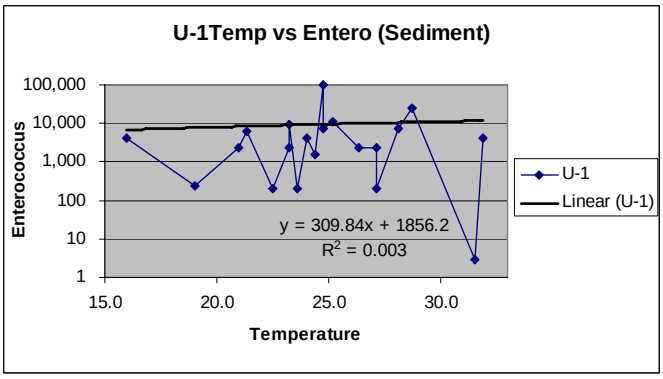


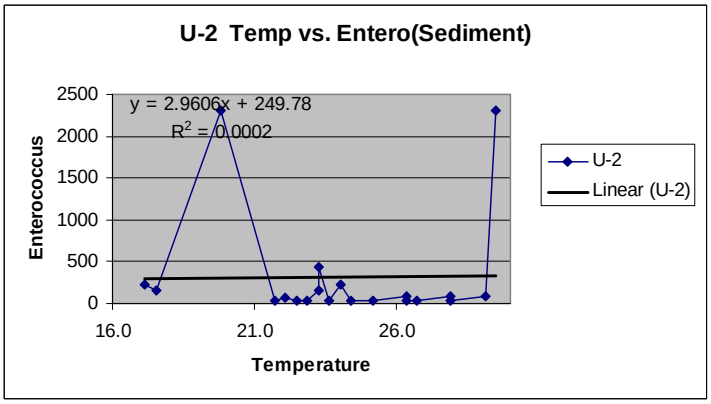








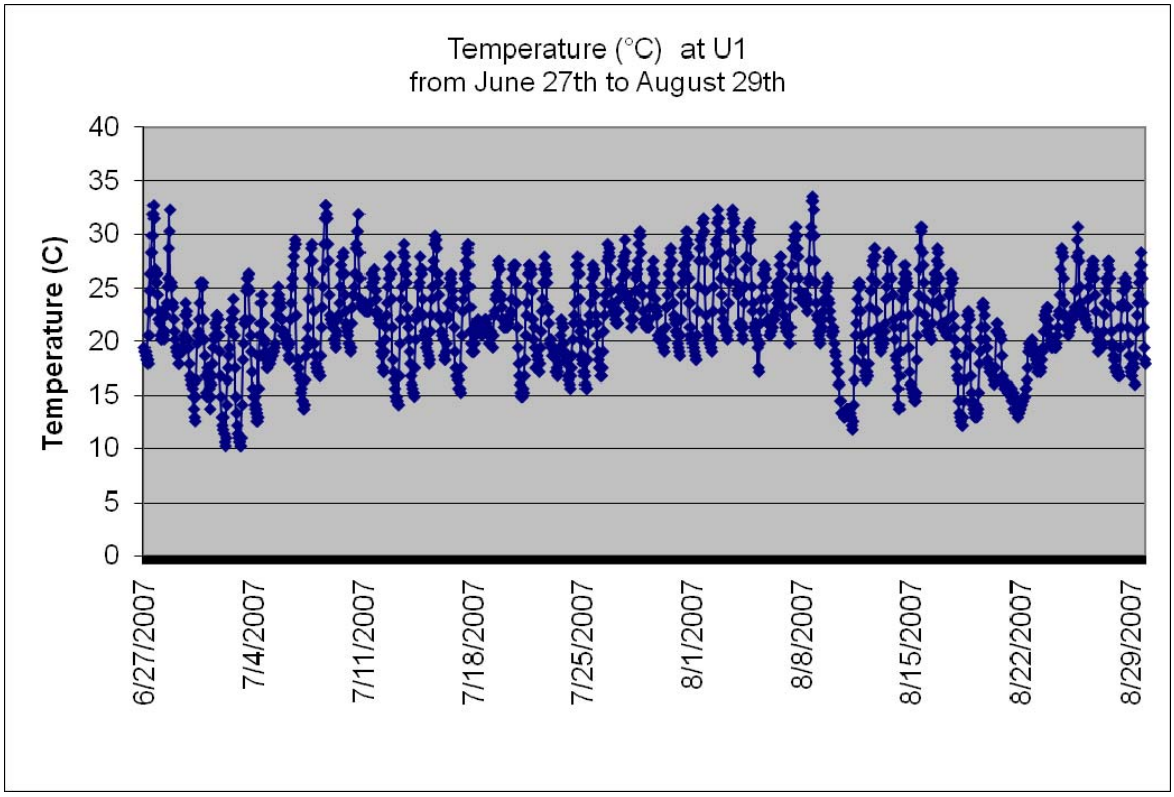


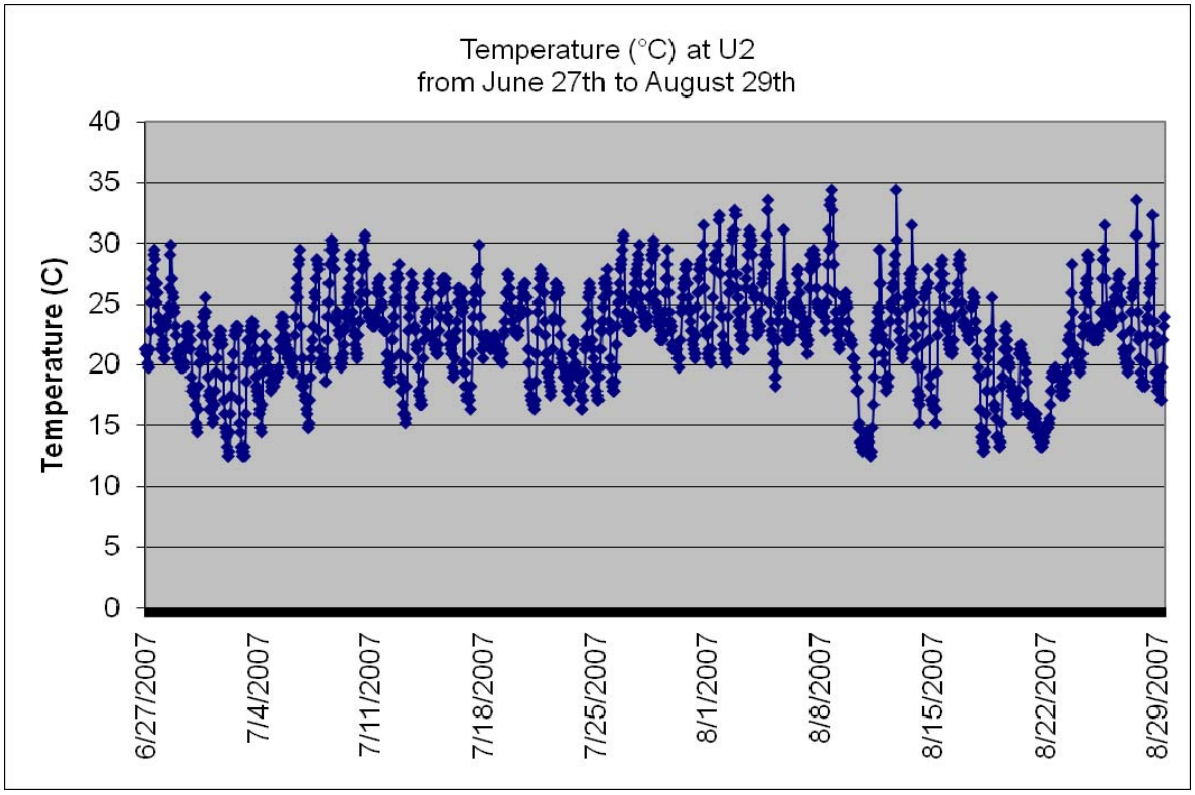


4. HOBO Continuous Temperature Data Recorders

IEC developed charts that showed the results from the two continuous temperature data recorders that were placed in the sediment at the two upstream locations. The examination of the data revealed that the temperature for the U1 sampling location ranged from 10.2º C to 33.6º C and the temperature for U2 sampling location ranged from 12.6º C to 33.6º C (See Figure 3).

