CITIZEN'S GUIDE TO BACTERIA MONITORING IN VERMONT WATERS

Scientific basis
Swim beach monitoring
Watershed-scale monitoring

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I. INTRODUCTION

So you think you want to start a bacteria monitoring program? There are a few things you should know about bacteria monitoring before jumping into it. There are two major reasons to implement a bacteria monitoring program. You may want to protect swimmers at a local beach or swimming hole from exposure to waterborne pathogens that may make them sick. Waterborne pathogens are disease-causing agents like viruses or bacteria. Alternately, you believe that a pollution source exists somewhere in your watershed, and you want to find it to clean it up. Depending on which of these applies, the design of the monitoring effort will differ. However, before we get into those details, let's talk about the science behind bacteria monitoring.

II. SCIENTIFIC BASIS FOR BACTERIA MONITORING

A) Indicator of potentially infectious pathogens

Bacteria monitoring is limited in the amount of information it can tell you. It is called bacteria monitoring because the amount of a certain bacteria is what is actually measured. The resulting measurement is used to infer the likelihood that the water contains human pathogens that would elevate the risk of contracting a swimming-related illness. This prediction is not without flaw, but it is the best available approach to date.

The primary indicator of fecal material in water used in most freshwater monitoring efforts is *Escherichia coli*[1]. Since *E. coli* is a constituent found in the intestines of humans and other warm-blooded animals[2], when found in rivers, lakes, ponds, streams, or drinking water, it means that somehow fecal material has made its way into the water. *E. coli* is therefore used as an indicator of potential fecal contamination of the water. While some strains of *E. coli* are pathogenic in and of themselves, the presence of *E. coli* is used in monitoring programs to indicate that other fecally transmitted pathogens like live viruses, bacteria, protozoans or worms may also be present. While fecally-contaminated water may have pathogens present, many times pathogens cannot survive outside the intestines for long periods of time and therefore are not alive (Schaechter, 1992). To be prudent, it

[1] Another indicator used to a lesser extent is Enterococci. Although Enterococci has some characteristics that make it a more useful fecal indicator than *E. coli*, *E. coli* is the more commonly used freshwater indicator of the two indicators approved for freshwater by the United States Environmental Protection Agency.

[2] Interestingly, microbiologists have not been able to isolate *E. coli* from the fecal material of beavers (Jones, 2002).

is assumed that if *E. coli* is present, live contagious viruses or pathogens may have been present in the source fecal material and thus may now be in the water.

B) How much *E. coli* is too much?

Well, one of the things we love about Vermont is swimming in the great outdoors and enjoying the wide variety of wildlife we share our state with. Naturally, some 'waste' from wildlife makes it into our waterbodies. But, this does not necessarily mean we are going to contract gastroenteritis or any other illness from swimming in these waters. The more fecal contamination that enters a waterbody, the more likely that human viruses and pathogens are going to be present. How do we know when the level of fecal contamination is high enough to increase our risk of illness to an unacceptable level? One way is to measure the amount of *E. coli* in swim waters and then record the number of people who become ill afterwards. This is repeated over and over under a wide range of *E. coli* measurements. The ensuing illness rate is then related to the amount of bacteria measured.

The U.S. Environmental Protection Agency (EPA) has used findings from epidemiological studies just like this to develop recommended criteria for water quality standards (U.S. EPA, 1986). EPA decided that 8 in 1,000 swimmers getting sick would be an acceptable level of risk, and set their most stringent single sample criterion at 235 *E. coli* organisms/100 milliliters (ml) of water, which corresponds to the statistical threshold beyond which this illness rate may be achieved. By the federal criterion, as long as *E. coli* derived from a single sample collection remains below 235 *E. coli* /100ml, waters are considered safe to swim in. The Vermont Department of Health uses this guideline in providing advice to beach managers on opening and closing beaches.

Now keep in mind that these studies were conducted at very populated, urban beaches, where some of the swimmers themselves were likely the source of the fecal contamination. These beaches were also under the influence of nearby sewage discharges. It is thought that human fecal material such as this is more likely to contain organisms that are pathogenic to humans than is fecal material from other animals. However, we don't yet have the scientific studies to tell us which warm-blooded animals carry organisms that are pathogenic to humans, and whether or not non-human fecal contamination is as likely to make people ill. Until results of such studies are available, we will have to assume that non-human sources present the same risk as human sources. In Vermont, we no longer have beaches that are subject to sewage discharge **and** have thousands of human swimmers like those studied by EPA.

So the presence of *E. coli* indicates that there may be pathogens in the water that may make humans sick, but it is not an actual measurement of those pathogens. This is important to keep in mind as you look at a standard like 235 *E. coli* /100 ml and think, hey why not make that standard zero? Zero is used for drinking water standards, but unless we want to exterminate all wildlife and chlorinate our waterbodies, we must coexist with some level of fecal contamination in our waterbodies. We tend to think that all bacteria are bad, but bacteria are as important a component of the ecosystem as they are of our own 'internal' fauna.

