©iStockphoto.com/budgetstockphoto

When Bacteria Call the Storm Drain "Home"

A study with implications for TMDLs and beach closures

BY GRETEL SILYN ROBERTS

With many new bacterial total maximum daily loads (TMDLs) statewide in California and the strict regulatory requirements they impose on dischargers, it is important to understand the true extent of how bacteria arrive, thrive, and die in urban environments. In southern California, with the "Total Maximum Daily Loads for Indicator Bacteria, Project I—Twenty Beaches and Creeks in the San Diego Region (Including Tecolote Creek)" now in place, many permittees are focusing on controllable anthropogenic sources and how best management practices (BMPs) might reduce loads and concentrations at compliance points within watersheds. However, our understanding of the microbial world indicates that even with the strictest of measures, in some cases these reductions may contribute little toward achieving compliance, particularly in wet weather. To gain a better understanding of the role indicator organisms play outside of the classic source control arena, the city of San Diego contracted Weston Solutions Inc. to perform a pilot study to investigate whether storm drains are a hospitable environment for biofilm containing fecal indicator bacteria (FIB).

Biofilms are matrices of bacteria and other microbes that form on and coat various surfaces. Biofilms form on solid surfaces exposed to a liquid (Characklis and Marshall 1990). Storm drains—with periodic urban flows, steady nutrient concentrations, and dark environments protected from ultraviolet (UV) light—are ideal environments for biofilm growth. Storm drain systems therefore have the potential to act as reservoirs for FIB and biofilm formation. During periods of high flow, biofilm sloughing can increase bacterial concentrations in storm drain flows, elevating bacterial levels in receiving creeks and estuaries. Fecal indicator bacteria (e.g., enterococci) that are able

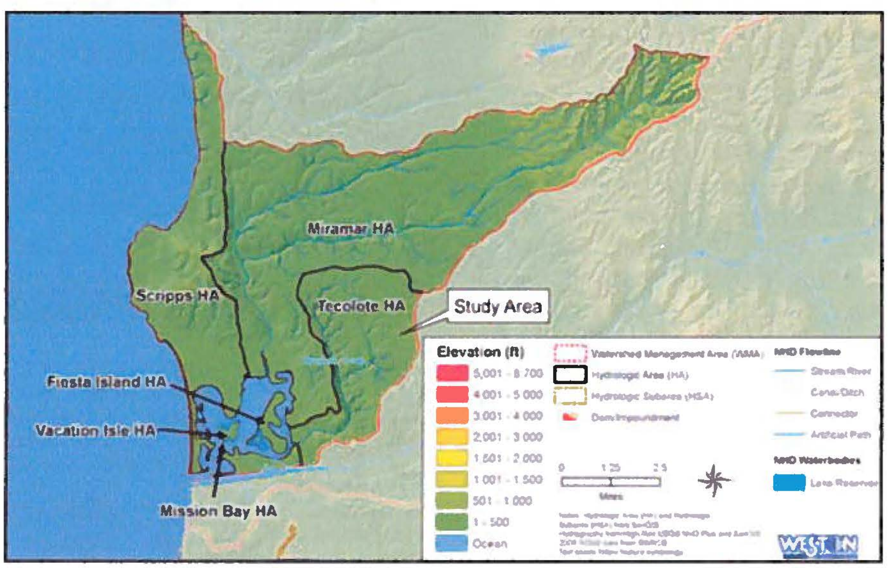

Figure 1. Mission Bay–La Jolla watershed management area in the City of San Diego

to grow in storm drain biofilms may be considered an environmental source of bacteria. As a consequence, increased concentrations of FIB in the receiving environment may not be caused by fecal sources in the watershed; however, the naturally occurring environmental sources may still contribute to noncompliance with water-quality objectives. To evaluate the origins of FIB, specifically enterococci, it is possible to speciate (identify the colony-forming unit to a species level) to determine whether the bacteria originates from a fecal source or an environmental source because certain species of enterococci are predominately associated with the human gut whereas others are not. This approach was used in the study to evaluate the bacterial content of storm drain biofilms.

To investigate the potential of storm drains to act as reservoirs for bacteria and biofilm formation, Weston performed a pilot study at a storm drain system in Tecolote Creek in the city of San Diego, CA.