Figure 3 – Temperature Logs from Two Upstream Locations





5. Bacterial Source Tracking

Host specific PCR.

a. Decription of the Approach. IEC sent samples to Biological Consulting Services Laboratories to analyze for the presence of DNA markers that specifically indicate the source of fecal pollution in a watershed. These methods use polymerase chain reaction (PCR) to identify DNA targets within the bacterial chromosome that have been shown to be indicative of specific sources of fecal pollution (i.e., human, bird, dog, deer). The DNA markers approach investigates the presence or absence of exact DNA sequences. These sequences are associated with the presence of markers for humans or a particular type of animal. For this project, BCS tested for the presence of birds, deer and dog as described in the approved workplan.

Overall, the DNA marker methods exhibit a high degree of sensitivity and specificity. Confidence in results can be increased if the markers are detected in multiple sample events and if backup tests are also positive. A positive result is considered to be as highly specific due to little or no cross-reactivity. Negative results should be confirmed due to particle and target distribution and low sample volumes.

The methods are highly specific for human fecal pollution. The Dog Bacteroides primer set has also shown specificity for all breeds of dogs tested, although many validation samples were collected from "dog parks" without knowing exact type of dog. The bird primer sets have been validated primarily on wading birds, shore birds, gulls and geese. Effectively, they are specific for flocking birds and would likely not detect an event from neighborhood sparrows or parakeets.

b) PCR Results. Results indicated that birds are significant contributors of bacterial pollution on-site. For two downstream sampling locations (D1 and D2), two of the four samples contained a DNA marker specific for *Enterococcus* originating from birds. In addition, one of the four sediment samples at U2S (sediment) had shown positive identification for *Enterococcus* from birds. The only water sample analyzed from U1 had also shown a positive identification for *Enterococcus* from birds. This finding is significant, as the DNA markers do not persist or reproduce in the environment and their presence indicates recent fecal pollution (explained further in DNA fingerprinting section, below). The fact that the marker was detected in the sediment suggests that at least some of the bacteria present there were deposited recently. One of the four water samples at D1 had shown positive identification for *Enterococcus* from humans. The presence of human fecal pollution is always a significant finding from both a public health and remediation standpoint. This result, however, was not confirmed in subsequent assays or by additional tests specific for human fecal pollution. Therefore, the result should be confirmed before significant human fecal pollution is suspected. All of the analyses or examinations for bacteroides from dog and *Enterococcus* from deer came up negative.

Table 4 - DNA Markers from Most Specific DNA Tests

Silver Sands Beach Study
DNA Markers from Most Specific DNA Tests

		Bird Enterococcus				Human Enterococcus				Dog Bacteroides				Deer Enterococcus			
Location	Type	27-Jun	10-Jul	14-Aug	29-Aug	27-Jun	10-Jul	14-Aug	29-Aug	27-Jun	10-Jul	14-Aug	29-Aug	27-Jun	10-Jul	14-Aug	29-Aug
U1	Sediment	ND	ND	ND		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
U2	Sediment	ND	**Yes**	ND		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
D1	Water	**Yes**	ND	ND	**Yes**	ND	ND	**Yes**	ND	ND	ND	ND	ND	ND	ND	ND	ND
D2	Water	ND	**Yes**	ND	**Yes**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
U1	Water				**Yes**												

Notes:
"Yes" indicates a presence of the indicated fecal pollution source was detected
"ND", not detected, indicates no presence of the indicated pollution source was detected
U1 and U2 - Upstream sampling locations of Creeks #1 and #2, respectively
D1 and D2 - Downstream sampling locations of Creeks #1 and #2, respectively
- indicates no sample was taken (samples were included in original sampling design, based on the funds available)

DNA Fingerprinting.

a) Description of Approach BCS Labs uses fingerprinting to examine the genetic relatedness of organisms isolated from a particular location. Because bacteria reproduce by binary fission, each progeny is genetically identical to the parent organism and, therefore, produces an identical DNA fingerprint. Some watersheds contain reservoirs of fecal indicator bacteria (i.e. *Enterococci*, E. coli) that regrow in the sediments. When these sediments are agitated (by wave action, rainfall, etc.) the organisms are resuspended into the water column and can lead to a false indication of recent fecal contamination. By fingerprinting the DNA of these suspended organisms, one can make presumptive determinations as to the nature of their presence (i.e. fecal source, environmental source). Highly clonal DNA would indicate regrowth, while highly heterogeneous fingerprints would initially indicate that the organisms are not genetically related. The latter result does not necessarily indicate the organisms are not regrowing nor does it indicate that they are not accumulating; however, it can be used as a tool to make decisions regarding future sampling and analyses. These tests reveal whether there is regrowth (identical DNA fingerprints) or new organisms being introduced to the area (different DNA fingerprints).

b) DNA Fingerprinting Results. DNA fingerprinting of *Enterococci* isolated from the sediment samples revealed a highly heterogeneous genetic population. The heterogeneous genetic population indicates that regrowth is not occurring. Specifically, a total of 45 sediment and 15 water column isolates were compared to each other and did not reveal any significant similarity in fingerprinting patterns. In addition, prior *Enterococcus* survival studies (Scott, T.M., et al., (2005)) revealed that *Enterococci* with host-specific markers typically do not stay in the environment in excess of three weeks. This suggests that most of the *Enterococcus* organisms originating from birds, which were identified in the creek sediments by host-specific PCR analyses, were deposited recently and have a true fecal link. These results indicate that the sediments might serve as a limited fecal indicator reservoir that could potentially have a deleterious impact on water quality.

VIII Conclusions and Recommendations

The study results show that temperature of both water and sediment has no significant impact on bacterial pollution. It also showed that the sediments in the upstream creek may potentially serve as a limited source of bacterial pollution. The results also indicate that bird pollution is a major contributor to the bacterial problems at the beach and on one occasion human pollution was highlighted as a source at one of the downstream locations (D1).

While the objectives of the study have been fully met and a fairly representative DNA-based set of analyses has been conducted for upstream sediment locations, we recommend having corresponding DNA-based water samples at upstream locations collected and analyzed in the future. Similarly, it would be beneficial to conduct a follow-up investigation to locate the specific source of human pollution discovered at the D1 downstream location.

