Removal of bacterial indicators of fecal contamination in urban stormwater using a natural riparian buffer

M.J. Casteel[1]*, G. Bartow[2], S.R. Taylor[3] and P. Sweetland[4]

[1]Water Quality Bureau, [2]Planning Bureau, and [3]Bureau of Environmental Regulation Management, San Francisco Public Utilities Commission, San Francisco, CA 94103, USA
[4]Department of Water and Wastewater Resources, City of Daly City, CA 94015, USA
**Corresponding author, email mcasteel@sfwater.org*

ABSTRACT
Little information is available on the ability of naturally-occurring riparian buffers to remove waterborne microbes from urban stormwater runoff. In the present study, bacterial indicators of fecal contamination were quantified in stormwater before and after intentional diversion to a natural riparian buffer (RB) adjacent to a lake located in the City of San Francisco, California. Analysis of lake water showed that levels of *Escherichia coli* and total coliforms increased significantly during storm events, indicating the presence of nonpoint sources of fecal contamination in the area surrounding the lake. When 0.44 million L (5,700 L/min) of stormwater was intentionally diverted and discharged to the RB, lake levels of *E. coli*, enterococci, and total coliforms were about 2-3 $\log_{10}$ (99-99.9%) lower than levels in stormwater. The RB was less effective in reducing bacterial levels during some major storms (>4 cm precipitation in <48 h) with higher inputs (several million L), but large amounts of uncontrolled surface runoff had been observed entering the RB and Lake Merced during those events. These results suggest that the RB evaluated in this study worked effectively to reduce the numbers of bacteria in stormwater from entering a body of water in an urban setting.

KEYWORDS
Enterococci; *Escherichia coli*; fecal contamination; riparian buffer; urban stormwater

INTRODUCTION
Lake Merced is a eutrophic lake located in the southwest corner of the City of San Francisco, California. It consists of four inter-connected basins (referred to as South, East, North, and Impound) which serve as an emergency source of non-potable water, and limited non-contact recreational activities (*e.g.*, boating, fishing) are permitted. Historically, water levels in Lake Merced have been replenished by rain and by seepage from ground-fed springs and creeks. In recent years, however, lake water levels have declined due to increasing groundwater extraction, and the management of stormwater runoff in the area surrounding the lake has become problematic because of changes and development in the urban landscape and infrastructure. Most stormwater in the area surrounding Lake Merced is collected by conveyance systems, but during major storms (i.e., 5-8 cm in 24-48 h or several cm in a short time period), increased stormwater volumes may overwhelm such networks, thereby running the risk of overflows and flooding, or being released untreated and in large amounts to the environment. In a simultaneous effort to raise water levels in Lake Merced and to better manage stormwater flows, intentional diversion of stormwater to the lake was proposed. However, since urban stormwater can contain numerous chemicals and microbes associated

with adverse environmental and public health effects (Gaffield *et al.*, 2003) as well as excess nutrients that can influence a body of water's trophic status, it was determined that stormwater would require some level of treatment before discharge to Lake Merced.

Included among the various methods to manage and treat stormwater are the use of vegetated areas of land or wetlands. These may be man-made or constructed areas (*e.g.*, constructed wetlands; vegetated filter strips; grassed swales), or may be naturally-occurring features such as riparian buffers. Numerous studies have shown that constructed wetlands and engineered buffer or filter strip systems may effectively remove waterborne contaminants from relatively constant inputs such as municipal and agricultural waste effluents, and some information is available on the effectiveness of constructed systems to remove stormwater pollutants (see for example Stenström and Carlander, 2001). In contrast, less information is available on the ability of natural systems such as riparian buffers to remove bacterial indicators of fecal contamination and other microbial contaminants from intermittent, high-volume flows such as those encountered during storm events in heavily urbanized areas. Such information would be valuable in determining the role that natural ecosystems play in stormwater pollutant fate and transport in the urban environment, and might assist urban planners in utilizing such features to manage and treat stormwater. In the present study, bacterial indicators of fecal contamination (*Escherichia coli*; *Enterococcus* spp.) and total coliforms were quantified in urban stormwater and in lake water, both before and after intentional diversion to a riparian buffer adjacent to the shoreline of Lake Merced's South Basin. This project is consistent with a global trend towards minimizing the input of stormwater into sewer systems and instead practicing various methods of stormwater management and treatment (Marsalek and Chocat, 2002).

