Part 1: Shoreline Surveys Using Standard Tests with an Innovative CEHA Laboratory.

Part 2: Microbial Source Tracking – Survival Assumptions Need Testing.





PCR amplification scheme. Many of the available RNase P RNA sequences are partial sequences obtained by PCR. The amplification primer sequences 59FBam and 347RRco (note that the latter is the complement of the sequence present in the RNA) are boxed, with anowheads indicating the polarity of the primers and lower-case nucleotides indicating linker sequences used for cloning. Sequences distal to the amplification primers are not available and are indicated by lines only.

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2 Illicit Connections









Sediment Vs. Water Column Samples

- 1. TMDL Site at Squankum (Rt. 195 & Rt. 524)
 - Fecal Coliform Counts

(Sampled 7/6/00)

	Water	Sediment	% Total Solids
1	210		
2		5,000	77.24%
3		2,300	80.39%
4		13,000	64.76%

Total **Phosphorus**

	(Sampled 9/21/00)				
	Water	Sediment	% Total Solids		
	(mg/L)	(mg/kg)			
1					
2	0.13	5318.00	22.4%		
3	0.30	928.00	70.0%		
4	0.18	2558.00	57.1%		

 Manasquan River at fishing access off Squankum – Yellowbrook Road (about 1 mile upstream of Site 1 – TMDL Site)

	(Sampled 7/6/00)				
	Water	Sediment	% Total Solids		
Central	210	8,000	80.27%		
East		23,000	72.30%		
West		5,000	79.86 %		

Fecal Coliform Counts

Total **Phosphorus** (Sampled 9/21/00)

Water	Sediment	% Total Solids
(mg/L)	(mg/kg)	
0.13	3573.00	65.1%
0.18	2438.00	42.0%
0.15	6677.00	34.8%

Percent total solids is the remainder of an aquatic sediment sample after the water has been evaporated. A sample that consists of silt and clay will have a lower percent total solids value than one consisting of sand and pebbles. Silt is preferentially eroded from streambanks. Fecal coliform adsorb and survive best on silt.

When stormwater runoff erodes streambanks, it also increases the numbers of fecal coliform in the sediment by providing greater amounts of silt in the streambed than would be present naturally. Coliform are resuspended into the water column during and after rainstorms.



Ramanessin Brook, Crawford Corners Rd., Holmdel: Spring 2003 RBA Downstream of PNC: NJIS 3 Holland Rd. Nature Trail: NJIS: 24







The map was developed in performing AUCEP digits data to compression with the MCHQs work, but the executivity product has not seen vertiled by the AUCEP and is not able autorate. Data security is includ by the excitivity of the acquires of the original data security.

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This map was developed in part, using NJDEP digital data, in conjunction with the MCHDs work, but this secondary product, has not been verified by the NJDEP and is not state authorized.

This map was prepared to recognize public and environmental health trends. Data accuracy is limited by the accuracy and scales of the original data sources

Site specific conditions should be field verified.

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EPA Method 1600 problems for Enterococcus

Aerococcus viridans growth at Ocean County beaches in 2004 (Feerst et al, 2002).

EPA: only count colonies >0.5 mm. Monmouth Park colonies less < 0.5 mm. confirmed for enterococcus during 96 hour test.

Staphylococcus growth in 2005 during dry weather with north or east winds, at 8:30 AM at sites near Sandy Hook and to a lesser extent other estuaries and lakes. The NJ Public Health Research Institute confirmed by PCR that the isolates are Staphylococcus but are not *S*. aureus. Likely S. haemolyticus or S. saprophyticus, in natural biofilms. For further discussion of Staph and marine growth see (Duan et al, 1995; Lee et al, 2003;)



Figure 5. E. faecalis ATCC29212



Figure 6. A. viridans ATCC



figure 12. Yellow staphylococcal growth on mEI agar, USEPA Method 1600



Figure 10. Samples A5AK0004 and A5AK0005 represent typical plates for Monmouth Park samples in October 2005. All colony diameters measured < 0.5 mm. Selected colonies were inoculated into confirmation media and mostly verified for enterococcus.

