Russian River Pathogen TMDL Monitoring Design:

A Technical Report to the North Coast Regional Water Quality Control Board

06-428-110 Project Report

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September 2009

Acknowledgements

Portions of this work were funded by a North Coast Regional Water Quality Control Board grant (SWRCB# **06-428-110**). The following individuals made contributions to the information contained herein Rachel Hutchinson, Michelle Jensen, Heidi Schott, Anja Wehrmann, Henry Calinchini, and Liz Bowen.

Please cite this report as follows:

Shilling, FM, JH Viers, ML Johnson. 2009. Russian River Pathogen TMDL Monitoring Design: a Technical Report to the North Coast Regional Water Quality Control Board. University of California, Davis. 42ppd.

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A Executive Summary

We are presently faced with overwhelming environmental change, which is manifest in every component of our biological systems and at every scale. The interwoven nature of environmental processes, ecosystem services and human needs requires that we pursue solutions that leverage advances in knowledge and technical capabilities with an explicit move to *synthesis* (Pfirman & NSF Advisory Committee for Environmental Research and Education. 2003). This *synthe*sis requires that we move beyond historic disciplinary boundaries and proceed with approaches that cross spatial, temporal, and organizational scales.

Because standard methods of measuring fecal contamination in water – specifically the quantification of fecal indicator bacteria (FIB) – do not identify the sources of the contamination, synthetic approaches are needed to quantify and source impairment. We present here a synthesis of strategies to more fully integrate monitoring for, and source assessment of, pathogen impaired waterbodies. Foremost, employed approaches must be suitable to answer questions being asked and, moreover, questions must be appropriate to the problem at hand.

Based upon the literature and our pilot monitoring program, the following are our recommendations for future monitoring in the lower Russian River watershed (and other streams and rivers in the North Coast region) that is suspected or shown to have impairment from fecal contamination:

1. The fecal indicator bacteria (FIB) species used in investigations and monitoring need to be suitable for question(s) being asked. For example, *Bacteroides* spp. and *Cryptosporidium* spp. are indicators of recent fecal matter inputs, but don't tell much about longer term loading because of their high rate of mortality in the environment. Conversely, *Escherichia coli* may live longer in the environment, but have been shown to naturalize and reproduce, confounding their utility as a reliable indicator.

2. The relationship between non-aqueous media (i.e., reservoirs, such as floodplain and benthic sediments and vegetation, of fecal material) and indicator bacteria are poorly understood. Contemporary studies have documented trans-seasonal holdover, transport from unknown sources, and cross-seasonal transmission of fecal bacteria to points of human contact. While complex, these relationships deserve greater scrutiny as pathways to pathogen impairment.

3. Potential public health impacts should be connected to definitive sourcing. Certain potential public health impacts (e.g., contamination of ground-water sources of drinking water) are not thoroughly examined in the watershed. Pathways from fecal contamination (via animal agriculture waste and septic system waste) to pathogen loading to human health have not been definitively identified, let alone quantified.

4. FIB monitoring should be used to determine effectiveness of Total Maximum Daily Load (TMDL) implementation, including complete spatial coverage and cross-seasonal and opportunistic (event driven) monitoring. The history of FIB monitoring in the watershed makes trends almost impossible to determine and potential source types and areas difficult to determine.

5. Index sites and timeframes for background condition and management actions must be identified so that there are both treatment conditions and reference conditions (Before After Control Impact experimental design). This will allow implementation management actions to be measured for effectiveness.

6. A strategic monitoring network is needed to permit spatio-temporal characterization of fecal micro-organism impairment, superseding the current approach, which is largely oriented toward timely beach closures. Known inputs (i.e., municipal wastewater, septic effluent, agricultural runoff) should be monitored closely for transmission of microbes through surface- and groundwater. Monitoring of these sources should also be conducted year-round to capture not only the recreational season (current focus), but through the wet season to capture first flushes, storm water pulses, and recession flows that largely condition downstream waterbodies.

Because *E. coli* is not a conclusive indicator bacteria (Power et al., 2005; Doyle & Erickson 2006; Ishii et al., 2006) or Enteroviruses (Noble et al., 2006), we feel that alternative methods must be employed to more accurately indicate level and source of fecal impairment and thus potential health hazards. As summarized in Field and Samadpour (2007), monitoring of actual pathogens in the environment is difficult, time consuming, expensive, and often inconclusive; thus surrogate measures, such as FIBs, are used almost exclusively. Recent advances in microbial source tracking (MST) allow sources of fecal contamination to be accurately identified and located; however, actual remediation is often directed at reducing FIBs, not pathogenicity or potential exposure. Moreover, if MST results point toward wildlife, it is not clear that remediation efforts would be helpful at reducing potential health hazards, let alone FIBs. While few studies have examined direct causative linkages between fecal inputs, pathogen contaminated water, and outbreaks of disease (see Olsen et al. 2002), it does not minimize the likelihood of previous or future outcomes.

Recent estimates of impaired waterbodies indicates that agricultural run-off is the leading source for rivers and streams, whereas urban and stormwater runoff is the leading source for beaches and shorelines and municipal point sources for estuaries (Arnone & Walling 2007). Our recent study (see Viers et al. 2009), however, found substantial urban and suburban runoff influence on riverine impairment by fecal bacteria, often three orders of magnitude higher than agricultural sources.

B Background

B.1 TMDLs and Monitoring

Waterbody listing for pathogens is the most common type of 303(d) listing, triggering close to 3,000 Total Maximum Daily Loads (TMDLs) in the U.S. (He et al., 2007). There have been several TMDLs approved recently for mixed-use watersheds in the Russian River vicinity (see descriptions for two of them below). The USEPA has developed guidance for the establishment of loads in listed waterbodies and contributing water-sheds/ways. Usually a basic understanding of the workings of the watershed is required, as well as primary pathways for human exposure. Although there is no national model TMDL to base new TMDLs, there have been approaches tried in different watersheds which, when drawn upon, can collectively inform new TMDL development. It is worth considering that a microbial water quality standard is generally a measure of a bacterial indicator organism, which is most often used as a surrogate to actual pathogenicity. Thus, developing a TMDL for supplementary indicators is also required if a use impairment (i.e. waterborne disease outbreak) would still exist even after the water body is in compliance water quality standards because indicator organisms do not reflect the presence of pathogen contamination with complete certainty (Arnone & Walling 2007).

B.1.1 TMDL Standards

A total maximum daily load is a planning tool operating on input and transport processes at a watershed scale, but focused on waterways as output and outcome environments (Ferguson et al., 2007). There are many possible pathways for pathogens contained in fecal material to come into contact with people, including recreation (primarily during the summer months) or drinking water. Inputs that can be regulated include agricultural area runoff, municipal discharge, septic system discharge, and urban storm-water runoff. Transport of fecal material, including pathogens, occurs primarily during winter storm events. Fecal material can be deposited during post-storm flow reductions and late winter seasonal reductions in flow. Beneficial use impacts can occur during any time of the year through recreational contact (primarily in the summer) and drinking water from ground or surface sources.

The contemporary standard for pathogen TMDLs is for developers to: 1) take into account the input environment (watershed) composed of non-point and point sources of fecal material, 2) measure bacterial indicators appropriate for the extent and timing of the inputs, 3) describe and measure the transport processes that lead to the loads in each waterway, 4) understand the timing and nature of the impact on beneficial uses, and 5) fully describe unknown factors because of inadequate knowledge and data about the system.

B.1.2 Pathogen TMDL Examples

Tomales Bay

In this TMDL, threats from fecal organisms to recreational contact (REC-1 and REC-2) and shellfish harvesting were the basis for the TMDL. Sources of problems included human and domestic animal waste entry into tributaries to the Bay. The detection methods for indicator bacteria were restricted to growth media-based approaches, which quantify the culturable fecal bacteria. Several potential pathogens were examined – Salmonella, coliphage, and *E. coli* H:O157. Some correlations were made in the background studies based on spatial or temporal proximity of problems (high concentrations) with particular tributaries or storm events. There were fecal bacterial concentration variations by tributary, predominant upstream land-use, and season. Human inputs to the system were thought to be primarily from failing septic systems, municipal runoff, and possibly wastewater treatment plants. Animal inputs to the system were thought to be primarily from domestic animals – grazing, dairies, and equestrian facilities. Hydro-dynamic modeling was conducted to target input tributaries for potential inputs to the Bay violating the SHEL standard. Finally, TMDL implementation was to include continuing *E. coli*/fecal coliform monitoring with medium spatial resolution (30 sites) and temporal resolution (weekly/monthly and event). Confounding temporal variables for this type of study include tides (Solo-Gabriele et al. 2000).

Napa River[1]

In this case, fecal coliform and Enterococci bacteria presented threats to REC-1 and REC-2 uses and were the basis of the TMDL. Sources of problems were much the same as in the Tomales Bay watershed, including the exceeding of standards from human and domestic animal sources. Detection was conducted using growth media.

The Kendall Tau statistic was used in non-parametric correlation analysis to relate wet season *E. coli* concentrations to various urban area parameters and dry season *E. coli* to percent cover of agriculture. In addition, most of the wet season delivery of fecal bacteria was through surface water pathways and in the dry season through ground-water pathways. Human inputs to the system were determined to be primarily from failing or inadequate septic systems, sewer lines, municipal runoff (thought to be very important), and possibly wastewater treatment plants. Animal inputs were primarily from grazing lands and confined animal feeding operations. The TMDL was based on the REC-1 standard as the use most likely to be impaired.

Charles River, Massachusetts[2]

This TMDL focused on fecal coliform and *Enterococcus* bacteria in a forested and residential watershed. Tributary watersheds/waterways were rated (e.g., Class A) according to goals for fecal bacterial concentrations. Inputs were thought to be primarily from various human waste disposal mechanisms, urban/storm-water runoff, domestic animals, and wildlife. Upstream areas are more contaminated than downstream areas and concentrations have generally been declining since 1989. The TMDL included river segment-specific potential causes in dry and wet seasons. Generally, there was a correlation

[1] http://www.waterboards.ca.gov/sanfranciscobay/napariverpathogentmdl.htm

[2] http://www.mass.gov/dep/water/resources/tmdls.htm#final

between level of development (undeveloped to urban/agricultural) and *E. coli* concentrations. Single family residential areas tended to have higher concentrations than commercial and industrial areas. Finally, wet season concentrations tended to be higher than dry season. Human inputs during the dry season were due to agriculture, failing septic and sewer systems, and illicit connection of sewer to storm drains. Fecal inputs during the wet season were domestic animal waste, storm-water runoff, and sewer overflows. Animal wastes were minor inputs from wildlife and some input from domestic animals in the dry season. One statistical analysis in the baseline study was correlation analysis of fecal coliform concentrations and rainfall (using Pearson's R and Spearman Rank Order Correlation). Current monitoring under this TMDL consists of filling gaps in information available for decision-making and measuring effectiveness of control measures and Best Management Practices. Hydrodynamic modeling using MIKE-21 was used to study actual and potential benefits from BMPs.

