Puget Sound Notes

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inside:

Ecology proposes changes to the state's bacterial standards

Fecal waste from animals and humans contains a wide range of pathogens that can make people sick. These pathogens include many species of bacteria, viruses and protozoa. Swimming, wading, fishing, boating and working in and around the water exposes people to pathogens that can cause illness.

It is not technically possible nor economically feasible to test for all of the pathogens present in our waters. Instead, we test for **indicator bacteria** (specific bacteria types that are excellent predictors of the safety of water for human contact.) The ability of these indicator bacteria to predict illness has been found by examining human exposure to a variety of water types around the world.

Setting standards for the bacterial quality of the water can reduce the chances of people getting sick. Washington State's criteria for bacterial pollutants is based on the use of fecal coliform as an indicator of contamination by humans and other warm-blooded animals. The current standards establish three levels of fecal coliform criteria (50, 100, 200 colonies per 100 milliliters of water) in both fresh and marine waters.

In response to urging by environmental groups, industries and the U.S. Environmental Protection Agency, the Washington State Department of Ecology conducted a technical evaluation on the current use of fecal coliform as a general indicator of pathogens. As a result, Ecology is proposing to revise the state's surface water quality standards by recommending the use of enterococci as the indicator bacteria. Its level of survival is similar to viral and protozoan pathogens and is considered to be a superior indicator for ensuring that waters do not contain unhealthy levels of bacterial pathogens, and thus will better protect people. *Enterococci* is also being recommended because it is the only indicator bacteria approved for use in both fresh and marine water by the EPA. By selecting *Enterococci* for our state criteria, we avoid requiring multiple indicators for rivers that drain into marine waters.

Ecology is proposing a criterion of 33/100 ml for all fresh waters and a criterion of 35/100 ml to protect water contact activities (swimming, boating) in marine waters. This standard is equal to the EPA recommended levels. Currently, marine waters have a higher standard of protection with 14 fecal coliform colonies/100 ml of water. This is to ensure that people who eat shellfish are protected from unreasonable health risks. Ecology plans to continue using fecal coliform at 14/100 ml for shellfish, since it is tied to other state and national shellfish protection programs.

Ecology project characterizing wastewater influent/effluent using the new bacteriological indicators:

Municipal sewage treatment plants generally use either chlorination or ultraviolet light to disinfect their wastewater prior to discharge. The effectiveness of these treatment methods in killing bacteria is uncertain in different situations (for example, with different levels of suspended sediment). Data show that the same treatment method may not be equally effective in treating different bacteria (fecal coliform, *E. coli,* and *Enterococcus*). *Enterococcus* appears to be one of the hardier bacteria and thus more resistant to treatment. Ecology would like to get more information on treatment effectiveness, appropriate sampling frequency and verification steps.

To get more information about effectiveness, frequency and cost of municipal sewage treatment, Ecology is beginning a project to collect data from several different municipal sewage treatment plants that use different treatment methods or have different conditions. They will collect both influent and effluent samples over the course of several weeks to months from at least six treatment plants. Each sample will be analyzed for all three indicators. Ecology will pay all of the sampling and analytical costs associated with this project, which are estimated to be about $20,000. Partnerships with some municipalities may allow the number of facilities to be increased.

The results will help Ecology determine the most appropriate bacterial indicator for the water quality standards, appropriate treatment design, and to estimate the additional costs, if any, associated with a change from fecal coliform to another bacterial indicator.

For additional information please contact Mark Hicks at Ecology (360) 407-6477.

A Case Study in Tillamook Bay, Oregon

Fecal source identification using antibiotic resistance analysis

Introduction by **Stuart Glasoe,**
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Tillamook Bay Studies by **Robert J. Bower,**
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Finding and controlling bacterial pollution is a challenging, sometimes maddening, task. The pollution sources are usually varied, numerous and widespread. The impacts to shellfish beds and other beneficial uses of water are measurable and often significant, yet are hard to link with known or suspected sources. And the work required to find and fix the sources is generally costly, intensive and unending. One author has wryly described this challenging task as follows (Center for Watershed Protection 1999):

- Bacteria happen.
- The usual suspects are rounded up.
- Examples are made of a few.
- Most get off scot-free.
- Bacteria still happen.
- The public is asked to avoid the area.
- Victory is declared.

While there is some truth in this lighthearted view, it's important to point out that professionals across the country are making progress in dealing with the complicated issues associated with bacterial contamination. This article discusses recent research in the field of bacterial source tracking and a particular methodology known as antibiotic resistance analysis (ARA). Collectively, these methods hold great promise for achieving meaningful and lasting results—not just hollow victories—in the battle to protect public health and water resources.

Tools for Fecal Source Identification

There is wide agreement that pollution prevention is the key to protecting water quality. But there is also consensus that resource managers need better tools and techniques to more quickly, reliably and affordably address existing and emerging problems associated with bacterial contamination. Traditional water quality monitoring techniques have long been used to measure ambient pollution levels, but have limited utility in distinguishing between different sources of pollution.

To aid in this effort, a number of methods are being explored to more accurately identify and trace sources of fecal contamination. Hagedorn (undated) has organized these methods into three basic groups: molecular, biochemical and chemical. The molecular methods are referred to as "DNA fingerprinting" and are genotypic in their approach, meaning they examine the unique genetic makeup of different strains or subspecies of bacteria. The biochemical methods are phenotypic in that they assess the unique characteristics and behaviors of different bacteria. And the chemical methods are designed to find chemical compounds closely associated with the bacteria. The most notable methods are outlined in Table 1, page 3 (Hagedorn undated, Sargeant 1999).

Despite continuing research and increasing use, no studies have yet been conducted comparing the different methods using a common set of bacterial isolates. Presently there is no indication that any one method will emerge as the best approach. Instead, the toolbox approach will likely remain intact as existing methods are refined and new ones are created (Hagedorn undated).

Several studies have been carried out in recent years using ARA, a relatively new technique in the environmental health field but with established roots as a routine, diagnostic procedure in the medical profession. In summary, this technique takes bacterial isolates (generally *Streptococci* or *Escherichia coli*) from known human and animal sources and analyzes their resistance to various types of antibiotics. Methods of statistical analysis are used to establish patterns of resistance for each of the sources, which are then used to identify unknown bacterial isolates taken from water samples in the natural environment.