The Tecolote Creek watershed is located within the Mission Bay–La Jolla Watershed Management Area (WMA) in San Diego (Figure 1). The Mission Bay–La Jolla WMA is approximately 43,244 acres and supports a variety of ecosystems and beneficial uses. The Tecolote Creek watershed runoff area covers more than 5,992 acres, which is approximately 14% of the Mission Bay–La Jolla WMA. Tecolote Creek discharges into the eastern portion of Mission Bay. The primary land uses within the contributing runoff area are residential (45%), transportation (21%), parks (18%), and public (8%). The remaining 8% consists of commercial, industrial, military, and vacant land uses. Tecolote Creek is listed on the State Water Resources Control Board 2006 303(d) list for indicator bacteria.

Materials and Methods

Artificial substrate (i.e., coupons) can be used in quantifying biofilm growth in natural environments (Långmark et al. 2007). In this study, two substrates were used in the comparison of biofilm growth as a reservoir for enterococci:

- Polyvinyl chloride (PVC), a thermal plastic polymer. This substrate was chosen as a positive control in biofilm development. PVC is extremely hydrophobic. One of the determining factors in initial bacterial adsorption to a surface is hydrophobicity (Characklis and Marshall 1990). High hydrophobicity in a surface's properties allows adsorption of similarly hydrophobic particles, including nutrients and bacterial cells. The initial adsorption of nutrients creates a viable surface for bacterial colonization and reproduction.
- Concrete, the most common substrate found in storm drain systems. The rough surface of concrete provides an ideal microenvironment for bacterial adhesion and growth.

PVC coupons were handmade using 1/4-inch PVC sheets. The PVC coupons were made as 2-inch-diameter circular stubs. Concrete coupons were made using builders' cement: build-

Figure 2. Examples of PVC (left) and concrete (right) coupons

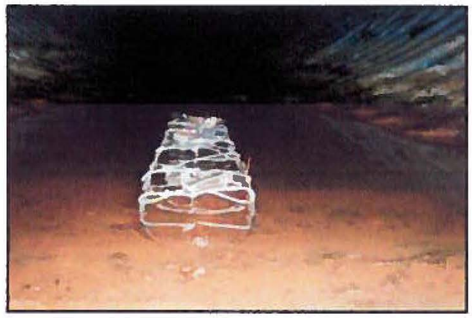

Figure 3. Coupon structure at site of upstream biofilm development experiment

Figure 4. Coupon structure at site of downstream biofilm development experiment

ers' cement was placed into 2-inch-diameter molds to form uniform concrete cylinders. An additional processing step was used to create equivalent surface roughness on both the PVC and concrete coupons. Coupons were abraded to a uniform roughness using a 600-grit abrasive wheel. Surface roughness is another key variable in biofilm development (Characklis and Marshall 1990); therefore, ensuring this variable was as constant as possible was important in the biofilm growth comparison experiment. Every attempt was made to ensure uniform surface roughness, but the concrete surface remained rougher than the PVC surface. Prior to the experiment, the concrete and PVC coupons were tumbled in distilled water for one week. This "weathering" process was designed to reduce the presence of any chemicals potentially leaching from the fresh cement mix. Each coupon had a final surface area of approximately 1.5 square inches (Figure 2).

The coupons were then suspended from the longitudinal cross section of either of one of two structures of 2-inch-diameter PVC pipe, specially built for the biofilm development experiment (Figures 3 and 4). The coupon structures were designed to provide a laminar flow of storm drain water directly on the base of each coupon. Both coupon structures were then autoclaved to ensure sterile conditions prior to deployment into the storm drain system.

Two sample locations were chosen from within the storm drain at Tecolote Creek. The first was upstream, within an enclosed storm drain pipe (Figure 3). The storm drain pipe was not exposed to sunlight and received constant urban runoff. This location was chosen as representative of the most common environment within the municipal separate storm sewer system (MS4), where pipes are enclosed and constantly wetted.

The second sample location was downstream, within an open concrete channel exposed to sunlight and a constant flow of water from the upstream sample location (Figure 4). The downstream sample location was chosen as representative of commonly constructed open concrete storm drain channels.