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Appendix 1

Site Map including Sampling Locations

Site Map and Sampling Locations

Appendix 2

Sampling Results

Interstate Environmental Commission

Silver Sands State Park - Run #1

Date 27-Jun-07

IEC Investigation # 16639

Weather Hot Hazy 89º F

High Tide 9:54 am at Bridgeport, CT
Low Tide 3:49 pm at Bridgeport, CT

Sampling Crew Kristen Barashatzky
Evelyn Powers
Gillian Spencer
Kristen Titmas

Rain in previous 24 hours 0"
Rain in previous 48 hours 0"

Water

Location	Time	Temp	Fecal	Entero	Salinity	Velocity	Direction	pH	Depth	TSS
	DST	C	mpn/100 ml	mpn/100 ml	PPT	ft/s	In or Out	S.U.	cm	ppm
U1-1	8:00 am	21.4	930	43	25.0	0.1	In	7.52		19.5
U1-2	10:06 am	21.0	93	23	26.8	0.1	In	7.86		
U1-3	12:10 pm	24.9	430	23	26.3	0.07	Out	7.47		
U1-4	2:05 pm	30.3	230	9	25.2	0.6	Out	7.54		
U2-1	8:45 am	25.5	15	9	29.7	0		8.03		110.6
U2-2	10:35 am	27.9	9	9	29.6	0.05	In	8.23		
U2-3	12:50 pm	30.4	9	<3	29.5	0.01	Out	8.69		
U2-4	2:30 pm	32.7	<3	<3	28.0	0.03	Out	8.80		
D1-1	8:30 am	19.5	93	9	26.6	2.14	In	7.69	105	3.9
D1-2	10:20 am	19.3	230	9	26.8	0.45	In	7.72	110	
D1-3	12:25 pm	23.2	93	4	26.7	0.86	Out	8.02	70	
D1-4	2:16 pm	26.5	150	23	26.0	0.83	Out	7.47	53	
D2-1	9:25 am	26.1	43	9	26.4	0.13	In	8.00	19	12.8
D2-2	11:10 am	26.3	23	9	30.9	0.14	In	8.37	23	
D2-3	1:40 pm	29.4	23	4	26.2	0.95	Out	8.80	13.5	
D2-4	2:50 pm	31.0	23	<3	26.2	0.62	Out	8.40	16	
B-1	9:12 am	27.4	93	23	28.5			7.85		19.0
B-2	10:55 am	23.5	93	4	26.7			7.98		
B-3	1:35 pm	23.2	43	<3	26.6			8.14		
B-4	2:40 pm	28.4	23	43	23.6	0.01	Out	8.24		

Sediment

Location	Time	Temp	Fecal	Entero
	DST	C	mpn/100 ml	mpn/100 ml
U1S-1	8:00 am	24.8	2,300	100,000
U1S-2	10:06 am	28.1	4,300	7,500
U1S-3	12:10 pm	31.9	430	4300
U1S-4	2:05 pm	31.5	930	<3
U2S-1	8:45 am	22.9	<30	40
U2S-2	10:35 am	26.3	<30	90
U2S-3	12:50 pm	27.9	<30	90
U2S-4	2:30 pm	29.5	<30	2300

Interstate Environmental Commission
Silver Sands State Park - Run #2

Date 10-Jul-07

IEC Investigation # 16641

Weather Humid Hazy 82º F

High Tide 8:04 am at Bridgeport, CT
Low Tide 2:09 pm at Bridgeport, CT

Sampling Crew Kristen Barashatzky
Caitlyn Nichols
Evelyn Powers
Gillian Spencer

Rain in previous 24 hours 0"
Rain in previous 48 hours 0"

Water

Location	Time	Temp	Fecal	Entero	Salinity	Velocity	Direction	pH	Depth	TSS
	DST	C	mpn/100 ml	mpn/100 ml	PPT	ft/s	In or Out	S.U.	cm	ppm
U1-1	6:45 am	23.0	75	23	27.1	0.11	In	7.52	49	35.0
U1-2	8:25 am	22.2	430	43	27.2	0.31	Out	7.66		
U1-3	10:00 am	22.9	230	430	27.3	0.58	Out	7.50		
U1-4	11:30 am	26.3	930	4600	27.3	0.08	Out	7.23		
U2-1	7:30 am	24.0	230	230	27.9	0.01	In	7.10		75.6
U2-2	9:00 am	24.7	430	230	27.9	0.0		6.94		
U2-3	10:40 am	27.6	43	4	27.9			7.21		
U2-4	12:15 pm	28.0	230	15	27.9			7.43		
D1-1	7:00 am	22.0	9	23	26.5	1.9	In	7.65	109	5.9
D1-2	8:40 am	21.2	210	930	27.4	0.95	Out	7.68	127	
D1-3	10:20 am	22.1	430	390	27.3	1.6	Out	7.69	109	
D1-4	12:00 pm	24.3	430	230	27.2	1.15	Out	7.60	7	
D2-1	7:55 am	21.7	23	23	27.2	1.15	In	7.77	56	15.5
D2-2	9:20 am	22.6	23	<3	27.3	0.92	Out	7.73	41	
D2-3	11:10 am	24.7	93	4	27.0	1.57	Out	7.61	24	
D2-4	12:35 pm	26.3	150	15	27.3	1.28	Out	7.62	17	
B-1	7:40 am	22.8	930	2400	27.0			7.75		25.9
B-2	9:10 am	23.5	430	2400	27.1			7.60		
B-3	11:00 am	24.0	2400	230	27.4			7.79		
B-4	12:50 pm	28.5	15	23	27.0			7.84		

Sediment

Location	Time	Temp	Fecal	Entero
	DST	C	mpn/100 ml	mpn/100 ml
U1S-1	6:45 am	23.2	230	2,300
U1S-2	8:25 am	24.4	930	1,500
U1S-3	10:00 am	25.2	46,000	11,000
U1S-4	11:30 am	26.3	4,300	2,300
U2S-1	7:30 am	22.5	<30	<30
U2S-2	9:00 am	23.6	<30	<30
U2S-3	10:40 am	25.2	<30	<30
U2S-4	12:15 pm	26.7	40	<30

Interstate Environmental Commission

Silver Sands State Park - Run #3

Date 7-Aug-07

IEC Investigation # 16647

Weather Partly Cloudy 81º F

High Tide 6:46 am at Bridgeport, CT
Low Tide 12:52 pm at Bridgeport, CT

Sampling Crew Kristen Barashatzky
Caitlyn Nichols
Gillian Spencer

Rain in previous 24 hours 0.01"
Rain in previous 48 hours 0.07"

Water

Location	Time	Temp	Fecal	Entero	Salinity	Velocity	Direction	pH	Depth	TSS
	DST	C	mpn/100 ml	mpn/100 ml	PPT	ft/s	In or Out	S.U.	cm	ppm
U1-1	6:45 am	24.7	430	43	0*			7.53		26.4
U1-2	8:35 am	25.0	230	150	27.9	0.5	Out	7.55	55	
U1-3	10:00 am	25.0	11,000	4,600	27.8	0.21	Out	6.98	46	
U1-4	11:15 am	28.3	4,600	1,500	26.2	0.26	Out	7.11	17.5	
U2-1	7:40 am	25.5	230	930	0.1*			7.56		28.7
U2-2	9:40 am	27.2	93	43	16.4			7.33		
U2-3	10:25 am	30.1	150	43	0.7*			7.45		
U2-4	11:35 am	29.9	430	43	12.4			7.55		
D1-1	7:05 am	24.6	23	43	0.1*	2.08	In	7.79	124	12.6
D1-2	8:45 am	24.6	23	93	27.9	0.61	Out	7.66	109	
D1-3	10:15 am	25.0	43	9	28	1.73	Out	7.20	76	
D1-4	11:25 am	26.4	230	230	27.4	1.81	Out	7.16	46	
D2-1	7:50 am	24.2	43	430	12.4	0.71	Out	7.68	64	8.5
D2-2	9:55 am	24.8	43	23	27.9	1.79	Out	7.37	21	
D2-3	10:35 am	26.3	230	430	27.9	2.66	Out	7.51	17	
D2-4	11:40 am	28.0	43	<3	27.5	1.26	Out	7.07	18	
B-1	7:35 am	24.7	430	430	12.2			7.82		20.3
B-2	10:00 am	25.7	43	4	18.0			7.58		
B-3	10:50 am	27.1	15	7	27.6			7.91		
B-4	11:50 am	29.9	21	<3	27.7			8.13		