B.1.3 Land-Use/Land Cover as Drivers

There are several major land-uses and point sources that could contribute fecal matter into tributaries of the Russian River. These include dairy and livestock operations, wastewater treatment facilities, sewer lines, septic systems, municipal area runoff, and manure applications on agricultural fields. Previously-published research has identified all of these sources as potential contributors of fecal material to waterways. These land-use related inputs differ in magnitude at certain times of the year and/or when facilities and best management practices are failing.

Animal agriculture, which is largely confined to cattle grazing and dairying in Sonoma County, but also include horses, sheep, and poultry (and conceivably other hobby animals, such as lamas), is likely to result in increased fecal bacteria into waterways due to overland flow and poor management of waste material. Additional inputs from animal agriculture includes the application of manure on fields. Many of the sub-watersheds in the southern end of the watershed have agriculture as the largest or one of the largest land-uses in the sub-watershed.

Rural residential development often is accompanied by on-site septic systems that vary in age and efficiency of capture and processing of input material. If and when these systems are over-whelmed or as they age, fecal matter may enter surface and ground waters.

Urban area stormwater runoff can contribute very high loads of fecal bacteria to waterways (Salmore et al., 2006), as can wastewater treatment plant effluent. In Maryland, urban areas are considered the primary contributors to waterway fecal contamination in mixed land-use (agriculture and urban) watersheds (Wickham et al., 2006). In one study in the Southern Appalachians, a stream contaminated with fecal bacteria while running through an urban area, became less contaminated once it ran through National Forest lands (Clinton and Vose, 2006). A massive survey of stormwater in urban Milwaukee found numerous entry points of fecal contamination into waterways[3]. City of Santa Barbara officials estimate that up to 80% of the sewer laterals in the city are defective[4].

B.2 Russian River Geography

The Russian River watershed is located predominately in Sonoma County, California with some of its headwaters reaching into southern Mendocino County. The mainstem of the Russian River is fed by many moderately sized creeks, including Mark West Creek, Atascadero Creek, Dry Creek, etc. The predominant land uses in the Russian River watershed include agriculture, urban, and natural or semi-natural habitats. Agricultural uses include both crop and animal agriculture. Crop agriculture is predominantly orchards and vineyards, with some nursery and truck crops. Animal agriculture is predominately beef and dairy production but does include some other operations such as poultry. Indirect impacts from agriculture include but are not limited to increased erosion caused by grazing, excess fertilizer changing the biogeochemistry of receiving waters, pesticides eliminating pollinators with adverse impacts to riparian communities, and increased invasibility of aquatic ecosystems due to excess nutrients in waterways, Urban land uses include low and high density residential and industrial; potential impacts to waterbodies include effluent from sewered and non-sewered (i.e., septic) wastewater treatment. Natural or semi-natural areas are commonly adjacent to highly urbanized areas and can be impacted by human uses (e.g., recreation, illegal dumping, encampments, etc.).

Previous studies have clearly shown relationships between urban, suburban, and exurban development and elevated FIBs. Such studies typically use measures of impervious surface (e.g., asphalt) or conversely measures of natural cover (e.g., forest) estimated by land cover data within a geographical information system (GIS) (Schoonover & Lockaby 2006).

B.3 Russian River Water Quality

Water quality analysis on the Russian River is an ongoing process that has been and is presently conducted by the North Coast Regional Water Quality Control Board, the Sonoma County Water Agency (SCWA), the Community Clean Water Institute (CCWI), Guerneville Wastewater Treatment Plant, among many others. These samples have been collected since the early 1980's and include water quality (pH, EC, etc.) and pathogen detection (*E. coli*, *Enterococcus*, etc.). Sampling occurs year round but is focused on the summer months when human beneficial uses, principally contact recreation, are at their highest.

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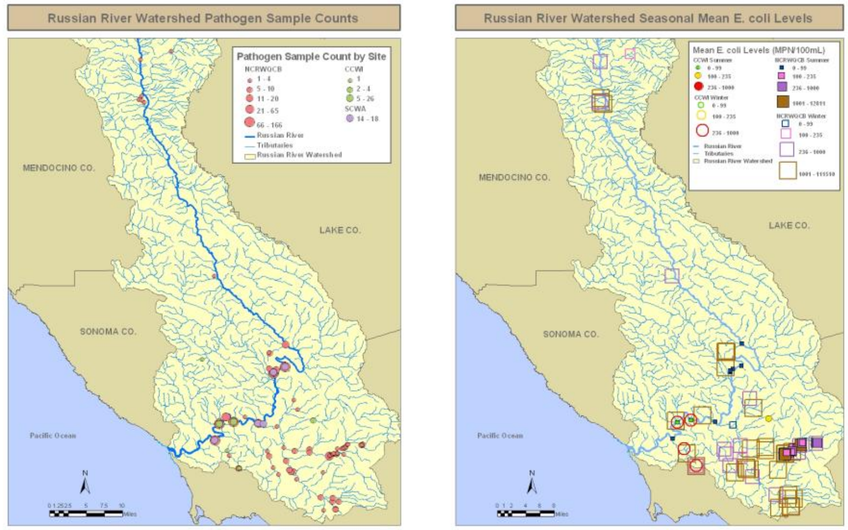
[3] Milwaukee Riverkeeper (http://www.milwaukeeriverkeeper.org/content/bacteria-testing)

[4] Waterkeeper Magazine Winter 2007 (http://www.waterkeeper.org/)

These sampling efforts are distributed throughout the entire watershed and include the mainstem and many of the major tributaries.

All available monitoring data in electronic form were assembled and compiled into an MS Access Database[5] in 2007 by UC Davis, under contract with the Regional Board. Subsequent monitoring data have been added as available.



A B

Figure 1. A) Number of sampling events to date at sites throughout the watershed. B) Mean *E. coli* concentrations for all sampling events.

B.3.1 Bacterial indicators used

In addition to fecal coliforms used in Basin Plan objectives, the fecal bacteria currently used to indicate fecal matter input into the Russian River are *Escherichia coli* (more commonly used) and *Enterococcus* spp. (less commonly used). These fecal bacteria are also the most commonly used around the world, though there have been several studies that point to problems with using these bacteria as generally reliable indicators of fecal input. In a nearby watershed (Tomales Bay), investigators have also used *Giardia* (Miller et al., 2007) and *Cryptosporidium* (Miller et al., 2008) to track fecal matter inputs to natural waterways from confined animal operations. To date, there has not been an evaluation within the watershed of when and where certain fecal bacteria genera and species should be used as indicators. Instead, the assumption has been that the two main indicators will be sufficient for all regulatory, public safety, and remediation needs.

[5] http://rrpp.ice.ucdavis.edu/

B.3.2 Spatial distribution of sampling and exceedance

B.3.2.a Watershed distribution

Samples have historically been taken by multiple entities along the main-stem of the Russian River and more widely across the lower/south-eastern watershed (figure 1). The sampling has been for multiple programmatic needs and has not necessarily been coordinated. Although widespread in the lower watershed, sampling intensity (figure 1A) has not corresponded to measured *E. coli* concentrations (figure 1B). For certain sub-watersheds, *E. coli* concentrations have been historically measured to be high, but this has not resulted in more sampling in the sub-watersheds. Land-uses (e.g., urban, agriculture) are distributed heterogeneously across the watershed, with most human uses in the lower watershed and most sampling sites in these areas.

B.3.2.b Waterway distribution

Once bacteria and other fecal organisms enter the environment they may survive and even grow in media that have the right physical and chemical conditions. These media include benthic sediments, benthic periphyton (e.g., attached filamentous green algae), and riverbank/floodplain soils where survival rates may be greater than in the water column (Sherer et al., 1992; Burton et al., 1987; Thomann and Mueller, 1987; Whitman et al., 2003; Hoyer et al., 2006; Sampson et al., 2006; Whitman et al., 2006). In waterways near and including the Russian River, investigators have found that fecal bacteria, including *E. coli*, appear to be deposited during wet season flows, along with fine sediments (Atwill et al., 2007). Pathogenic organisms, including indicator bacteria like *E. coli* may have increased survival times if they are protected from sunlight and extreme temperatures, for example when attached to algae within thick mats (Whitman et al., 2003). Enteric and pathogenic bacteria and viruses may survive for months in benthic sediments, increasing the chance of resuspension and health impacts

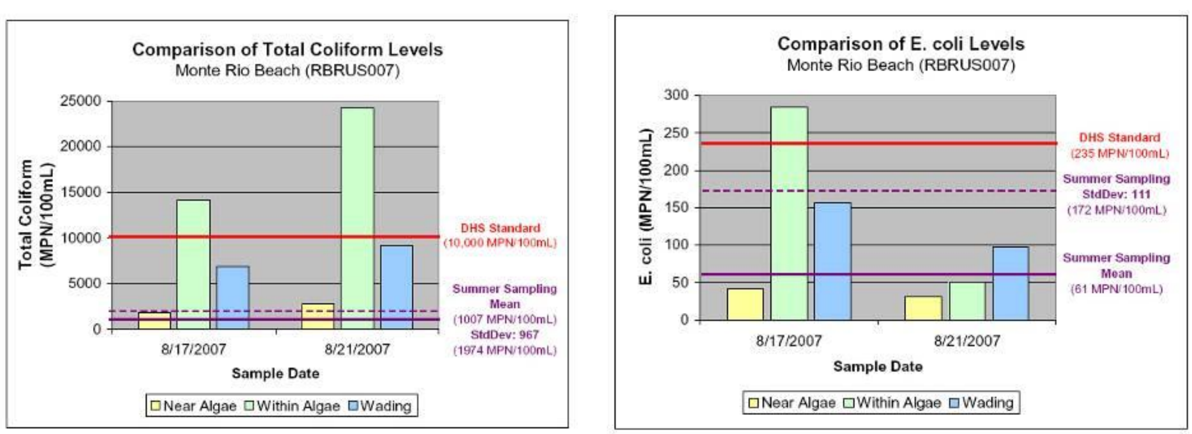


Figure 2. Comparison of *E. coli* concentrations among water column and algal compartments at a recreational beach.

(Burton et al., 1987; Craig et al., 2004; LaBelle and Gerba, 1980; Roper and Marshall, 1979; and Sherer et al., 1992). In some cases, resuspension of sediments can result in higher measured concentrations of fecal bacteria than municipal outfalls to recreational beach areas (Noble et al., 2006). These bacteria can become health threats to people through direct contact with water, *E. coli* contaminated fish (Hansen et al., 2008), and shellfish contamination.

E. coli and *Enterococcus* have been found to be associated with *Cladophora sp*. in surface waters and beach sands (Whitman et al., 2003). These bacteria can survive 6 months at 4°C in dried algae, suggesting that these algae are an important secondary source of fecal bacteria.

