

October 11, 2022

**FINAL REPORT:**  
Tracing Fecal Contaminants and Copper  
From Watershed Sources to the Ocean



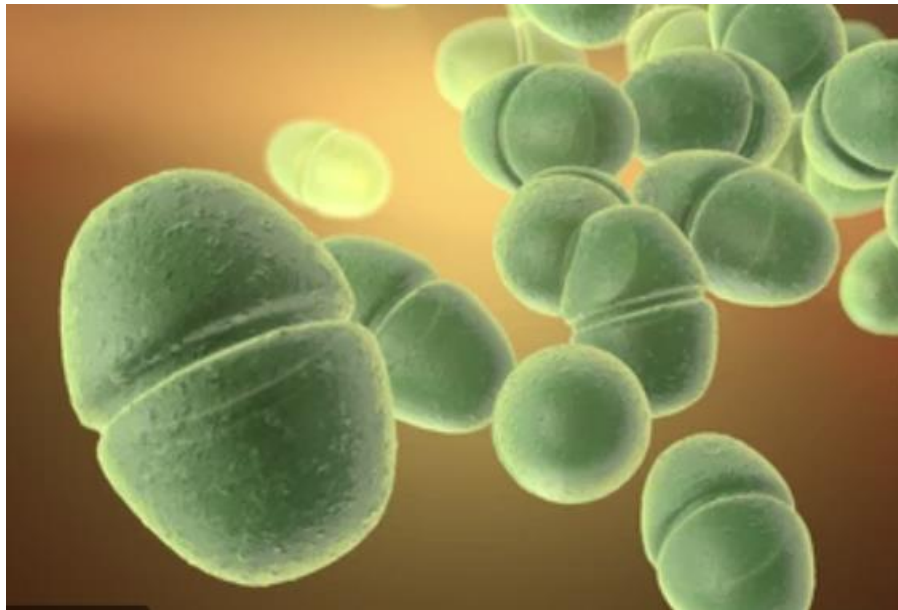
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Submitted to:

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## Executive Summary

This report describes the results of monitoring performed from 3/1/2018 to 3/28/2022 to trace potential sources of fecal indicator bacteria (FIB) and copper exceedances in ocean receiving water adjacent to the 4th Avenue storm drain that discharges from the City of Carmel-by-the-Sea (Carmel) into Carmel Bay.

Since the program's beginning, samples have been collected from six watershed sites (WS), three building rain downspouts, the large storm drain (SD) at the end of 4<sup>th</sup> Avenue, a nearshore receiving water site (RW) directly off the SD, and an ocean water site (OW) north of the receiving water site. Nine storms have been sampled. Not all sites were sampled in each storm, as results from previous samples guided program changes to focus on existing sites or add new sites to delineate areas requiring management actions. WS2 was consistently sampled at its discharge into SD. Subwatershed WS1 was not sampled after 3/20/2019 and subwatersheds WS3, WS3a, and WS3b were not sampled before 3/16/2020.

Measurements included concentrations of *Enterococcus* (upon which water quality benchmarks are based) and dog, gull, human, and anthropogenic (dog + human) fecal bacteria, and dissolved and total copper. The contributions of WS2 and its subwatersheds to SD discharges were estimated by converting concentration data into load estimates (concentration x watershed acreage).

### Watershed Loads and Sources

Contributions of loads from WS2 to SD differed among fecal indicators. On average, WS2 contributed 46% of *Enterococcus* loads from SD, indicating broadly distributed sources across the entire SD watershed. Loads of dog, gull, human, and anthropogenic fecal bacteria from WS2 averaged 4 – 51,000 times greater than loads from SD, indicating potent sources for these fecal bacteria within WS2. WS1 loads of dog fecal bacteria averaged 72% of WS2 loads and none of the other fecal bacteria loads from WS1 exceeded 27% of WS2 loads, suggesting that WS1 could be an important source of dog fecal bacteria. Loads of *Enterococcus*, gull, and human fecal bacteria from subwatershed WS3 to WS2 averaged 84%, 13%, and 48%, respectively of WS2 loads. Loads of dog and anthropogenic fecal indicators from WS3 were 5.1 and 2.4 times greater than from WS2, identifying WS3 as the major contributor of these indicators into WS2 and SD. Loads of dog and human fecal bacteria from WS3a averaged 2 – 24 times greater than those from WS3. Dog and gull fecal bacteria were not measured in WS3b, but loads of human fecal bacteria from this subwatershed averaged 45 times greater than from WS3. These results identify WS3a and WS3b as important subwatersheds for management actions to reduce loads of dog, human, and anthropogenic fecal bacteria into SD.

Following high concentrations of human fecal bacteria in WS2 on 3/13/18, the sewer line under Junipero Street between 1<sup>st</sup> Avenue and 2<sup>nd</sup> Avenue was relined in mid 2020. Concentrations of human fecal bacteria before (T1) and after (T2) the sewer was relined were not statistically different, probably due to small numbers of samples, but concentrations in T2 remained several orders of magnitude below the value seen on 3/13/18. Robust

concentrations of human fecal bacteria in WS3, WS3a, and WS3b suggest continuing sources of human fecal bacteria in those subwatersheds.