Sediment

Location	Time	Temp	Fecal	Entero
	DST	C	mpn/100 ml	mpn/100 ml
U1S-1	6:45 am	23.2	230	9,300
U1S-2	8:35 am	27.1	350	2,300
U1S-3	10:00 am	27.1	430	210
U1S-4	11:15 am	28.7	2,300	24,000
U2S-1	7:40 am	22.1	40	70
U2S-2	9:40 am	26.3	<30	<30
U2S-3	10:25 am	27.9	<30	<30
U2S-4	11:35 am	29.1	<30	90

* Meter error

Interstate Environmental Commission

Silver Sands State Park - Run #4

Date 14-Aug-07

IEC Investigation # 16649

Weather Sunny Breezy 68º F

High Tide 12:34 am at Bridgeport, CT
Low Tide 6:55 am at Bridgeport, CT

Sampling Crew Caitlyn Nichols
Evelyn Powers
Gillian Spencer

Rain in previous 24 hours 0"
Rain in previous 48 hours 0.02"

Water

Location	Time	Temp	Fecal	Entero	Salinity	Velocity	Direction	pH	Depth	TSS
	DST	C	mpn/100 ml	mpn/100 ml	PPT	ft/s	In or Out	S.U.	cm	ppm
U1-1	7:00 am	19.4	430	2,100	24.3			7.58	8	87.3
U1-2	8:30 am	21.4	430	230	27.9			7.41		
U1-3	10:00 am	20.2	930	43	25.7			8.04		
U1-4	11:20 am	24.0	43	23	28.0			7.99		
U2-1	7:35 am	19.8	39	23	28.4			7.33		35.9
U2-2	9:00 am	21.0	93	23	28.5			7.42		
U2-3	10:35 am	22.5	39	7	28.7			7.45		
U2-4	12:05 am	25.5	93	4	28.7			7.67		
D1-1	7:15 am	20.0	930	930	25.1	0.42	Out	7.31	18	19.0
D1-2	8:50 am	20.3	430	430	24.2	0.06	Out	7.35	11	
D1-3	10:15 am	22.8	43	3	28.1	3.01	In	7.96	66	
D1-4	11:42 am	24.6	9	<3	27.9	3.13	In	7.93	*	
D2-1	7:50 am	20.6	23	93	28.2	1.4	Out	7.31	16	8.9
D2-2	9:10 am	21.2	75	43	28.1	2.38	Out	7.26	18	
D2-3	11:00 am	23.3	93	9	28.2	0.13	In	7.22	31	
D2-4	12:20 am	24.1	7	<3	28.1	2.48	In	8.02	94	
B-1	8:00 am	20.0	15	<3	27.5			7.64		15.1
B-2	9:36 am	21.9	43	4	27.4			7.97		
B-3	11:10 am	24.4	930	43	27.8			8.04		
B-4	12:35 am	24.3	21	9	28.2			7.98		

Sediment

Location	Time	Temp	Fecal	Entero
	DST	C	mpn/100 ml	mpn/100 ml
U1S-1	7:00 am	19.0	230	230
U1S-2	8:30 am	21.3	90	6,400
U1S-3	10:00 am	24.0	930	4300
U1S-4	11:20 am	24.8	4,300	7,500
U2S-1	7:35 am	17.5	40	150
U2S-2	9:00 am	21.7	40	40
U2S-3	10:35 am	23.2	<30	150
U2S-4	12:05 am	24.4	40	<30

* 41 cm from top of tunnel to water - current was moving to fast to get measurement since ruler kept on bending.

Interstate Environmental Commission
Silver Sands State Park - Run #5

Date 29-Aug-07

IEC Investigation # 16650

Weather Sunny

High Tide 12:08 am at Bridgeport, CT
Low Tide 6:27 am at Bridgeport, CT

Sampling Crew Kristen Barlikas
Caitlyn Nichols
Gillian Spencer

Rain in previous 24 hours 0"
Rain in previous 48 hours 0"

Water

Location	Time	Temp	Fecal	Entero	Salinity	Velocity	Direction	pH	Depth	TSS
	DST	C	mpn/100 ml	mpn/100 ml	PPT	ft/s	In or Out	S.U.	cm	ppm
U1-1	6:00 am	19.0	4,600	230	22.6		In	7.25	5	101
U1-2	8:00 am	20.2	11,000	230	27.9		Out	7.31	6	
U1-3	9:45 am	23.6	1,500	430	26.5	0.26	In	7.63	17.5	
U1-4	11:00 am	24.1	9	9	27.9	0.17	In	7.79	65.25	
U2-1	7:20 am	21.1	430	150	20.3			8.08		59.8
U2-2	8:40 am	23.0	430	93	13.9			7.29		
U2-3	10:10 am	23.8	2,400	43	28.3			7.69		
U2-4	11:25 am	23.5	430	9	27.8			7.72		
D1-1	6:15 am	20.2	390	930	11.4	0.92	In	7.37	25	23.1
D1-2	8:20 am	21.0	2,400	2,400	23.8	0.22	In	7.07	19	
D1-3	10:00 am	23.2	4	7	27.9	2.4	In	7.60	64	
D1-4	11:15 am	23.7	43	<3	27.9	3.3	In	7.65	64.5	
D2-1	7:30 am	21.2	430	230	28.1		Out	6.97	11	13.5
D2-2	8:50 am	22.6	930	120	27.9	0.68	Out	7.19	16	
D2-3	10:15 am	23.5	230	390	28.1	0.09	In	7.01	31	
D2-4	11:30 am	23.8	43	9	28.0	2.93	In	7.83	67	
B-1	6:40 am	21.1	230	7	20.3			8.08		15.8
B-2	9:00 am	22.3	150	23	27.7			7.77		
B-3	10:25 am	23.5	75	4	27.9			7.85		
B-4	11:35 am	24.8	93	24	28.1			7.53		

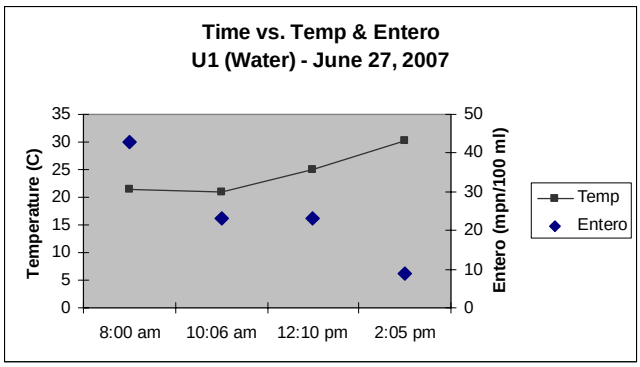
Sediment

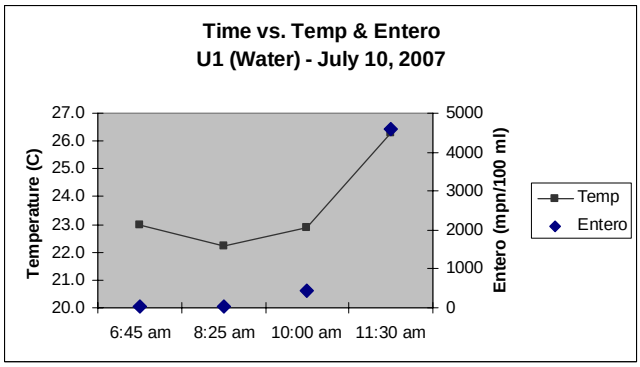
Location	Time	Temp	Fecal	Entero
	DST	C	mpn/100 ml	mpn/100 ml
U1S-1	6:00 am	16.0	430	4,300
U1S-2	8:00 am	21.0	230	2,300
U1S-3	9:45 am	22.5	90	210
U1S-4	11:00 am	23.6	150	200
U2S-1	7:20 am	17.1	230	230
U2S-2	8:40 am	19.8	40	2300
U2S-3	10:10 am	23.2	90	430
U2S-4	11:25 am	24.0	930	230