In the Russian River, *E. coli* were found associated with live benthic algae and benthic sediments at recreational beaches during the summer (Figure 2). Although this association was not characterized fully, it is consistent with associations reported in the scientific literature.

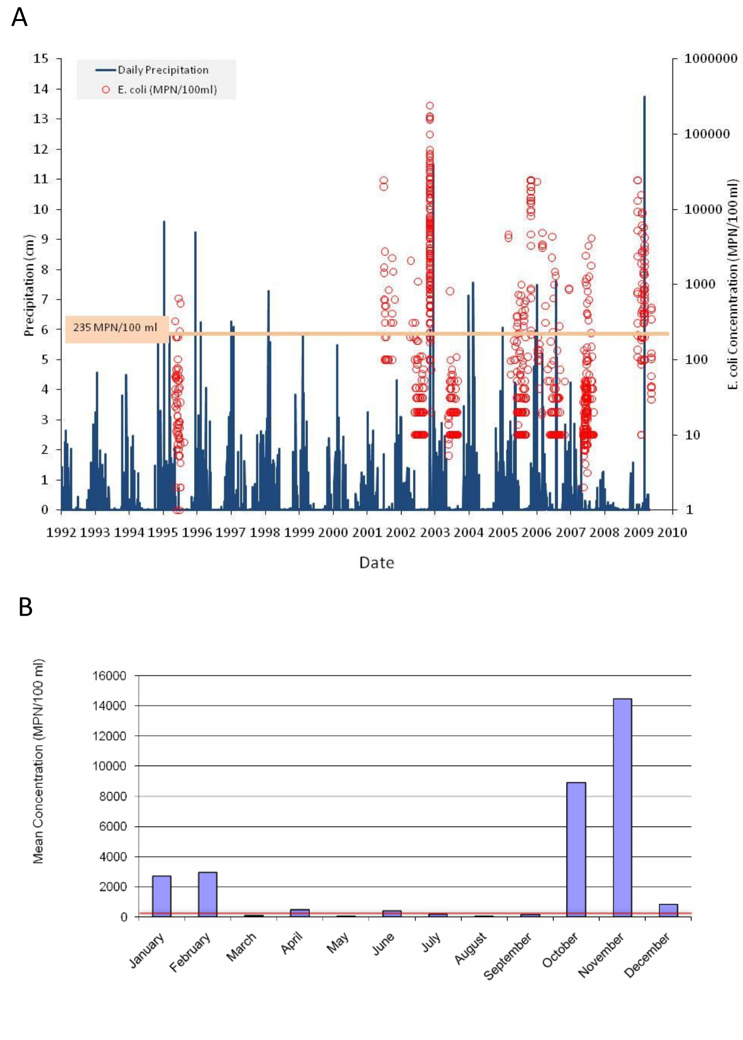


Figure 3. *E. coli* monitoring at all sites throughout the watershed. A) All *E. coli* concentrations found in the watershed. B) Monthly average *E. coli* concentration for all sites in the watershed. The red line corresponds roughly to the legal threshold for *E. coli* in surface waters (235 MPN/100 ml).

B.3.3 Temporal trends of sampling and exceedance

E. coli sampling has tended to be concentrated around certain storm events, or summer sampling periods (figure 3A). There have also been relatively long intervals without sampling (e.g., 1995, not shown, to 2001), possible due to funding constraints. The highest concentrations have been during the early parts of the storm season (e.g., October and November) when the first flush storms occur (figure 3B). The sampling has been a combination of periodic (weekly) and event sampling, which each have different goals. Periodic sampling is intended to capture condition at regular intervals to evaluate general condition and to alert water quality managers to exceedances. Event sampling has been conducted historically because, storms tend to cause elevated fecal bacteria concentrations in streams, especially the first storms of a season. These different goals result in essentially two different, but effectively comparable data sets. They complement, but do not replace each other.

B.4 Current Strategies / Recommendations for Improvement

B.4.1 Frequency / Timing

Current sampling strategies are unlikely to capture certain aspects of fecal bacteria input. Although bacteria concentrations tend to be lowest in the late Spring and Summer, this is also the time period of primary concern for human contact (REC1 and 2). Regular sampling (e.g., biweekly or monthly) may reveal spatial or temporal trends over many years, but is unlikely to be helpful in determining when and where fecal bacteria inputs are occurring. Previous monitoring reveals that most inputs are occurring on two primary occasions: the first large storms of the rainy season (fall) and large storms later in the season when the landscape is saturated, runoff from agricultural areas is likely and septic systems may fail. Storm sampling frequency should occur during all three hydrologic phases of a typical winter storm (rising, peak, and falling). Because the most critical inputs may be during storms, sampling for source detection should also occur during storms.

An important question is: How often should sampling occur for exceedance and source detection to be detectable? Exceedances (>235 MPN/100 ml) detected through single grab samples are common during the rainy season for most sub-watersheds with agriculture or urban land-uses. During the summer, exceedances are rare, though potentially more important because of human recreation. Lab processing delay times may be the most important factor in determining timing of sampling. For example, if a commercial lab takes 2 days to process samples (from sample delivery to reporting values), then Wednesday sampling allows for warnings to take place prior to weekends when recreation is likeliest to be greatest. Because of variation during the summer and the frequency of exceedances (at least monthly), weekly sampling is the minimum frequency recommended. However, little is known about the actual quality of waters during the weekend period when exposure is highest, due to contracted lab closure. Thus, to move beyond the minimum frequency it is necessary to establish background rates of exceedance over long time periods, which is mostly well established for recreational beaches in the lower Russian River. It is also necessary to increase both the rate of frequency and spatial density of samples taken to determine hydrologic pathways of impairment. Lastly, monitoring must also cover more episodic events, such as storm events, late winter pulses, and importantly holiday weekends that increase likelihood of exposure and detrimental health outcomes.

B.4.4 Bacterial Indicators

Current fecal bacteria indicators commonly used in the Russian River watershed are fecal coliform, *E. coli*, and *Enterococcus* spp. These indicators meet legal standards, but are not suitable for rapid detection of sewage releases and land-surface source analysis. In order to meet the separate needs for source detection (spatial), animal types, and timing of impact, sampling should be conducted for *E. coli*, *Enterococcus*, and *Bacteroides* spp. *Bacteroides* spp. has been proposed and used as an indicator of recent fecal matter input (Kiksdal, 1985; Bernard et al., 2003). Recent research has shown that survival of this genus of enteric bacteria varies from 2 days to 2 weeks, depending on temperature and the presence of protozoan grazers (Bell et al., 2009).

E. coli may survive for months after introduction into the environment (e.g., > 6 months on Lake Michigan beaches; Whitman et al. 2003), so does not make an ideal indicator of recent fecal material input. Other bacteria, such as *Bacteroides* spp. may make better indicators of recent fecal material input. Both *E. coli* and *Bacteroides* strains specific to particular vertebrate hosts may be identified based on their DNA. Anderson et al. (2005) found that bacteria from different host organisms may survive in the environment at different rates, complicating the interpretation of linking strains found in the environmental samples with specific vertebrate hosts. This problem can be addressed by also sampling and identifying strains of bacteria with very short survival rates in the environment (e.g., *Bacteroides* spp.). A combination of *E. coli*, *Enterococcus* spp., and *Bacteroides* spp. sampling and identification may provide answers to multiple questions about long-term fecal matter loading, host organisms, and recent fecal matter inputs. Ideally, quantitative and/or qualitative DNA finger-printing (i.e., real time quantitative polymerase chain reaction) would be used for strain identification and Colilert/Enterolert types of tests for measuring concentrations of *E. coli* and *Enterococcus* spp., respectively. In addition, using genera that have close associations with the problem being investigated (e.g., *Cryptosporidium* or *Giardia* for confined animal discharges) will ensure that the fecal inputs are indicated appropriately.

B.4.5 Water Quality Surrogates (N&O isotope ratios)

As described in Cabana and Rasmussen (1996) (Figure 4), human populations are strongly correlated with elevated δ15N (a measure of the ratio between two isotopically stable forms of N and a standard) due to concentration of the heavy N isotopes in human diet (and other high trophic consumers). When combined with the δ18O isotopic signature from nitrate, as described in (Kendall & McDonnell 1998), the

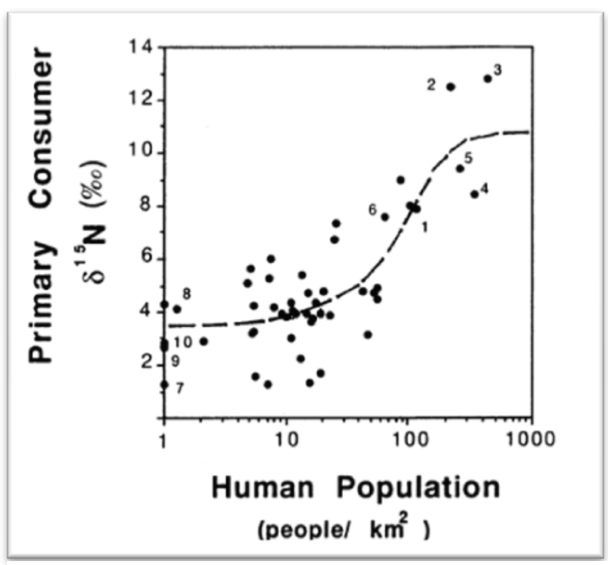


Figure 4 Relationships between elevated 15N and human population (taken from Cabana & Rasmussen 1996)

separation of sources of NO_3- become evident. Using such natural abundance stable isotopic ratios in biota and sediments provide a useful indicator for tracing the spatial and temporal impacts of sewage-derived nitrogen in aquatic ecosystems (Savage 2005). The use of $\delta^{15}N$ analyses to track sewage was particularly effective in the Savage (2005) study as the wastewater had an isotopically distinct $\delta^{15}N$ signature relative to marine $\delta^{15}N$ values and because sewage N dominated N inputs to the bay for several years. Because nitrogen stable isotope ratios can be sampled for contemporaneous inputs, the potential is there to map sewage inputs using the anthropogenic nitrogen signal over watershed space and time. Floodplain and bank sediments could be mined for information about recent and older deposits, while regular and storm event sampling could provide information about more immediate inputs.

Similarly, Steffy & Kilham (2004) examined the applicability of stable-isotope analysis as an indicator of anthropogenically based inputs of excess N in aquatic systems. They found marked difference in the d15N at all trophic levels in food webs in between areas that used septic tank systems and those areas that were connected to public sewers. Stream sites located in areas of septic systems had higher d15N values in each trophic level by as much as 10‰, and concluded that improperly functioning septic systems were contributing large amounts of N carrying the 15N signature of human sewage. Agricultural and urban land uses have been repeatedly shown to be primary contributors of NO_3- as determined by stable isotope analysis of δ15N and δ18O (see Figure 5; Mayer et al. 2002).