As with other source identification methods, study design is crucial. Here are several important considerations in carrying out ARA:

- The number of source samples must be sufficiently large to accurately represent the fecal sources in the study area and to produce statistically reliable patterns of resistance.
- The number of drugs used must be adequate to, once again, yield reliable patterns of resistance. Between nine and 13 drugs were used in three recent studies (Hagedorn, et al. 1999; Wiggins, et al. 1999; Moore and Bower 2000).
- The selected drugs must be tailored to the human and animal populations in the study area.
- Generally, the more drugs used in the analysis, the higher the average rate of correct classification (ARCC), due in part to the fact that some drug combinations will produce better classifications of bacterial isolates than others.
- Study design and results are shaped fundamentally by the questions you choose to answer. For example, do you want to identify and classify all contributing sources, or simply differentiate between human and animal sources? In one study, the ARCC increased from 74 percent based on the

classification of six sources (human, beef, dairy, chicken, turkey, wildlife), to 84 percent when isolates were pooled into four categories (human, cattle, poultry, wildlife), and ultimately to 95 percent when isolates were pooled into two categories (human and animal) (Wiggins 1996).

In short, technical specialists need to ensure that the integrity of any analysis is not compromised by poor design or the pursuit of quick, easy answers.

The Tillamook Bay Studies

Purpose, Methods and Background

The overall purpose of this study was to characterize the major sources of fecal *Streptococci* contamination in the Tillamook Bay watershed in western Oregon by statistically analyzing antibiotic resistance patterns. The first step in the implementation of this technique is to identify the major potential fecal pollution contributors in the area of interest. In the Tillamook Bay watershed the primary sources are dairy cattle and humans. It was also deemed necessary to account for the potential contributions of wild animals (elk, beaver, birds, etc.) to establish the background of fecal pollution in the watershed. Wild animal samples were analyzed as a potential third source of fecal pollution.

The next step is to establish the antibiotic profile or fingerprint for each of these fecal sources (human, dairy, wild) to be used in comparison to the unknown river samples. Human samples were collected from wastewater treatment plants in Tillamook and Garibaldi, Oregon. Samples were collected from dairy cattle at several farms

Table 1. Methods for Identifying and Tracking Sources of Fecal Contamination

Molecular Methods *(genotypic analysis of bacteria)*	**Basis**
Ribotyping	ribosomal RNA are isolated to create distinctive bands or fingerprints for different sources
Pulse Field Gel Electrophoresis	distinguishes bacterial DNA using low-voltage, oscillating electrical current to separate bands
Randomly Amplified Polymorphic DNA	identifies unique polymorphisms within the DNA of fecal bacteria
Biochemical Methods *(phenotypic analysis of bacteria and other host organisms)*	
Antibiotic Resistance Analysis	bacteria from different hosts have unique patterns of resistance to various antibiotics
Bacteriophage/Coliphage Indicators	different phages (bacterial viruses) are characteristic of different hosts and pollution sources
Sterol Analysis	humans and other organisms have distinctive types and quantities of sterols (fatty acid constituents found in cell walls & membranes)
Fecal Bacteria Ratios	humans and animals carry unique ratios of different types of stomach and intestinal bacteria
Streptococcal Population Profiles	compositions of fecal *Streptococcus* group species differ among animals
Species-Specific Indicators	some bacterial strains (e.g., *Streptococcus bovis*) are associated with humans or certain animals
Bacterial Nutrition Patterns	bacteria have different nutrient requirements and use carbon and nitrogen differently for energy and growth
Chemical Methods *(Unique chemical tracers of human sources of pollution)*	
Optical Brighteners	brighteners in laundry detergents are persistent in the environment and are associated with human sources
Caffeine Detection	caffeine passes through the digestive system and is largely characteristic of human sources
Fluorescent Dye Tracing	dye introduced to sewage system and detected in the environment establishes pathway
Other Methods	
Site-Based Water Quality Monitoring	targeted/segmented monitoring identifies pollution areas and sources

in the Tillamook watershed. Wild fecal samples were collected from water in the forest-agriculture interface where upstream influences of both dairy cattle and human contamination were not expected to be present.

Source samples were processed by isolating and antibiotically screening fecal *Streptococci* bacteria. Antibiotic screening is a process that records the ability of isolated bacteria to grow on a culture inoculated with selected antibiotics. Nine discriminant antibiotics were selected because of their wide clinical use for bacterial infection control in animal and human populations. A profile of each isolate's response to the battery of antibiotics is established. The resistance profiles produced by each source group of isolates (wild, human, dairy) creates the group profile against which unknown samples will be compared.

Once the profiles or fingerprints for each source (wild, human, dairy) were established, river water samples were collected during a winter storm event, as well as seasonal water samples for five rivers, over a one-year period. These samples contained fecal organisms of unknown origin. They were antibiotically screened in the same manner as the major source groups from which the group profiles were created.