Table 1. Early Biofilm Development—Deployment and Harvesting of Coupons in Tecolote Creek

Deployment Date	Collection Date	Duration of Biofilm Growth	Samples Collected	Analyses	Comments
March 4, 2009	March 11, 2009	1 week	Surrounding water Upstream biofilms Downstream biofilms Sediments MS4 surface biofilms	Enterococcus quantification	Coupons were lost during a small storm event.
April 9, 2009	April 23, 2009	2 weeks		Enterococcus quantification	Coupons deployed. Significant sediment buildup was observed around coupons.
	May 6, 2009	4 weeks		Enterococcus quantification and enterococcus speciation	Some trash and sediment buildup were observed around coupons.
	May 18, 2009	6 weeks		Enterococcus quantification	Fine sediments, leaf litter, and organics were observed around coupons.
February 5, 2010	February 17, 2010	2 weeks	Biofilms and surrounding water	Enterococcus quantification	Early biofilm development reassessment
	March 2, 2010	4 weeks			
	March 31, 2010	8 weeks			Post-storm sample
	April 8, 2010	10 weeks			Post-storm sample

The coupon deployment and retrieval schedule is presented in Table 1. Immediately after harvesting, coupons were placed in 100 mL of phosphate buffer saline (PBS) solution and were sonicated in the field to remove biofilm from only the lower surface of each coupon. In addition to coupon retrieval, naturally occurring biofilms attached to sediments and plants were also collected for assessment of enterococci presence. All samples were placed on ice, transported to the laboratory, and enumerated for enterococci using multiple tube fermentation (Method SM 9230B). After four weeks of growth, isolates of enterococci from sediments, surrounding water, and biofilms from the concrete coupons were identified to a species level using the VITEK 2 Compact Identification System (bioMérieux Inc.). VITEK 2 Gram Positive Identification cards are used in conjunction with the VITEK 2 Compact system for the automated identification of microorganisms. The organisms identified are dependent upon the cards utilized.

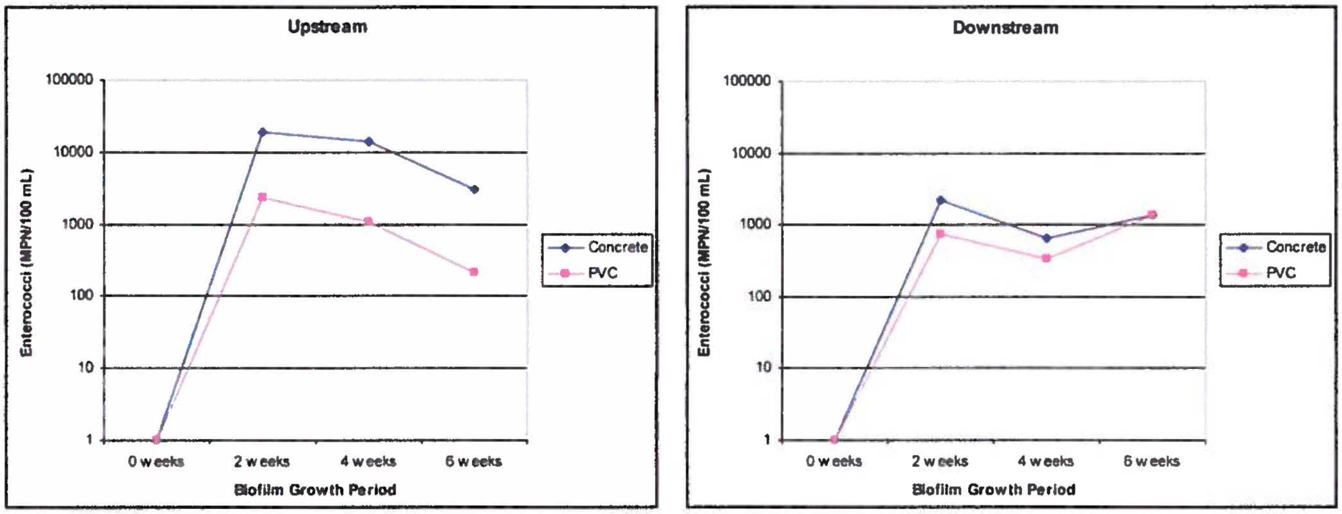

Figure 5. Early biofilm growth on different substrates upstream (left) and downstream (right) of the storm drain mouth

Results

Early Biofilm Growth. Rapid biofilm development was observed within the first six weeks of deployment (Figure 5). The data collected during the early biofilm development portion of the study are provided as geometric means in Table 2. Results of a t-test analysis showed no significant difference between enterococcus on the concrete surface compared to the PVC surface.

