

# Fluorometry as a Bacterial Source Tracking Tool in Coastal Watersheds, Trinidad, CA<sup>1</sup>

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## Abstract

Bacterial counts have long been used as indicators of water pollution that may affect public health. By themselves, bacteria are indicators only and can not be used to identify the source of the pollutant for remediation efforts. Methods of microbial source tracking are generally time consuming, labor intensive and expensive. As an alternative, a fluorometer can be used to measure fluorescence in natural creeks as an indicator of concentrations of optical brighteners found in laundry detergent. In this way, fluorescence can be used as a tool for identifying failing or malfunctioning onsite wastewater treatment systems. Fluorometry was used in watersheds draining into northern California's Trinidad Bay in Humboldt County, in conjunction with bacterial sampling and measurement of rainfall and turbidity for correlation and comparison. Results showed that optical brighteners, when coupled with rainfall and turbidity data, can accurately predict whether bacterial standards will be exceeded within individual watersheds. Fluorescence of optical brighteners was also shown to be a useful tool for discerning the most impacted watersheds and tributaries for more detailed investigation of individual bacterial pollution sources.

*Key words:* bacteria, fluorometer, optical brighteners, septic, source tracking, wastewater

## Introduction

Four types of bacteria are routinely used to assess and regulate water quality in terms of human health: total coliforms, fecal coliforms (a subgroup of coliforms), *Escherichia coli* (a specific fecal coliform) and *Enterococcus*. These bacteria types are found in fecal matter, in the presence of enteric pathogens, they have good survivability in the environment, and are relatively easy to isolate and identify (McCorquodale et al. 1996). Current methods of bacterial testing take 24 to 48 hours to resolve, which is problematic for posting and removing public health warnings in a timely manner (Ely 2006). It is also impossible to determine the source of pollution using just the Most Probable Number (MPN) of the indicator bacteria that most tests provide. Human fecal contamination poses the greatest threat to public health because the associated pathogens are more transmittable. Because the indicators are found in the environment and in the feces of other warm blooded animals, the human health threat is not known even when their numbers are high.

In order to implement actions to improve water quality, the specific source (e.g. pets, livestock, septic systems) and its location, need to be identified. Source tracking is the term for a process used to identify the origin of fecal contamination in water. Unfortunately, most methods currently in use tend to be time consuming, expensive

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and / or unreliable and are not practical for small government jurisdictions or volunteer groups to use (Stoeckle 2005). Optical brighteners (OBs) are an emerging chemical method of source tracking human bacterial pollution from septic systems. These organic compounds are added to laundry detergents and other products where they absorb UV light and re-emit that energy in the visible blue spectrum. When added to white textiles, or paper, it makes the objects appear less yellow and more blue, giving them a 'whiter' appearance (Hagedorn et al. 2005). OBs are added to 97 percent of detergents sold in the U.S. Because OBs are readily adsorbed to particles, including soil, a properly functioning onsite wastewater treatment system (OWTS), or septic system, should remove nearly 100 percent of any OBs discharged to the system (Dates 1999). In addition, OBs do not readily biodegrade in the environment, unless they are exposed to sunlight (Hagedorn et al. 2005). Recent studies (e.g. Hagedorn et al. 2003, Hartel et al. 2007) show that monitoring OBs with a fluorometer holds promise as a relatively inexpensive source tracking method for suspected bacterial pollution of septic origin (Hartel et al. 2008).

The first goal of this study was to test the viability of using a fluorometer to detect the presence of OBs to confirm and track OWTS pollution in the greater Trinidad area; consideration was given to both spatial and temporal patterns. The fluorometer was used as a source tracking tool both to confirm anthropogenic sources of bacterial pollution and to identify and prioritize areas contributing the greatest amount of bacteria. The second goal of this study was to evaluate the effectiveness of this relatively new, cheap and quick method of identifying and locating human sources of bacterial pollution that can be used by even small municipalities and volunteer monitoring groups and to provide recommendations for its use.

## Study area

This study took place in and around the City of Trinidad in Humboldt County, California (*fig. 1*). The study area consists of a pre-defined project area related to water quality and watershed management / planning grants received by the City of Trinidad. The entire area consists of seven watersheds, four of which were utilized in this study – Mill, Parker, Luffenholtz and Joland Creeks – as well as beach and seep samples. The entire planning area contains a total of 6,498 acres and 32.7 miles of stream. This area is characterized by heavily wooded, steep slopes, particularly near stream channels and ocean bluffs, interspersed with flatter plateaus. Baseline water quality monitoring efforts have shown periodic, widespread bacterial contamination as high as 60 times, or 6000 percent of the State's contact recreational standards<sup>4</sup>.

