THE UNIVERSITY OF RHODE ISLAND
COLLEGE OF THE ENVIRONMENT AND LIFE SCIENCES

Bacterial Monitoring

URI WATERSHED WATCH, Cooperative Extension
College of the Environment and Life Sciences (CELS)
Department of Natural Resources Science (NRS)
Coastal Institute in Kingston, 1 Greenhouse Road, Kingston, Rhode Island 02881-0804

Kelly Addy, Elizabeth Herron, and Linda Green

URIWW-2, March 2003 (updated May 2010)

Why Monitor Bacteria?

Is it safe to swim in the water? That's a question we often hear when we say we are monitoring a favorite swimming spot. Researchers and regulatory agencies have determined that one way to answer that question is to conduct bacterial monitoring. They do this to identify the human health risk associated with recreational water contact. The bacteria selected for water quality monitoring rarely cause human illness directly; rather the presence of these bacteria *indicates* that fecal contamination may have occurred and pathogens may be present in the water. Pathogens are microorganisms that cause illnesses; they may be viruses, bacteria or protozoans. Direct measurement of these pathogens, such as giardia, cryptosporidium, and Norwalk virus, is expensive and impractical because:

- There are innumerable types of pathogens that may be in waterbodies; it would be impossible to check for all these pathogens.
- The presence of one pathogen may not indicate presence of others.
- Generally, simple laboratory techniques do not exist to measure pathogens.

Bacterial monitoring is a practical method to determine the potential health risk of water exposure. Bacteria are microscopic, single-celled organisms that can be found in virtually any environment. Bacterial indicators of pollution are the species found in the intestines of warm-blooded animals, including humans, where many pathogens also originate. Indicator bacteria in a waterway come from many sources (Figure 1), e.g., animal droppings, faulty or leaking septic or sewage systems, combined sewage overflows (CSOs, see Box 1), stormwater runoff, boat sanitary waste and disturbed sediments.

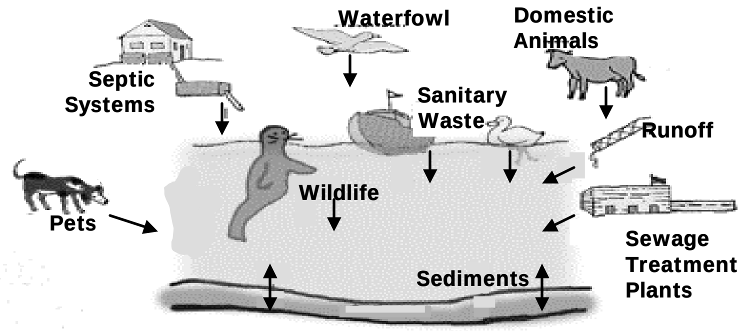


Figure 1: Potential sources of bacteria to a waterway (from Ely, 1997).

What bacterial indicators are monitored?

Bacterial indicators should meet as many of the criteria listed in Box 2 as possible to ensure safe swimming water. Water quality monitors screen water samples most frequently for fecal coliform (F.C.), *Escherichia coli* or enterococci as bacterial indicators (see Box 3 for details). These indicators are prevalent in the intestines of warm-blooded animals and associated with fecal contamination. Total coliforms are a group of closely related bacteria, fecal coliforms are a subgroup of total coliforms and *E. coli* are a specific species of F.C. bacteria (Figure 2). Enterococci are another group of bacteria unrelated to the coliforms.

Box 1: Combined Sewage Overflows (CSOs)

Combined Sewage Overflow systems carry storm water from roadways and untreated sewage from home and businesses in the same pipes. On a dry day, all this waste water is treated by the sewage treatment plant. However, on very rainy days, the sewage treatment plant may not be able to treat all the water and may need to release some untreated waste water into waterways. CSO control plans are in progress in the Providence area to minimize these inputs to Upper Narragansett Bay.

Box 2: Criteria for a good bacterial indicator (adapted from Ohrel and Register, 2001).