C) Vermont's standard

In 1986, the United States Environmental Protection Agency published its national Ambient Water Quality Criteria for Bacteria document (EPA, 1986). States were strongly encouraged to adopt the criteria in the document or ones more stringent. In 2002, and again in 2010, EPA issued updated guidance for implementing the 1986 criteria (see EPA, 2002a). EPA recommends that States set their freshwater quality standard to correspond to an illness rate of 8 illnesses per one-thousand swimmers. EPA's studies (discussed above) tell us that a 5-sample geometric mean of 126 *E. coli* /100ml, or a single sample of 235 *E. coli* /100 ml, indicates a likely illness rate of 8 per 1000 swimmers. EPA will allow States to establish standards that correspond to illness rates up to 14 illnesses per thousand swimmers (five-sample geometric mean of 548 *E. coli* /100ml or a single sample in excess of 1,021 *E. coli* /100ml) for swimming in freshwater (see Section 4.1.1 of EPA, 2002a). States can use the geometric mean (see below) of at least five samples collected during a 30-day period, or use a single sample value, or a combination of both to determine whether a waterbody meets the criteria.

It is not until their adoption as part of a state's water quality standards that the criteria become legally binding (EPA, 1986). Many years ago, Vermont adopted a water quality standard for *E. coli* bacteria for Class B waters that is far more strict than EPA's recommendation. Vermont's Class B standard is 77 *E. coli* /100ml in a single sample. ***This is the most stringent standard in the nation.*** Based on EPA's epidemiological studies, Vermont's standard level equates to an illness rate less than four per 1,000 swimmers.

To be precise about it, the meaning of Vermont's standard is as follows. At 77 *E. coli* /100 ml, we can be 75% certain that 3.4 persons in 1,000 will get sick, assuming that they are swimming at a beach with very heavy use, that is influenced by some level of waste discharge. To be 95% certain that 3.4 individuals per 1000

swimmers would become ill, the single sample value would increase to 187 *E. coli* /100 ml.

For a variety of reasons, the highly strict nature of Vermont's present standard produces an impractical situation in terms of assessing real risks to swimmers and determining where bacterial pollution is a real issue. In fact, recent local studies (Sargent and Morrisey, 2000; Moir, 2003) tell us that under moderate rainfall, *E. coli* will be found in waters running off of completely undisturbed, forested watersheds at levels in excess of 77 *E. coli* /100ml. The Watershed Management Division is re-evaluating Vermont's Class B water quality standard in light of these newly emerging scientific findings, and consistent with the Health Department, suggests that beaches be posted when samples exceed 235 *E. coli* /100ml.

D) No instantaneous measurement exists

Why use *E. coli* as the indicator? There are plenty of other indicators of fecal contamination, however, *E. coli* is inexpensive and relatively easy to measure. The drawback to *E. coli* is that the test result cannot be conveyed to swimmers at the time the sample showing a standard violation is collected. Currently, there is no instantaneous test to directly measure for fecal contamination and thus *E. coli* is our best bet. It can take days to get the results of a water test since culturing and enumeration alone can take up to 24 hours from the time of sample collection (U.S. EPA, 2000). Thus, by the time the beach can be closed to swimming, the swimmers who were present when the elevated *E. coli* sample was taken are long gone. This type of sampling does not provide any protection to the swimmers who were present at the time of sampling, or to those swimming within 24 – 48 hrs after the sampling occurred. It can provide protection if the bacterial source is an ongoing one, like failing septic systems. However, many times, bacteria levels will rise sporadically in response to passing waterfowl or other wildlife. A beach sampled just at that time might become closed to swimming 24-48 hours later, even though the transient contamination has passed, and the water may, in fact, be perfectly clean.

E) *E. coli* concentrations vary over short distances and times

Depending on where you sample at a beach, you will end up with very different *E. coli* readings. In a Massachusetts beach study, sampling at ankle depth yielded consistently higher readings than sampling at waist level, which gave higher readings again than samples collected at chest level (Doolittle, 2002). The same study found that sampling in the morning produces higher readings than sampling at high noon, because *E. coli* is degraded by sunlight. Now, keep in mind this does not

mean that the pathogen levels are degraded by sunlight, but that the indicator bacteria levels are. According to this study, there was more variation in the level of bacteria measured as you went out from shore than there was from left to right along the shore. The study also showed higher *E. coli* concentrations when the wind was onshore. The patchy and episodic nature of *E. coli* in the environment makes it very difficult to monitor efficiently.