Copper loads from WS2 averaged 50% of those from SD, indicating broadly distributed sources within the entire SD watershed. Copper loads from WS3 averaged <40% of WS2 loads, which is also consistent with broadly distributed sources. Copper concentrations in watershed and SD samples were dominated by dissolved copper, indicating that the use of treatment devices that focus on sediment removal would not be very effective at reducing copper loads to the receiving water.

Total copper concentrations were significantly higher in watershed and SD samples than in ocean samples (RW and OW), indicating the high copper concentrations in RW samples originate from sources on land. Samples collected from copper roof gutters and downspouts revealed higher concentrations of copper than measured in watershed or SD samples. The downspout samples also were dominated by dissolved copper suggesting the high dissolved copper loads in watershed and SD samples arise from rainfall flowing across architectural copper (i.e., derived from buildings and infrastructure).

### Stormwater Effects on Receiving Water

Receiving water concentrations of dog, human, and anthropogenic fecal bacteria were significantly associated with SD loads, although other unmeasured factors affecting RW concentrations also were evident. OW also significantly affected RW concentrations of anthropogenic fecal bacteria indicating sources other than the 4<sup>th</sup> Avenue storm drain.

Estimates of the contributions by SD loads to RW concentrations suggested that stormwater discharges contributed loads of *Enterococcus* that often exceeded both Ocean Plan and ASBS objectives. While there is no direct comparison between the *Enterococcus* measurements (CFU/100ml) and animal fecal bacteria (gc/ml) data, it was found that dog, gull, and anthropogenic fecal bacteria from SD exceeded background (10 gc/ml) in several RW samples. Three of the five samples that could be analyzed for SD effects on RW concentrations exceeded *Enterococcus* Ocean Plan and ASBS 85<sup>th</sup> percentile benchmarks. These three samples had low calculated dilution factors for SD discharges that ranged from 3.2 to 5. Two of the exceeding samples were associated with high SD loads of anthropogenic fecal bacteria and both were due to dog fecal bacteria. The third exceeding RW *Enterococcus* concentration was associated with high gull fecal bacteria. The same three samples that exceeded *Enterococcus* Ocean Plan or ASBS 85<sup>th</sup> percentile benchmarks also exceeded the benchmarks for copper concentrations. These results suggest that management actions aimed at decreasing loads of dog fecal bacteria from the watershed, and in particular subwatershed WS3, should be prioritized to reduce anthropogenic (dog) fecal bacteria. In addition, watershed-wide management actions to reduce loads from architectural copper are needed to reduce copper exceedances in receiving water.

## Introduction and Background

City of Carmel-by-the-Sea (Carmel) participated in the Central Coast Regional Monitoring Program (Regional Program), for the period 2014-2016, which measured water quality in stormwater discharges and ocean receiving waters in conformance with Special Protections for Areas of Special Biological Significance (ASBS) adopted by the State Water Resources Control Board (SWRCB). The Regional Program identified several exceedances of water quality criteria for copper and fecal indicator bacteria (FIB) associated with stormwater discharged from Carmel into the Carmel Bay ASBS. As a result, Carmel proposed additional follow-up monitoring to help identify the potential sources of the exceedances. This report describes the results from the third and fourth years of monitoring to trace potential sources of FIB exceedances as well as sources (particulate or dissolved) contributing to copper exceedances.

From a regulatory standpoint it is assumed that FIBs detected in stormwater and in ocean receiving water are associated with human fecal waste. However, these FIBs, including *Escherichia coli* and *Enterococcus spp.*, are present in the intestines of all warm-blooded animals and could come from a variety of different non-human sources. The importance of various sources of fecal bacteria to the FIB signal, typically measured using selective media to culture either *E. coli* or *Enterococcus*, is not clear. Parsing anthropogenic (i.e., human and pet-associated) sources of fecal matter from wildlife sources (i.e., birds, ruminants, raccoons, etc.) becomes important in situations where there are persistent exceedances of the Ocean Plan guidance for FIBs because studies demonstrate that human health risks associated with wildlife fecal inputs are inherently lower than those associated with human fecal inputs (Schoen and Ashbolt 2010, Soller et al. 2010). Accordingly, the SWRCB has emphasized that dischargers are not responsible for reducing FIBs from wildlife sources. Because the regulatory compliance endpoint is ocean receiving water, which is influenced by fecal inputs from wildlife sources all along the coastal zone, and from within ocean water, it is of interest to determine the fraction of the FIBs measured in ocean water that may be comprised of non-human fecal sources. In addition to potentially being diluted by wildlife FIBs, the anthropogenic FIB signal may be further diluted by cross-reactivity with bacteria endemic to ocean water. This latter concern is particularly associated with fluorescent substrate methods such as Enterolert (Budnick et al. 1996, Adcock and Saint 2001, Sercu et al. 2011).