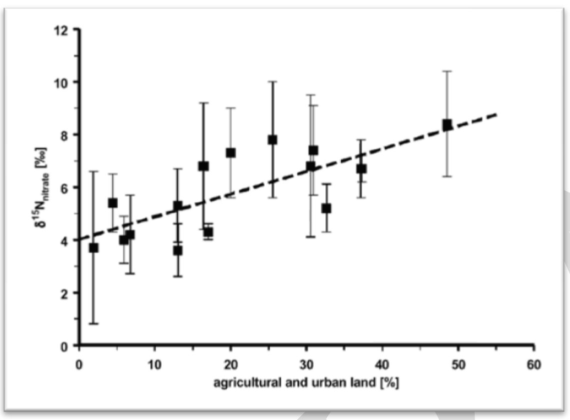


Figure 5 Mean δ15N ratios of riverine nitrate as a function of agricultural and urban land uses (taken from Mayer et al. 2002)

C Strategies

C.1 Bacterial Quantification Strategy

A critical function of fecal indicator bacteria is that they both allow tracking of fecal matter inputs to waterways and reliably indicate when potentially pathogenic organisms that are transmitted in feces may be present in recreational or drinking waters, posing a public health risk. Recently, there have been many studies examining this question, with the primary finding being that “it depends” whether or not this critical function is fulfilled by fecal indicator bacteria. In one study of coastal Southern California (Noble and Fuhrman, 2001), concentrations of regularly monitored fecal indicator bacteria were not significantly related to the presence of enteroviruses. The investigators did find that when suites of bacterial indicators were used, a weakly predictive relationship was found with enteric viral pathogens.

However, there was still the problem that these viral pathogens could be present in contact recreation waters when fecal bacteria concentrations were low. This cautionary note is intended to remind investigators of fecal pathogens that there is currently no silver bullet strategy to safely and reliably indicate water quality for recreational and consumptive uses. Rather, a suite of approaches is needed to both determine sources and loading of fecal microorganisms and to identify waterways and ground waters with impaired uses.

The primary strategy that we recommend here is one of triangulation. Unless known pathogenic microorganisms are being directly identified and quantified in environmental samples, the monitoring program should use multiple fecal indicator bacteria and other animal waste indicators in source tracking and load quantification, as well as to determine if regulatory thresholds are being exceeded. We describe most of the commonly-used fecal indicator bacteria, but new research may provide tools to rapidly and accurately measure presence and quantity of pathogenic microorganisms. We don't suggest abandoning these commonly-used bacteria. We do recommend that their role be recognized as individually indicative of different aspects of fecal matter input across varying time frames and of potentially different source types.

C.2 Bacterial Identification Strategy

Bacteria are a normal and natural part of all terrestrial and aquatic ecosystems and perform a vital decomposition function by mineralizing and cycling nutrients through the ecosystem. In many instances, they also provide a food source for small invertebrates and form the basis for some food webs. There may be other species of wildlife that contribute intestinal bacteria to the environment including (but not limited to) waterfowl (ducks and geese), raccoons, otters, ground squirrels, and in some locations deer. In addition, there may be companion animal (dogs and cats primarily) that could contribute intestinal bacteria to the environment. Consequently, there are expected to be many species and large numbers of bacteria in any environmental sample that is collected.

Typically, monitoring methods used for detecting potential pathogenic microorganisms in environmental waters are based upon cultivation and enumeration of fecal indicator bacteria (i.e. fecal coliforms, *E. coli*, and fecal *Enterococci*). Currently, there is increasing interest in the potential for molecular fingerprinting methods to be used not only for detection but also for identification of fecal contamination sources (see Simpson et al., 2002). Molecular methods have been applied-to study the microbial ecology of environmental systems for years and are now being applied to help improve our waters by identifying problem sources and determining the effect of implemented remedial solutions. Management and remediation of water pollution would be more cost-effective if the correct sources could be identified. We provide here an outline of the main methods that either have been used or have been suggested for use in microbial source tracking and some of the limitations associated with those methods.

As discussed in a recent review, Field & Samadpour (2007), describe microbial source tracking (MST) as one approach that can be used to identify the sources and level of pathogen impairment in watersheds, and has been used in for TMDL determinations. Recent advances have allowed for greater discrimination between microbial strains by using universal genetic makers (Figure 6). This approach has all of the basic elements for determining the effectiveness of Best Management Practices (BMP) implemented in a watershed in reducing non-indigenous pathogens entering and exiting a BMP implementation zone, thus allowing managers to calculate effectiveness for a particular set of circumstances (waterways (see Smith & and Perdek, 2004).

Method	Advantage(s)	Disadvantage(s)
Fecal coliform/fecal streptococous ratio	Easy to perform; may be useful for recent contamination	Variable survival rates of fecal streptococci can alter ratio
Bifidobacterium sp.	Sorbitol fermenters may be human specific	Low numbers present in environment; variable survival rates; culture methods not well-defined
B. fragilis HSP40 bacteriophage	Very human specific; easy to perform	Not present in sewage in some areas
F+ RNA bacteriophage	Groups are well-correlated with source; easy to perform	Unreliable in marine and tropical waters due to variable survival rates
Human enteric virus	Human specific; Direct monitoring for pathogen circumvents need to use indicators	Low numbers in environment; labor-intensive; more sensitive methods needed
MAR	Rapid; can be used to discriminate isolates from multiple animal sources	Requires reference database; may be geographically specific; isolates that show no antibiotic resistance cannot be typed
PFGE	Extremely sensitive to minute genetic differences	May be too sensitive to broadly discriminate for source tracking
BOX-PCR	Rapid; easy to perform	Reproducibility a concern; reference database required; may be geographically specific
Ribotyping	Highly reproducible; some methods useful for classifying isolates from multiple sources	Labor-intensive; reference database required; may be geographically specific; variations in methodology exist
Bacteroides-Prevotella molecular marker	Does not require culturing of organism; PCR method is rapid, easy to perform	Little is known about survival and distribution in water systems; currently not applicable to all animals
Caffeine	Useful for assessing impact from human sewage	Minute quantities in the environment make sensitivity an issue; requires expensive analyses
Fecal sterols and/or stanols	Some sterols/stanols have greater specificity for humans and/or animals	Present naturally in sediments; requires expensive analyses; Low prevalence makes sensitivity an issue

Figure 6 Advantages and Disadvantages of microbial source tracking methods (taken from Scott *et al.* 2002)

The pilot study that was part of this study focused in part on identifying bacteria from the genus *Bacteroides*. These bacteria are found as a normal component of the intestinal fauna of all warm-blooded animals and are voided in feces. It is estimated that these bacteria compose as much as 50% of the fecal material produced by animals (Lamendella et al. 2006). These bacteria are not *E. coli* nor are they related to *E. coli*. *Bacteroides* and *E. coli* both inhabit the intestinal tracts of animals and are voided together in fecal material. A major difference between the two is that *Bacteroides* are obligately anaerobic, i.e. they cannot live for any length of time in the presence of oxygen and they cannot reproduce in the presence of oxygen. Field et al. (2003) in a recent review indicated that *Bacteroides* can persist up to 14 days at temperatures of 4^{o}C and only 4-5 days at temperatures of 14^{o}C.

E. coli are not obligately anaerobic and may persist in the presence of oxygen in the environment for long periods of time after being voided. They are also known to reproduce in the environment and can be mobilized from the sediment into the water column (see review in Field et al. 2003). The conditions under which these processes occur are not well understood and will require additional research. However, detection of *E. coli* indicates that fecal contamination may have occurred in the past, but the contamination may have occurred weeks or even months prior to sampling. Detection of *Bacteroides* indicates recent (days) contamination by fecal material. Consequently, one of the objectives of the pilot project was to examine the relationship between the presence of *E. coli* and *Bacteroides* in the samples.

C.4 Temporal Trends Strategy

Previous investigations have revealed that antecedent precipitation is correlated with increased surface water concentrations of fecal bacteria and protozoa in agricultural and rural-urban watersheds in California and elsewhere (Sinclair et al., 2009; Miller et al., 2008; Miller et al., 2007; Lewis et al., 2005). Other correlative factors include surface water flows, presence of animal agriculture, time since last rain, overall intensity of rainy season, and suspended sediment concentrations. These relationships suggest that for mixed land-use watersheds, the occurrences and magnitudes of fecal bacteria mobilizations and violations of water quality standards depends in part on early wet season and overall wet season precipitation. In addition, different mobilization and transport pathways may exist for different fecal indicator micro-organisms (e.g., *E. coli* vs. *Enterococcus* spp.), suggesting that each may have unique causative factors and appropriate sampling regimes.

Historical values for fecal indicator bacteria *E. coli* and *Enterococcus* spp. concentrations, collected by the Regional Board and others, were compared with the month samples were taken and antecedent precipitation

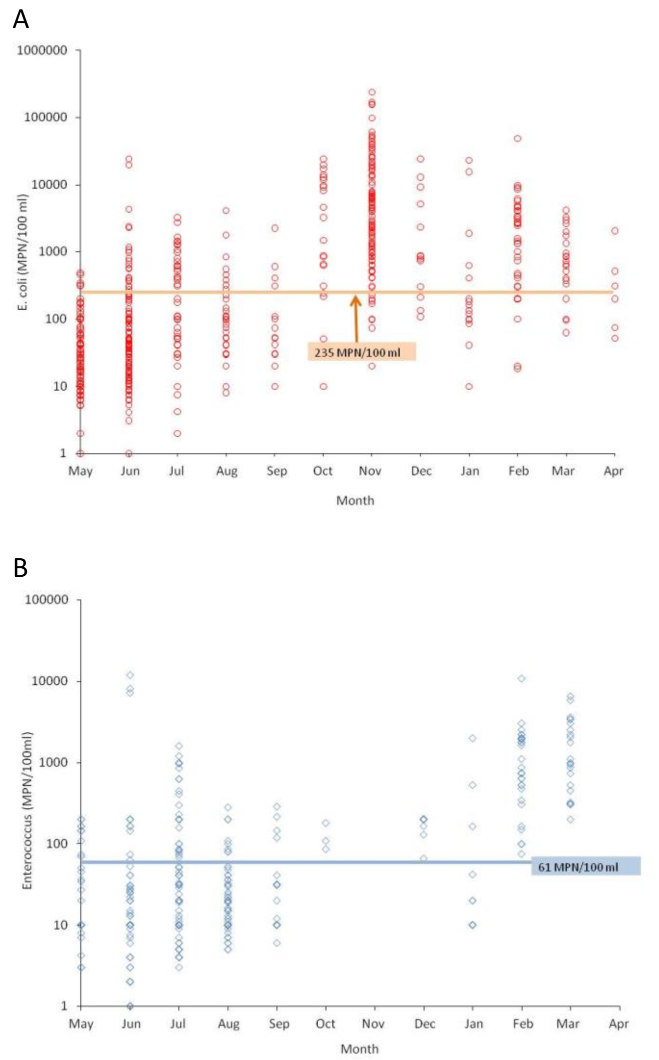


Figure 7Range of *E. coli* (A) and *Enterococcus* (B) concentrations during each month for samples collected between 1995 and 2009.

conditions, to determine if wet season rains results in greater concentrations of these bacteria in surface water. In general, *E. coli* and *Enterococcus* concentrations were higher in the wet season (Figure), though the patterns were different for each type of bacteria. Bacteria concentrations were also compared with the amount of precipitation that had fallen prior to sampling to investigate possible relationships.