However, a t-test analysis showed that the difference between upstream and downstream was significant, with the upstream location having higher enterococcus concentrations. The upstream location appeared to provide a better growth environment than the daylighted downstream location for the concrete-grown biofilms, sediments, and naturally occurring biofilms. This may be due to UV-light-induced inactivation of bacterial cells or increased predation as reported in published literature (Schultz-Fademrecht et al. 2008, Långmark et al. 2007). Natural sediments collected from the surrounding habitat and naturally occurring biofilms were also found to be significant reservoirs of enterococci.

Table 2. Geometric Mean Values for Early Biofilm Growth in the Municipal Separate Storm Sewer System

Substrate	Units	Downstream (daylight)	Upstream (dark)
Concrete coupon biofilm	MPN/inch²	280	2,095
PVC coupon biofilm	MPN/inch²	157	183
Natural biofilm	MPN/gram-wet weight	2,753	3,505
Sediment	MPN/gram-dry weight	173	2,266

MPN: most probable number

Enterococcus Speciation of Storm Drain Water Surrounding Biofilms. Figure 6 presents the results of the species composition found in the water during the biofilm growth experiment. Water discharging from the storm drain appears to be the primary inoculation mechanism for biofilms within the MS4 system. These flows provide not only the inoculating bacteria but also important sources of moisture and nutrients. The results from 60 isolates suggest that over 80% of species found in the stormwater were associated with two fecally associated sources of enterococci. Both *E. faecium* and *E. faecalis* are enterococcus species associated with the intestinal flora of warm-blooded animals. These species are represented by the red solid fill and black border in Figure 6. These results suggest that the storm drain system transported isolates of fecal origin into the MS4 system and provided an inoculation mechanism for biofilms.

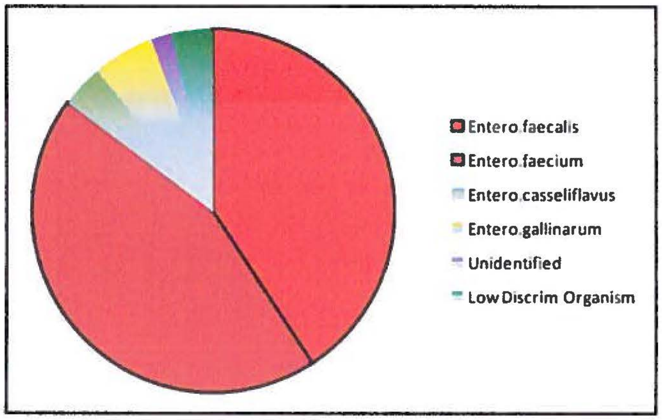

Figure 6. Enterococcus speciation of isolates from storm drain water during biofilm growth

Enterococcus Speciation of Biofilms Grown on Concrete. Figure 7 presents the results of the speciation analysis conducted on isolates collected from the concrete coupon biofilms during early biofilm development. The results show that 30% of isolates originated from likely fecal sources (*E. faecalis* and *E. faecium*). These species are represented by the red solid fill and black border in Figure 7. One-third of the species originated from likely environmental sources (*E. casseliflavus, E. gallinarum, and P. pentasauceus*) (Badgley et al. 2010a). These environmental sources are represented by the graded fill in Figure 7. The presence of one isolate identified as *S. epidermidis*, a species closely correlated with human flora, may be the result of

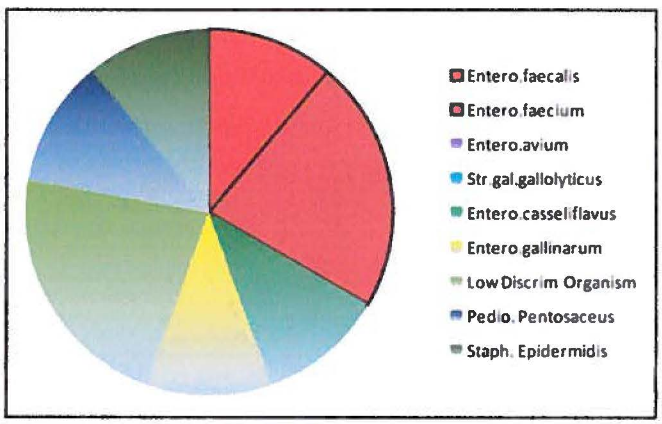

Figure 7. Enterococcus speciation of isolates from early storm drain biofilms grown on concrete

cross contamination during processing (Queck and Otto 2008). Of the isolates, 22% could not be identified (low discrimination organisms), but were likely *E. mondii*, an environmental species, which was not identified through the VITEK method.