MATERIALS AND METHODS

Study site. The study site is located (approx. latitude / longitude = 37º42'56" / -122º29'40") at the South Basin of Lake Merced in San Francisco, California, USA. The South Basin of Lake Merced (hereafter referred to as Lake Merced) has a surface area of approximately 66 ha, is on average 4.5 m deep, and has a capacity of about 2.6 billion L. The riparian buffer evaluated in this study is on average 46 m in width (range, 32-55 m) as determined by professional survey analysis and is part of the continuous shoreline of the lake. Stormwater is diverted from the Vista Grande Canal (an open, brick-lined channel that runs parallel to the west shoreline of Lake Merced) into a continuous deflection separator (CDS) unit (designed to remove trash, large debris, and other waterborne material). From the CDS, stormwater is then channeled via an underground tunnel and discharged to the riparian buffer via a 91 m long, 46 cm diameter PVC pipe containing 6.4 cm diameter perforations spaced evenly at 0.76 m intervals for the entire length of the pipe. Vegetation of the riparian buffer consists of two general series (scrub and wetlands), which includes a $\leq$ 21 m band of dense, shrubby vegetation (predominantly California blackberry (*Rubus ursinius*), swamp knotweed (*Polygonum amphibium*), and stinging nettle (*Urtica dioica*)) and a $\leq$ 39 m band of California bulrush (*Scirpus californica*) in shallow water. Soil of the riparian buffer is typical of the surrounding area and consists of sandy loam, sand, and loamy sand.

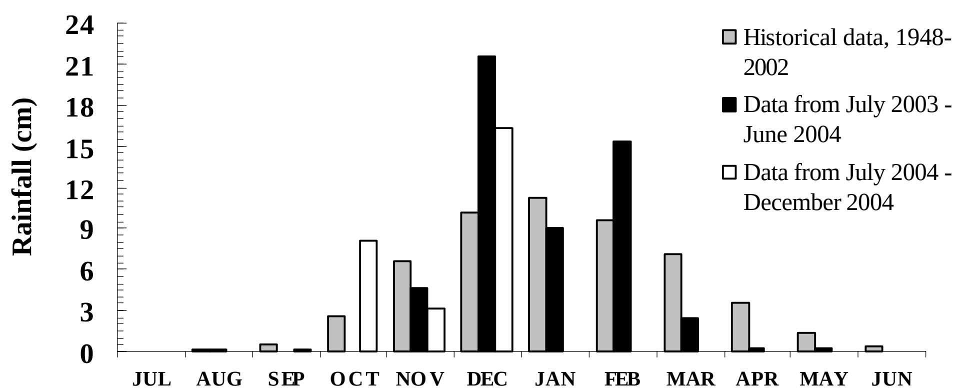
Sampling, bacteriology, and water quality analyses. Grab samples of stormwater and lake water were collected during storms and within 24 h following discharge to the riparian buffer, respectively, transported to the laboratory, and analyzed within 6 hours of collection. Lake samples were collected at the shoreline (n=3) and at locations 40 m (n=2) and 80 m (n=1) offshore and the results averaged (see below). Stormwater samples were collected at the intersection of the Vista Grande Canal and CDS (see above). For bacteriological assays, lake and stormwater samples were serially diluted in sterile buffered water (APHA, 1998), and replicate 100 mL portions were mixed with Colisure media (IDEXX Laboratories Inc., Westbrook, ME, USA) for the detection of *E. coli* and total coliforms or Enterolert media (IDEXX) for the detection of enterococci. Mixtures were transferred to IDEXX enumeration vessels (i.e., QuantiTray 2000) and incubated at 35°C for 24-48 h (Colisure) or 41°C for 22-24 h (Enterolert). Most Probable Numbers of bacteria were determined by enumerating positive wells in QuantiTrays by visual inspection, including examination under 365 nm ultraviolet light, per manufacturer's instructions. Water quality parameter analyses of lake and stormwater samples were determined in accordance with standard methods (APHA, 1998).