In order to examine the contribution of anthropogenic sources of FIBs in Carmel's storm runoff and receiving water, this project used microbial source tracking (MST). This technique takes advantage of the specificity of quantitative polymerase chain reaction (qPCR) probes to unique source bacteria associated with human and other animal fecal matter. A primary objective of this study was to examine whether ocean receiving waters that had *Enterococcus* exceedances were 1) dominated by human and dog waste sources which were discharged by stormwater, or whether these receiving waters were dominated by 2) wildlife waste sources that were either discharged by stormwater or already present in the ocean water. Furthermore, statistical approaches were used to estimate the maximum human and dog contributions to receiving water *Enterococcus* concentrations.

A second objective was to trace copper from the watershed to the ocean as previous stormwater sampling has demonstrated ocean receiving water copper concentrations above

Ocean Plan objectives (ASBS report, Applied Marine Sciences, 2016), and to determine the amounts of copper in the dissolved and particulate phases to support decisions about management practices.

## Approach

### Sites

Since the beginning of the Carmel stormwater monitoring program in 2018, watershed samples have been collected from six locations (Table 1 and Figure 1). Sites WS1, WS3, WS3a, and WS3b were sampled in order to delineate subwatershed areas in WS2 that could account for high concentrations measured in the WS2 discharge. The areas drained by each subwatershed are shown in Figure 2. Samples collected at WS2 include discharges from all subwatersheds and samples collected at WS3 include discharges from WS3a and WS3b. WS2 represents 53% of the entire SD watershed and spans areas both within and outside of the City limits. Subwatersheds WS1 and WS3 represent 40% of the entire WS2 watershed. WS1 collects runoff from outside of the City limits. Subwatersheds WS3a and WS3b account for 73% of the WS3 subwatershed and are located uphill of the only location in the watershed where a sanitary sewer main is located above the storm drain. Sampling to investigate sources of copper was performed in December 2021 and March 2022 at WS2, 4<sup>th</sup> at Monte Verde, and from downspouts at City Hall, Sunset Center, and Scout House (Figure 1).

**Table 1. Locations and dates of sampling from 2018 – 2022.**

Station	Description	Latitude	Longitude	Acres	Dates Sampled <sup>a</sup>
WS1	Subwatershed 1	36.5633 N	121.9189 W	10	1, 2, 3, 4
WS3a	Subwatershed-3	36.5606 N	121.9190 W	10	5, 6, 7, 8, 9
WS3b	Subwatershed-3	36.5612 N	121.9191 W	12	5, 6, 7, 8, 9
WS3	Subwatershed-3	36.5611 N	121.9202 W	30	5, 6, 7, 8, 9
WS2	Watershed-2	36.5600 N	121.9220 W	95	1, 2, 3, 4, 5, 6, 7, 8, 9
SD	Storm Drain @ 4 <sup>th</sup> Ave.	36.5564 N	121.9310 W	180	1, 2, 3, 4, 5, 6, 7, 8, 9
RW	Receiving Water	36.5564 N	121.9314 W	-	1, 2, 3, 4, 5, 6, 7, 8, 9
OW	Ocean Water	36.5566 N	121.9315 W	-	2, 3, 4, 5, 6, 7, 8, 9

<sup>a</sup> 1 = 3/1/18, 2 = 3/13/18, 3 = 1/17/19, 4 = 3/20/19, 5 = 3/16/20, 6 = 1/28/21, 7 = 3/10/21, 8 = 12/13/21, 9 = 3/28/22

Not all sites were sampled in each sampling event (Table 1), and not all analyses were performed in each sample (Appendix A). Sampling at subwatershed WS1 was discontinued after it was deemed to have relatively lower concentrations of dog and human fecal bacteria compared to WS2. Sampling of subwatersheds WS3, WS3a, and WS3b was initiated to identify areas with high contributions to fecal bacteria concentrations in WS2.