Figure 8 presents the results of enterococcus species identification from mature biofilms grown on concrete coupons. The results show that fecal-associated species (represented by the red solid fill and black border in Figure 8 for *E. faecalis* and *E. faecium*) contributed over 40% of the biofilm population. However, the biofilm showed a significant presence of nonfecal species; almost 40% of the species were associated with a strain of enterococcus commonly found in birds (*Streptococcus gallolyticus*), while 20% were likely to be species associated with soils,

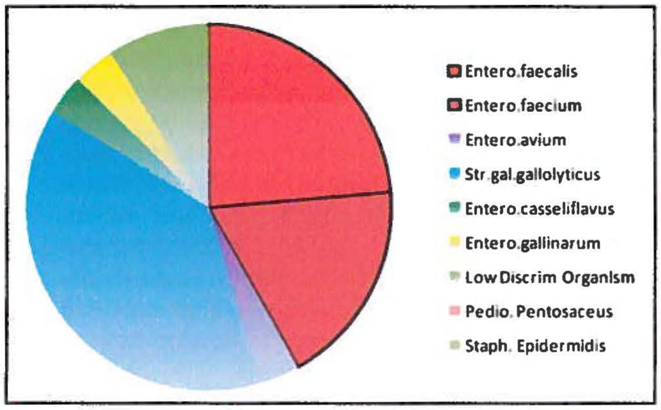

Figure 8. Enterococcus speciation of isolates from mature storm drain biofilms grown on concrete

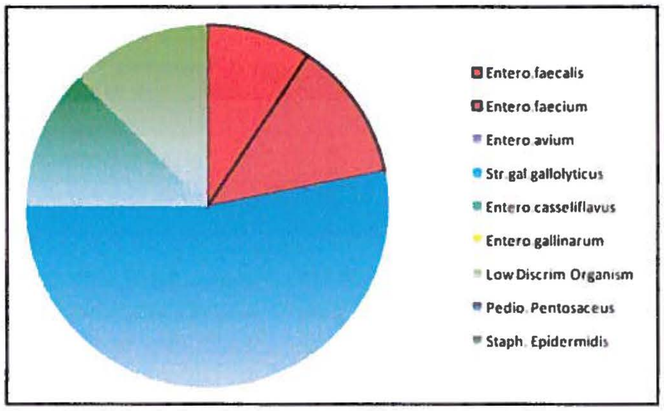

Figure 9. Enterococcus speciation of isolates from mature storm drain biofilms grown on PVC

plants, and other environmental sources, including *E. casseliflavus*, *E. gallinarum*, *E. avium*, and *E. mondii*.

Isolates collected from biofilms grown on PVC coupons showed similar results with only 20% of species associated with fecal origins (Figure 9). The majority of the 32 isolates collected from PVC coupons were *Streptococcus gallolyticus*, a strain of enterococcus commonly found in birds.

Mature Biofilm Growth and Stabilization. The results of the mature biofilm growth experiment showed that enterococci continue to inhabit biofilms in the MS4 system. However, a t-test analysis showed that enterococci densities in early biofilms were significantly higher than those observed in mature biofilms. This is shown in Figure 10; enterococcus concentrations in biofilms decreased as the biofilm matured. This finding may be a function of season, with higher flow rates and colder water temperatures during winter months (October through February). This hypothesis is supported with the water column results, which show some decrease in concentrations at weeks 36 and 40 (December and January, respectively).