III. DESIGNING A BACTERIA MONITORING PROGRAM

A) Monitoring bacteria to protect swimmers

You may be feeling a little disheartened after reading the above facts about *E. coli's* limitations. This does not mean that it is not a useful indicator for protecting the health of swimmers, it just means it isn't the instantaneous and absolute test you may have thought it to be. However, there are effective ways to use bacteria monitoring to proactively protect swimmers. By coupling routine monitoring with a rainfall threshold study, one can monitor changes in *E. coli* concentrations at a swim beach and develop the capability to predict when wet weather conditions are likely to result in elevated *E. coli* concentrations. Other threshold studies can also be useful depending on the nature of the primary *E. coli* threats to your waterbody.

1) Routine monitoring

Take samples at your beach on a weekly basis (or more often), making sure to get 5 samples per month. There will be a delay of a day or so after sampling before the results are available. If the result indicates a value above the guideline of 235 *E. coli* /100ml, the Watershed Management Division recommends resampling immediately to ensure the result was not simply transient. During this time, the swim beach can be kept open, can be posted with a warning, or closed. For reasons discussed above, using Vermont's standard of 77 *E. coli* /100ml will result in the need for more frequent resamplings and possibly lengthier closures while resample results are processed.

Beach closures should be reported to your Town Health Officer. For guidance on how to post your swim waters see the Vermont Department of Health website, or Chapter 5 of EPA's National Beach Guidance (USEPA, 2002b). A listing of Vermont Town Health Officers is also posted on the VT Department of Health website. California also has some good guidance in Section 2.6 of their Draft Guidance for Fresh Water Beaches document (CADHS, 2001). Both of these documents are posted on the web and their respective websites can be found in the references section of this document.

2) Use of the geometric mean

A geometric mean is a measure of the average concentration that accounts for extreme variability in datapoints. A geometric mean is appropriate to keep track of the long-term condition of beaches and swim waters. Use the geometric mean of the 5 monthly samples to look for any trend in *E. coli* over time, and to indicate where chronic contamination is evident. Are you seeing an increase each year in the average monthly geometric mean? EPA recommends a geometric mean not exceed 126 *E. coli*/100ml. Perhaps you aren't exceeding EPA's recommendation, but you are consistently close. Perhaps you perceive an increasing trend and you want to look for reasons why. In those cases you may want to develop a bacteria monitoring approach to identify the pollution sources in your watershed (see Section III-C of this document).

Calculating a geometric mean is a simple affair. The equation is as follows:

$$\text{Geometric mean} = (r1 \times r2 \times r3 \ldots x \ldots rN)^{1/N}$$

Where: r is the *E. coli* result per 100 ml for samples 1, 2, 3, through the N^{th} sample; and,
N is the number of samples collected.

For example, you have monitored a river site over the course of five weeks, keeping track of prior weather conditions. Table 1 shows the resulting data, and a sample calculation:

Table 1. Example data table for tracking *E. coli* concentrations

Sample	*Prior weather*	*E. coli* /100ml	Calculation
Week 1	No rain 2 weeks	22	**Step 1:** 22 x 234 x 17 x 188 x 77 = 1,266,881,616 and **Step 2:** $(1{,}266{,}881{,}616)^{1/5}$ = ***66.2*** *Note: To do step 2 in an Excel spreadsheet, you would put the following in a cell:* =1266881616^0.2
Week 2	2" hard rain last night	234	
Week 3	No rain since prior to week 2 sample	17	
Week 4	Geese everywhere this morning	188	
Week 5	1.5" rain over the past three days	77	

3) Where to sample

To select your sampling sites, identify your most populated swim area(s) and take your samples there. To protect the largest number of swimmers, focus your

sampling in the area and depth at which most of your swimmers recreate. If your beach is frequented mostly by children, sample at knee depth. If this is largely an adult area, chest-depth is more appropriate. More than one sampling station may be appropriate if there are multiple popular swimming areas. Ideally, you'd take replicate samples so that you have a second sample against which to check a high reading. **This is very useful to avoid an unwarranted swimming advisory posting or beach closure**. Multiple sampling sites and/or replicates will, however, increase the cost of the monitoring program.

At your selected sampling sites, wade out to the chosen depth to take the sample, being careful not to disturb the sediment too much. Bacteria levels are higher in the sediments, so taking readings at ankle depth will yield higher values as will samples that have the bottom sediments stirred up into them (Meals, 2001; Moir, 2003). Take the samples in the morning, before the day's sunlight degrades the *E. coli* present. If you can only take your measurements midday, then do so consistently over time (e.g., if you sample midday, always sample midday). ***Make sure that you have coordinated your sampling schedule with your laboratory.*** You will need to make sure that the samples arrive on ice at the lab such that they can be processed within their six-hour hold time.