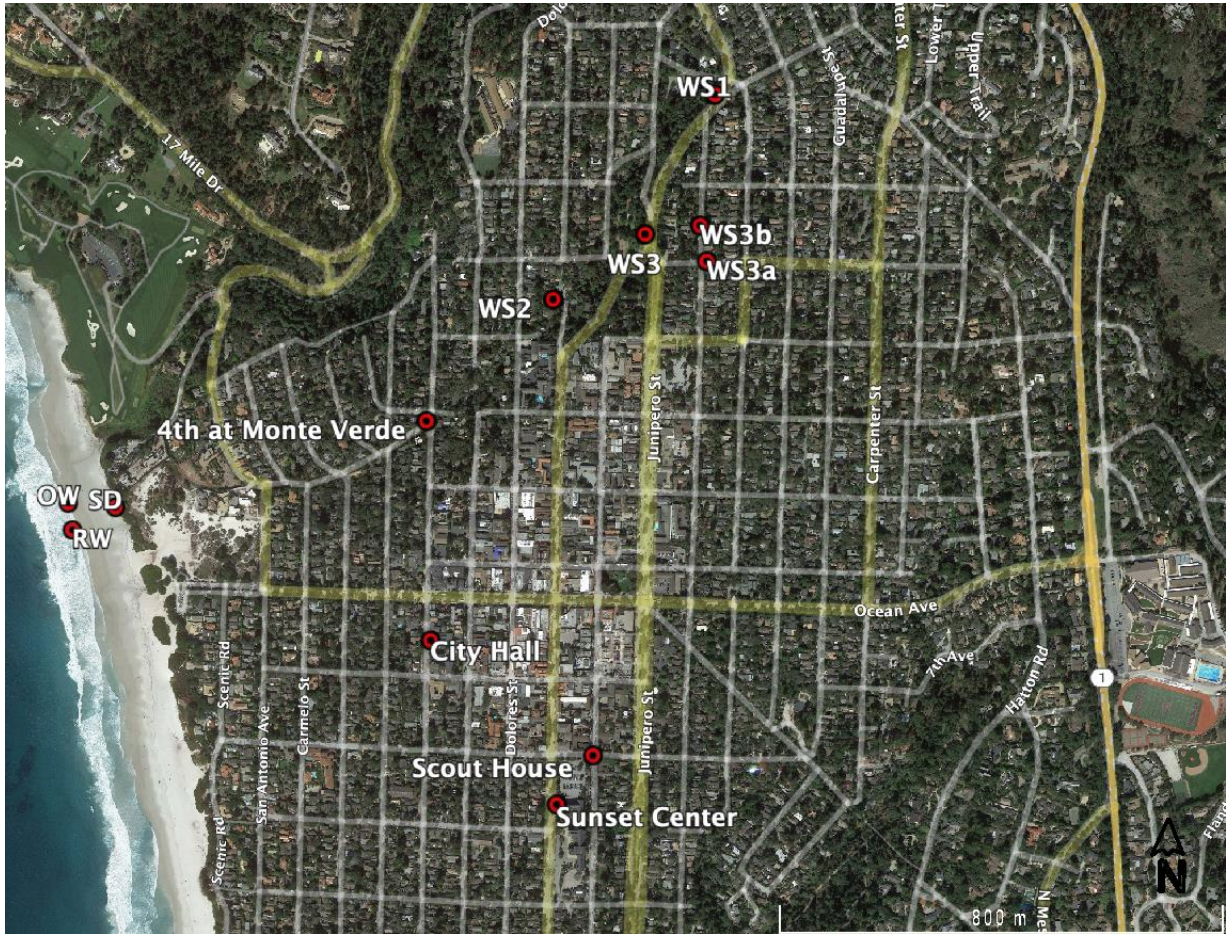


Figure 1. Carmel sampling sites. Sites as follows: watershed 1 (WS1), watershed 2 (WS2), watershed 3 (WS3), watershed 3a (WS3a), watershed 3b (WS3b), 4<sup>th</sup> at Monte Verde, City Hall, Scout House, Sunset Center, 4<sup>th</sup> Avenue storm drain (SD), ocean receiving water (RW), and ocean water 50 meters away from the receiving water (OW).