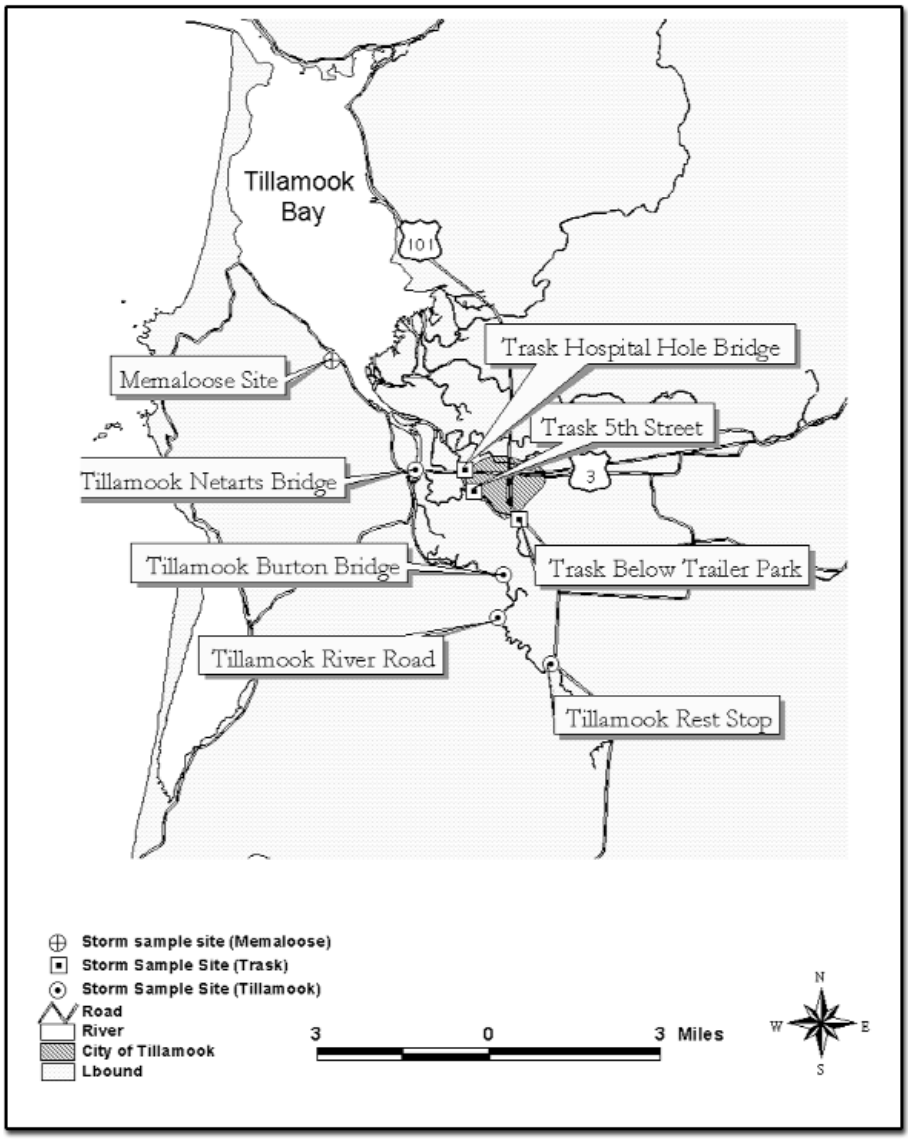

Figure 1. Map of Tillamook Bay Storm Sampling Locations

A statistical comparison was then made between the antibiotic resistance patterns recorded in the sources and the unknowns. This statistical technique sorts each of the unknown isolates into one of the sources (wild, human, dairy) based on their antibiotic resistance profiles. The final product is a distribution (percentage) of the unknown isolates by sources (wild, human, dairy) in each unknown sample.

The ARCC for the samples of the three sources was determined to be 83 percent for the entire database (830 isolates), with individual rates of 73 percent for human isolates, 88 percent for wild isolates, and 89 percent for dairy isolates. The antibiotic resistant profiles of individual sources achieved acceptably high levels of classification (i.e., comparable to other studies).

Winter Rainstorm Event

River samples were collected twice during the storm at seven sites along the Trask and Tillamook rivers with an additional site on the Memaloose Slough (Figure 1, above). To develop a clear understanding of the pollution sources affecting the sampling sites, ARA was used to determine the distribution of sources for each sampling site. To quantify the concentration of fecal contamination in each storm sample, water samples were collected in conjunction with the ARA-processed samples. A total of eight fecal coliform samples were collected at each location in approximately 12-hour increments between February 27, 1998 to March 3, 1998. Of these eight samples, two were collected to coincide with ARA processed samples.

Oregon's Department of Environmental Quality has established concentration standards of 400 colony forming units (cfu)/100 ml for a single sample for the recreational uses of water.

Based on the fact that both fecal coliform (used to measure concentration of contamination) and fecal *Streptococci* (used in the ARA to identify contamination

sources) are members of the enteric bacterial family the following equation was used to estimate the concentration of fecal contamination from each source in each ARA processed storm sample.

Distribution x Fecal Coliform Bacteria Concentrations

$$D_{d,h,w} \times FCB = Q_d$$

$D_{d,h,w}$ = Source Distribution of 'dairy,' 'human,' wild' (%)
FCB = Fecal coliform bacteria concentrations (cfu/100 ml)
Q_d = Quantified distributions (cfu/100 ml)

The data clearly show that the dairy or human sources contribute a majority of the fecal coliform isolates in all samples that exceed the water quality standards and that no one source is solely responsible for all of the fecal coliform contamination observed in the samples collected for the studies (Table 2). The data also shows us that wild sources consistently contributed small fractions of the fecal coliform isolates observed in the storm samples.

The storm sampling data also appears to support the

Table 2. Source Distribution Quantified with Fecal Coliform Concentrations.

Location	Date	Distribution x FCB Concentrations cfu/100ml (% Distribution)			Total Fecal Coliform (cfu/100 ml)
		Dairy	Human	Wild	
Tillamook River, HWY 101 Rest Stop	2/28/98	220 (28)	360 (50)	160 (22)	720
Tillamook River, HWY 101 Rest Stop	3/3/98	32 (17)	152 (80)	2 (3)	190
Tillamook River,River Road	2/28/98	118 (49)	96 (40)	26 (11)	240
Tillamook River, River Road	3/3/98	51 (58)	36 (42)	0 (0)	87
Tillamook River, Burton Bridge	2/28/98	22 (38)	34 (60)	1 (2)	57
Tillamook River, Burton Bridge	3/3/98	70 (47)	77 (51)	3 (2)	150
Tillamook River, Netart HWY Bridge	2/28/98	23 (33)	47 (67)	0 (0)	70
Tillamook River, Netart HWY Bridge	3/3/98	125 (66)	45 (23)	21 (11)	190
Trask River, Trailer Park	2/28/98	256 (69)	74 (25)	40 (6)	370
Trask River, Trailer Park	3/3/98	13 (22)	41 (72)	3 (6)	57
Trask River, 5th Street	2/28/98	525 (73)	165 (23)	31 (4)	720
Trask River, 5th Street	3/3/98	220 (37)	357 (59)	23 (4)	600
Trask River, Hospital Hole	2/28/98	484 (63)	194 (25)	97 (12)	775
Trask River, Hospital Hole	3/3/98	253 (62)	148 (36)	9 (2)	410
Memaloose Slough, Boat Landing	2/28/98	67 (74)	19 (21)	5 (5)	90
Memaloose Slough, Boat Landing	3/3/98	102 (68)	46 (31)	2 (1)	150

hypothesis that nonpoint sources of fecal bacteria may increase as rainfall increases; increases in fecal coliform concentrations generally coincided with the increase in river flow. Figure 2 (page 6) plots eight fecal coliform samples in conjunction with two ARA-processed samples in relation to the times they were collected during the storm. The quantified samples appeared to have been collected before and after the anticipated peak in fecal coliform concentration.