Fecal coliform and enterococcus fail as EPA "IDEAL SOURCE IDENTIFIERS:"

<u>Rate of Decay</u>: "...no growth under any conditions." <u>Abundance in primary vs. secondary habitat</u>: "...bears a significant resemblance to that found in contaminating fecal material." (EPA's "Microbial Source Tracking Guide Document" (June 2005))

Because coliform and enterococcus survive and grow in sediments, seaweed, etc, and because of the specificity problems with Method 1600, we need to pursue Microbial Source Tracking techniques but: PAST LESSON: FECAL COLIFORM/FECAL STREPTOCOCCUS RATIO

- "The failure of the fecal coliform/fecal streptococcus (FC/FS) ratio for fecal pollution source tracking is a lesson to heed in the current pursuit of microbial source tracking methods ...
- Initial assumptions about the comparable survival of coliforms and streptococci proved invalid ...
- The lesson identifies the importance of <u>testing survival assumptions for MST</u> Source Identifiers before methods are widely applied." (EPA, 2005).

The EPA states in "Microbial Source Tracking Guide Document" (June 2005):the horizontal transfer of resistance genes between bacteria in the environment is potentially confounding to source identification when there is "extensive regrowth of recipients in the environment;" then goes on to review studies that demonstrate how aquatic sediments can support significant bacterial survival and regrowth.

<u>HGT</u>

Shiga-toxin producing E. coli (O157:H7) is most famous example of HGT taking place in the cow gut. <u>Jack-in-</u> <u>the-Box Deaths.</u>

The bacteriophage genome (the prophage) survived within a host bacterium without lysing.

Remaining slides will focus on:

Mar vs. Bacteriophage as human indicators.

Using PCR to confirm Method 1600 results and the implications of using PCR.



E. coli (O157:H7) adhering to intestinal cells



MAR Testing



MAR ASSUMPTIONS IGNORE THE POTENTIAL FOR HGT IN SURVIVOR CLONES

Assumes that the primary environment of the bowel is equivalent to secondary environments like water or sediment.

EPA is funding "library" studies for "human indicators", but what about testing what happens when to the bacterial species that best adapt to the secondary environment. What species survive and why? What happens to their pathogenicity? Do the environmental clones have a different resistance pattern than the original clones in the feces?

DNA Persists in the Environment

DNA is protected from degradation by adsorbing to detritus, humic acid, and in particular, clay and sand particles. **Half lives** in freshwater and marine water are 3 to 5 hours, with high values of 45 to 83 hours on the ocean surface, and extremely high values of 140 and 235 hours for the **marine sediment (10 days)** (HO, 1998).

POLLUTANTS FOUND IN SEDIMENTS PROMOTE HGT

Mutants of E. coli that selected for resistance to **pine oil** also showed resistance to multiple antibiotics (tetracycline, ampicillin, chloramphenicol, and nalidixic acid) (Moken et al , 1997).

In routine laboratory procedures for genetic transformation, **heavy metals** ... are used to greatly increase the competence of cells for transformation (HO, 1998). Antibiotic resistant genes, ... have **heavy metal** resistance, such as mercury and antiseptics like **ammonia** compounds (White, 2000).

Elevated temperature **PAHs**, **PCBs and pesticides** cause prophage induction in natural populations suggests that such processes could in part be causing the elevated phage abundances seen in eutrophic estuaries, particularly in the summer months (Paul et al, 1999)

ANTIBIOTICS THEMSELVES AS HGT PROMOTERS – Are AR bacteria more efficient survivors?