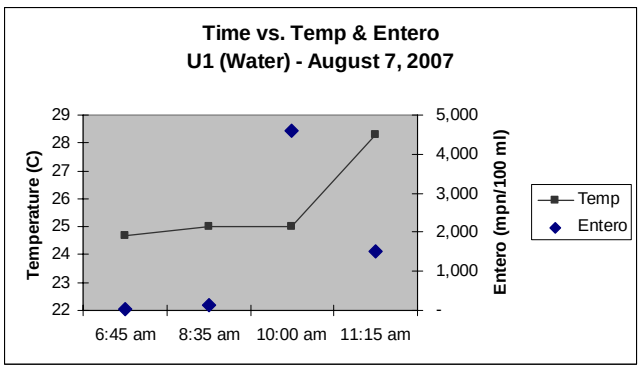
Appendix 3

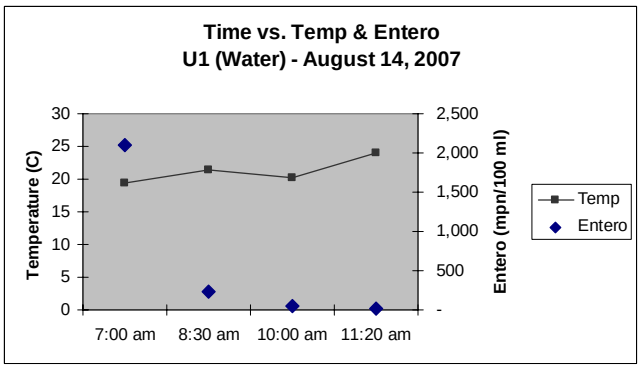
Time vs. Daily Temperature and *Enterococcus* Graphs