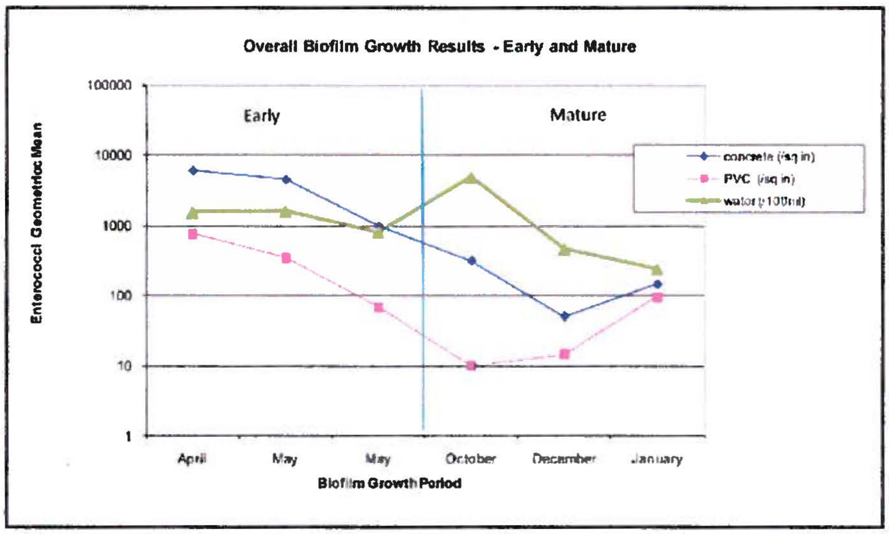

Figure 10. Comparison of enterococcus enumeration on biofilms (MPN/in.²) and concentrations in surrounding water (MPN/100 mL) using standard methods

Alternatively, the enterococcus concentration decrease may be a function of increased predation of the biofilm ecosystem, with protozoa and nematode activity increasing with biofilm growth. A stabilization of biofilms would be expected after a period of maturation, with factors such as predation and sloughing acting as key control mechanisms (Characklis and Marshall 1990).

Comparison of Substrates. A comparison of geometric means (Table 3) showed that, for the duration of the experiment, enterococcus numbers averaged 627 MPN per square inch on the concrete coupons while the average density on the PVC coupons was significantly lower at 79 MPN per square inch. The concrete coupon results were assumed to represent bacterial growth in standard reinforced concrete pipe commonly used in MS4, and the PVC coupons were assumed to represent bacterial growth in standard PVC pipe.

A t-test comparison of data showed a significant difference between bacterial densities on concrete coupons compared to PVC coupons, with significantly lower densities of bacteria associated with biofilms grown on PVC. A potential reason for this difference is the variation in surface area among the substrates, with a greater surface area on the concrete inherent to the composition of the matrix. Greater and more frequent sloughing, caused by the planar and hydrophobic surface of PVC, could be expected, resulting in less biofilm development on PVC pipe.

Impact of Wet Weather Flows. One objective of the mature biofilm growth experiment was to assess the potential sloughing of biofilms during periods of high flow in storm drains (e.g., during wet-weather events).

Figure 11 illustrates the enterococcus geometric mean densities on concrete biofilms during a period of high flow. Samples were collected prior to the first wet event of the season in Tecolote Creek at week 29 (October 27, 2009). The first subsequent rainfall event of the season occurred on December 13, 2009, and another biofilm collection

Table 3. Geometric Mean Values for Mature Biofilm Growth in the Municipal Separate Storm Sewer System

Substrate	Units	Early	Mature	Overall
Concrete coupon biofilm	MPN/inch^2	2,095	131	627
PVC coupon biofilm	MPN/inch^2	183	24	79

was undertaken on December 14, 2009. The next rainfall event occurred on January 18, 2010. Biofilm samples were collected on January 12, 2010, prior to that rain event. A t-test comparison of data showed a significant difference between bacterial densities on the concrete coupon before the storm (week 29) and after the storm (week 36) with a significant decrease in enterococcus numbers after the storm. Prior to the storm, enterococcus numbers on concrete were on average 313 MPN per square inch; after the storm event, enterococcus numbers decreased to 50 MPN per square inch. These results suggest some sloughing of live cells from concrete coupons during rain events and periods of increased flow. The increase in enterococcus numbers in the month after the storm event suggests biofilm regrowth.

In addition, speciation of enterococci was undertaken during the assessment of storm flow impact. Isolates were collected from concrete biofilms immediately before the first storm of the season and again immediately after the storm. Additional samples were collected one month after the storm but prior to the next significant rain event.

The results, presented in Figure 12, show that the pre-storm mature biofilm is composed of predominantly environmental strains, with approximately 40% of the isolates originating from fecal strains. Immediately after the storm, the enterococcus population not only decreased but also changed in terms of species composition. Over 80% of the isolates, one day after the storm, originated from fecal strains. One month after the storm, the biofilm population was once again dominated by environmental strains of enterococci. These results suggest that sloughing during high-flow conditions removes a significant enterococcus population from the storm drain system and that the majority of that sloughed biofilm consists of environmental strains of enterococci. The biofilm then appears to be re-inoculated with fecally associated enterococcus species from the stormwater, which may be slowly outcompeted during biofilm regrowth.