Data reporting and data analyses. Most Probable Numbers (MPN) of *E. coli*, total coliforms, and enterococci were log-transformed and are reported as $\log_{10}$ MPN/100 mL. Average bacteriological values are reported as the geometric mean and are presented with their corresponding 95% confidence limits (CLs). Bacterial reductions by the riparian buffer are reported as the percentage difference between $\log_{10}$ levels in stormwater and in Lake Merced. Water quality parameter values are reported as arithmetic means with corresponding standard deviations (SD). Some bacteriological and water quality data were examined for statistically significant ($P \leq 0.05$) differences between type of water sample or by dates using t-tests and one-way analysis of variance (ANOVA; followed by the Tukey-Kramer Multiple Comparisons Test) (InStat v3.0, GraphPad Software, San Diego, CA, USA).

RESULTS AND DISCUSSION

The management of stormwater-associated flooding and uncontrolled runoff is problematic in San Francisco, where monthly rainfall amounts have exceeded 15 cm/month in recent years (Figure 1). San Francisco and its outlying area is classified as a coastal Mediterranean climate with dry mild (temperature) summers generally lasting from May to September, followed by a period of increasing precipitation in the form of rainfall from October to April.

Figure 1. Monthly rainfall totals (cm), City of San Francisco



For the purposes of the present study, the 'dry season' in San Francisco is defined as the interval from 1 May to 30 September, during which time rainfall totals a monthly average of $\leq$1.4 cm (Figure 1; historical data from 1948-2004, Western Regional Climate Center, Reno, NV, USA). The storm season as defined in this study is the period from 1 October to 30 April, during which time monthly rainfall historically has ranged from about 2-11 cm.

Urban stormwater, defined as rainwater that is unable to penetrate the ground and which runs over urban surfaces (Marsalek and Chocat, 2002), can contain numerous chemical and pollutants such as metals, herbicides, pesticides, and fertilizer residues, and is considered one of the predominant causes of surface water eutrophication and impairment. Typical water quality parameters, such as dissolved oxygen, pH, turbidity, chlorophyll-a and nutrients (particularly total phosphorous) can be measured in stormwater and in receiving waters at different times of the year in order to determine the extent to which surface waters are impaired and possibly affected by storm runoff, and such data for Lake Merced are shown in Table 1. Technically classified as a shallow lagoon, Lake Merced is considered eutrophic (USEPA, 2000), a determination supported by elevated levels of chlorophyll-a, Secchi depth transparency readings of <3 m, and elevated total phosphorous levels, which for all analyses performed from 2000-2003 were 19-30 µg/L, ≤0.55 m, and 0.2 mg/L, respectively. Turbidity and total phosphorous levels in stormwater were appreciably higher than in lake water generally, although there was little difference between levels of dissolved oxygen and pH. These results suggest that stormwater may play at least some role in seasonal changes in Lake Merced's water quality, but other environmental factors, including wind and sunlight, may also play contributing roles to its overall water quality conditions.

Table 1. Characteristic (average (± SD)) water quality parameters (param.): dissolved oxygen (diss. O_2), pH, turbidity (turbid.), chlorophyll-a (chloro.-a), Secchi disc (depth), and total phosphorous (P) in Lake Merced and stormwater, 2000-2003

Quality param. (units)	**Lake Merced**		**Storm-water**	**Quality param. (units)**	**Lake Merced**		**Storm-water**
	Dry season	**Storm season**			**Dry season**	**Storm season**	
Diss.O_2 (mg/L)*	7.2 ± 2.5 (n=32)	7.9 ± 1.9 (n=33)	7.6 ± 2.3 (n=4)	chloro.-a (µg/L)*	30 ± 9.4 (n=68)	19 ± 10.0 (n=70)	n/a
pH*	8.1 ± 0.3 (n=72)	8.1 ± 0.3 (n=72)	7.9 ± 1.1 (n=2)	Secchi depth (m)	0.49 ± 0.09 (n=18)	0.55 ± 0.15 (n=18)	n/a
Turbid. (NTU)*	15 ± 3 (n=67)	12 ± 5 (n=70)	51 ± 33 (n=11)	Total P (mg/L)*	0.2 ± 0.05 (n=68)	0.2 ± 0.04 (n=70)	0.4 ± 0.5 (n=22)

*average of values from samples collected at surface and at depths of 1.5, 3.0, and 4.5 m; n/a=not applicable