Good Bacterial Indicators Are:

Present whenever intestinal pathogens are present
Useful in fresh and marine waters
Alive longer than the hardiest intestinal pathogen
Found in a warm-blooded animal's intestines
Analyzed with an easy testing method
Directly correlated with the degree of fecal contamination

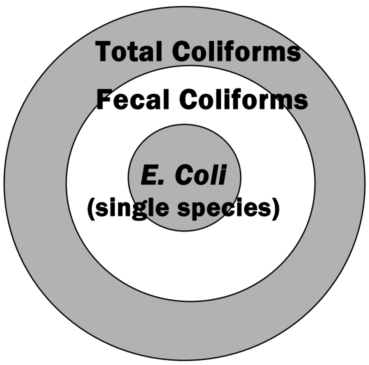


Fig. 2 (left): Breakdown of coliform bacteria (adapted from Ely, 1998).
Fig. 3 (bottom left): Filtering a water sample for the Membrane Filtration Method.
Fig 4 (below): Checking for positive bacterial results using the MPN method (Photo by IDEXX).

Box 3: Common Indicator Bacteria.

Total Coliforms and Fecal Coliforms:

Total and fecal coliforms have been used as bacterial indicators since the 1920's. Total coliforms (T.C.) as a general group are *not* particularly useful in terms of estimating human health risks because they can also be found in soil and plants naturally. Fecal coliforms (F.C.), a subgroup of the total coliforms, are considered a more useful indicator of human health risk because they are more often associated with fecal sources, even though a few non-fecal species exist, and are widely used to test recreational waterways and to classify shellfish waters.

***E. coli* and Enterococci**

E. coli and enterococci are bacteria that occur primarily in the intestinal track of warm-blooded animals. The US Environmental Protection Agency (US EPA) and other researchers have found better correlations between swimming-associated gastrointestinal illness and *E. coli* and enterococci in fresh waters, and with enterococci in marine waters than with T.C. or F.C. (US EPA 2002).

How are bacteria monitored?

Since bacteria are everywhere, great care must be taken to avoid contamination when collecting water samples for analysis. Water sample containers must be sterile and non-toxic. Plastic bottles that have been autoclaved (an autoclave is like a giant pressure cooker where objects are sterilized inside a chamber at high temperature and pressure) are most frequently used. Because water sampling devices usually can't be sterilized, samples are collected directly into the sampling container - basically scooped into the bottle. Monitors open the container just before sampling, being careful not to touch the inside of the container or the lid with anything other than the water. The sample container is closed immediately after collection, with samples stored on ice until delivery. Samples should be analyzed within six hours of collection. In the laboratory, samples are most commonly processed with either Membrane Filtration (MF) or multiple tube fermentation methods. The multiple tube fermentation (or the proprietary IDEXX tray version) method yields the Most Probable Number (MPN) of bacteria and is commonly referred to as MPN.

The MF method is a well-established method approved by most federal and state agencies to assess bacterial concentrations. Water is pulled through a filter that traps all the bacteria from the sample (Figure 3). The filter is then placed in a petri dish with growth medium and incubated at a specific temperature. The resultant bacterial colonies that grow are visible to the human eye and easily counted. Varying the type of growth medium, temperature and incubation periods help laboratories to isolate particular species of bacteria.

Unlike MF methods, MPN methods don't provide a specific count of bacteria. Rather, they are based on a statistical probability that the sample contained a certain number of bacteria based on a series of test tube analyses with water and species specific liquid media that positively identify the presence of the indicator bacteria. The IDEXX method, approved by US EPA and the RI Department of Health (RIHealth), substitutes a plastic tray and sealer for the tubes, and relies on their own reagent designed for specific bacteria indicator species (including total coliform, *E. coli* and enterococci) (Figure 4).

URI Watershed Watch (URIWW) uses an MF method with mTec media to assess fecal coliform, the indicator species required by the National Shellfish Sanitation Program (NSSP) for classifying shellfish waters, for some samples. Enterococci is analyzed with the IDEXX MPN method for all of the sites monitored through URIWW as an indication of safety for recreational uses. The URIWW analytical laboratory is certified by RIHealth for both methods, and reports results to RI DEM and RIHealth.

What are the water quality standards for bacteria?

Current RI water quality regulations use enterococci for recreational contact (swimming) and F.C. for shellfish waters and as an overall indicator of water quality. RI bacterial standards for recreational use in fresh and salt waters are listed in Table 2. The standards include single sample maximum values, as well as standards based on the geometric mean of multiple samples. Information on specific regulations can be found at the RI DEM website (http://www.dem.ri.gov/pubs/regs/index.htm#WR). To learn more about how areas designated or licensed as swimming beaches are monitored see the RIHealth site (http://www.ribeaches.org/). The US EPA criteria (US EPA, 1986, 2002) are based on *E. coli* and enterococci as the bacterial indicators (Table 2).