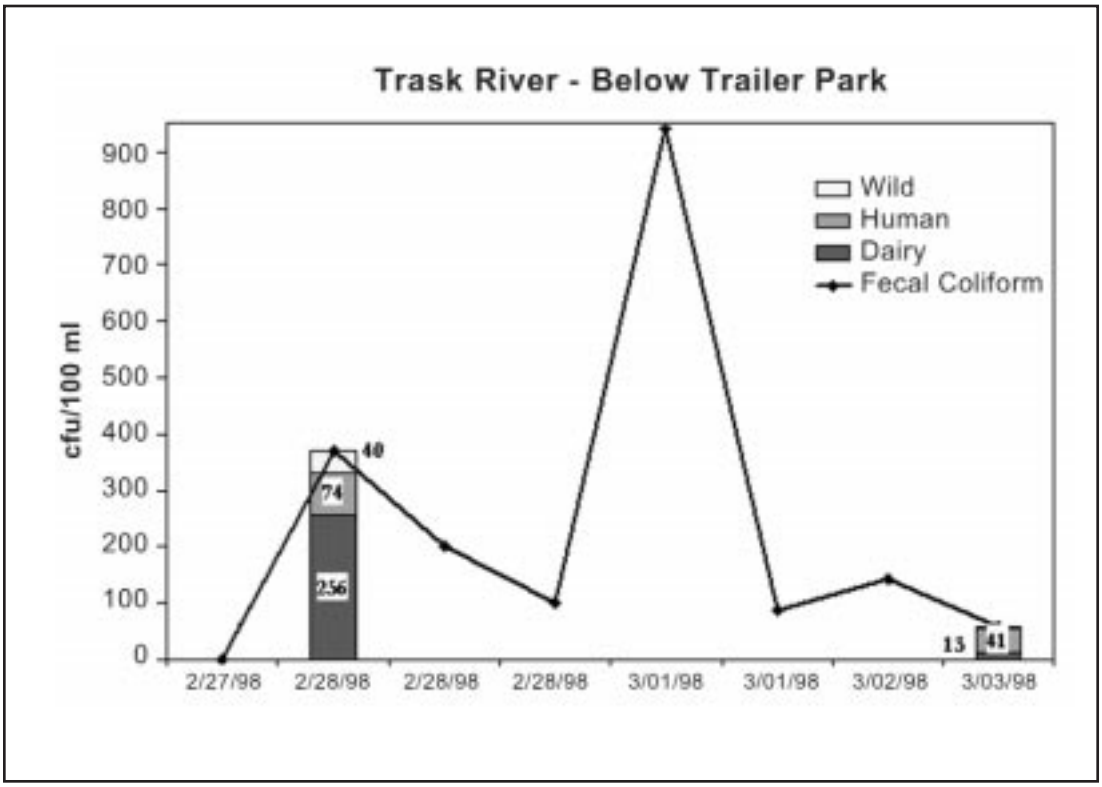

Figure 2. Quantified Distributions and Fecal Coliform Data at TRA BTR Site

One possible explanation for dairy sources appearing as the major source during the rising limb of fecal concentration, might be that they are a nonpoint source. Contamination from nonpoint sources can be expected to increase as the hydrograph rises because the rising hydrograph reflects flow contributions from overland flow. Observe that wild isolates, also reflecting a nonpoint source, also appear to be high in the first sample corresponding to the increases in dairy.

The second ARA sample appears to have been collected after the storm-driven fecal coliform peak on March 1, 1998 and may indicate a return to pre-storm fecal coliform conditions. Observe that human sources are the major contributor to contamination in the smaller second sample. This would be consistent with the idea that human sources of fecal pollution (e.g., wastewater treatment plants) might show up as more consistent fecal coliform concentrations (as treatment plant outflow is not greatly affected by storm events).

Seasonal River Study

Samples were collected near the mouths of the Miami, Kilchis, Wilson, Trask and Tillamook rivers in approximately six-week intervals from December 1997 through December 1998 (Figure 3, page 7). Samples were processed using the ARA technique to record changes in each of the rivers' fecal source distributions on a seasonal basis. Fecal *Streptococci* were enumerated to estimate the concentration of contamination in each sample.

The seasonal data provide some interesting insights into the chronic fecal pollution problems that occur in the Tillamook watershed. Data were similar among the rivers. Results for the Miami River are presented as a representative example.

As with the storm sampling, the data generally showed that either dairy or human sources consistently were the majority of the source distributions. For example on the Miami, the human sources made up the majority in six of eight samples (Figure 4, page 8). Also consistent with the storm study data was that wild sources were consistently a smaller percentage of the contamination in all of the river samples collected.

Dairy and human source distributions appear to change over the study (Figure 4). The percentage of human isolates decreases gradually from April to June and then gradually increases from June to December. This might give the impression the dairy sources are most prevalent during the early summer months while human sources are most prevalent in the fall and winter. However, by reviewing the concentrations observed over the seasons we reach some other conclusion.

Miami River fecal *Streptococci* concentrations attributed to each of the three sources are shown in Figure 5 (page 8).

The data showed that concentrations of all sources were generally low in the winter, spring and summer and higher in late summer and fall. In the majority of the rivers, there was a peak in fecal *Streptococci* concentrations around the September 1998 sampling. A majority of these September 1998 samples showed a human source

majority with only the Tillamook showing dairy as the majority. After this apparent basin-wide peak in human source isolates was observed, Tillamook river flow and rainfall data were analyzed to determine any environmental causes (data not shown here). There were no notable changes in either the rainfall or river flow during the period that these samples were collected. The reason for this increase in fecal streptococci concentrations is unknown at this time.