Discussion

The results of this study are similar to those found in published literature, which suggests that natural and anthropogenic waterway ecosystems provide ideal habitats for biofilm growth, and that FIB form a quantifiable portion of that biofilm community (Balzer et al. 2010, Schultz-Fademrecht et al. 2008). Based on the results of this study, biofilms containing enterococci appear to develop well on storm drain structures, with concrete providing a better habitat for biofilm development than PVC. This may be attributable to the reduced surface area of PVC, together with the increased likelihood of biofilm sloughing from the PVC surface. The implication of these results is that the concrete pipes used in most municipal storm drain systems are a hospitable environment for bacteria and microbe growth and subsequent biofilm formation.

In addition to the differences noted in colonization of substrates, the species composition in early biofilm development is not solely from enterococci of fecal origin. The speciation of enterococci from the water column suggests that biofilms in storm drain systems are likely initially inoculated by both storm drain flows and surrounding reservoirs (such as plants and sediments). Other research has found similar results with the Enterococcus species *casseliflavus*, *faecalis*, faecium, *hirae*, and mundtii appearing frequently both temporally and spatially in environmental water samples (Badgley et al. 2010a). Presence and changes in enterococcus species composition also have been found to be strongly influenced by the environmental conditions, with pigmentation of the species being linked to survival in sunlight (Maraccini et al. 2011) as well as the presence of plant material in sediments (Badgley et al. 2010b).

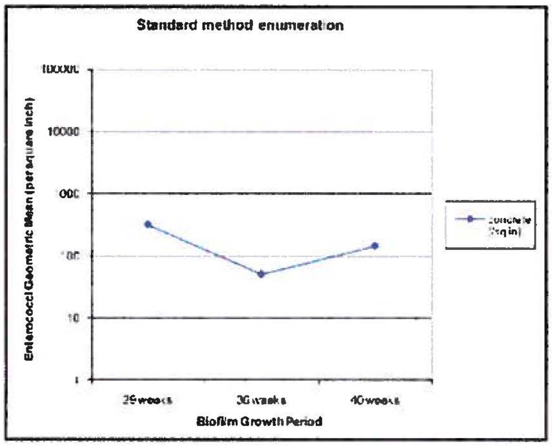

Figure 11. Impact of storm flows on biofilms

Although early biofilm development was dominated by fecal species from the water column, steady increases in environmental enterococci species, as the biofilm matured, demonstrated the changes in species composition over time. The comparison of early biofilms and mature biofilms suggests that enterococci numbers from the biofilm mass stabilized after approximately two months. The reduced presence of enterococci in the mature biofilms suggests predation and competition by other microbial fauna.

In addition, the reduction in enterococci as the biofilm matured suggests the existence of complex, diverse microbial fauna in the storm drain biofilm. An important factor often overlooked in water-quality improvement studies is that the presence of biofilms often reduces nutrients, suspended solids, and metals (Cardinale 2011), thus improving water quality. Therefore, engineered designs that retrofit storm drains to enhance microbial community presence could improve water quality. Although this study did not investigate the benefits of naturally occurring biofilms in storm drains, the results suggest that environmental species of enterococci outcompete fecal species. Further study of the benefits of storm drain ecosystems is necessary to fully understand how such ecosystems might enhance overall water quality.

The results of the biofilm assessment during wet-weather flows have significant implications for TMDL compliance. It was found that both biofilm mass and enterococci numbers decreased significantly during wet weather, suggesting significant sloughing during high flows. One of the most important findings of this study was that species diversity and composition was changed significantly as a result of wet-weather flows. The enterococcus species diversity within the biofilm decreased as a result of significant sloughing, to be replaced by a population made up almost entirely of the species comprising the surrounding water column. The maturity and diversity of the enterococcus species in the biofilm recovered after one month of growth. The dominance of certain strains within the storm drain biofilms suggests that these persistent enterococcus populations are not directly related to pollution events. The "indicator bacteria" form the basis of our predictive ability to assess potential human health risk. The fact that fecal enterococci are only present for a short period of time within the biofilm matrix, to be outcompeted by environmental species, would suggest that current wet-weather water-quality criteria over estimate risk.