4) When to sample

The days of the week when samples are collected depends on use patterns at your beach. EPA recommends testing during peak use (EPA, 1986), but as shown in section II D, results of such samples will not protect the maximum number of swimmers. Alternately, you can take the sample(s) a couple of days before your busiest days of the week, so that you can get the results back in time to take any necessary action, including resampling and/or posting as deemed necessary. The speed with which laboratory results are reported can in part guide the timing of sampling. If your laboratory has a fast turnaround time, sampling can be done closer in time to peak beach usage.

Many beach managers and watershed associations post signs showing the results of all of their *E. coli* sampling over the course of the swimming season, where practical. This provides swimmers with a good base of information to guide their own decisions as to whether they should swim. Such postings should include text on the risks implied by the presence of *E. coli* in excess of the chosen standard, as well as the limitations of using *E. coli* as an indicator of beach swimming suitability. The Cities of Burlington and South Burlington, and the Town of Colchester, post information about *E. coli* testing, as well as all of their beach testing results, on-line at www.burlingtonecoinfo.net.

5) How to sample; collection, preservation, and storage

Follow the instructions for sampling provided by the laboratory performing the analyses. In general, samples should be collected in sterile plastic containers which have been stored in plastic bags away from dirt or other potential contaminants. Your laboratory typically will provide sample containers that are appropriate. Make sure that your hands are clean prior to sampling to avoid contaminating the sample. Store the samples in a cooler, dark and on ice during transit. Ideally, samples should be kept as cool as possible (say 36°F, but not frozen), but of course the samples you collect from the swim beach will not be this cold! A University of Wisconsin-Milwaukee study showed that wet ice is better for transporting E. coli samples than cold packs (Brooks, 2003). Apparently, cold packs do not keep the samples cold enough and some growth of the bacteria occurs within the 6-hour hold time when cold packs are used instead of ice. In this same study, samples on wet ice actually were found to be able to be kept longer than the 6-hour hold time. Samples should be delivered to the laboratory to begin processing within 6 hours. Samples should not be analyzed if this time is exceeded, or if the samples are not kept on ice (U.S. EPA, 2000).

The following is a step-by-step protocol for collecting a sample for *E. coli,* broken down into protocols for beaches on lakes vs streams. (*note: If your laboratory's guidance differs from these, follow your laboratory's guidance.*)

Lake Bathing Beach Sampling:

1. *Wading*: Try to disturb as little bottom sediment as possible. In any case, be careful not to collect water that has sediment from bottom disturbance. Wade out to where the water is 3' deep. *Boat or dock*: Carefully reach over the side and collect the water sample away from the side of the boat or dock where the water depth drops to 3' deep.
2. Remove the cap from a sterile collection bottle without touching the inside of the cap or the inside of the bottle.
3. Grip the bottle at the base and plunge it in a downward motion into the water to a depth of 12 inches.
4. Using a forward sweeping motion (so water is not washed over the hand into the bottle), invert the bottle and bring it to the surface.
5. Empty it slightly to leave approximately one inch of air at the top.
6. Re-cap the container, then label and store it on ice at a temperature between 39° and 45° F. It is better to use wet ice rather than cold packs.

7. Transport the bottle to the laboratory as soon as possible after sampling. (Modified from EPA, 2003 and VTDEC, 1989 to be consistent with VTDOH in 2012)

Stream or River Sampling

In general, sample away from the streambank in the main current. Never sample stagnant water. The outside curve of the stream is often a good place to sample, since the main current tends to hug this bank. In shallow stretches, carefully wade into the center current to collect the sample. A boat will be required for deep sites. Try to maneuver the boat into the center of the main current to collect the water sample.

Where possible, go to the centroid of flow in a river at a depth of three feet of water, face upstream, and allow any disturbance to flow downstream. Plunge bottle mouth down into the water to avoid introducing surface scum to a depth of one foot below the surface. If this is not possible, the sampling depth should be a minimum of 15 to 30 cm (6 to 13 inches) below the water surface in the centroid of flow, or at the depth relevant to the swimwater under evaluation.

1. Label the bottle with the site number, date, and time.

2. Remove the cap from the bottle just before sampling. Avoid touching the inside of the bottle or the cap. If you accidentally touch the inside of the bottle, use another one.

3. *Wading*. Try to disturb as little bottom sediment as possible. In any case, be careful not to collect water that has sediment from bottom disturbance. Stand facing upstream where the depth is 3' deep. Collect the water sample on your upstream side, in front of you. You may also tape your bottle to an extension pole to sample from deeper water. *Boat.* Carefully reach over the side and collect the water sample on the upstream side of the boat.

4. Hold the bottle near its base and plunge it (opening downward) below the water surface. If you are using an extension pole, remove the cap, turn the bottle upside down, and plunge it into the water, facing upstream. Collect a water sample 12 inches beneath the surface. If this is not possible, the sampling depth should be a minimum of 6 to 13 inches below the water surface in the centroid of flow, or at the depth relevant to the swimwater under evaluation.