While the measurement of quality parameters (such as those shown in Table 1) in stormwater and in receiving bodies of water may be useful in monitoring a lake's trophic status and water quality conditions, such data is generally not useful for assessing the scope and magnitude of microbial pollution in surface waters impacted by stormwater. In addition to chemical contaminants, urban stormwater runoff may also include fecal material and bacteria (Ellis and Wang, 1995) from mixing with municipal wastewater or septic discharges, or from domestic and wild animals. To assess the extent of stormwater runoff-associated fecal contamination in Lake Merced, bacteriological analyses were performed on lake samples for levels of indicators of fecal contamination (*E. coli*) during the dry season, during the storm season, or in stormwater itself. Bacteriological analyses also included total coliforms as an overall indication of the microbial quality of water.

As shown in Table 2, geometric mean levels of *E. coli* and total coliforms in Lake Merced during the dry season (for years 2000-2003) ranged from 1.2-2.0 and 2.4-3.1 $\log_{10}$ MPN/100 mL, respectively. Levels of *E. coli* in Lake Merced during the dry season remained relatively constant (i.e., were not significantly different) each year from 2000-2003, and were also the same as *E. coli* levels measured at a point in time during a winter month (December 2003) with little reported precipitation (data not shown). The levels of total coliforms in Lake Merced during the dry season also were essentially the same in 2001 and 2003, but were significantly higher (P=0.0074) compared to 2000 and 2002.

Table 2. Geometric mean levels ($\log_{10}$ MPN/100 mL) of *E. coli* and total coliforms in Lake Merced during the dry season, 2000-2003

Date	# samples (n)	$\log_{10}$ *E. coli* MPN/100 mL (95% CLs)	$\log_{10}$ total coliforms MPN/100 mL (95% CLs)	Date	# samples (n)	$\log_{10}$ *E. coli* MPN/100 mL (95% CLs)	$\log_{10}$ total coliforms MPN/100 mL (95% CLs)
2000	n=22	1.7 (1.4-2.1)	2.7 (2.5-2.9)	2002	n=2	1.2 (0-6.0)	2.4 (0.44-4.3)
2001	n=22	2.0 (1.8-2.2)	3.1 (3.0-3.3)	2003	n=6	1.3 (0.82-1.7)	3.1 (2.9-3.4)

Levels of *E. coli* and total coliforms in Lake Merced during storm events in 2002-2003 are shown in Table 3. During these storms, in which rainfall ranged from <1 to several cm in a 48 h period, stormwater was not intentionally diverted to Lake Merced, although there may have been uncontrolled runoff from the surrounding area to the lake.

Table 3. Geometric mean levels ($\log_{10}$ MPN/100 mL) of *E. coli* and total coliforms in Lake Merced following storm events (no intentional diversion of stormwater)

Date	Total rainfall *(cm)	# samples (n)	$\log_{10}$ *E. coli* MPN/100 mL (95% CLs)	$\log_{10}$ total coliforms MPN/100 mL (95% CLs)
12/15/02	3.3	n=2	3.2 (3.0-3.4)	3.7 (3.5-3.8)
12/20/02	5.2	n=2	2.3 (1.5-2.9)	3.7 (3.6-3.9)
12/22/02	0.18	n=2	2.0 (1.0-2.7)	3.0 (2.7-3.3)
12/31/02	1.5	n=2	3.2 (3.0-3.4)	4.0 (3.8-4.1)
1/10/03	2.8	n=2	3.3 (3.0-3.5)	4.0 (3.8-4.1)
1/23/03	0.28	n=2	3.8 (3.7-4.0)	4.6 (4.4-4.7)
2/25/03	1.2	n=2	2.0 (1.0-2.7)	3.1 (2.8-3.3)