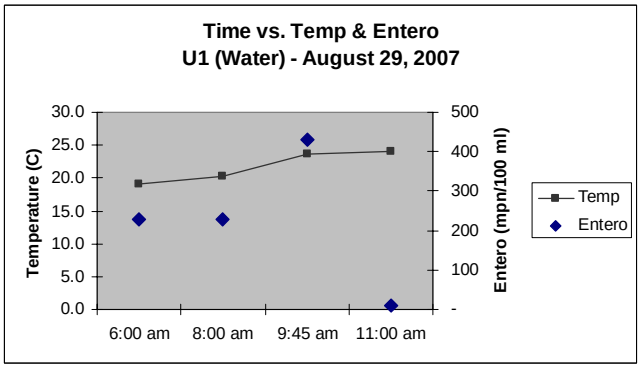
U1 - Water



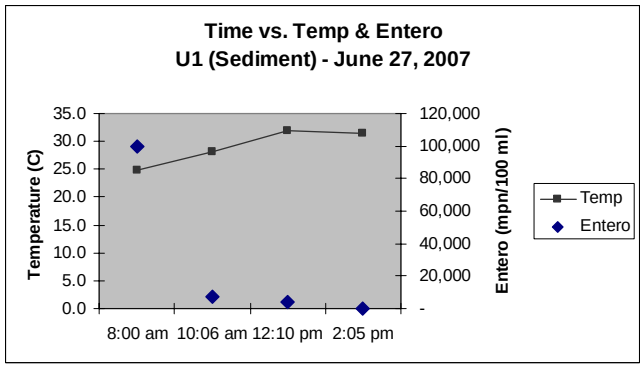


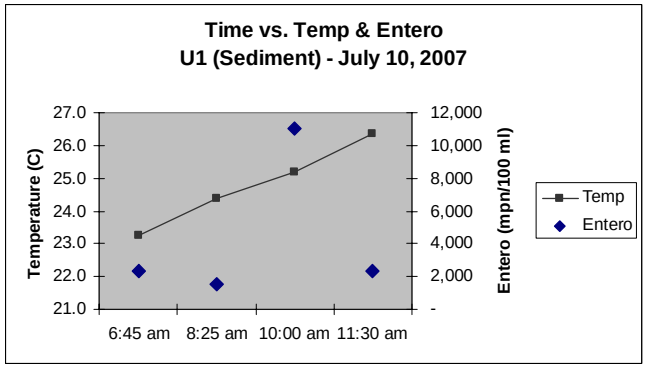


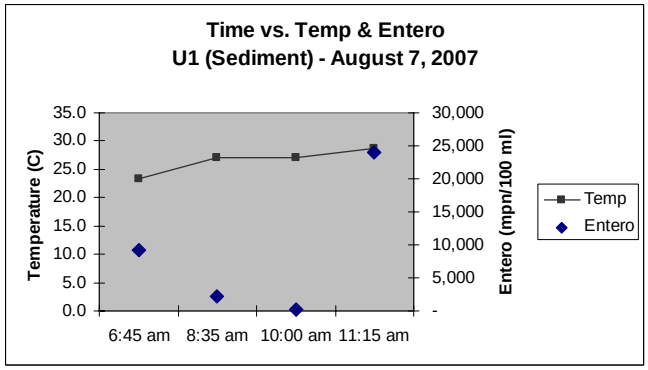


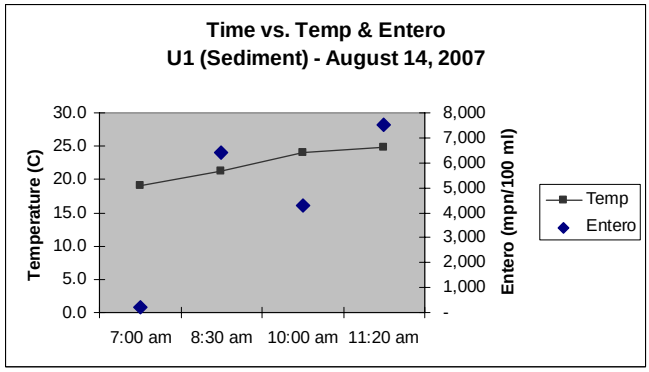


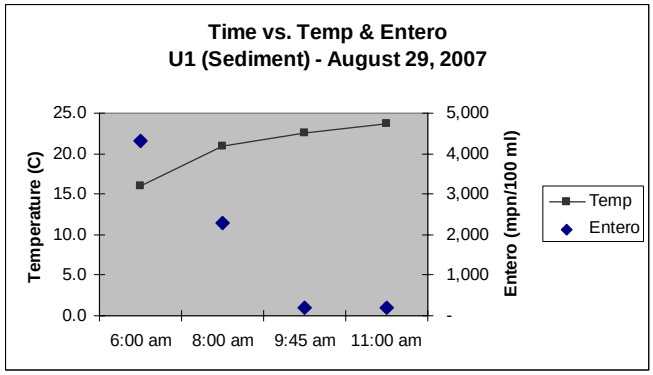
U1S - Sediment



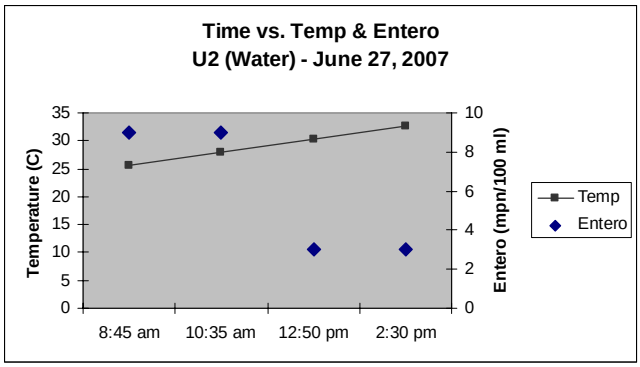


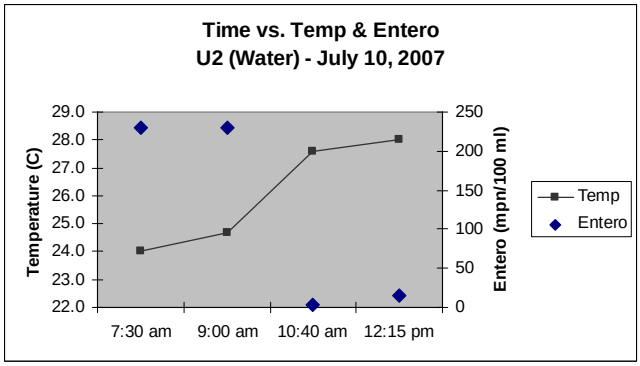


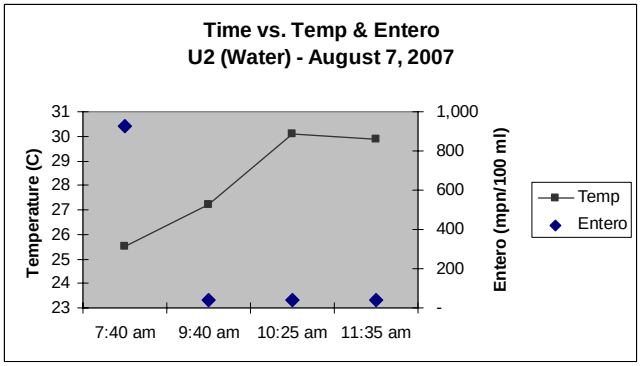


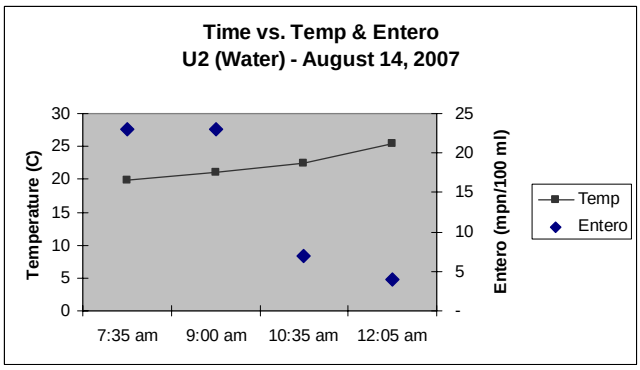


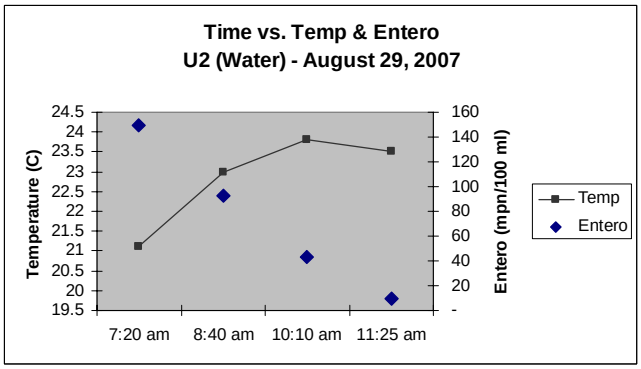
U2 - Water



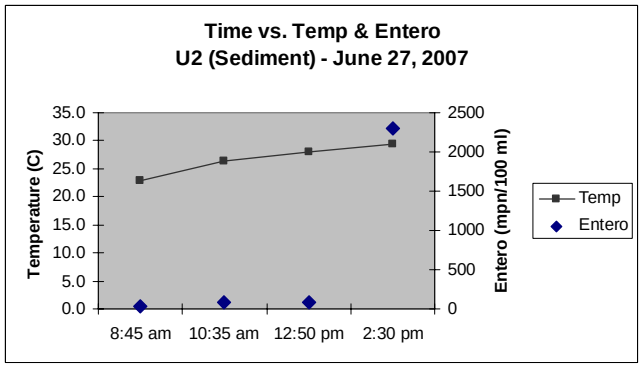


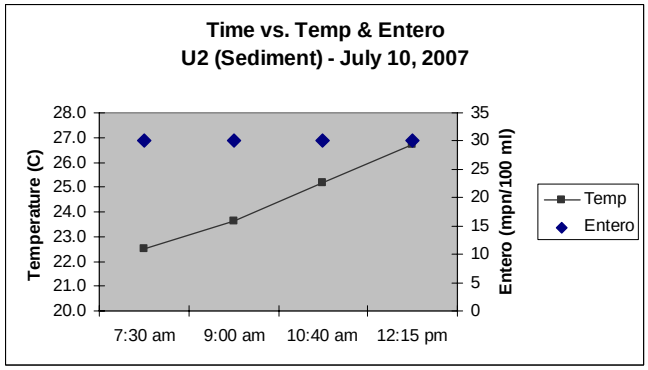


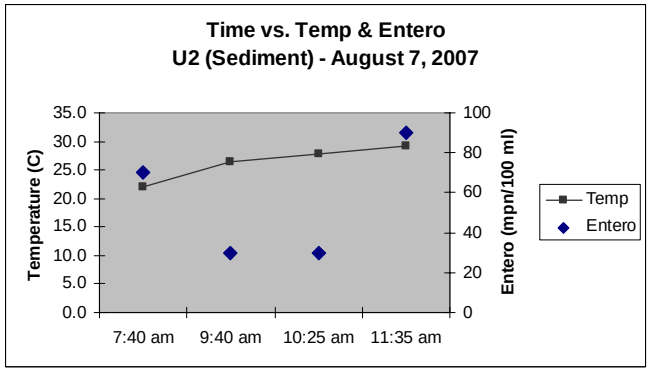


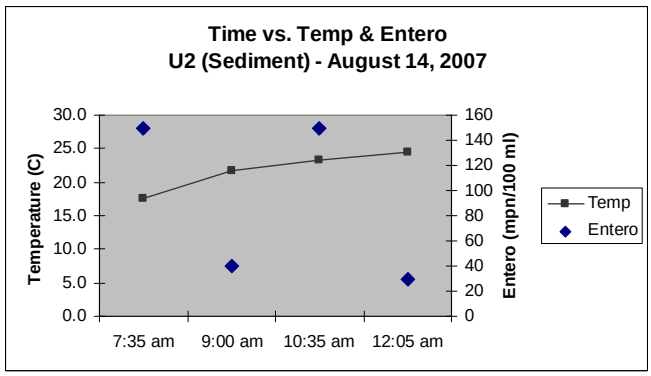


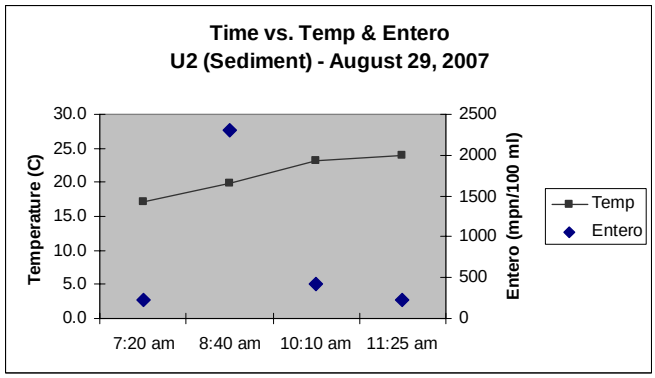
U2S - Sediment



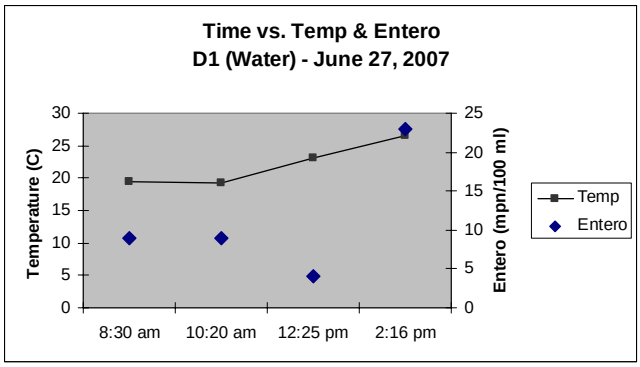