5. Turn the bottle underwater into the current and away from you. In slow-moving stream reaches, push the bottle underneath the surface and away from you in an upstream direction.

6. Leave a 1-inch air space. Do not fill the bottle completely (so that the sample can be shaken just before analysis). Recap the bottle carefully, remembering not to touch the inside.

7. Fill in the bottle number and/or site number on the appropriate field data sheet. This is important because it tells the lab coordinator which bottle goes with which site.
8. If the samples are to be analyzed in the lab, place them in the cooler for transport to the lab.

(Modified from U.S. EPA, 1997 to be consistent with VT DOH in 2012)

EPA, 1997 summarizes some recommended field quality assurance/quality control procedures that are important to include in your sampling program. These steps should be used whether you are conducting lake or stream sampling:

- "Field Blanks. These should be collected at 10 percent of your sample sites along with the regular samples. Sterile water in sterilized containers should be sent out with selected samplers. At a predetermined sample site, the sampler fills the usual sample container with this sterile water. This is labeled as a regular sample, but with a special notation (such as a "B") that indicates it is a field blank. It is then analyzed with the regular samples. Lab analysis should result in "0" bacteria counts for all blanks. Blanks are used to identify errors or contamination in sample collection and analysis.

- Field Duplicates. These should be collected at 10 percent of your sampling sites along with the regular samples. A field duplicate is a duplicate stream sample collected at the same time and at the same place either by the same sampler or by another sampler. This is labeled as a regular sample, but with a

special notation (such as a "D") that indicates it is a duplicate. It is then analyzed with the regular samples. Lab analysis should result in comparable bacteria counts per 100 mL for duplicates and regular samples collected at the same site. Duplicates are used to estimate sampling and laboratory analysis precision."

B) Threshold studies

1) Rainfall threshold studies

The most health-protective program would allow you to close a beach before any swimmers were exposed. Unfortunately, no instantaneous test exists yet. However, we can measure rainfall events and determine when to have preventative closings based on the relationship between the amount of rain and *E. coli* concentrations. Rainfall washes fecal material off land in the watershed into rivers and lakes. Stormwater runoff was responsible for the majority of Burlington-area beach closings during the 2000-2001 period, and is recognized nationally as a common source of *E. coli* (NRDC, 2002). By conducting a rainfall threshold study, we can determine the relationship between rainfall quantity and *E. coli* levels, and post or close beaches when a threshold amount of rain has occurred, and *E. coli* standards violations can be expected.

A rainfall threshold study involves taking *E. coli* several times during a rain event and for a day (or more) after. Do this for several different rain events (5 events would be a good number to start), recording the amount of rain (in inches) that has fallen during the storm by the time the sample is taken. This should be done across rain events of different duration and magnitude. Then review the results by creating graphs of rainfall quantity and *E. coli* concentration for each storm. If runoff during rain events causes *E. coli* levels to exceed Vermont's single sample standard, look to see how much rain it took for the level to exceed this number. Then look to see how long it took for the level of *E. coli* to diminish below the standard. Questions to ponder include: Does this happen for every rain event, or only during events exceeding a certain intensity?; Is the rainfall quantity at which the *E. coli* level exceeds the standard consistent? Table 2 and Figure 1 show hypothetical results for one storm event.

Table 2. Sample data table for a rainfall threshold study.

Date and Time	Inches rainfall	*E. coli* concentration
7/16/03 8:00a	0.2	6
7/16/03 11:00a	1.2	16

7/16/03 2:00p	2.5	60
7/16/03 5:00p	3.7	179
7/16/03 8:00p	4	99
7/17/03 8:00a	4.1	18
7/17/03 11:00a	4.1	0
7/17/03 2:00p	4.1	2