URIWW is state certified to analyze samples for F.C., *E. coli* and enterococci.

Table 2: Bacterial standards for recreational water uses by RI[1] and EPA

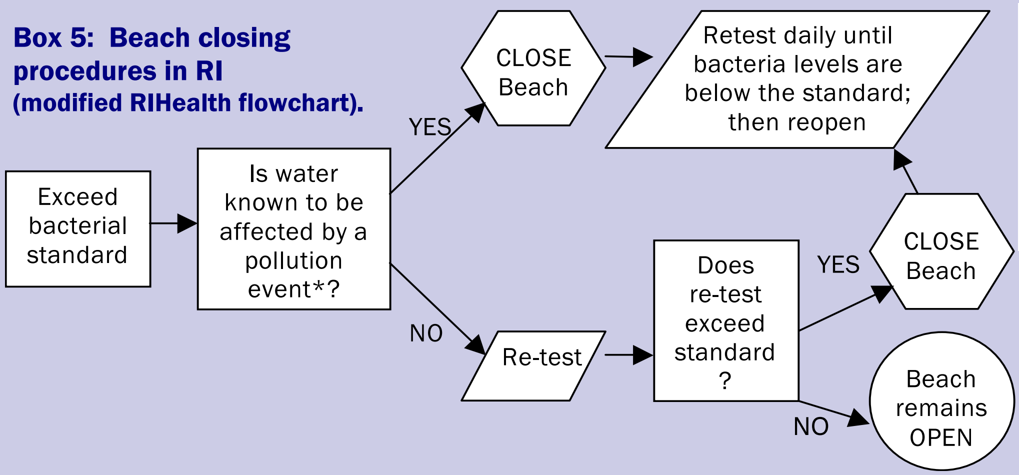
Water Type	Current RI Standard[1]		EPA Criteria[2]	
	——— count per 100 ml water sample ———			
Freshwater	Enterococci - Single Sample	61	Enterococci - Geometric mean	33
	Enterococci - Geometric mean	33 / 54*	*E.coli*	126
Saltwater	Enterococci - Single Sample	104	Enterococci - Geometric mean	35
	Enterococci - Geometric mean	35	* = non-designated beach	

[1] For more details on RI water classes and other bacterial standards please see RI DEM: www.dem.ri.gov/pubs/regs/index.htm#WR
[2] From US EPA 1986 and 2002.

Box 4: Who monitors bacteria in RI?

The RI Department of Health requires monitoring at all of RI's licensed salt and freshwater beaches and posts subsequent beach closings (Box 5). The RI Department of Environmental Management (DEM) Shellfish program collects samples regularly in shellfish waters and conducts sanitary surveys. RI DEM, with help from URI Watershed Watch (URIWW) and other volunteer monitoring programs, monitors bacterial levels in additional water bodies. URIWW monitors waterways for indicator bacteria to augment the state's dataset and to point out potential areas of concern.

Box 5: Beach closing procedures in RI (modified RIHealth flowchart).



** Pollution event may be a storm related CSO discharge.*

Corner illustration from the University of Wisconsin Cooperative Extension

What do bacterial standards mean?

Recreational contact with waters at or above standard levels of indicator bacteria does not mean you will definitely get sick; however, your chances of getting sick are increased. The US EPA criteria are based on health risk of contracting gastrointestinal illnesses (EPA, 1986). More research is needed on the risk of contracting upper respiratory and skin ailments from recreational water contact. RI's procedures for beach closings are outlined in Box 5.

As in any facet of water quality monitoring, the US EPA and other water quality professionals emphasize the importance of repeat analysis of waterbodies for indicator bacteria. Single samples may give the most recent information about the water quality and a basis on which to post beach closings or advisories for potential health risks. However, repeat sampling should be conducted to determine variability in indicator bacterial levels or if a chronic contamination problem exists.

What can YOU do to minimize the amount of bacteria entering waterways?

- Have your septic system inspected and pumped regularly.
- Properly dispose of pet waste.
- Don't feed waterfowl.
- Pump out boat waste at approved pumping stations.
- Support community plants to construct or upgrade sewage treatment plant and eliminate CSO's

What can be done about high bacteria levels?