Monthly E. coli and Enterococcus Concentrations

E. coli and *Enterococcus* spp. are often assumed to indicate the presence of fecal inputs in the same way. However, we found that *E. coli* concentrations tended to be highest during the first storms of the year and *Enterococcus* concentrations were highest during spring storms and not during the first storms (Figure 7A,B). This difference suggests that either these two bacteria types are indicating different fecal input types (septic vs. confined animal), or different bacterial sources (fresh sewage vs. naturalized), or a combination of the two. The explanation most consistent with the literature is that 1) *E. coli* concentrations are highest during early storms because the sources are on the surface of the landscape and easily mobilized into streams (e.g., from paved areas), and/or the sources are in-stream already (e.g., in algal mats) and are mobilized by the disturbance of the first rains. 2) *Enterococcus* concentrations are highest later in the wet season because these bacteria are leaching into surface waters from ground-water and saturated soil sources, including flooded septic systems and overwhelmed surface ponds and treatment facilities. If these two fecal indicator bacteria are to remain the foundation of source tracking, health-related monitoring, and management action effectiveness, then differences between the responses of these bacteria to the wet season should be determined.

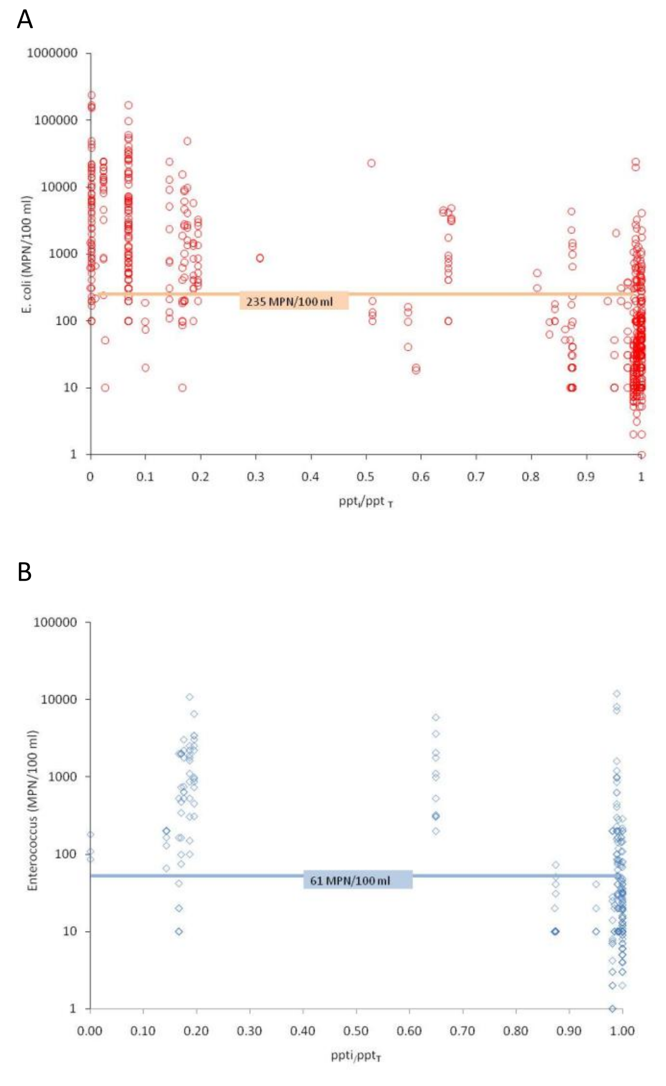


Figure 8A) *E. coli* concentrations compared to the corresponding proportion of precipitation that had fallen by the time each sample had been taken. B) *Enterococcus* concentrations compared to the corresponding proportion of precipitation that had fallen by the time each sample had been taken.

Antecedent Rainfall and Bacteria Concentrations

The amount of rainfall may determine the release of fecal bacteria into waterways through surface runoff, overwhelmed sewage systems, and disturbance of in-stream bacteria sources. We compared bacteria concentrations to antecedent rainfall to determine if there was a correlation between the two (Figure 8). In general, both *E. coli* and *Enterococcus* concentrations declined with increasing antecedent precipitation during the water year (beginning October 1), which roughly reflects the time between the least rainfall at the beginning of the water year, through summer, when the most rainfall has preceded. The line on each graph shows the regulatory threshold concentrations for each fecal indicator bacteria type.

Many sites within the Russian River watershed have been sampled in the last 15 years, but they can be quite different from each other and these differences may affect the appearance of the graphs. In order to determine how concentrations of each fecal indicator bacteria is responding to storms during the wet season, it would be necessary to sample multiple characteristic sites within the watershed during multiple storms over several years. This has not been accomplished to date, but is critically-needed.

C.4.1 Detecting change from sample to sample

Bacterial sampling is typically conducted through grab samples collected during a periodic or event sampling. Generally, one grab sample is collected at a specific location and the measured concentration of fecal bacteria in the sample is considered to represent the actual water-body concentration of bacteria. Unfortunately, this is not often the case. For example, during the rainy season sampling conducted by UC Davis (2008-09), the average difference between duplicate *Enterococcus* spp. samples was 63%, with a range of 0% to 250%. Although, there were no statistical differences across orders of magnitude ($p=0.47$; $n=11$). Single or duplicate sampling of water-bodies for contaminant concentrations is a common strategy and is even recommended by state and federal agencies. This strategy fails to do two things – accurately reflect bacterial concentrations in the water-body at the time the sample is taken and permit differences to be detected over time, space, or before and after management activities are conducted to improve water quality. Two samples will always be different, but the observer will not be confident in which is more likely to be the accurate measurement.

To detect change and accurately measure conditions, at least 3 samples should be taken and preferably more. This minimum number allows a central tendency (mean or median) to be calculated, which is more likely to reflect the actual concentration. The range among $\geq$3 samples also allows the analyst to determine if more samples are needed. For example, if with 3 samples, the concentration range is two-fold and spans the legal threshold, then more samples will need to be taken to determine whether or not the threshold has been statistically crossed. For comparisons among multiple events/days, $\geq$3 samples allows differences to be accurately determined based on comparison among central tendencies. Single or duplicate samples usually cannot be used to compare among pairs of sites or pairs of days and for most monitoring comparisons because the analyst cannot determine how likely it is that the values represent the "true" concentration of fecal bacteria.

As described in D.3 below, trends over long time periods are usually best determined using formal trends analysis techniques. However, with sufficient number of samples per sampling event (i.e., $\geq$3), changes over short time frames, such as during a storm, can be detected. This is important if these changes are of immediate regulatory or public safety concern.

C.4.2 Detecting change over first flush and seasons

Previous research has found that peaks in *E. coli* concentrations can depend on the type of waterway (position in the watershed) and antecedent rainfall (Gentry et al., 2006). In the present study, we found that historically, the earliest storms (in October and November), even the small ones, result in high concentrations of fecal indicator bacteria (Figure 7). The increase appeared to be so sensitive to the "first flush" that we were not able to detect and characterize the rising curve of *E. coli* concentration with precipitation (Figures 7,8), with the caveat that the data were not collected for that purpose. Both graphs (Figures 7A and 8A) also indicate that the primary increase in *E. coli* concentrations occur in the fall and early winter. After these early storms, there is a tendency for gradually reduced concentrations through the remainder of the winter and through the spring.

For *Enterococcus*, this sensitivity was not as apparent, with increases in concentration appearing to take place as the wet season wears on (Figures 7B), but no apparent relationship to proportion of annual rainfall (Figure 8B). However, there were insufficient data for early storms to be sure one way or the other about the relationship between first flush and this indicator genus.

One of the primary gaps in monitoring to date is characterization of storm-caused increases in fecal indicator bacteria. Storm event-related sampling should include investigations at headwater reaches on tributaries, tributary mainstems, and river mainstem sites. These investigations would include sampling before, during, and after storms for several days to determine when peak concentrations and loads of fecal bacteria occur. This combination of spatial and temporal thoroughness should provide sufficient information about the importance of the first flush in generating the very high concentrations of *E. coli* in the early rainy season and possible sources of material.

C.4.3 Detecting change over water year(s) – seasonal trends analysis

A critical need in water quality is to be able to detect changes in specific parameters over years, while correcting for seasonal dependence of the parameters. Many changes in aquatic conditions are linked to annual seasons, through the water cycle, seasonal changes in land-use, and change in allochthonous and autochthonous production. The Seasonal Kendall test (Kendall, 1975; Hirsch et al., 1982) is a non-parametric test for inter-annual monotonic trend in environmental data, while correcting for seasonality. The test makes comparisons among years, by comparing data from similar seasons. The null hypothesis for the test is that there is no change over time. Pair-wise comparisons are made of water quality values over time. Values later in time, that are larger than values earlier in time are recorded as pluses, values later in time that are smaller than values earlier in time are recorded as minuses. The test statistic is the difference between the sum of pluses and minuses, where positive value indicates an increase in the parameter over time. The Seasonal Kendall test statistic is calculated as a summation of the Mann-Kendall test statistics from each seasonal period (Hirsch et al., 1982). The probability of rejecting the null hypothesis (no change over time), p, is also calculated. The total variance of the Seasonal Kendall test statistic is estimated as the sum of the seasonal estimates of variance. Statistical significance, which is obtained from a standard normal distribution, is reported for the standardized Seasonal Kendall test statistic (Hirsch et al., 1982).

The utility of this approach in the Russian River and specifically for the Russian River Pathogen TMDL is both in detecting proximate causes of heavy loading (e.g., natural precipitation cycles) and in detecting change following remedial action. It is unlikely that the Regional Board will be able to state with any certainty how effective remedial actions are without carrying out formal trends analysis. A core strategy should be to collect data in such a manner as to permit trends analysis to be carried out in order to measure programmatic effectiveness.

C.4.4 Detecting change in winter/spring to anticipate summer trends

Wet season tributary and mainstem base-flow may have lower fecal bacterial concentrations than during and after storms, but may still represent a significant proportion of wet-season transport of fecal bacteria. In addition, as storm flows subside, fecal-bacteria-containing sediments may be deposited in-stream or on banks and floodplains and function as reservoirs for fecal bacteria later in the year. In other words, differences in storm and base flows may represent deposition (as well as death and disintegration) of fecal bacteria within the system. Wet season base-flow sampling should be regular (weekly) and distributed in a way to capture transport from potential source areas to potential deposition areas.