The major land uses in the study area are timber production and rural residential. Much of the lower watersheds consist of residential development with small pockets of commercial and public land, and the upper watersheds are all commercial timberland. The residential development is concentrated around the communities of Trinidad and Westhaven. All residences in the study area utilize onsite wastewater treatment systems (OWTS), including septic systems, to dispose of wastewater (*fig.*

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<sup>4</sup> Parker, T. 2008. **Trinidad-Westhaven coastal watershed program: preliminary watershed assessment**. Unpublished report, prepared as part of the requirements for a Proposition 50 Watershed Planning Grant from the State Water Resources Control Board to the city of Trinidad. City of Trinidad, Trinidad, CA.



Figure 1—Project location and vicinity.

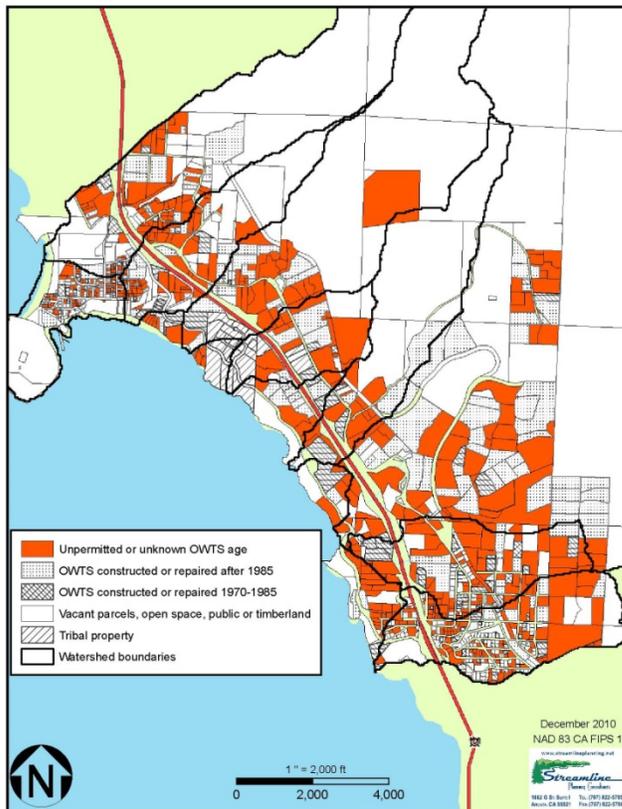
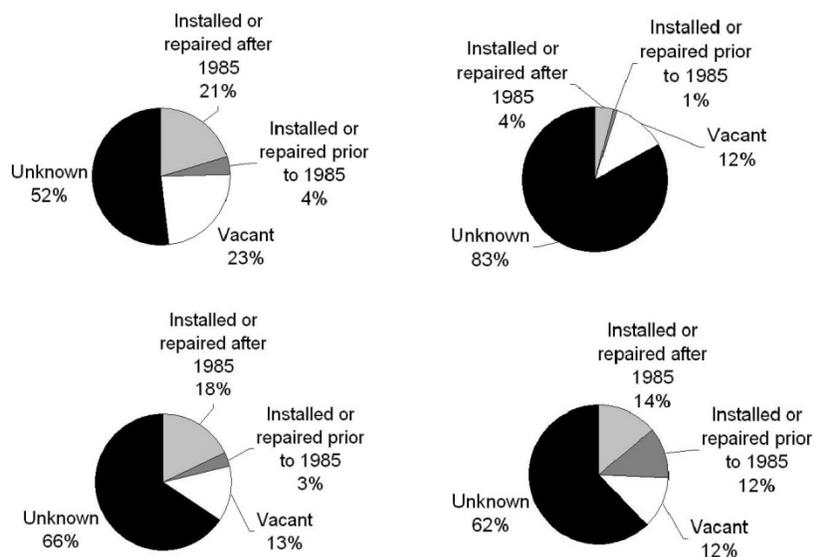


Figure 2—Permit data gathered from the Humboldt County Division of Environmental Health showing age of OWTS or lack of permit data, Trinidad, CA.

2). There are approximately 1,000 developed parcels in the study area. Many are less than  $\frac{1}{4}$  acre in size, much smaller than current regulations require for use of an OWTS. Most of these parcels were created and developed prior to current regulatory standards (*fig. 3*), and therefore the OWTS are often unpermitted (built prior to permit requirements) or otherwise do not meet current requirements (City of Trinidad 2010).