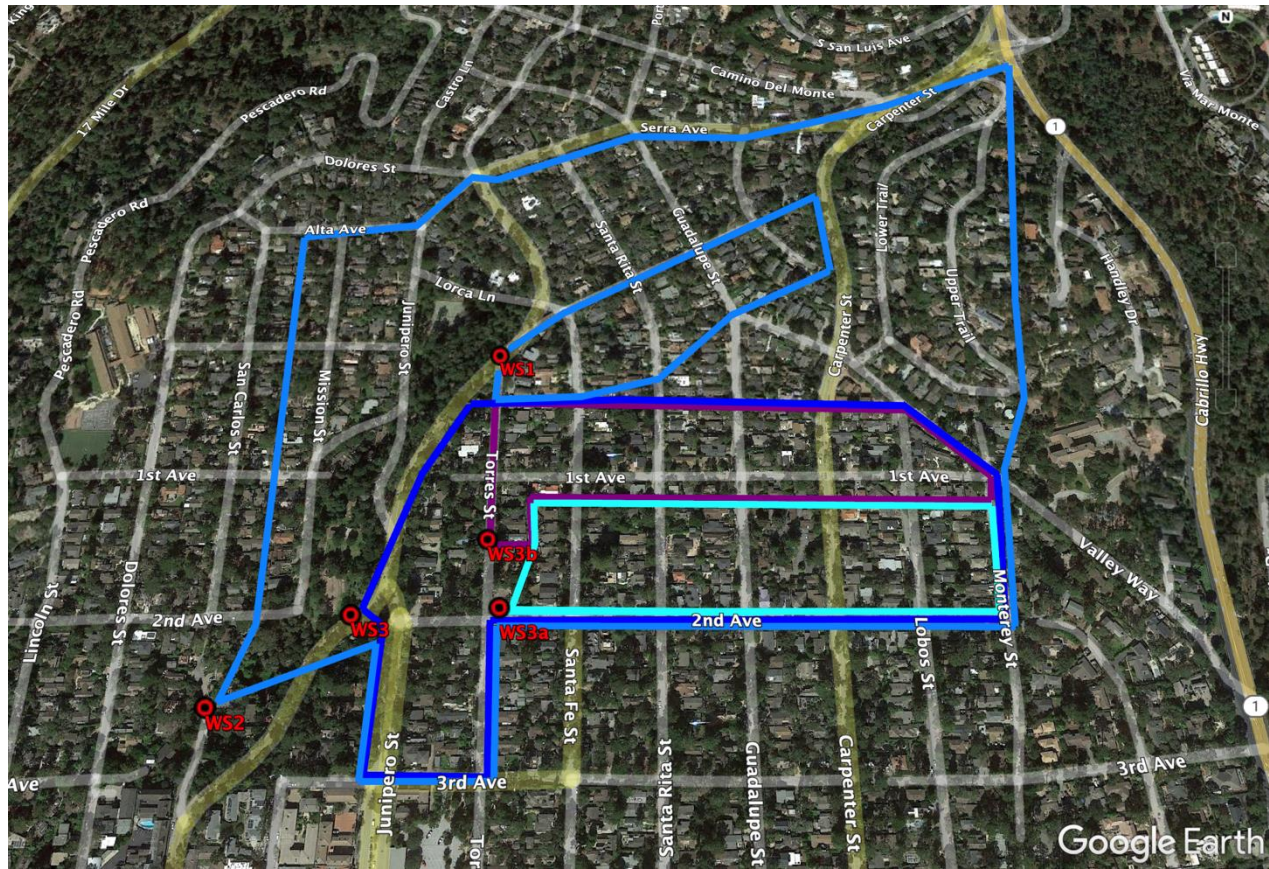


Figure 2. Subwatershed areas sampled within WS2.

#### Analytical Methods

The following analytical methods were employed on the samples:

- 1) **Enterococcus enumeration** was performed on all samples using membrane filtration followed by *Enterococcus* culturing and enumeration (Messer and Dufour 1998) by Cel Analytical, Inc.
- 2) **qPCR analyses** were also performed by Cel Analytical, Inc. on all samples using primers specific to species of the Bacteroidales order associated with dog (DogBact), gull (LeeSeaGull), and human (HF183) feces to quantify the amount of DNA associated with each source (Bernard and Field 2000, Fogarty and Voytek 2005). Human qPCR was performed according to USEPA Method 1696 using human marker HF183 specific to Bacteroides genera.
- 3) **Trace metal (TM) analyses**, including total and dissolved trace metal quantification, were performed on all seven sites from all three storms by Physis Environmental Laboratories, Inc. The amount of copper associated with particulate matter was computed by subtracting dissolved concentrations from total concentrations.

## Data Analysis

Data analysis began with aggregating data from all sites and all sampling events. This analysis utilized a load index that was calculated by multiplying the concentration (CFU/100ml or gc/ml) in each sample by the watershed acreage (Table 1). Rainfall was not included in the load index because it consists of a single amount applied to all watersheds in each sampling event, it was assumed to affect each watershed equally. Due to the large range in values measured among parameters, data are presented in log-scale graphs.

The sum of data obtained during the monitoring program was analyzed to search for temporal and spatial variation that could uncover the major sources of *Enterococcus*, fecal indicator bacteria and copper associated with exceedances of Ocean Plan and ASBS water quality benchmarks in nearshore receiving water. Our objective was to cast a wide net that would not disqualify results due to the limited numbers of samples that were available given restricted municipal resources and developing drought conditions. Accordingly, a liberal criterion has been used to qualify a statistical result as significant with a probability of  $< 0.10$ .

Average percentages of loads from watersheds and subwatersheds into each successively lower watershed or subwatershed were calculated for each sample to identify the largest upstream sources. This involved calculating mean contributions of SD loads from WS2, mean WS2 contributions from WS1 and WS3, and mean WS3 contributions from WS3a and WS3b. WS2 and WS3 samples were analyzed for differences between samples collected before (T1) and after (T2) the sewer line along Junipero (located upstream of WS3 and downstream of WS3a and WS3b) was relined. Linear regressions were performed to determine whether RW concentrations were significantly influenced by SD and OW. Linear regressions were also used to examine the relationship of SD discharges to RW concentrations and exceedances, as well as to partition effects of SD and OW on RW concentrations.

## Results and Discussion

### Watershed Loads

#### *Enterococcus and Fecal Bacteria from Dogs, Gulls, and Humans*

Estimating the contribution of dog and human fecal bacteria to exceedances of *Enterococcus* in receiving water samples is challenging. It requires establishment of statistical relationships between the qPCR results for dogs + humans and *Enterococcus* concentrations in stormwater drainages and discharges into the receiving water. Finally, the approximate dilution of storm drain discharges in receiving water samples must be known to estimate the percentage of receiving water *Enterococcus* due to the storm drain. Moreover, designing management actions to reduce dog + human contributions to exceedances requires information about whether dog or human fecal bacteria predominate in receiving water samples that exceed *Enterococcus* objectives.