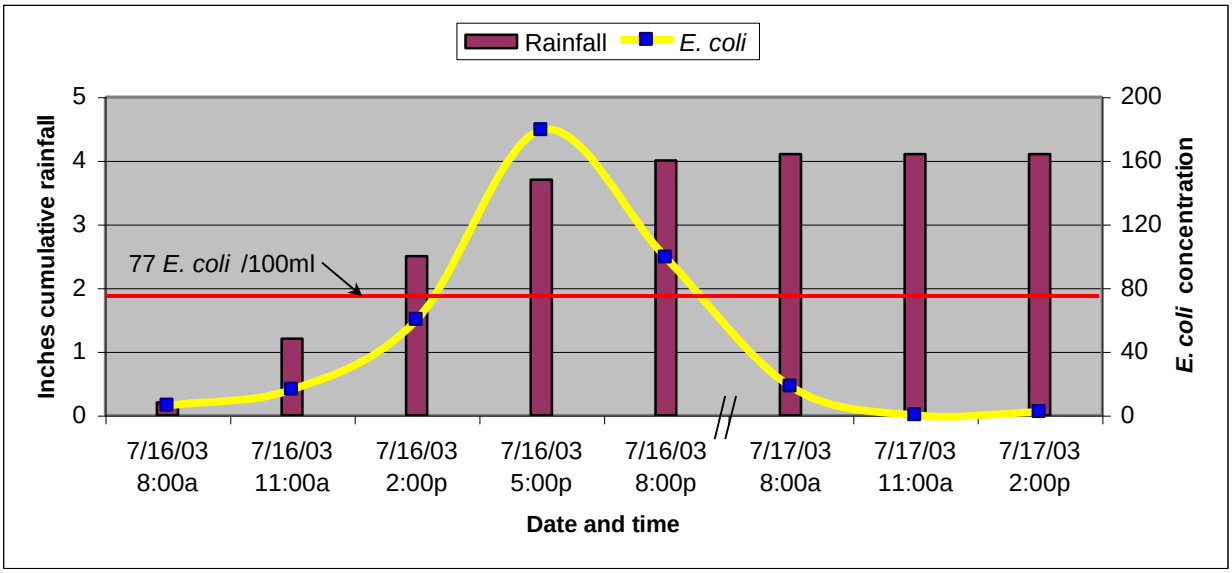


Figure 1. Illustration of *E. coli* concentrations in relation to cumulative rainfall for one storm event.

This information can be used to set proactive and preventative closings by closing the beach every time you have a rain event of a sufficient amount to cause a violation of the standard. Using the example in Figure 1, and if you were using the Vermont standard of 77 *E. coli* /100ml, the beach would be closed as approximately two inches of rain had fallen, and would a remain closed until the next morning, presuming that rain had ceased during the night. Remember that several rainfall events should be included in a rainfall threshold study; the more, the better. Doing a rainfall threshold study will require that a rain gauge be installed at the swim beach, and rainfall measurements recorded as part of the beach management program. Rainfall threshold studies done for beaches located on rivers should also include data on streamflow as well as rainfall.

When designing a rainfall threshold study, it is important to have a longer-term record of rainy and dry spells previous to the beginning of the study. This will help in the interpretation of the resultant data set. For example, you may find that a rain event following a long spell of dry weather results in your highest levels of bacteria in the water. Whereas in a scenario where you have a well established contamination source, after multiple rain events, you may find that the rain event

you happen to study, has lower than expected bacteria levels since most of the fecal material was delivered during the previous rain events.

Depending on how often rain causes a violation of the standard (and how big that violation is), you may want to try to find the source of the contamination. In that case, you will want to design a study to identify the pollution sources in your watershed (see section III C of this document).

2) Migratory bird threshold study

Some waterbodies in Vermont experience heavy influxes of waterfowl over short periods of time during migrations. If your waterbody experiences such influxes of waterfowl during a time that coincides with swimming use, then you may want to do some increased *E. coli* monitoring at this time and perform bird counts simultaneously. This way you can determine if there is a relationship between a certain number of waterfowl and *E. coli* violations, thereby allowing you to post preventative swimming advisories whenever you observe that threshold number of waterfowl present. Bird migration is a good example of a threat that can be identified, but that a community may not wish to control due to the habitat value the water resource provides.

3) Other possible threshold studies

Depending on the nature of a waterbody, specific *E. coli* threats may be identified and preventative-closing criteria may be developed specific to that waterbody, affording better swimmer protection. A study similar to the rainfall study can be designed in order to address a myriad of potential threats. Migratory waterfowl is one example, but given your assessment of the threats to your waterbody you can apply the same study design to other threats not mentioned here.

C) Monitoring bacteria to identify pollution sources in your watershed

It is important to note that monitoring bacteria in the swim water is only one facet of an effective program to prevent and reduce the occurrence of waterborne disease in swimmers where routine and recurring violations of standards are evident. While monitoring bacteria lets you know when bacteria levels are high and how frequently they exceed standards, it does not address the cause for the elevated bacteria counts.

In addition to creating a health risk, fecal contamination delivers nutrients to the aquatic environment. While a natural background level of fecal material is part of

the ecological process[3], when humans alter the environment such that excess fecal material makes its way into waterbodies, it can be considered pollution. Bacteria monitoring at the watershed scale is a bit different than that done at beaches. It is important to do both wet and dry weather sampling in order to best identify where the fecal contamination is coming from and how best to address it.