**Figure 3**—Percentages of OWTS by age in (from left to right, top to bottom) Mill, Parker, Luffenholtz and Joland Creeks watersheds. ‘Unknown’ indicates there is no file information for those parcels, which means they were installed prior to 1970 or without permits.

## Materials and methods

For this study we used an Aquafluor™ handheld fluorometer and turbidimeter (Turner Designs, Sunnyvale, CA). The UV-445 wavelength filter was chosen based on the recommendation that it is the most ideal setting (available) for detecting OBs while eliminating other sources of fluorescence such as hydrocarbons and organic matter (Hartel et al. 2007, Turner Designs 2007).

Samples were taken from five different watersheds: beach/seeps, Mill Creek, Parker Creek, Luffenholtz Creek and Joland Creek, on 18 different dates starting on September 17, 2008 and ending April 15, 2010 (*fig. 4*). Samples were taken at various times and locations with varying weather, seasons and conditions in an attempt to get a broad view of data patterns. Field crews started at the mouth of the creeks, or another easily identifiable starting point such as a culvert. Samples were taken approximately every 100 feet upstream, and also at tributaries and other inlets. Bacterial tests included total coliform, *E. coli* and *Enterococcus*. Daily rainfall was collected from records at the city’s water plant.



**Figure 4**—Sampling locations and transects in this study, Trinidad, CA.

All calibrations, sampling, testing and data management occurred according to a quality assurance project plan prepared in accordance with State Water Quality Control Board guidelines. The fluorometer was calibrated with Tide liquid detergent and distilled water. Samples were taken in accordance with Standard Operating Procedures. Measurements for OBs were taken in the field according to the manufacturer’s instructions. Turbidity readings were taken immediately following the fluorescence reading for most samples. Bacteria samples were also taken at the same time and delivered to the Humboldt County Public Health Lab.

Data recorded were as follows: sample point identification, watershed, date, OB first reading, OB second reading, total coliform, *E. coli*, *Enterococcus*, turbidity, rainfall in the past 24 hours, rainfall in the past 3 days (72 hours), rainfall in the past 2 to 3 days (24 to 72 hours) and rainfall in the past week. In addition to the directly sampled data, several derived data categories were utilized. This included the difference between the first and a second OB reading (called ‘difference’) and whether the bacterial results were in exceedance of the state contact recreational standards.

## Results

The results showed consistent patterns between sampling events and watersheds with each watershed having a consistent ranking for each of the water quality parameters tested (*table 1*).

**Table 1**—Median values of variables and their ranks of all data sampled between September 17, 2008 and April 15, 2010 from five watersheds, Trinidad, CA.

Water-shed	Optical		Total		<i>E. coli</i>		<i>Enterococcus</i>		Turbidity	
	Brighteners		Coliform							
	Med.	Rank	Med.	Rank	Med.	Rank	Med.	Rank	Med.	Rank
Beach	1.1	5	31	5	1	5	1	5		
Mill	8.0	4	424	4	10	3	6	4		
Parker	17.0	1	1935	1	31	1	121	1	50.8	1
Luffen-										
holtz	12.6	2	504	3	20	2	41	2	23.6	2
Joland	8.6	3	697	2	10	3	20	3	20.5	3

The most promising statistical results were obtained using logistic regressions to predict if bacterial counts would exceed state standards for contact recreation using OBs, rainfall and turbidity as the independent variables (*table 2*). The bacterial standards are as follows: total coliform 10,000 MPN/100 ml; *E. coli* 400 MPN/100 ml; and *Enterococcus* 104 MPN/100 ml. These regressions were run with all the data together, starting with the following independent variables – OBs, difference, turbidity, rain 1-day and rain 2-3 days. Total coliform and *E. coli* could not be analyzed, because there were too few bacterial exceedances. For the same reason, logistic regression could not be used on the data for each individual watershed.

**Table 2**—Result of logistic regression analysis for the aggregated data from five watersheds in Trinidad, CA sampled between September 17, 2008 and April 15, 2010.

Independent Variables	Non-exceedances		Model
	Exceedances Correctly Classified	Non-exceedances Correctly Classified	
OB 1st <sup>a</sup> , Difference, Turbidity, Rain 1- day, Rain 2-3 days	82.4%	96.1%	0.860 - 0.237*Diff + 0.219*OB_1st - 2.14*Rain_1_day + 1.22*Rain_2_3_days - 0.0536*Turbidity
OB 1st, Turbidity, Rain 1-day, Rain 2-3 days	82.4%	96.1%	0.917 + 0.215*OB_1st - 2.13*Rain_1_day + 1.23*Rain_2_3_days - 0.0536*Turbidity
OB 1st, Turbidity, Rain 1-day	76.5%	96.1%	1.73 + 0.194*OB_1st - 2.03*Rain_1_day - 0.0508*Turbidity
Turbidity, Rain 1- day	61.8%	98.0%	3.33 - 0.831*Rain_1_day - 0.0486*Turbidity
Turbidity	58.8%	98.0%	2.92 - 0.0494*Turbidity
OB 1st	38.6%	83.6%	2.80 - 0.170*OB_1st

<sup>a</sup> OB 1st refers to the first optical brightener or fluorescence reading.