*Enterococcus* loads from all samples suggested sources distributed broadly over the entire SD watershed (Figure 3). WS2 consistently had the second greatest load of *Enterococcus*, followed by subwatershed WS3, WS3a, and WS3b, except for the samples from 3/28/22, when SD and



WS2 had lower loads than WS3. WS1 had the lowest load on each of the three dates it was sampled.

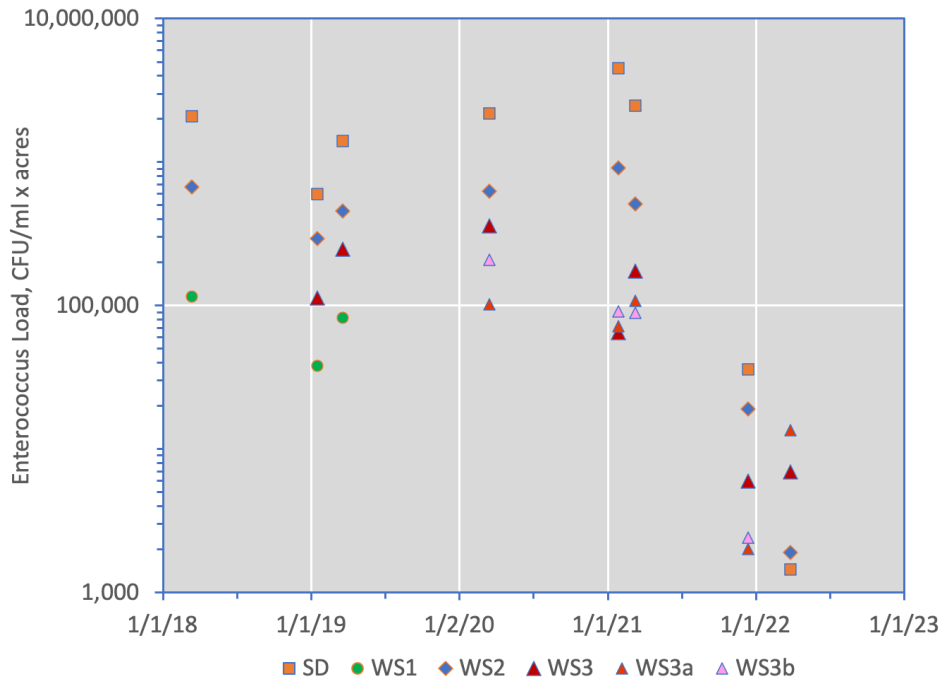


Figure 3. *Enterococcus* load indices for each site.

While gull fecal bacteria were not analyzed as consistently through time as were *Enterococcus*, they displayed greater spatial and temporal variation than did *Enterococcus* (Figure 4). Gull fecal bacteria were frequently not detected in WS3 samples and WS2 sometimes had similar or greater loads than did SD.

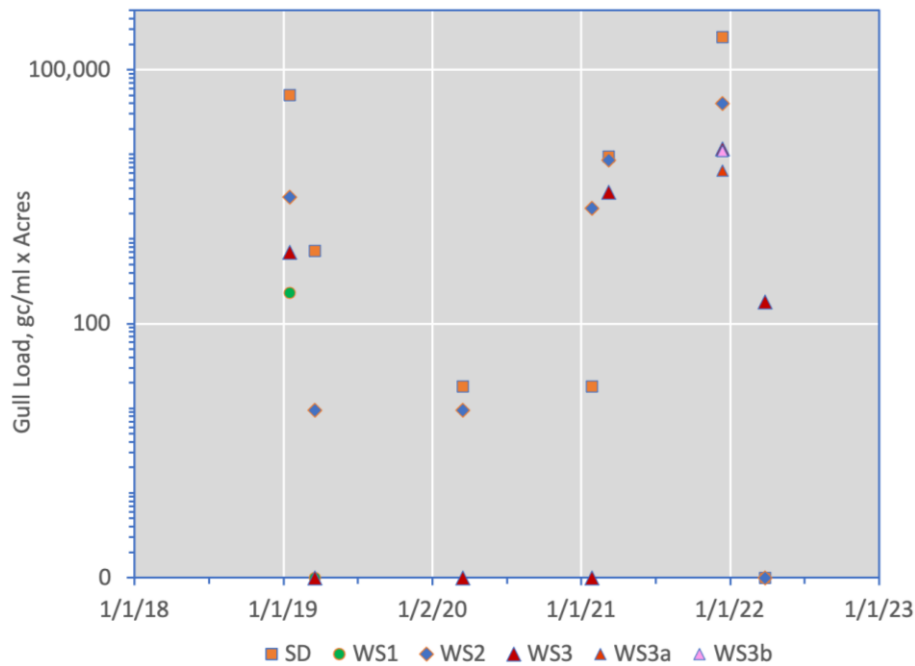


Figure 4. Gull fecal bacteria load indices for each site.