Conclusion

Understanding the sources of fecal contamination in quality-limited waters could greatly enhance our ability to restore and protect the water quality of these systems. The ARA technique as applied in the Tillamook Bay studies demonstrates a strong potential for use in water quality monitoring programs aimed at differentiating the sources of fecal pollution.

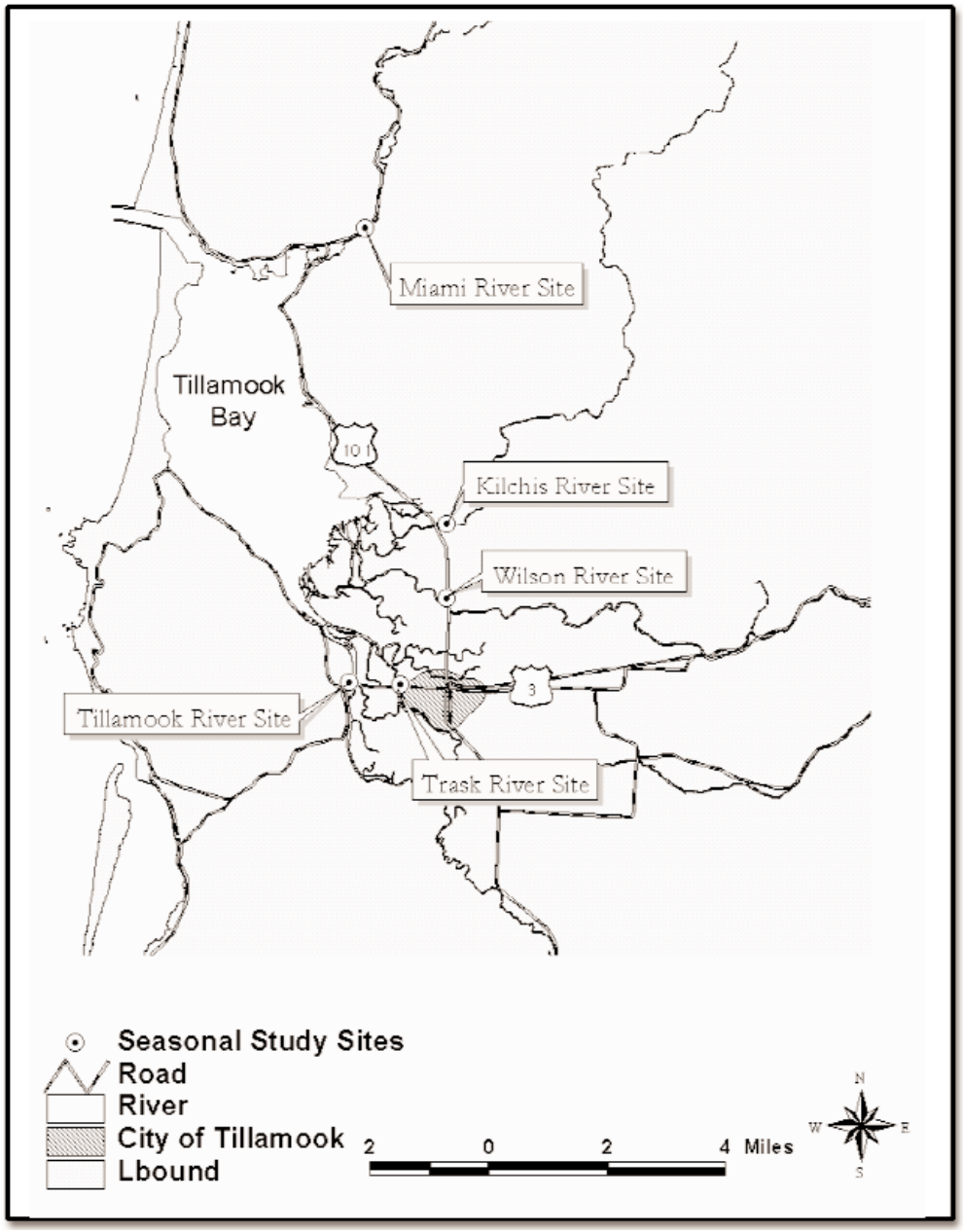

Figure 3. Map of Five-River Seasonal Sampling Locations

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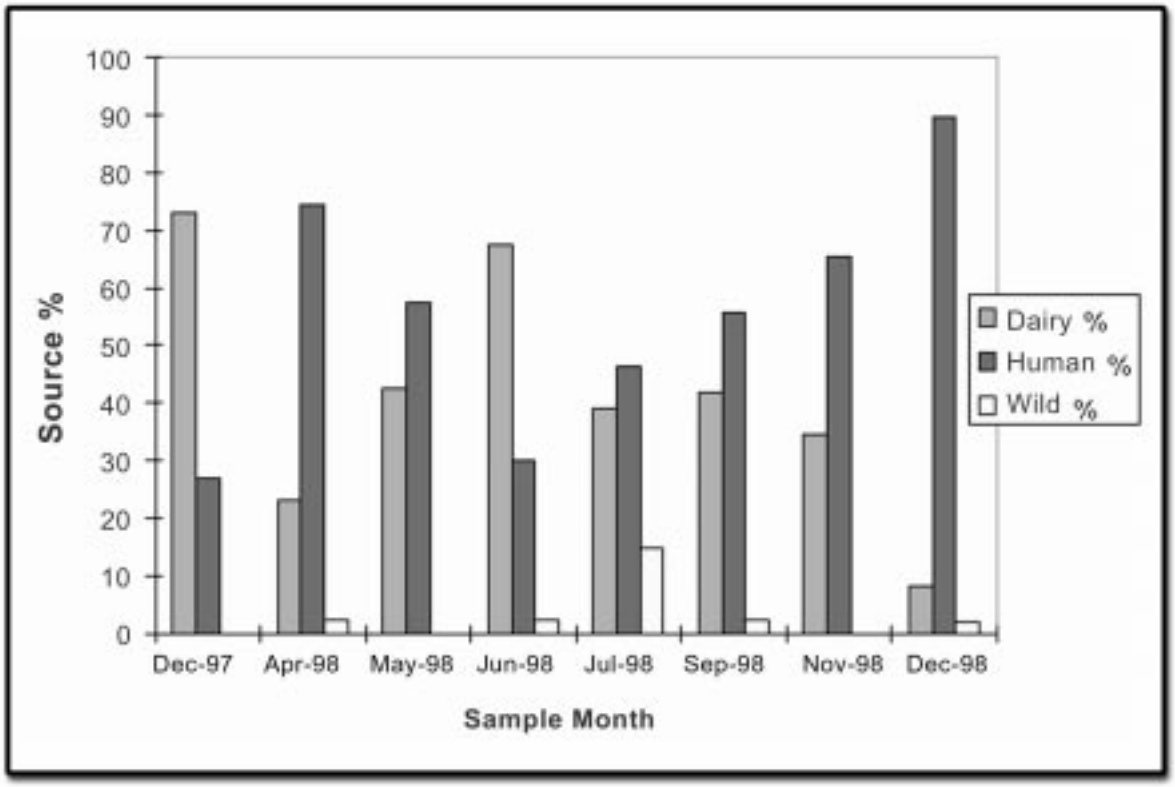