Linear regressions between OBs and the three different bacteria measurements essentially showed no direct relationship. Multiple regression was more successful. Based on the results of variable selection routines performed, three of the variables were consistently the best predictors of bacteria, are easy to collect, and provide good power for the multiple regression models: OBs, turbidity and rain 1-day (24 hours). We used these three variables as independent variables in multiple regressions against each of the three bacterial variables for each watershed with good predictive results (*table 3*). We did not run this analysis for the beach / seep dataset due to the lack of both turbidity readings and rainfall. For Mill Creek we used only OBs and

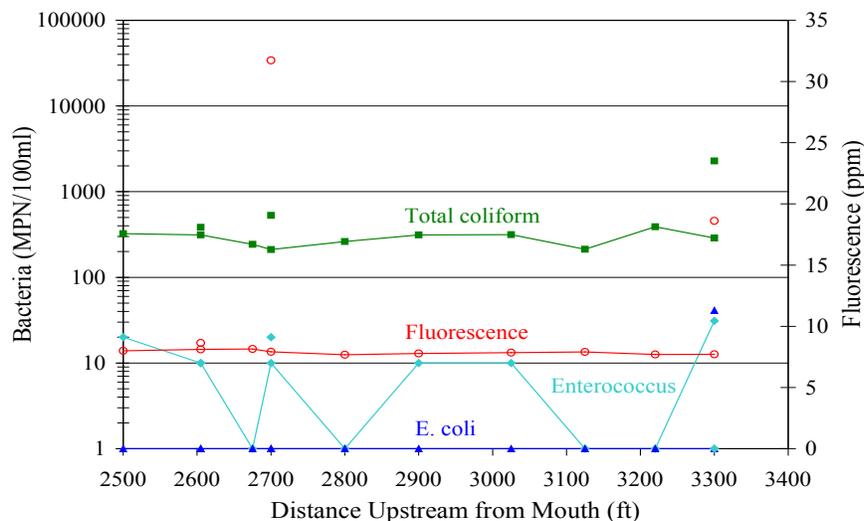
rain 1-day since there were no turbidity samples taken.

**Table 3**—Results of multiple regression analysis from four watersheds in Trinidad, CA sampled between September 17, 2008 and April 15, 2010.

Mill Creek	Model	r <sup>2</sup>
Total coliform	104.5+ 40.5*OB_1st <sup>a</sup> + 28,681.6*Rain_1_day	0.83
<i>E. coli</i>	0.33+ 0.67*OB_1st+ 345.6*Rain_1_day	0.77
<i>Enterococcus</i>	-53.5+ 6.83*OB_1st+ 2,493.2*Rain_1_day	0.80
<b>Parker Creek</b>		
Total coliform	1,393.9-56.6*OB_1st+ 527.1*Rain_1_day+ 9.87*Turbidity	0.48
<i>E. coli</i>	-39.4+ 1.28*OB_1st+ 41.8*Rain_1_day+ 0.78*Turbidity	0.78
<i>Enterococcus</i>	1.41-0.30*OB_1st+ 116.5*Rain_1_day+ 0.82*Turbidity	0.71
<b>Luffenholtz Creek</b>		
Total coliform	NA	
<i>E. coli</i>	12.9-6.74*OB_1st+ 66.5*Rain_1_day+ 2.41*Turbidity	0.59
<i>Enterococcus</i>	46.2-25.2*OB_1st+ 220.6*Rain_1_day+ 7.62*Turbidity	0.68
<b>Joland Creek</b>		
Total coliform	4,687.9-773.1*OB_1st+ 5,109.6*Rain_1_day+ 106.9*Turbidity	0.84
<i>E. coli</i>	-184.6+ 27.1*OB_1st+ 648.3*Rain_1_day- 2.89*Turbidity	0.91
<i>Enterococcus</i>	-92.2+ 7.57*OB_1st+ 836.1*Rain_1_day+ 0.27*Turbidity	0.88

<sup>a</sup> Refers to the first reading of optical brighteners / fluorescence

We graphed the sample results from the longitudinal stream profiles to provide visual clues as to pollution sources within the watersheds. The following chart (*fig. 5*) is one selected example (out of a total of 10 transects). Regardless of the statistical relationships between the variables, these transects may provide a more reliable analog for what is actually happening in the environment. They remove the influence of rainfall, because each transect was taken on a single day under the same conditions. Anomalous points can be looked up in the field notebook for clues as to what is causing the differences.