Loads of both dog and human (i.e., anthropogenic) fecal bacteria from WS2 were often greater than or similar to loads from SD (Figure 5 and Figure 6). This suggests that there are sources of anthropogenic fecal bacteria throughout the SD watershed, with potent sources in WS2.

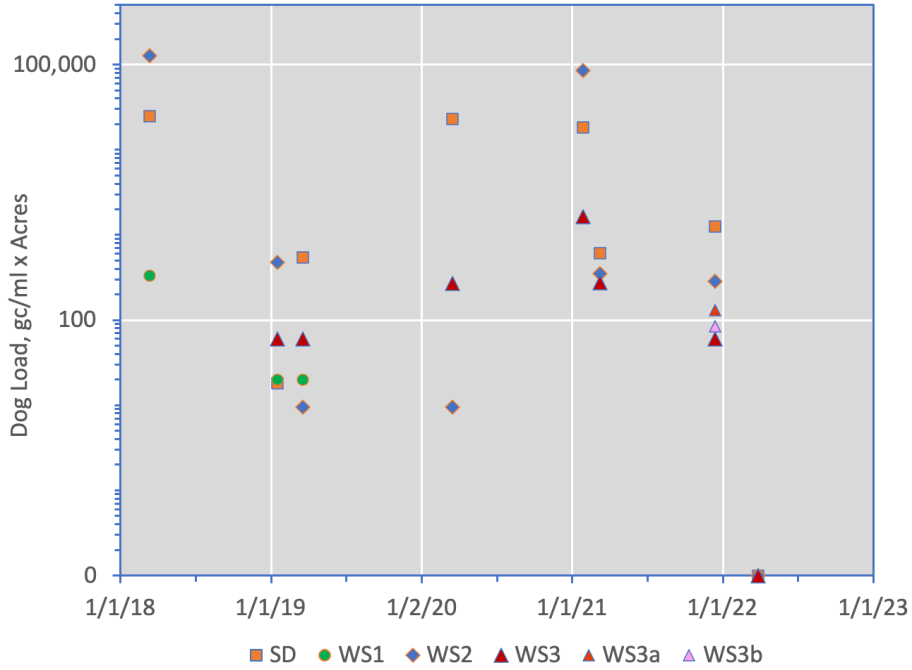


Figure 5. Dog fecal bacteria load indices for each site.

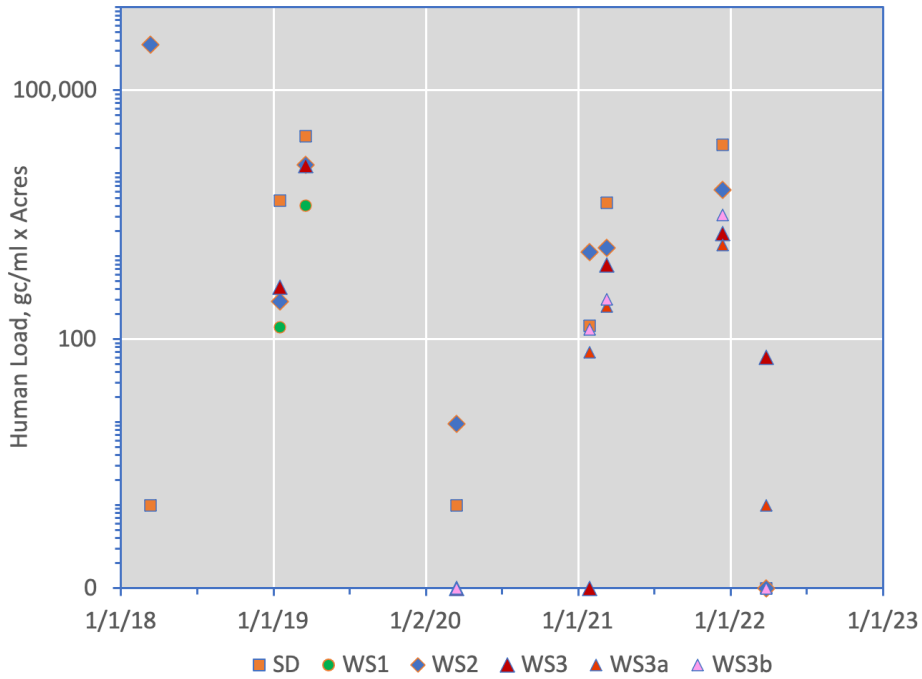


Figure 6. Human fecal bacteria load indices for each site.