Figure 4. Source Distribution for Miami River Samples

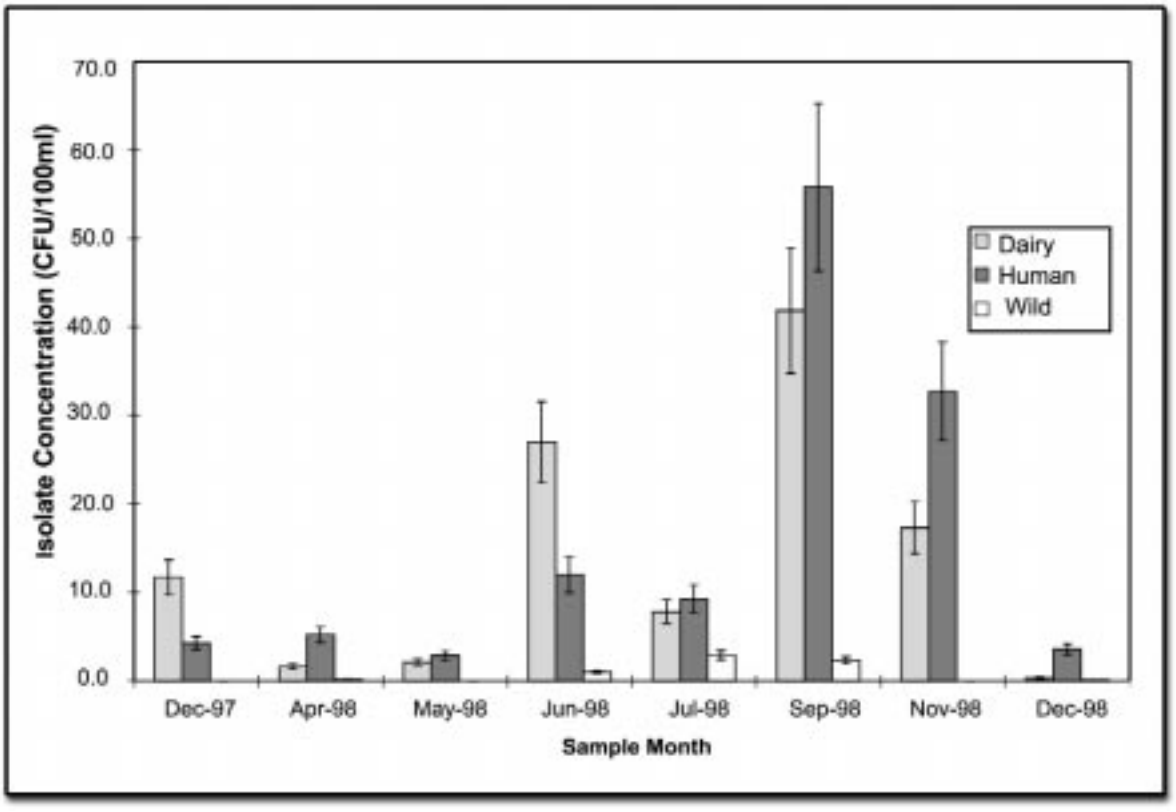

Figure 5. Miami River sources quantified

Factors affecting the survival of viruses in marine sediment and seawater

By **Jay Vasconcelos**, Environmental Microbiologist
WaterTrek Consulting Services

Introduction

Marine environments such as Puget Sound are potentially contaminated with a great variety of pathogenic microorganisms, including more than 140 known serotypes of enteric viruses. Unlike enteric bacteria, viruses are more resistant to environmental stress and conventional wastewater treatment practices, including activated sludge, post chlorination and ultraviolet radiation. Moreover, in contrast to bacteria, viruses are obligate intracellular parasites, which cannot grow or multiply outside a living host. Therefore, once in the marine environment, viruses have a finite life expectancy. Unfortunately, there are multiple environmental factors that can extend survival, all of which are temporally dependent.

Since the 1960s, fecal coliform bacteria have been the microbial tool of choice used to monitor the level of pollution in marine estuaries and shellfish growing water. Fecal coliforms have been used by regulatory officials with the full knowledge that this bacterial indicator does not adequately predicate the occurrence or survival of enteric viruses in the marine environments. Several laboratory studies have shown that enteric viruses can remain viable for up to 130 days in cold seawater, which is longer than fecal coliforms under similar environmental conditions. Consequently, the use of fecal coliforms as a predictor of virological water quality has limited value.

Although fecal coliform may be an imperfect indicator of enteric bacterial contamination, it has questionable value as an indicator of human viral contamination. The three most substantiated criticisms voiced against fecal coliforms as virus indicators have been:

1) during several shellfish-associated outbreaks, enteric viruses have been detected in seawater meeting current FDA fecal coliform standards;
2) fecal coliforms, as currently employed, cannot distinguish between fecal pollution originating from human sources versus non-human sources; and
3) commercial shellfish, self-cleansing practices (depuration) employed on the East Coast have failed to consistently eliminate enteric viruses from oyster shell stock as quickly as bacteria.