These results imply that it may be virtually impossible to attain compliance with southern Californian TMDLs during periods of wet weather because

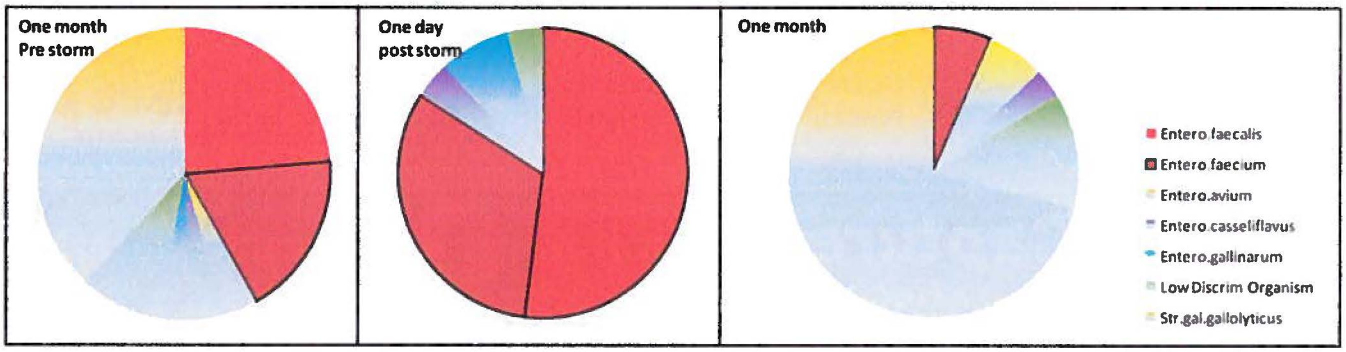

Figure 12. Impact of storm flows on biofilm species composition

of the potential discharge of biofilms from storm drains into receiving waters. Enterococci are prevalent throughout the environment, with species found in sediment (Badgley et al. 2010a), water columns (Balzer et al. 2010), and plants (Badgley et al. 2010b). Add to that the vast network of storm drain systems in urbanized areas and the expanse of the potential enterococci habitat becomes apparent. The ubiquitous nature of these communities means that engineered approaches such as UV disinfection that aim to eliminate bacterial presence to achieve water-quality compliance are unlikely to succeed.

Acknowledgements

Grateful acknowledgement is extended to the following organizations for their support of this project: the city of San Diego, Transportation and Storm Water Department, and the city of San Diego Public Utilities Department, Environmental Mentoring and Technical Services Division, Marine Microbiology and Vector Management Laboratory.

References

Badgley, B. D., B. S. Nayak, and V. J. Harwood. 2010a. "The Importance of Sediment and Submerged Aquatic Vegetation as Potential Habitats for Persistent Strains of Enterococci in a Subtropical Watershed. *Water Research* 44(20): 5857–66.

Badgley, B. D., F. I. Thomas, and V. J. Harwood. 2010b. "The Effects of Submerged Aquatic Vegetation on the Persistence of Environmental Populations of Enterococcus spp." *Environmental Microbiology* 12(5): 1271–81.

Balzer, M., N. Witt, H. C. Flemming, and J. Wingender. 2010. "Faecal Indicator Bacteria in River Biofilms." *Water Science and Technology* 61(5): 1105–11.

Cardinale B. J. 2011. "Biodiversity Improves Water Quality Through Niche Partitioning." *Nature* 472(7341): 86–9.

Characklis, W. G., and K. C. Marshall, eds. 1990. *Biofilms* (Environmental and Applied Microbiology Series). John Wiley & Sons.

Långmark J., M. V. Storey, N. J. Ashbolt, and T. A. Stenström. 2007. "The Effects of UV Disinfection on Distribution Pipe Biofilm Growth and Pathogen Incidence Within the Greater Stockholm Area, Sweden." *Water Research* 41(15): 3327–36.

Maraccini, P. A., D. M. Ferguson, and A. B. Boehm. 2011. "Diurnal Variation in Enterococcus Species Composition in Polluted Ocean Water: A Potential Role for the Enterococcal Carotenoid in Protection Against Photoinactivation." *Applied and Environmental Microbiology*, November 2011.

Queck, S., and M. Otto. 2008. "Staphylococcus Epidermidis and Other Coagulase-Negative Staphylococci." In *Staphylococcus: Molecular Genetics*. Caister Academic Press.

Schultz-Fademrecht C., M. Wichern, and H. Horn. 2008. "The Impact of Sunlight on Inactivation of Indicator Microorganisms Both in River Water and Benthic Biofilms." *Water Research* 42(19): 4771–9.

Gretel Silyn Roberts *is a senior project manager with Weston Solutions Inc.*