C.5 Aquatic Compartment Strategy

Previous research has demonstrated that fecal bacteria can become associated with various compartments of waterways and the adjacent floodplain. These compartments are: benthic sediment, benthic periphyton, water column, floodplain/bank sediment, floodplain and ground-water, and remnant riparian ponds. The strategy proposed here extends traditional water column monitoring to include these other compartments of aquatic ecosystems.

C.5.1 Partitioning the compartments

The compartments proposed for investigation are distinct, but interacting parts of the aquatic ecosystem. Although the compartments can be sampled separately, it should be kept in mind that they do interact with each other in different ways at different times of the year. The primary interactions are listed here:

1) As Winter and Spring rains cease and creek and river runoff decline, suspended material will tend to leave the water column and settle to the benthos and/or floodplain. Settlement rates depend on particle size and water velocity. Bacterial and other microbial particles may be associated with larger particles and settle faster than when alone. As Spring turns into Summer, water column loads of suspended fecal microbes will settle to the benthos and remain there until they die and decompose or become resuspended. Sediments settled in the benthos and floodplain during the last wet season should be sampled for fecal bacteria that could either function as a reservoir that interferes with summer season sampling or pose a health risk to people.

2) Bacteria and other microbes can adhere to suspended or benthic particles. In the benthos, plants (periphyton), wood, and sediment particles provide many surfaces on which to stick. As periphyton (primarily algae) grows from Spring into the Summer, it provides both an elaborate set of surfaces and a potential food source for live bacteria. Fecal microbes may leave the water column by adhering to growing algae, and may themselves grow and multiply given sufficient food and warm enough temperatures. These bacteria may be released as the algae is disturbed or dies. Attached or floating periphyton should be sampled for attached fecal bacteria that could either function as a reservoir that interferes with summer season sampling or pose a health risk to people.

3) As wet season rains start again in the late Fall, fecal bacterial loads in the water column may originate from the land through surface runoff and from the benthos through resuspension. Although agricultural waste and septic systems may provide high concentrations in runoff from the land, it is possible that resuspension of the benthos in certain areas can also provide its own contribution. Monitoring should be conducted that differentiates between resuspension of benthic bacteria and new inputs.

C.5.2 Water column

Sampling depth in the water column can determine the relative concentrations measured at a site (Kleinheinz et al., 2006). Because fecal bacteria concentrations in the water column above undisturbed sediments tend to be highest near the surface, grab samples should be taken consistently within the top few inches of water in order to capture the highest occurring concentrations at a site.

C.5.3 Benthic sediment

Fine benthic sediment (mud, silt, sand) can act as a reservoir for fecal bacteria. One scenario that is likely in the Russian River is that winter and spring rains and runoff mobilize fecal bacteria on the landscape and in smaller tributaries. These bacteria may be free-living or attached to sediment and organic particles. As the hydrograph declines, suspended inorganic and organic sediment will be deposited in slower moving waters in the river and its tributaries. The fecal bacteria thus become part of the benthic microbial community until they are disturbed and re-distributed, or die/decay. Population decay rates vary, but have been reported in situ and in laboratory experiments as being dependent on ambient temperature and sediment characteristics (e.g., Craig et al., 2004).

Our approach to understanding the source-transport, deposition, and survival of benthic fecal bacteria consists of two parts. The first is to sample fecal bacteria attached to suspended sediment during Spring storm sampling events. The second is to sample benthic sediment-attached fecal bacteria (BSAFB) as the hydrograph declines going into the early summer. This will be accomplished by sampling in-stream sediments at one site that is downstream of reaches and tributaries that were found to be high in fecal bacteria during rainy season storm events. To account for variability, sampling will be done in transects across the waterway and along a depositional reach. Simultaneous bacterial samples will be taken from the water column. Both quantitative and strain identification measurements will be conducted.

This approach will provide us with information about the relative impact of sediment-bound bacteria in the water column and the benthos, compared with free-living fecal bacteria in the water column. It will inform us about the animal sources of fecal bacteria found in the sediment. Finally, it will tell us about spatial variability in benthic sediment fecal bacterial concentrations.

C.5.4 Bank and floodplain

Most California waterways flood at some point and interact with their floodplain. This interaction includes deposition of sediment, which may stay deposited for months or years, or may be resuspended and re-distributed in shorter time intervals. Fecal bacteria have been found in terrestrial sediments near waterways (beaches, river-banks) that are periodically inundated. These bacteria are sometimes replenished regularly and in some cases become naturalized and grow as part of the microbial community. There are reported cases of the naturalized fecal bacteria growing in shore sand and being the most likely source of the bacteria detected at the site (Kon et al 2007), making simultaneous sampling of surface and pore water, bank, and benthic sediments all the more important.

For beach and shore sediments and potentially for different points across still water, fecal bacteria concentrations can vary depending on where the sample is taken (Kleinheinz et al., 2006). To account for this, sites where beach/shore sediments are to be sampled, or where water is relatively still, samples should be taken in a transect across the site. Byappanahal et al (2003) found that *E. coli* concentrations peak within the first 2 m of the water's edge, then declined with distance, roughly correlated with soil moisture. They also found that benthic sediment and floodplain sediments had much higher *E. coli* densities than waterways or sols outside of the floodplain.

Our proposed strategy is to sample the floodplain from the edge of the waterway to the approximate outer edge of the regularly-inundated floodplain.

C.5.5 Benthic periphyton

Many studies have shown that bacteria can reversibly attach to living and dead periphyton/algae in various waterbodies. Because algae can provide food to the bacteria, when other environmental conditions are right, these attached bacteria may also grow. Therefore, fecal bacteria may become isolated from the water column and either persist or even grow/multiply in attached or floating algae. The ramifications of this phenomenon for monitoring is that water column sampling may miss this important reservoir of fecal bacteria. The consequences of this phenomenon for human health is that contact with the algae may result in exposure to fecal bacteria and disturbance or disintegration of the algae may result in high concentrations of fecal bacteria in the water.

Our approach to understand the potential impacts of fecal bacteria association with periphyton is to conduct an in-depth investigation of this association as benthic algae grows in the Russian River and its major tributary, Laguna de Santa Rosa. We will carry this investigation out in a place that is downstream from monitoring sites with high water column concentrations of fecal bacteria during early 2009. The rationale for this is that the main source of algal-attached fecal bacteria (AAFB) is the water column. We will sample benthic and floating algae (if occurring) along the river course and in transects at one large riffle and run complex. We will conduct sampling that provides 1) an absolute estimate of AAFB concentration (CFU/g algae and CFU/m^2 benthos), 2) identification of AAFB *E. coli* strains, and 3) spatial variability at the site, among stretches and among individual samples. We will also sample benthic sediment and water column for concentrations and strains of fecal indicator bacteria and measure basic environmental parameters (light and water temperature).

This approach will provide us with information about the relative importance of algal stocks as reservoirs and sources of fecal bacteria (compared with the water column). It will also inform us about the potential animal sources of the AAFB and how these compare to the water column. Finally, it will provide information about spatial variability, which will be useful for future studies of AAFB.

C.6 Sub-watershed Strategy

For the pilot monitoring study that accompanied this monitoring design (see Viers et al., 2009), we considered the following information in determining the classes and distributions of 13 proposed sites:

1) previous sampling intensity (# of sampling events), 2) previously-high *E. coli* concentrations, 3) previously-monitored location, and 4) representation of residential, agricultural, and wild-land sub-watershed land-covers.

1) Sampling counts among the existing tributary sample sites we analyzed closely, ranged from 1 (low intensity) to 49 (high intensity). We selected sites that had this wide range in order to cover places that were consistent wet season problems as well as places that may be problems, but sampling intensity was very low.

2) Previously-measured mean concentrations for the wet season varied among existing tributary sampling sites from 495 MPN/100 ml to >20,000 MPN/100 ml. We selected sites that were well above the WQO and that met other criteria.

3) We tried to select as many previously-monitored sites as possible, but in some cases had to choose new locations that appeared likely to have reasonable stream access.

4) We chose 1 to 3 sites per major land-cover types. We segregated sites by predominant adjacent and upstream/up-watershed land-cover in order to get a measure of the potential relative contributions from these different land-cover types. The land-cover and land-uses categories we considered were (Table 1): High density/urban residential and commercial development, moderate to low density residential development (i.e., suburban), very low density rural-residential development; animal (e.g., dairy and crop agriculture (e.g., vineyards), and semi-natural lands largely composed of forests.

Table 1. Monitoring stations and associated dominant land-uses (see Viers et al., 2009).

Project ID Code	NCRWQCBID	Site Name	Land-use	Notes
114ATASC01	RBATA004	Atascadero Creek at Bodega Hwy	AGRICULTURAL	
114COPE01	RBCOP001	Copeland Creek	URBAN	
114GREEN01	RBATA001	Atascadero Creek at Green Valley Rd	AGRICULTURAL	Mixed ex-urban
114LAGU01	RBLAG005	Laguna de Santa Rosa	AGRICULTURAL	
114LAGU02	114LAGU02	Laguna de Santa Rosa below Santa Rosa Creek	URBAN	Mixed suburban and agriculture
114LGVARR	CCGVC00	Lower Green Valley Creek	AGRICULTURAL	Mixed ex-urban
114MARK01	CCMWC004	Upper Mark West	SEMINATURAL	
114MILL01	CCMIL001	Mill Creek	SEMINATURAL	
114SROSA1	RBSRC004	Prince Memorial Greenway	URBAN	
114VINE01	RBFOS001	Foss Creek	URBAN	
114WEST01	UCDRRPP002	Lower Dry Creek	SEMI-NATURAL	Agricultural site, semi-natural watershed
114WILD01	UCDRRPP001	Upper Santa Rosa Creek	SEMI-NATURAL	Suburban site

D Methods

An important study in the region of the Russian River to consider is one conducted by UC Davis and other scientists for the Regional Board (Atwill et al., 2007). In this study, the authors describe appropriate fermentation and chromogenic techniques for enumerating fecal bacteria. They also describe sampling regime considerations that they measured to impact fecal bacterial concentrations. These included a non-linear dependence on antecedent rainfall, a correlative relationship of benthic fecal bacteria with fine sediments, and a linear relationship with depth of sampling.

D.4 Temporal Trends Methods

Measuring change is a critical part of any stewardship or regulatory program. This critical step allows for effective resolution of water quality problems because management approaches can be measured for their relative effectiveness and project/programmatic effectiveness can be determined. There are a few very basic steps necessary to measuring change among seasons and among years. They are the same basic steps for accurate determination of success and measurement of program performance. These relate to sampling intensity and statistical analyses.