Survival in Sediments

Many studies since the early 1970s have found that marine sediments play a major role in the survival and distribution of both pathogenic enteric viruses and bacteria. The final resting place of wastewater-associated enteric viruses is the bottom sediments in and around the discharge point diffuser of wastewater treatment plants, regardless of the level of treatment employed. Once introduced into the water column, viruses rapidly adsorb to both organic and inorganic particulates and settle to the bottom becoming an integral part of bottom sediments.

Although the settling process removes viruses from the water column, it does not cause inactivation of those viruses. The lack of any inactivation means the viruses associated with these sediments retain their infectivity and can cause disease if brought into contact with humans. Virus-laden particulates accumulate in the upper layers of bottom sediments thereby becoming concentrated at much higher levels than overlying water. Sediments protective to viruses have been reported to contain 10 to 10,000 times more viruses per unit volume than overlying water. As a consequence, marine sediments represent the most important reservoir of viruses, contributing to the long-term survival, transport and potential uptake by bottom-dwelling finfish and shellfish. Recontamination of the water column can often occur during increased river flows, tidal currents, storms or human activities such as dredging and recreation. All these natural- and human-based activities can lead to re-suspension of virus-laden particulates and transported to other locations distances away from the original point source outfall.

Not only do viruses persist in marine sediments for long periods of time, they also tend to remain viable and infectious while attached to particulate matter. If particle-bound viruses remain infectious for extended periods, their re-suspension and hydro-transport could serve as a major vehicle for virus dissemination to nearby shellfish beds.

Viruses generally persist longer attached/adsorbed to sediment particles and appear to remain viable longer than fecal coliforms and other surrogate indicator bacteria. The one notable exception may be *Enterococci* bacteria, which have be shown by Environmental Protection Agency studies to mimic virus survival times in marine waters. These differences in survivability may help to explain the continual low-level isolation of viruses from oysters collected from approved growing areas meeting current FDA fecal coliform regulatory standards.

Consideration of the data relating to adsorption of viruses to solid particulates shows that the physical and chemical make-up of bottom sediments play a key role in the retention and survival of viruses. Certain types of sediments appear to adsorb and retain viruses more strongly than others, notably those containing a higher proportion of fine clay composition. Adsorption of virus-

es to small particles is enhanced by the presence of divalent cations like Mg++, Mn++ and Ca++, all common constitutes of seawater. This in itself may help explain the higher adsorption rates reported in marine versus freshwater sediments.

Although the protective mechanisms by which enteric viruses survive and remain infective in sediments have not been clearly shown, many theories have been postulated. Among the known factors that may prolong survival in sediments are:

1) cold temperatures in the range of 5-10ºC;
2) association with small organic solids;
3) physical entrapment within particles;
4) stabilization of electrostatic forces on the surface of virion capsids;
5) the adsorption and neutralization of virus inactivating agents; and
6) sediments compositions high in clay content.

Sediment particulates could also act as a simple buffer, absorbing toxic chemicals present in surrounding interstitial water. Ironically, sediments located in highly polluted locations may enhance virus survival in at least three ways. First, high levels of *E. coli* bacteria in polluted sediments may enhance survival by providing an adsorption site for viruses, indirectly acting like any other biocolloid. Second, bottom sediments high in soluble organics from polluting sources could further enhance virus survival by acting as an adsorbing agent to chemical inactivating agents, such as heavy metal compounds. Third, an abundance of diverse organic matter provides an opportunity for increased viral adsorption offering less space for surface interaction with inactivating substance, a feature that may result in protection of the virion structure.

Laboratory studies have shown that certain physiochemical factors affecting viruses' retention onto the surface of sediment-bound solids can indirectly enhance viral survival. Virus retention and resulting survival is mainly dependent upon four factors, which include salt concentration (salinity), pH, organic content, sediment composition, texture and type of surface.

Salinity, measured in parts per thousand (ppt), appears to have a considerable effect on the amount of viruses that will adsorb to solid surfaces. Studies on the Gulf Coast have shown that enteroviruses adsorb well above 5 ppt but poorly below 5 ppt. High salt concentrations in the form of cations will tend to enhance attachment to particles and thus help prolong survival. Viruses retained in the upper fluffy layer could be eluted by low salinity river water during rainfall events and then hydrotransported downstream to other locations.

Normally, the pH of freshwater lakes and rivers varies between 6 and 9 units compared to surface seawater, which is about 8.3. The various gaseous and solid constituents in the sediments usually maintain seawater pH immediately above bottom sediment at about 7.5. Low pH enhances viral adsorption to particulates while high pH results in elution or de-adsorption. A multitude of studies have shown that nearly 100 percent of all culturable enteroviruses tested remained adsorbed to sediments in the pH range of 6.0 to 9.0.

The organic matter found in sediments has a profound influence on the persistence of viruses. However, virus-particle adsorption bonding is apparently a complex interaction and subject to continual ongoing changes. Although the process is not clearly understood, several investigators have demonstrated that the ability of organic sediments to protect viruses from inactivation in the nearshore environment is only limited by concentrations of animal protein, polysaccharides, humic and fulvic acids all of which are usually found at either the mouth of a river or within the affected zone of a wastewater treatment plant outfall. These constituents apparently can compete with viruses for binding sites on both organic and clay particles. Notwithstanding this competition, most studies have demonstrated that viruses adsorb to organics quite

Department of Health weighs in on fecal coliform

Public health officials are concerned about human pathogenic viruses in clams, oysters and mussels near human sources of pollution. Due to the many difficulties of monitoring viruses of concern, the state Department of Health monitors only fecal coliform bacteria in shellfish growing waters, in accordance with national guidelines and requirements.

The National Shellfish Sanitation Program requires that the area around any wastewater outfall be closed to the commercial harvest of shellfish. In addition to commercial harvest areas, the Department of Health also establishes closure zones around outfalls near recreational shellfish beaches. Recreational harvesters can obtain information about those areas from the Department, Washington State Department of Fish and Wildlife fishing regulations, and their local health department. The size of shellfish closure zones around outfalls in Washington State is based upon such factors as wastewater treatment plant performance and flows, and characteristics of the receiving water such as dilution, dispersion, stratification and current speeds. These closure zones are premised upon potential upsets that can occur within the treatment plants rather than upon normal operating conditions.