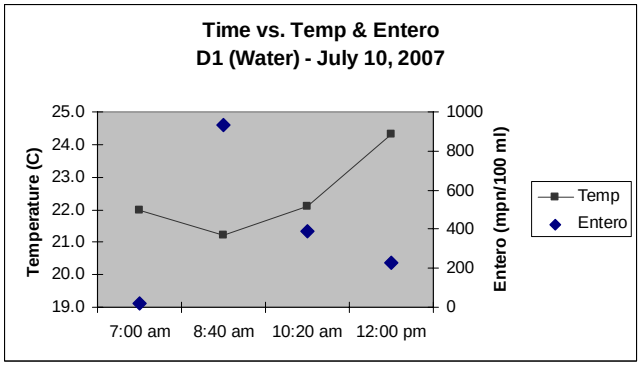


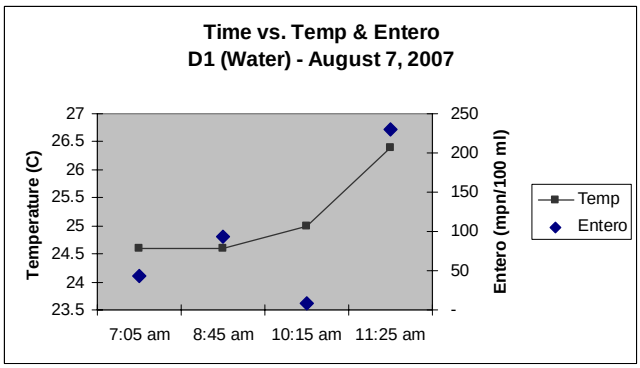


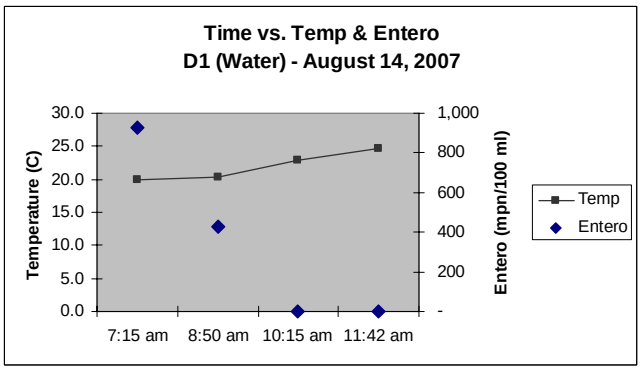


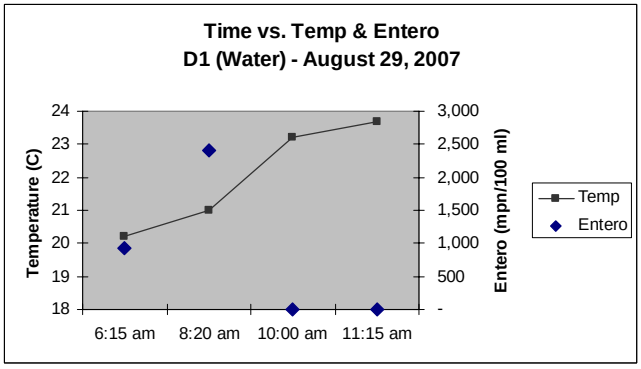
D1 - Water



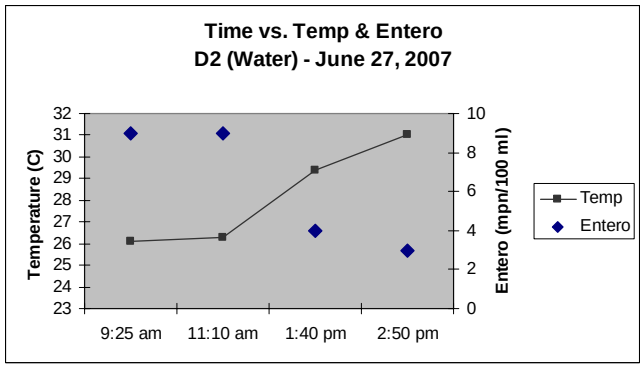


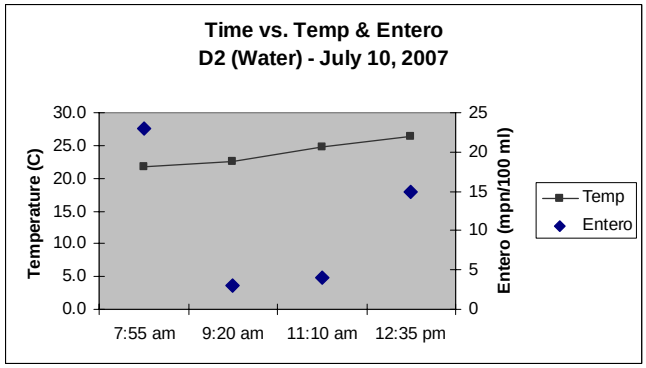


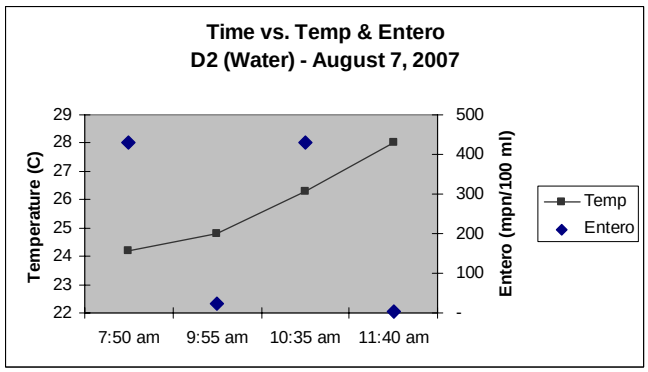


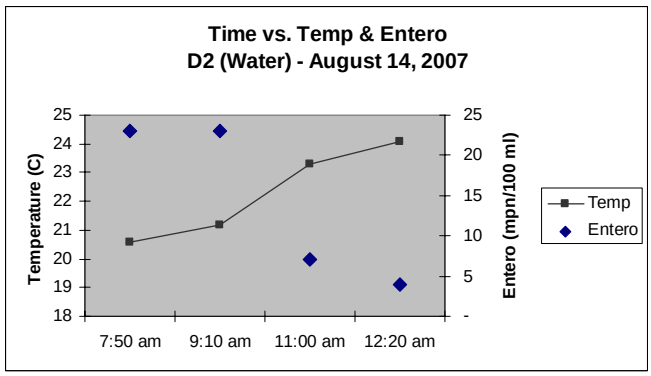


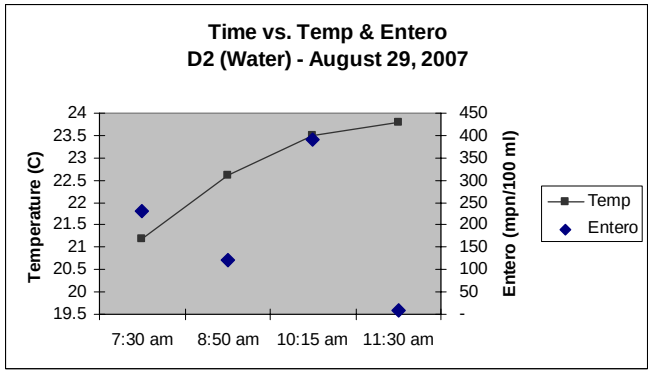
D2 - Water



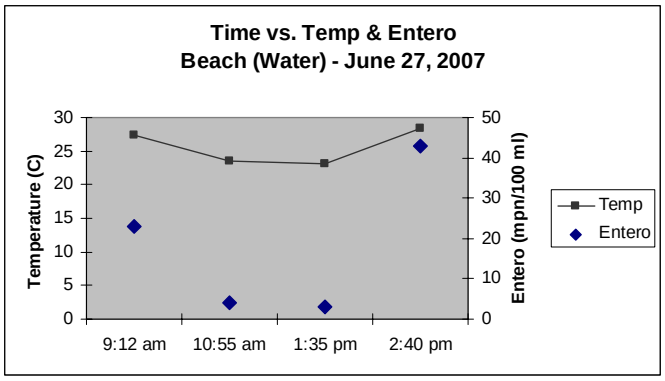


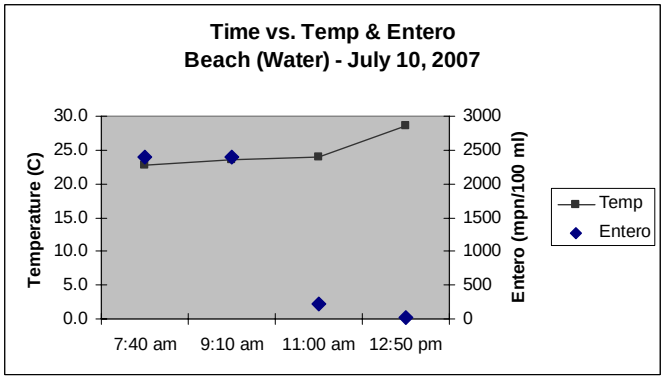


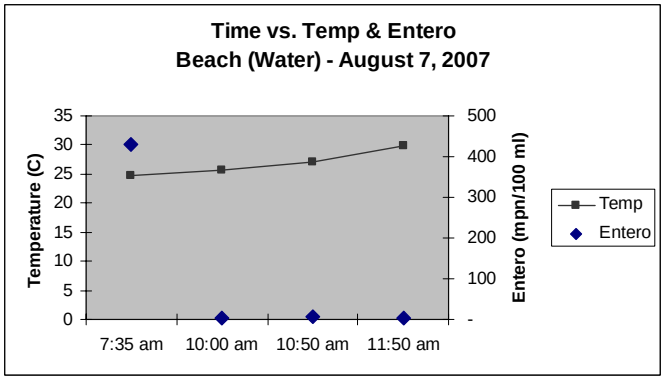


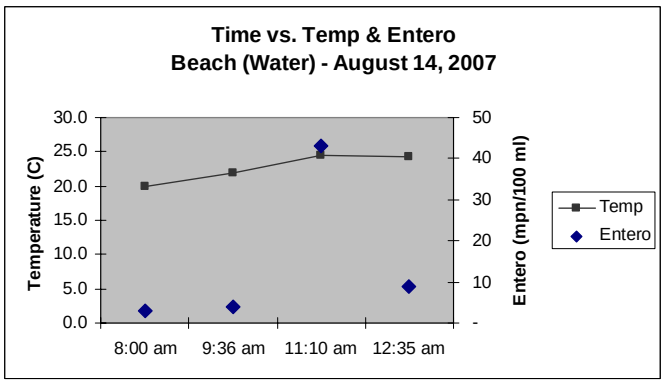


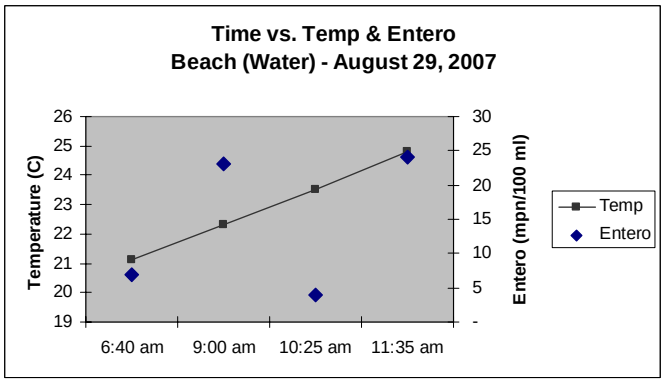
Beach - Water


Time vs. Temp & Entero
Beach (Water) - June 27, 2007
Temperature (C)
Entero (mpn/100 ml)
30
25
20
15
10
5
0
50
40
30
20
10
0
9:12 am
10:55 am
1:35 pm
2:40 pm
Temp
Entero


Time vs. Temp & Entero
Beach (Water) - July 10, 2007
Temperature (C)
Entero (mpn/100 ml)
30.0
25.0
20.0
15.0
10.0
5.0
0.0
3000
2500
2000
1500
1000
500
0
7:40 am
9:10 am
11:00 am
12:50 pm
Temp
Entero


Time vs. Temp & Entero
Beach (Water) - August 7, 2007
Temperature (C)
Entero (mpn/100 ml)
35
30
25
20
15
10
5
0
500
400
300
200
100
-
7:35 am
10:00 am
10:50 am
11:50 am
Temp
Entero


Time vs. Temp & Entero
Beach (Water) - August 14, 2007
Temperature (C)
Entero (mpn/100 ml)
30.0
25.0
20.0
15.0
10.0
5.0
0.0
50
40
30
20
10
-
8:00 am
9:36 am
11:10 am
12:35 am
Temp
Entero


Time vs. Temp & Entero
Beach (Water) - August 29, 2007
Temperature (C)
Entero (mpn/100 ml)
26
25
24
23
22
21
20
19
30
25
20
15
10
5
-
6:40 am
9:00 am
10:25 am
11:35 am
Temp
Entero