The antibiotic **tetracycline** acted as an 'aphrodisiac' for a number of bacteria, enhancing transfer frequencies up to 100-fold" in the gut (Steinbrecher et al, 2003)

Resistance plasmids encoding for many antibiotic resistance genes were transferred between pathogenic and non pathogenic Gram negative bacteria in several environments, including sea water. In the presence of tetracycline concentrations that were not high enough to kill the bacteria, the rate of gene transfer between Vibrio cholerae and Aeromonas salmonicida increased 100 times (Moriarity, 1999)

BACTERIOPHAGES AS PROMOTERS (TRANSDUCTION) in the presence of Turbidity

Turbidity: transduction frequencies were found to be enhanced as much as 100-fold in the presence of particulates (Ripp et al, 1995)

A marine phage host isolate is capable of transferring an antibiotic resistant plasmid among bacterial hosts ... **up to 13 trillion transduction events per year** could occur in the Tampa Bay estuary ... the presence of suspended **particulates** in the water column facilitates transduction by bringing the host and phage into close contact with each other (Jiang et al, 1998)

Bacteriophages themselves evolve by horizontal gene transfer and recombination; as many as 108 bacteriophages per ml. in aquatic environments, so that **one third of the total bacterial populations is subjected to a phage attack every 24 hours** (Ho, 1998)

BIRDS FECES LEADING TO MISCLASSIFICATION

Canada geese using manure lagoons at farms shed farm-animal antibiotic resistant bacteria (Cole et al, 2005).

ANIMAL ANTIBIOTIC GIVES FALSE POSITIVE FOR HUMAN ANTIBIOTIC

A antimicrobial drug used as an additive in animal feed in Europe (avoparcin) caused the poultry to have, and is associated with the human prevalence of, vancomycin resistant enterococci. So a residual of an animal antibiotic (that the people ate when they ate the animal meat) gave a positive test for a human antibiotic in feces. **Can other animal antibiotics produce 'false' positives as a human antibiotic after ingestion, or after release to the environment?** (McDonald et al, 1997)

These examples may account for why MAR results can exceed 100% after the different source contributions are totalled.



MALE SPECIFIC F+RNA Bacteriophage

Four types, Type 2 most is consistent in the literature for humans and pigs.

More human strains are found in domestic sewage than in human feces (Scott et al, 2002).

There is limited but general agreement that the F+RNA coliphage (unlike the somatic coliphage) does not reproduce in the environment because the pilus that serves as the site of attachment for virus most efficiently forms at 81 degrees F (25 C) or above (Scott et all, 2002; Woody, 1995).



Sewage can exceed 81 degrees; shallow aquifers could reach 81 degrees or higher, but phage do not reach log phase of growth (Woody, 1995).

Furase found that Group 2 survive preferentially at lower temperatures as compared with other groups (Cole et al, 2003).

The only lab doing it in NJ is the DEP's because it is a difficult technique.

PCR_RNA



Polymerase Chain Reaction: not as "human indicator" but to confirm species of interest.

RNA vs. DNA – study of bacterial mass

"freshwater and marine bacterioplankton assemblages are often numerically dominated by cells that are inactive or dormant, and that active cells usually constitute only a small portion of the bacterial community." (due to grazing or infected by viruses) (del Giorgio et al, 1995). See also Haglund et al, 2002.

<u>First study has evaluated the ability of PCR analysis of enterococcus</u> to predict GI illness in swimmers – but: further studies needed because this tested DNA and noted that since viable organisms were not necessary, no die-off caused by UV sunlight was observed in the afternoon, but was observed in the culture based enterococcus (Wade, 2006).

Which of 19 entero species to analyse: Faecium and Faecalis cause most human disease

Once survivor species are identified, then can do pathogenicity studies on clones:

Strains of E.coli better adapted to external environment than GI habitat (Gordon, 2002); greater virulence may result in a decreased ability to thrive in secondary environments (decreased conservation of energy) (Mouslim et al, 2002).

The simplicity of PCR will make it grassroots driven unless government takes the lead.

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