D.4.1 Detecting change from sample to sample

It is often critical to determine the difference between two spatially or temporally related samples, or differences between before and after treatments. The simplest way to detect change in this way is to compare groups of samples taken in these different spatial and temporal locations. There are several tests available that are similar in their performance. The most familiar are the Student's t-test and analysis of variance (ANOVA). These two tests compare the central tendency (usually mean) of groups of samples, where the basis of a difference is determined using the variance around the mean. In order to determine the mean and variance for individual spatial or temporal locations, or to compare among these locations, it is necessary to take 3 or more samples.

D.4.2 Detecting change over first flush

There are several ways to compare samples between different times of the year (or different parts of the watershed). One is to compare the central tendencies among seasons/months/events using the t-test for pairwise comparisons and analysis of variance (ANOVA) for comparisons between or among sample sets. Another is to compare the frequency of exceeding a threshold (e.g., 235 MPN/100 ml) using a contingency table-based analysis, such as Chi-square or Fisher's Exact Test. Two examples of these tests are given below, using *E. coli* concentrations for the Russian River and the commercial software JMP 8.

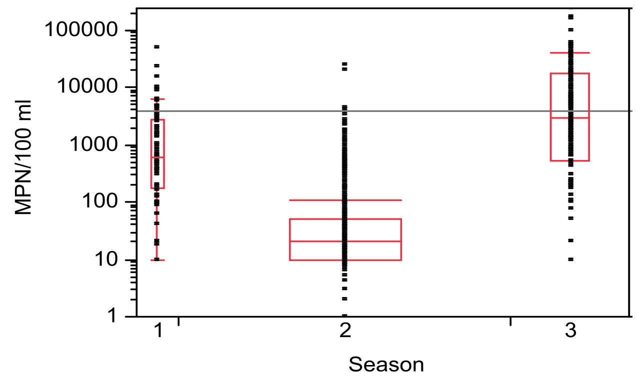


Figure 9Box-plot of means and variance and distribution of all *E. coli* concentrations across seasons: 1 – Winter/Early Spring, 2 – Summer, 3 – Fall/Early Winter.

1) ANOVA example: All *E. coli* concentration data (n=998) for all sites between 1997 and 2009 were grouped and sorted according to season – fall/early winter (October – December); winter/early spring (January – April); and summer (May – September, figure 9). Concentrations for each season were compared to values for the other two to determine if there were seasonal differences. There was a significant difference (P<0.001) among all seasons. There was a significant difference between summer and fall/early winter (P<0.001) and between winter/spring and fall/early winter (P<0.001), but not between summer and winter/early spring (P=0.21).

Conclusion: *Fall/early winter concentrations were significantly greater than summer or winter/early spring; winter/early spring concentrations were not greater than summer concentrations.*

2) Fisher's Exact Test: As with ANOVA analysis, all *E. coli* concentration data (n=998) for all sites between 1997 and 2009 were grouped and sorted according to season – fall/early winter (October – December); winter/early spring (January – April); and summer (May – September). Each season was compared to the other two to determine if there were seasonal differences in exceedance of the 235 MPN/100 ml standard. Summer-time samples had a significantly lower rate of exceedance than fall/early winter AND winter/early spring. Winter/early spring had a significantly lower rate of exceedance than fall/early winter. This pattern is similar to the comparison among means using the ANOVA, except that for this test, there was a difference between summer and winter/early spring. This is probably due to the fact that each analysis compared different seasonal conditions: ANOVA was used to compare means and Fisher's Exact Test was used to compare frequencies of exceedance.

Conclusion: *Fall/early winter concentrations were significantly greater than summer and winter/early spring; winter/early spring concentrations were greater than summer concentrations.*

D.4.3 Detecting change over water year(s) –trends analysis with seasonal adjustment

To detect changes in environmental condition, such as water quality, it is important to conduct time series analysis, measuring trends over years, while correcting for seasonal variations. One of the most common and robust tests is the Mann-Kendall test, also called the Seasonal-Kendall test. For each season, a single value is selected for use in the Seasonal-Kendall test. For seasons with multiple values and similar number of samples per season, a mean or median value can be calculated to represent the season. For seasons with multiple values and different rates of sampling, the most central value with respect to time is selected to represent the season. In contrast to the use of a mean or median to represent seasons with multiple values, this selection rule maintains a more constant variance in seasonal values for data records where the sampling frequency has changed over time. The maintenance of relatively constant variance during the testing period is desirable because more accurate statistical tests are likely under these conditions.

No example is given here for the Russian River because there are relatively few years with complete representation across seasons. In other studies of trends in water quality, 5 years of data with representation within all seasons is a minimum standard. In order to say anything about long-term trends in bacteria concentrations in response to remedial management and regulatory actions, it will be critical to regularly collect bacteria concentration data at reference and treatment sites.

D.4.4 Detecting change in winter/spring to anticipate summer trends

One possible cause of higher summer concentrations of fecal bacteria is higher Spring concentrations of the bacteria. The idea here is that bacteria that enter the waterways in the Winter and Spring survive until Summer and function as the reservoir for suspended fecal bacteria at recreational beaches. There have been investigations showing that sediment storage of *E. coli* could lead to viable bacteria entering the water column through later natural or human-induced turbulent resuspension (Craig et al., 2004). We looked for such a causative relationship between lumped antecedent Winter/Spring bacterial concentrations for a given rainy season and concentrations for the following Summer and could not find one. However, only five comparisons were possible for the last 15 years because of the inconsistent monitoring across the entire year. This relationship is important to investigate because it helps expose possible causes of exceedances of legal thresholds and can be used to predict recreation conditions in the Summer.

Dependency of Summer bacterial loading on Winter and Spring loading can be determined by comparing concentrations from various combinations of rainy season months with early or whole Summer average concentrations. Careful attention should be paid to the sites involved in this comparison. Because of the likely mechanism for the connection between antecedent Spring conditions and Summer conditions, it is reasonable to compare Summer sampling data to Winter/Spring data that are from upstream sites. The comparison can initially be made using a standard t-test or analysis of variance, assuming normal distribution of the data. If the data are not normally distributed, then the comparison could be made using a contingency table based test, such as the Fisher's Exact Test. This type of test uses proportions or frequencies rather than absolute numbers. Another way to look for correspondence between winter/spring concentrations and summer concentrations, is to use a linear regression to compare the relationship between winter/spring and summer concentrations across several years for hydrologically connected sites.

D.5 Aquatic Compartment Methods

Microbes living in feces may survive hours to months in the various receiving media in a watershed (soils, water column, benthic sediments). The faster these microbes die, the less risk they pose in to human health. The slower they die and if they can grow outside host animals (Desmarais et al., 2002; Solo-Gabriele et al., 2000), the greater risk they pose to human health. Oocysts (dormant early life stage) of *Cryptosporidium* and *Giardia lamblia* can survive for 2 to 6 months in river water at cold and ambient temperatures (Medema et al., 1997; Adam, 1991; Bingham et al., 1979). Temperature is apparently the major limiting factor for virus and coliform bacteria survival in soils, with an estimated doubling of the die-off rate for each 10 ^{o}C rise (Gerba and Bitton, 1984; Reddy et al., 1981; Sampson et al., 2006). Temperature is also the dominant factor affecting virus survival in freshwater, with greater survival occurring at lower temperatures. Enteric viruses can survive from 2 to more than 188 days in freshwater (Novotny and Olem, 1994). In addition, different strains of fecal coliform bacteria may survive at different rates outside of the host organism and the distribution of bacterial strains initially present in fecal matter changes over time in the environment (Anderson et al., 2005).

The sections below describe the general methods for monitoring fecal indicator bacteria in different compartments of the riparian – from aquatic to terrestrial environments. The "pilot" described was a one-time monitoring event on June 9, 2009, where water, sediment, and aquatic plant material were tested for *E. coli*. Please refer to figure 10 below.

D.5.1 Surface water

Methods used for pilot: Grab water samples were taken from the creek (moving water 1-2 m from the bank) and from the off-channel pond. Surface vegetation was carefully moved aside before sampling. In both cases, care was taken to not disturb benthic sediments. Samples were collected directly in the commercial lab's sample bottles and kept on ice until delivered to the lab for processing within 6 hours.

Additional method details:

The most common sampling procedures, and the ones used in the Russian River watershed, are to take grab samples below the surface of a water-body, briefly store and transport the sample on ice, and grow culturable bacteria in challenging growth conditions in order to isolate the intestinal bacteria *E. coli* and/or *Enterococcus* spp.

D.5.2 Benthic sediment

The following methods describe sampling benthic sediments from deep and shallow water and are adapted from (Whitman et al., 2003 and Ferguson et al., 2005).

Method used for pilot: Benthic fine sediment (<0.1 cm grain size) was collected using a benthic sediment core sampler (2" diameter). The sampler was washed with deionized water and ethanol between each sample. Core samples were manually pushed out of the sampler and the top 5 cm sub-sampled and the sample placed into a Ziplock bag. Samples were kept on ice until processed within 6 hours. Ten grams of each sample was weighed an put into a 50-ml plastic tube and 45 ml of distilled water added. Samples were vortexed for 2 minutes and centrifuged at 550 Xg for 2 minutes. The supernatant was decanted into sterile sample bottles. For each sediment sample, the wash, vortex, centrifuge, and decanting steps were repeated and the supernatants combined into one sample (~90 ml) stored on ice. Viable *E. coli* in each sample were enumerated by the commercial lab within 24 hours of sample collection.

Additional method details from literature:

In shallow water, benthic sand can be collected under a 0.5 m2 quadrat, to 2 cm depth. Fifty grams of benthic sand is shaken in 100 ml PBW, 2 min, and allowed to settle. (80-100% recovery rate). Bacteria in supernatant sampled for counts and identification.

In deeper water, benthic sediment can be grab sampled using Van Veen (e.g., Ferguson et al., 2005) or Ekman grab samplers, or other sediment sampling devices. The sampler should be rinsed with ambient surface water between samples or ethanol (Kon et al., 2007). About 75g of sediment is scraped from the top 2 cm of the sediment sample into a sterile sample bottle/container (Ferguson et al., 2005).

Bacteria are extracted from sediments by suspending about 10 g of sediment in 100 ml 1% (w/v) sodiummetaphosphate and sonicated for 30s at a rate below cell lysis levels. The supernatant after settling is used for bacterial quantification and identification. Bacterial concentrations can be expressed as CFU/g dry or wet weight sediment or 100 ml water. [method from Ferguson et al., 2008] Alternatively, bacteria can be separated from sediment by suspending replicate 25 g samples in 75 ml sterile peptone water (0.1%) and bath-sonicating for 10 min, stirring, then re-sonicating for 10 min. The supernatant is used for bacterial enumeration and identification. The results are expressed as CFU/ g dry weight sediment.