Human waste enters marine waters from several routes in addition to the discharge of wastewater treatment plants, such as failing septic tank systems, stormwater runoff, wastewater collection systems, and boater discharges. Health assesses shoreline conditions and monitors fecal coliform bacteria in marine waters at all commercial and recreational shellfish beaches. All recreational and commercial shellfish areas must pass a review of potential pollution sources and meet the shellfish water quality standards or be closed to harvest.

well and survive under all conditions of salinity and pH tested. Light, fluffy organic materials occupying the upper portion of bottom sediments present the best environment for virus preservation and hydro-transport.

In addition to mud and other organics, marine sediments comprise a host of inorganic constituents, consisting mainly of clays, silts, sand, pebbles, cobbles and even small boulders. Of these, clay fractions of montmorillonite and kalinite appear to play a major role in both the adsorption and protection of viruses in sediments. Sand, pebbles and minerals play less of a role, while the function of silts is still unknown at this time. Sediments high in organic mud would normally indicate a protracted survival time, while inorganic clastic sediments high in sand, rock fragments and minerals such as quartz, feldspars and heavy metals would indicate a shorter survival time. Quartz and feldspars are usually the most common minerals in clastic marine sediments, representing about 15 percent of the total ocean bottom.

For years, many researchers have emphasized the importance of clay minerals in the retention and protection of viruses in sediments. It is now apparent that no single mechanism is responsible for adsorption of viruses to clay particles, even though some processes such as hydrogen bonding, hydrophobic interaction and anionic/cationic exchanges may be the predominant factors in any clay-virus sediment interaction. Virus adsorption to clay particles is almost an irreversible process in nature requiring extensive processes in the laboratory to release (de-adsorb) them. The persistence and hydro-transport of viruses by the sediment route is closely related to their adsorption onto clays and other sediment particulates. A more thorough understanding of these basic interactions is needed before we can predict the fate of pathogenic enteric viruses in sediments and their role in disease transmission through shellfish grown atop or near this environment.

The accumulation of sediments in an estuary is dependent on several shape and texture (fabric) properties including size, porosity and permeability. Fabric properties refer to the spatial arrangement of particles in a sedimentary deposit over time. Of the many aspects of fabric arrangements, particle size, shape, porosity and packing are most important. Packing is related to the spatial density of particles while porosity is the percentage of the total volume in the void space in the total arrangement of particles. Sediment particles hold and collect seawater and consequently act as ionic or hydrophobic binding sites for viruses. Viruses binding to external, internal and convoluted surfaces of sediment particles are a complex interaction and subject to change (attachment and release) with major physiochemical alterations in hydrogen ion concentrations (measured as pH) and cations at the binding sites.

Survival in Seawater

The contamination of coastal and inland seawater by human enteric viruses occurs mainly through the disposal of wastewater effluents, failing on-site septic systems, disposal of waste from boats and river water contamination upstream with either treated or untreated domestic wastewater. Unlike enteropathogenic bacteria and protozoan parasites, enteric viruses do not originate from wild or domestic fecal contamination. Human enteric viruses are highly host-specific and usually spread in the environment by the water route. If an outfall effluent is located miles from shore in relatively deep water, viruses can be carried for great distances. Studies carried out in Galveston Bay, Texas and in the Mediterranean coastal waters off Tel Aviv, Israel have documented that some viruses can be carried as far as 1,500 m from the discharge point. In strong currents or heavy swells, enteroviruses have been recovered as far as eight miles from the discharge point. These examples demonstrate the importance of human-virus contamination in marine waters and underscore the limitations we should place on fecal coliform indicators when evaluating both recreational and shellfish growing waters.

Although all pathogenic microorganisms are transient residents in the water column for relatively short periods of time, enteric viruses appear to survive longer than bacteria under similar conditions. The considerable dilution effect of seawater often hinders the detection and quantification of human viruses from the water column. Add to this the short residence time in the water column compared to that of the microlayer or bottom sediments, one can see why subsurface seawater is considered a poor location for sampling viruses.

The virucidal powers of seawater have been a phenomenon reported by many marine microbiologists throughout the world. Early studies in the 1960s demonstrated for the first time that exposure of human enteric viruses (poliovirus) to natural seawater for 17 days could result in a three-fold reduction when compared to freshwater. Since then, laboratory studies have shown that virus survival is controlled by a host of chemical, physical, environmental and biological factors. Of these, water temperature and solar radiation are perhaps the most important. Other specific factors include osmotic stress from seawater, predation by marine bacteria, protozoan flagellate, enzymes produced by marine bacteria and heat-labile substances in seawater.

Of all the factors controlling the fate of viruses in seawater, water temperature has repeatedly been demonstrated to be the most decisive. The inactivation of wastewater enteric viruses is always higher as water temperature increases. Conversely, inactivation rates decrease as natural temperatures decline, leading to increases in survival

times. The time necessary for a two-log10 (99 percent) reduction in certain enteroviruses, like echovirus 6, has been reported to be 40 to 90 days at 3-5ºC, nine days at 22-25ºC and <five days at 37ºC. Similarly, other important enteric viruses like HAV have been shown to persist longer in seawater at 5ºC as opposed to 25ºC. Seasonal changes, primarily responsible for changes in temperature, may also be accompanied by changes in biological and chemical components which can further influence the fate of viruses in natural seawater.

Sunlight in the form of ultraviolet (UV) radiation is well known to have a detrimental effect of the survival of viruses in seawater and is only limited by surface depth and water clarity. The damaging effect of UV on non-native bacteriophages (viruses that infect bacteria) has been reported to penetrate tens of meters in clear seawater. Sunlight increased viral decay rates significantly, no matter what the origin or source of the bacteriophages. Unfortunately, far less is known about the effects of sunlight on human enteric viruses. It has yet to be determined if UV solar radiation plays any significant role in virus declines in West Coast or Puget Sound waters.

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