Repeat measurements should be performed to determine if there is a long-term bacterial problem within a waterway and to identify any seasonal variation in bacterial levels. Waterbodies surrounded by summer communities can experience a surge in bacteria levels due to increased near-shore population and the resultant waste during the summer. In addition, warmer water may protect bacteria and promote growth. Conversely in the winter, bacteria tend to die off in cold waters. It may also be useful to sample for bacteria following storm events when CSOs may overflow and runoff may wash fecal waste from the land into water. These seasonal and storm event data often guide agencies in advisory protocols and may help to track bacterial sources.

If a chronic bacterial problem is diagnosed, sanitary shoreline surveys are usually conducted to determine the source of the bacteria. Such a survey involves an investigator looking throughout the watershed for evidence of failing septic systems, broken sewer pipes, and storm drains discharging water during dry weather. Additional observations of large congregations of waterfowl, wildlife, farm animals or pets are noted. Dyes or tracers may be used to determine the pathways of some potential pollutants. Once the source of the high levels of bacteria is determined, remediation action can be taken.

There have been many advances in bacterial source tracking using state-of-the-art microbiological techniques. Researchers compare the DNA of the bacteria in the water sample with DNA of known sources of fecal contamination. It is important to note that the US EPA no longer allows broad exemptions to their regulations for waterbodies that have identified the source of high bacteria loads as non-human (US EPA, 2002). More research needs be conducted on the potential of human health risk from the exposure to non-human fecal contamination. Therefore, the goal of this DNA fingerprinting is to help identify the source of the contamination for remediation purposes.

In some instances where bacterial contamination cannot be resolved, most likely due to economic or social restraints, EPA allows states to deem waterbodies suitable for only secondary recreational contact. Activities such as canoeing or motor boating are allowed because water contact and immersion seem unlikely.

For further information on bacterial monitoring:

Ely, E. 1998. Bacteria Testing Part 1: Methods Primer. *The Volunteer Monitor*. Volume 10, No. 2.

Ely, E. 1998. Bacteria Testing Part 2: What Methods do Volunteer Groups Use? *The Volunteer Monitor*. Volume 10, No. 2.

Heufelder, G. 1997. Interpreting Fecal Coliform Data: Tracking Down the Right Sources. *The Volunteer Monitor.* Volume 9, No. 2.

Miceli, G.A. 1998. Bacterial Testing Q & A. *The Volunteer Monitor*. Volume 10, No. 2.

Ohrel Jr., R.L. and K.M. Register. 2001. *Volunteer Estuary Monitoring: A Methods Manual*. Second Edition. U.S. EPA. This manual is available on-line at: www.epa.gov/owow/estuaries/monitor/

Turin, D. and M. Liebman. 2002. Keeping Posted: Communicating Health Risks at Public Beaches. *Journal of Urban Technology* 9:45-69.

US EPA. 1986. *Ambient Water Quality Criteria for Bacteria-1986*. EPAA440/5-84-002.

US EPA. 2002. *Draft Implementation Guidance for Ambient Water Quality Criteria for Bacteria*. EPA-823-B-02-003.

RI DEM Office of Water Resources: http://www.dem.ri.gov/pubs/regs/index.htm#WR

RI DOH's Bathing Beaches Program: http://www.ribeaches.org/

Issues of the *Volunteer Monitor* : www.epa.gov/owow/volunteer/vm_index.html

http://www.usawaterquality.org/volunteer/Ecoli/

To learn more about the URI Watershed Watch program or their bacterial monitoring, please contact:

Elizabeth Herron, Program Coordinator
Phone: 401-874-4552
Email: emh@uri.edu
Linda Green, Program Director
Phone: 401-874-2905
Email: lgreen@uri.edu
www.uri.edu/ce/wq/ww/

Kelly Addy, M.S., Elizabeth Herron, M.A., and Linda Green, M.S., are members of the Dept. of Natural Resources Science, College of the Environment and Life Sciences, University of Rhode Island. Contribution #3983 of the RI Agricultural Experiment Station, with support from RI Cooperative Extension, RI Department of Environmental Management, and RI Seagrant. Cooperative Extension in Rhode Island provides equal opportunities without regard to race, age, religion, color, national origin, sex or preference, creed or handicap.

Sea Grant Rhode Island