*includes total amount of rainfall 24 h before and during date shown

As shown in Table 3, geometric mean levels of total coliforms in Lake Merced during storm events ranged from 3.0-4.6 MPN/100 mL (overall mean, 3.7 $\log_{10}$) while levels of *E. coli* ranged from 2.0-3.8 MPN/100 mL (overall mean, 2.8 $\log_{10}$). Compared to background levels of *E. coli* in Lake Merced during the dry season (Table 2), levels of *E. coli* in the lake during the storm season were significantly higher (P=0.0075). Total coliforms also were present in significantly higher (P=0.0078) concentrations in lake water during storms compared to lake samples collected and analyzed during the dry season. Comparison of data in Tables 2 and 3 suggests that fecal contamination of the lake had increased during winter storms in San Francisco, perhaps because of uncontrolled non-point surface runoff from surrounding areas. This is supported by analyses that enumerated *E. coli* and total coliforms in stormwater during the storm seasons of 2002 and 2003; as shown in Table 4, mean stormwater levels of total coliforms were approximately 1-2 orders of magnitude higher than in lake water. Stormwater levels of *E. coli* also were higher than lake levels, at about 4.4 $\log_{10}$ MPN/100 mL, which are similar to the average level (4.3 $\log_{10}$ MPN/100 mL) of fecal coliforms as reported in the Nationwide Urban Runoff Program (USEPA, 1983).

Table 4. Geometric mean levels (log_{10} MPN/100 mL) levels of *E. coli* and total coliforms in stormwater

Date	# samples (n)	log_{10} *E. coli* MPN/100 mL (95% CLs)	log_{10} total coliforms MPN/100 mL (95% CLs)
2002	n=4	4.3 (3.4-5.3)	5.2 (5.0-5.3)
2003	n=4	4.4 (3.3-5.4)	5.3 (5.1-5.5)

While the bacteriological analyses performed from 2000-2003 had enumerated *E. coli* and total coliforms in Lake Merced and in stormwater, total coliforms may occur naturally in the environment and therefore are considered poor indicators of fecal contamination. In addition, bacterial cells are generally less persistent in the environment than are other fecal microbes, such as enteric viruses and parasites, but enumeration of such microbes by infectivity assay is usually not performed for routine and rapid assessment of microbial water quality, required for projects such as the one described in this paper. In contrast, enterococci bacteria are increasingly used to monitor the water quality of recreational and ambient waters, including environmental water matrices impacted by urban runoff from storms (Noble *et al.*, 2003). Enterococci may be more resistant in the environment than are fecal coliforms and *E. coli*; for example, some evidence exists that enterococci might be more stable in stormwater-impacted lake water than *E. coli* (Jin *et al.*, 2004). Hence, enterococci were included with *E. coli* and total coliforms in determining bacterial removal in stormwater by the riparian buffer described in this study. Following the determination of background levels of bacteria in both storm and lake water (Tables 2-4), bacteriological analyses were performed before and after intentional discharge of stormwater to the riparian buffer located at Lake Merced. As shown in Table 5, bacterial levels in Lake Merced were about 1-3 log_{10} (90-99.9%) lower than in stormwater following the measured release of several hundred thousand to several million L of stormwater.

Table 5. Geometric mean levels (log_{10} MPN/100 mL) of *E. coli*, enterococci, and total coliforms in lake and stormwater (SW) following intentional diversion of stormwater to a riparian buffer (RB)

Date & total rainfall (cm)*	Vol. SW diverted to RB (L /min)	Sample	log_{10} *E. coli* MPN/100 mL (95% CLs)	log_{10} enterococci MPN/100 mL (95% CLs)	log_{10} total coliforms MPN/100 mL (95% CLs)
2/25/04 (4.1 cm)	4.1×10^6 L (1,900 L/min)	Stormwater	3.8 (0-4.2)	3.9 (0-4.3)	>4.4 (n/a)
		Lk. Merced	2.8 (1.2-4.4)	2.8 (1.7-4.0)	4.1 (3.7-4.5)
		% reduction	90%	92%	50%
3/26/04 (1.3 cm)	0.44×10^6 L (5,700 L/min)	Stormwater	4.2 (4.1-4.4)	4.2 (4.0-4.3)	>4.4 (n/a)
		Lk. Merced	1.6 (1.3-1.8)	0.6 (0.4-0.8)	2.8 (2.7-2.9)
		% reduction	99.7%	99.97%	97%
12/8/04 (4.8 cm)	0.41×10^6 L (280 L/min)	Stormwater	4.1 (4.0-4.1)	4.0 (3.7-4.1)	5.8 (0-6.5)
		Lk. Merced	1.6 (0.89-2.3)	1.3 (0.79-1.9)	3.6 (3.3-3.8)
		% reduction	99.7%	99.8%	99.4%
12/27/04 (6.2 cm)	13.6×10^6 L (5,113 L/min)	Stormwater	5.0 (4.8-5.2)	4.4 (4.2-4.6)	5.9 (0-6.7)
		Lk. Merced	3.7 (3.1-4.2)	2.9 (2.1-3.7)	4.0 (3.7-4.4)
		% reduction	95%	97%	98.7%