**Figure 5**—Upstream transect on Mill Creek showing longitudinal trends. These samples were taken on 11/17/08 with 0.0 in of rainfall in the previous three days. Note that points without lines indicate seeps or tributaries draining into the main channel.

## Discussion

When OBs were included with rainfall and turbidity as independent variables in multiple or logistic regressions with the bacteria indicators the results showed strong predictive value. The logistic regression analysis showed that these variables can be used to predict exceedances of bacteriological standards (*table 2*). Multiple regression was improved when the data was analyzed separately for each watershed (*table 3*). These results could be used to protect public health by allowing warnings on beaches to be posted immediately after rainfall.

Patterns of OBs and bacteria were consistent with expected priority areas based on land use and watershed characteristics. The results of this study showed that each creek had consistent rankings when considering the median values of each variable (*table 1*). These rankings can be explained by the land use characteristics of each watershed. For example, of the four creeks, Mill had the lowest medians. The upper and lower portions of the Mill Creek watershed are mostly undeveloped timber and park land. It also has a high percentage of undeveloped and open space land, large residential lot sizes and, maybe most importantly, the lowest percentage of old and unpermitted onsite OWTS (*fig. 3*). Similarly, Parker Creek had by far the highest median values and also has the greatest percentage of old and unpermitted OWTS (*fig. 3*). Parker Creek is also densely developed with a high percentage of impervious surfaces and the greatest diversity of land uses. These correlations are strong indicators that the pollution is in fact coming from failing or poorly functioning OWTS. Similar to this study, Hartel et al. (2007) consistently showed high fluorescence and high bacteria when the source was human/residential.

Based on past water sampling results, it was known that higher bacteria counts occur after rain, particularly if there was some prior dry weather. This study confirmed that both bacteria and OBs were influenced by rainfall. Rainfall in the

previous day (24 hours) was the best predictor of bacteria counts. Rainfall in the previous 2 to 3 days (24 to 72 hours) was also a useful predictor as was turbidity. However, rainfall also increases the amount of turbidity and organic matter in the water. Since organic matter fluoresces and can interfere with OB readings (Hartel et al. 2007) it may be beneficial to conduct fluorometry based source tracking during summer low-flow periods. Unfortunately, our study found that there was a lack of variability in bacteria counts during low-flow periods, possibly because the samples were diluted at a ratio of one to ten in order to pick up the higher bacteria counts, which are of greatest concern and interest.

The longitudinal creek profiles may provide the best information for source tracking individual failing OWTS or at least the worst contributing neighborhoods or subwatershed. *Figures 5 and 6* show points that form peaks or valleys for one or more constituents. The points can be identified in the graphing program, and then looked up in the field notebook for further information and / or investigated in more detail in the field. Some generalizations can be made when looking at the longitudinal profile data. Points with high levels of bacteria and OBs should be considered priority areas for further investigation of OWTS failures. High bacterial readings with low OBs could either indicate animal sources of bacteria, or an OWTS source that lacks laundry capabilities. High OBs with low bacteria likely indicate a greywater source, or a high concentration of organic matter.

The Mill Creek transect (*fig. 5*) shows sharp spikes of both bacteria and fluorescence in tributaries at 2700 and 3300 ft. The high point at 2700 ft was associated with a seep draining into the creek from an area occupied by an illegal settlement of trailers. Since the time these samples were taken, the County Division of Environmental Health investigated and condemned the site partially based on inadequate sewage disposal. The high point at 3300 ft was taken from a small tributary draining into the main channel at that location. This tributary drains a small residential neighborhood that would be a good candidate for further investigation.

This study helped confirm that OWTS are the cause of bacterial contamination in the study area creeks. This study also showed that a fluorometer was a viable tool in an easy to use form over a wide variety of conditions that is applicable to other government jurisdictions and non-profit organizations that are involved in water quality monitoring, but that do not have the funds for traditional bacterial source tracking. One of the important things this study showed was that the results are much more accurate when considered within a single watershed rather than the entire study area. OBs can provide a quick, cheap and easy method to help characterize different watersheds and to prioritize areas for further investigation and remediation that are contributing the most pollution. Other variables do have to be considered, including the natural variability of the system, seasonality and rainfall.

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