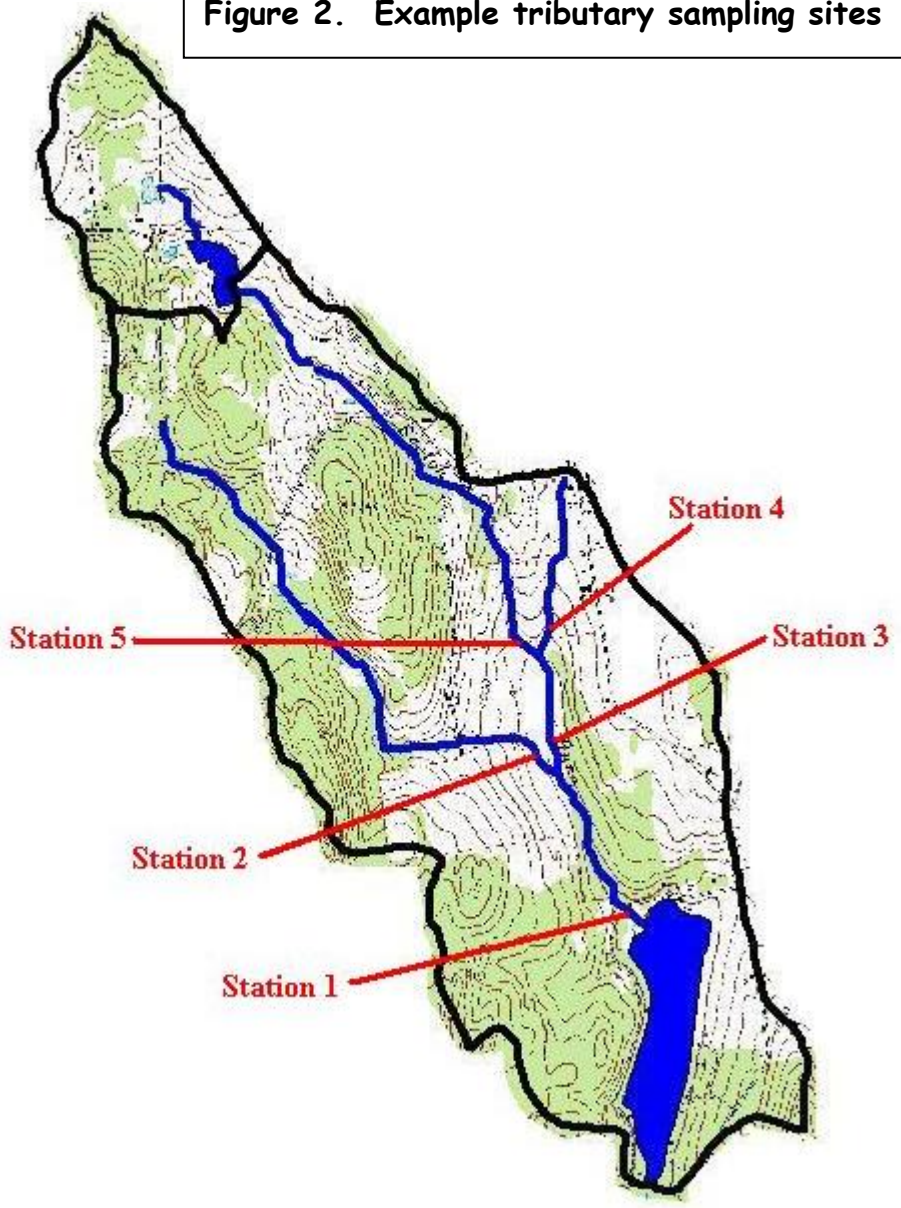
1) Stream survey and site selection

Choosing appropriate sites is critical to being able to interpret and use your data after it has been collected. Begin with a topographic map and determine the boundaries of the watershed, looking for all the places where streams come together (Figure 2). Then perform a 'stream walk.' Beginning at the outflow of the watershed, work your way upstream along the streambanks, noting where smaller streams feed into the river or stream you are walking along, and looking for potential fecal sources. These sources might include areas of pet waste accumulation, concentrations of manure, or obvious areas of wildlife activity. Make sure to obtain landowner permission prior to walking streams, since this provides for open dialog amongst neighbors, and gives an opportunity to inform local watershed residents about the water quality problem you are addressing.

Depending on the watershed size, you may want to walk up each smaller stream as well. Establish sampling locations just upstream from where the smaller stream feeds into the main stream. Ideally, you would want to measure as many of these junctions as possible. Since each stream flows from different parts of the watershed, if you measure each separate stream you can then find out which ones have the highest *E. coli* levels and focus on those smaller sub-watersheds to find the source of contamination (e.g., stormwater runoff, concentrated areas of wildlife use, a failing manure pit, etc.). You will want to sample in both dry and wet weather conditions, and you will want to sample each sampling location each time you do a sampling 'run.' During dry weather, continuous sources can be picked up more readily if they are the problem, since the signal is not diluted by rainfall. During wet weather, other contaminant sources that may only increase during rain events can be picked up.