*Total rainfall 24 h before and during dates shown; n/a = not applicable

Levels of total coliforms in lake water were only 50% (0.3 log_{10}) lower than in stormwater following the storm on 2/25/04, but like total coliform levels in stormwater on 3/26/04, reductions may have been greater than observed, as the upper limits of detection for the assay on both dates had been reached. The bacterial reductions shown in Table 5 are similar to values observed for other storm discharge events evaluated during this study. Reductions following discharge of stormwater to the riparian buffer on 10/26/04 (total rainfall = 3.1 cm; diversion of 0.76 x 10^6 L at 3,154 L/min) and 1/7/05 (total rainfall = 1.5 cm; diversion of 12.4 x 10^6 L at 4,321 L/min) of *E. coli*, enterococci, and total coliforms ranged from 99.4-99.6% (2.2-2.4 $\log_{10}$), 99.9-99.97% (3.1-3.6 $\log_{10}$), and 99.2-99.4% (2.1-2.2 $\log_{10}$), respectively (data not shown). As shown in Table 5, overall fecal bacterial reductions, ranging from about 99.7% (2.5-2.6 $\log_{10}$) for *E. coli* to 99.8-99.97% (2.7-3.6 $\log_{10}$) for enterococci, were greater when lesser amounts (e.g., 0.41-0.44 x 10^6 L) of stormwater was discharged to the riparian buffer. In contrast, bacterial reductions were less, at <90-99%, when the volume of diverted stormwater to the riparian buffer was increased to >10^6 L. However, these larger diversions usually coincided with more intense storms (>4 cm in 48 h or less), and large amounts of uncontrolled surface runoff had been observed entering the riparian buffer and Lake Merced during some of those events. Therefore, the effectiveness of the riparian buffer to remove bacteria from stormwater appeared to decrease somewhat because of the higher stormwater inputs, both from controlled and uncontrolled sources. However, the apparent ability of the riparian buffer to remove enteric microbes such as *E. coli* and enterococci in stormwater is supported in part by previous findings, in which fecal coliforms and oocysts of the protozoan parasite *Cryptosporidium* in simulated rain water were significantly reduced (1-3 $\log_{10}$) by similar treatment systems (vegetated filter or buffer strips) (Coyne *et al*., 1998; Atwill *et al*., 2002).

CONCLUSIONS

Numerous studies have shown that constructed wetlands, vegetated filter strips, and other carefully engineered systems may effectively remove biological and chemical contaminants from domestic or agricultural wastewater effluents. In contrast, less information is available on the ability of naturally-occurring riparian buffer systems to remove waterborne microbial contaminants from urban stormwater. In the present study, bacterial indicators of fecal contamination were quantified in stormwater and in lake water impacted by stormwater runoff, including analysis of samples taken before and after intentional discharge to a riparian buffer. In general, lake bacterial levels were <2 $\log_{10}$ (<99%) lower than in stormwater following discharge of several million liters of stormwater. Greater reductions (about 2-3 $\log_{10}$ or 99-99.9%) were observed following discharge of lesser amounts of stormwater, at several hundred thousand liters. The amounts of stormwater diverted to Lake Merced in this study are relatively small in comparison to total runoff volumes generated during the storm season in San Francisco, and natural riparian buffer systems such as the one described here may become overwhelmed under extreme conditions, such as during periods of intense rainfall or following larger inputs of stormwater. Other limitations include the fact that the precise mechanism(s) of bacterial removal from stormwater by such systems is difficult to determine conclusively. However, these results suggest that a naturally-occurring riparian buffer worked effectively to reduce the numbers of bacterial indicators of fecal contamination in stormwater from entering a body of water in an urban setting. Riparian buffers or zones are commonly used to control nonpoint source chemical pollution and for helping solve eutrophication problems in urban watersheds, and the present study demonstrates that riparian buffers may also be useful for reducing or preventing the contamination of surface water with fecal bacteria that can be present in urban stormwater runoff.

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