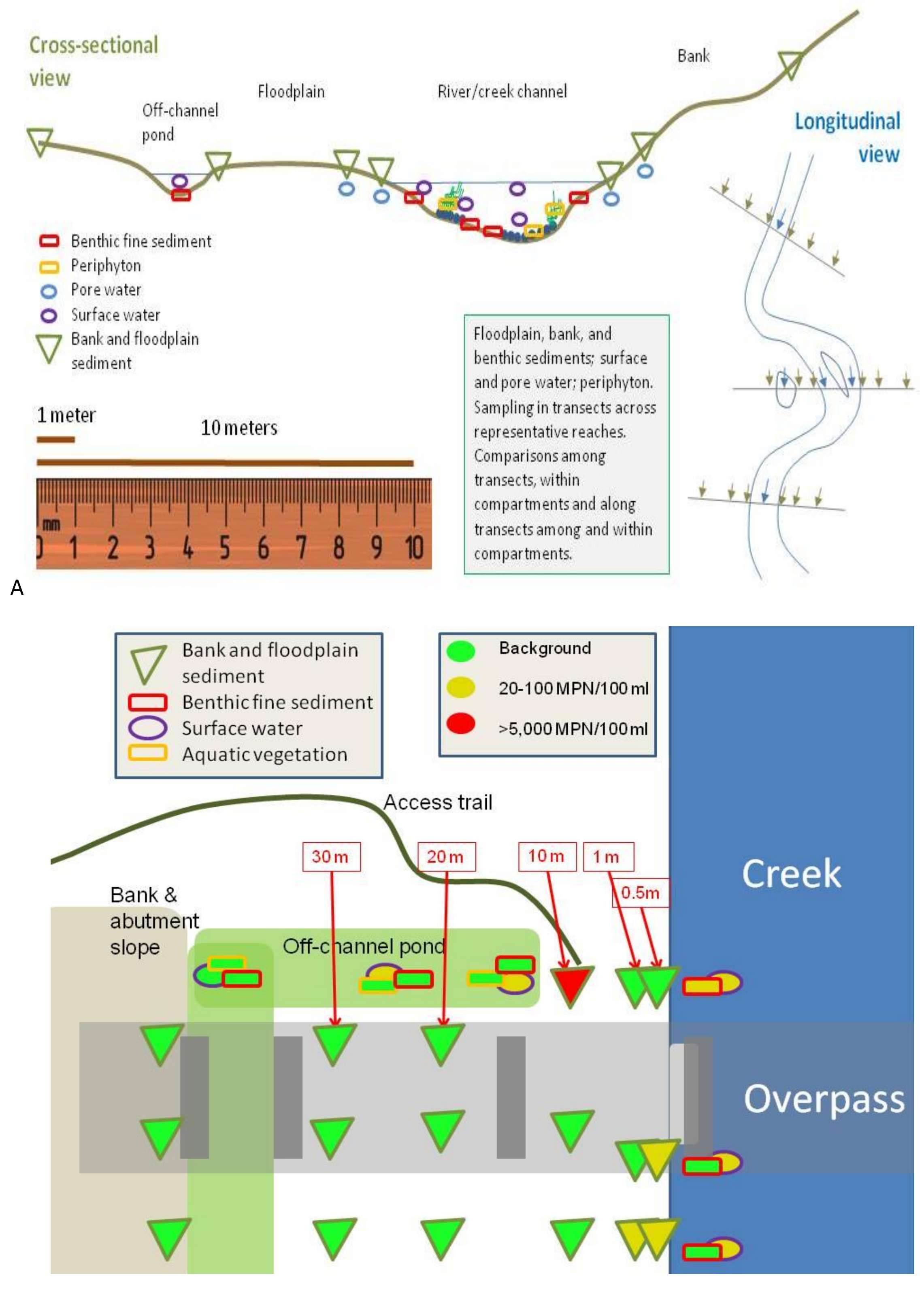


Figure 10 A) Theoretical profiles for riparian/floodplain transects and sampling for fecal bacteria; B) Actual compartment sampling on June 9, 2009 at "LAGU02", Laguna de Santa Rosa Creek.

D.5.3 Bank and floodplain

The following method is adapted from Byappanahal et al. (2003), Ishii et al. (2006), and other studies.

Method used for pilot: Floodplain and river-bank soil/sediment was collected by clearing surface vegetation and the top 0.5 to 1 cm of sediment and removing the soil using a trowel. Three holes were dug within 10 cm of each other and the soil from each hole combined as one composite sample in one Ziplock bag, which was put on ice until processing within 6 hours. The trowel was washed with deionized water and ethanol between each sample site. Bacteria were removed from the sediment/soil samples using the same method as for benthic sediment (above).

Additional method details from literature:

River-bank and floodplain sediments are collected along transects perpendicular to the waterway channel. Multiple transects per site can be sampled to improve understanding of spatial variability. Sites can be established comparing "clean" and discharge locations (Solo-Gabriele et al., 2000). Sampling is conducted starting at 1 m from the edge of flowing water and thereafter 5 m, 10 m, and 20 m from the edge of the channel (e.g., Ishii et al., 2007). Fewer (at shorter distance) or additional (at greater distance) samples may be needed to cover the floodplain. For example, Solo-Gabriele et al. (2000) had 6-in intervals. Samples are taken within the top 0-10 cm of the soil surface (Ishii et al. 2006) and large organic debris removed. Ishii et al. (2007) took 3 sub-samples at each spot on the transect and combined them to create a composite sample. Donovan et al. (2008) used clean metal trowels for sampling and placed samples in sterile plastic containers. Ishii et al. (2006) used coring tubes for sampling and store samples in plastic bags. Five sub-samples are taken at each transect distance and the sub-samples pooled to create one sample. Samples are stored on ice until treatment, usually within 24 hours.

Ishii et al (2006) extracted 10-20 g of soil in 95 ml 0.1 M gelatin-ammonium phosphate solution, while shaking at 280 rpm on a wrist-action shaker for 30 min. Solo-Gabriele et al. (2000) followed the settling step with filtering through a nylon filter (28 μm pore-size) to remove larger particles. Serial dilutions were performed and *E. coli* enumerated using Colilert. Ishii et al. (2006) uses PBS (pH) containing 0.01% gelatin for the serial dilutions. Moisture content (%) of the soil is determined by drying a known fresh weight of soil and re-weighing. *E. coli* concentrations are expressed as CFU/g dry weight soil/sediment or CFU/g wet weight (Donovan et al., 2008).

Interstitial water in beach and floodplain sediments can be sampled by digging a hole at sampling depths at increasing distances from a body of water (or other sampling strategy) using a sterilized shovel. The hole is dug until water invades the hole, which is the water sampled using a bottle or syringe. Sediments at the bottom of the hole where the interstitial water sample is taken can be simultaneously sampled using a sterile scoop. Concentrations are expressed as CFU/100 ml pore water.

D.5.4 Benthic periphyton

Method used for pilot: Floating aquatic vegetation (duck weed, *Lemna* sp.) was sampled from the surface of the off-channel pond using forceps. The forceps were washed with deionized water and ethanol between each sampling. The sampled vegetation was placed in a Ziplock bag and put on ice until processing within 6 hours. Two grams of vegetation was removed from the bag and placed in a blender with 100 ml distilled water. The sample was crudely homogenized for 15 seconds in the blender and the homogenate centrifuged for 2 minutes at 550 X g. The supernatant was decanted into a sterile sample bottle and put on ice. Viable *E. coli* in each sample were enumerated by the commercial lab within 24 hours of sample collection.

Additional method details from literature:

There are two basic methods, usable independently or together, to sample *E. coli* or *Enterococcus* spp. from attached or floating algae/periphyton.

Method 1 is adapted from KSoll et al. (2007). In this case, because of the likelihood of inter-substrate variation (e.g., among rocks) in amounts of periphyton, multiple replicate samples should be taken. In Ksoll et al, 8 sub-samples, 7 samples per sampling event and location were taken. Individual rocks from a cobbled substrate can be chosen randomly at each site, where a site can be a riffle or other location with larger rocks (>5 cm diameter). The rocks are removed from the water in order to sample a small area of each rock.

To sample the attached algae/periphyton: 1) bristles from a scrub brush are glued to the plunger of a 60 ml syringe, 2) the needle end of the syringe is cut off, 3) the syringe is applied to the rock and the plunger/scrubber used to loosen material, 4) loosened material is washed off into a funnel and sample container, and 5) material attached to bristles is removed and added to the sample. Samples are stored on ice in the dark for transport, most analyses should be completed within 18 hours of sampling.

To process samples for bacterial counts/identification: 1) replicate samples are diluted to 200 ml with filtered, autoclaved lake water and homogenized 10 s with a blender, 2) Tween 80 is added to 0.25% (final concentration) to each sample and the sample sonicated for 3 min. These samples may now be used for counts. 3) DAPI-staining of filtered bacteria can be used for direct bacterial count for each sample. In Ksoll et al. (2007), fecal coliform were counted using m-Fecal coliform agar and counting dyed colonies. Ksoll et al. also took individual *E. coli* isolates from periphyton sample by plating and DNA-fingerprinting using the horizontal, fluorophore-enhanced repetitive-polymerase chain reaction (HFERP) method and known wildlife and human markers.

To process samples for algal mass: 1) ash-free dry weight and dry weight are determined by passing samples through pre-weighed filters, drying, weighing, ashing and re-weighing, and 2) chlorophyll concentration is determined through ethanol or acetone extraction and spectrophotometry.

Using this method, *E. coli* or *Enterococcus* concentrations can be expressed as CFU/cm2 of rock surface sampled, or CFU/g algal mass.

Method 2 is adapted from Whitman et al. (2003). In this case, algae samples are collected from all substrate under a 0.5 m2 quadrat. The algae could be separate occurrences or combinations of floating, attached, and stranded algae. All algae is removed and collected under the quadrat. One gram of algae is crudely homogenized, placed in ~1/10 dilution phosphate buffered water (PBW, pH 6.8), vigorously shaken for 2 min, then centrifuged for 2 min at 653 g. The supernatant is filtered and filters placed on *E. coli* and *Enteroccus* selective media. Or, the supernatant, containing detached *E. coli* and *Enterococcus*, could be used in other identification and counting protocols. Whitman et al. report a 59% recovery rate for *E. coli* attached to algae. The *E. coli* and *Enterococcus* spp. counts are expressed as CFU/g algal material.

Because mat thickness and water temperature can affect colonization and survival of *E. coli* and *Enterococcus* spp., periphyton that is sampled should be assessed for type (e.g., *Cladophora* sp.) and thickness. Water temperature should also be taken at the site of sampling.

The two methods can be combined by conducting the rock sampling in Method 1 with the quadrat-based sampling in Method 2, where a quadrat is randomly placed at a monitoring site and the substrate below sampled for random rocks. The quadrat placement either in transect across the waterway or longitudinally along the riffle could be based upon random numbers. Where rocks (5-50 cm) are not present for sampling, attached periphyton may still be sampled. Where large wood is a common substrate, attached algae could be scrubbed free and sampled as in Method 1.

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