[3] Fecal material from wild animals is a natural component of the aquatic environment. The bacteria present in fecal material are actually themselves an important constituent of the very bottom of the food chain.

Figure 2. Example tributary sampling sites



2) Taking a closer look

Let's say you've identified a particular stream where you are finding consistent elevated *E. coli* measurements after rain events. Then you'll want to look for the source, if you had not already found it via your stream walk. It is possible that you won't find a readily discernible source. The contamination you observe may come from fecal bacteria that exist in the streambed from a historical source. In some circumstances, streambeds can be hospitable places for fecal bacteria to persist

(Moir, 2003). When the sediment is stirred up by storm events, elevated bacteria levels in the water column can result. Keep this in mind as you interpret the data from your study. It will help you to prioritize your efforts, enabling you to focus your fecal contamination abatement efforts on the known sources instead of spending a lot of effort looking for a source that may no longer exist in the watershed. Also, the Watershed Management Division has a 'Citizen Lake and Watershed Survey' Instruction manual that can help guide you in your hunt for fecal sources in your watershed (VT DEC, 2000). For a copy of these instructions contact the Watershed Management Division at 802-241-3777.

3) Septic surveys

A common perception in Vermont is that failing septic systems are a large source of fecal material, particularly to lakes. Determining the potential contribution of potentially failing septic systems is a tricky proposition, and is known as a 'sanitary survey' (although it is hardly sanitary). Historically, testing of septic systems was accomplished using dye tablets, which were flushed down the toilet in a shoreline property with follow-up visual monitoring over the next several days to identify if and where dye may be leaching into the adjacent water. The Watershed Management Division's prior history with successful sanitary surveys is mixed at best. That said, in collaboration with others in the Agency of Natural Resources, the Watershed Management Division may be able to assist in implementing a sanitary survey. Additional information regarding sanitary surveys is also available in Chapter 4 of EPA's draft Ambient Water Quality Criteria for Bacteria (Appendix G, EPA, 2002).

These shoreline sanitary surveys can be very significant and costly undertakings that can engender ill will among neighbors and property owners. If you believe that you are in a situation that necessitates a sanitary survey, please contact the Watershed Management Division to discuss the data results on which you are basing this need; the Division will want to become involved at this point.

You may have heard about a new technique that is being developed to identify the source (dog, human, cow, goose, etc.) of fecal contamination you are observing in a waterbody. This new technique is called Microbial Source Tracking (MST). Essentially, MST analyzes the genetic fingerprint of the *E. coli* itself, to identify the organism that produced the fecal material containing the *E. coli*. Currently, there are different genetic techniques and approaches being developed for this purpose. This approach is still in the developmental stage, although it is likely to be a very valuable and powerful tool for identifying fecal contamination sources in the near future. If you are interested in learning more about this evolving

approach, the United States Geological Survey has a helpful website on the topic http://water.usgs.gov/owq/microbial.html.

If you've identified sources of fecal material in your watershed and have determined that the sources are something beyond your control or will take a while to get under control, then you can use a threshold study tailored to the particular source to determine when to set preventative beach closings until controls can be developed.

D) Cleaning up identified fecal sources

Once you have identified the sources of fecal contamination in your watershed or at your beach, there are helpful resources to aid you in controlling them. Depending on the source, there are different options available to you. Contact the Watershed Management Division for information and appropriate resources.

E) Collaboration with the Watershed Management Division

If through your routine monitoring program you identify a swim beach that you believe is being contaminated by fecal material above natural wildlife levels and resulting in violations of the Vermont standards, then please contact the Division. It is the role of the Division to track where impairments to uses of Vermont's waters exist, and to work to diagnose and mitigate the source of these impairments. The Division can consult on the design of your program, and can also assist in implementation of watershed-based projects. The Division presently offers three grant programs intended to assist Vermont citizens in improving water quality. These are the EPA-funded "319" Water Quality Grant Program, the Vermont Watershed Grants Program (which is funded by the VT conservation license plates), and the Water Quality Laboratory Services Grants program for Volunteer Organizations. More information about these programs is available at http://www.vtwaterquality.org.

IV. CONCLUSION

While the precision of the information offered by bacteria monitoring may not be what you may previously thought it was, such monitoring provides effective information when used properly, with an understanding of its limitations. This document has outlined three ways that bacteria monitoring can be used. 1) It can be used as a routine monitoring tool at swim areas to detect (possibly) an episodic increase in bacteria levels. This monitoring won't protect the swimmers that were exposed, but can prevent further exposure. 2) It can be used to set rainfall thresholds, so that when you get a certain amount of rainfall, you can close a swim

area to prevent exposure to the anticipated elevated levels of *E. coli* in the runoff. 3) It can be used to hone in on the pollution sources in the watershed, so that efforts can then be made to reduce the sources of fecal contamination.

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