WS3 loads of both dog and human fecal bacteria were sometimes greater than those from WS2 and loads from WS3a and WS3b also sometimes exceeded those from WS3 indicating sources in WS3a and WS3b. Very high loads for human fecal bacteria from WS2 on 3/13/18 suggested a large source. As the municipal sewer line runs above the storm drain under Junipero Street, just uphill from the WS3 sampling location, relining of the sewer line was performed in 2020 after the 3/16/20 sampling event. Both parametric and nonparametric statistical tests revealed that there is no statistically significant difference in mean concentrations between WS2 and WS3 samples collected before and after the infrastructure improvement (Table 3), which is likely due to high variability among samples and low numbers of samples (i.e., 4 samples before and after). Nevertheless, the concentrations of human fecal bacteria measured in WS2 have been orders of magnitude below that measured on 3/13/18.

**Table 3. Results of tests for differences in human fecal bacteria in WS2 and WS3 samples from before and after sewer relining in 2020.**

Site	Test	Degrees of Freedom	Adjusted R <sup>2</sup>	ChiSquare	Probability
WS2	ANOVA	7	0.0066	-	0.3458
	Wilcoxon	1	-	0.1898	0.6631
WS3	ANOVA	6	-0.1476		0.5361
	Wilcoxon	1		1.1842	0.2765

Tests for the effects of dog, gull, human and anthropogenic fecal bacteria on *Enterococcus* concentrations in SD samples were performed to determine whether potential effects of receiving water exceedances could be due to one or more of the tested animal sources. Stepwise linear regressions revealed that concentrations of *Enterococcus* in SD samples are associated with dog fecal bacteria [ $Enterococcus\ CFU/100ml = 110(dog\ fecal\ bacteria,\ gc/ml) + 5072$ ; adjusted R<sup>2</sup> = 0.356] and none of the other animal sources of fecal bacteria were significantly associated with *Enterococcus* concentrations in SD samples.

The contributions to SD loads from WS2 differed substantially among the measured microbial indicators (Table 4). WS2 contributed 46% of *Enterococcus* loads from SD, which closely matches the percentage of SD watershed covered by WS2 (i.e., 95 acres/180 acres = 53%), confirming the observation from Figure 3 that *Enterococcus* sources are broadly distributed across the SD watershed. WS2 loads of dog, gull, human, and anthropogenic fecal bacteria were 3.6 – 513,000 times greater than SD loads, further indicating that SD loads of these fecal bacteria largely originate from WS2. Standard errors with magnitudes similar to means reflect large variations among individual values.

Within WS2, loads of *Enterococcus* and fecal bacteria from WS1 averaged 16% – 36% of WS2 *Enterococcus*, gull, human, and anthropogenic fecal bacteria loads (Table 4), which are greater

than the 10.5% of WS2 area covered by WS1. Additionally, the average contribution of WS1 dog fecal bacteria to WS2 loads was 72% suggesting it was an important source of dog fecal bacteria. The contribution of WS3 loads to WS2 ranged from 84% for *Enterococcus* to 257 times greater for gull fecal bacteria. Loads of dog, human, and anthropogenic fecal bacteria from WS3 were 5, 86, and 88 times greater than from WS2, which were all notably greater than the 33% of WS2 covered by WS3.

**Table 4. Means and standard errors for percentages of bacteria indicator loads from SD, WS2, and WS3 represented by loads from each subwatershed.**

		<i>Enterococcus</i>	Dog	Gull	Human	Anthropogenic
Percent of SD from WS2	Mean	0.46	5.29	21.6	512879	3.63
	SE	0.13	3.62	21.2	1.49	2.71
Percent of WS2 from WS1	Mean	0.16	0.72	0.36	0.27	0.18
	SE	0.02	0.84	0.32	0.14	0.10
Percent of WS2 from WS3	Mean	0.84	5.13	257.3	86.24	88.14
	SE	0.47	3.98	257.1	85.63	85.33
Percent of WS3 from WS3a	Mean	0.86	2.17	0.55	23.68	0.35
	SE	0.31	-	-	23.16	0.22
Percent of WS3 from WS3b	Mean	0.73	NA	NA	45.01	0.12
	SE	0.21	-	-	43.50	0.08

### Copper

Copper loads suggested broadly distributed sources among SD, WS2 and sub watersheds WS3, WS3a, and WS3b (Figure 7). SD always had the greatest loads, followed by WS2, WS3, WS3a, and WS3b. WS2 always had the second highest loads of copper followed by subwatersheds WS3, WS3a, and WS3b. Subwatershed WS1 discharged much smaller loads of copper than the other measured sources.

Percentages of copper in the dissolved fraction ranged from 35 – 88% and exhibited an inverse spatial pattern, compared to total copper (Figure 8). SD often had the lowest percentage of dissolved copper suggesting higher particulate loads compared to WS2 and its subwatersheds.

As with *Enterococcus*, the percentages of SD copper loads coming from WS2 were comparable to the respective watershed areas (i.e., WS2 = 53% of SD; Table 5). WS1 had much smaller loads of copper that would be expected if its load were proportional to area (i.e., 10% of WS2). The percentage of WS2 copper loads contributed by WS3 were marginally greater than the 32% expected if loads were proportional to watershed area. Copper loads from WS3a and WS3b into WS3 were greater than 33% and 40% expected if loads were proportional to watershed area.

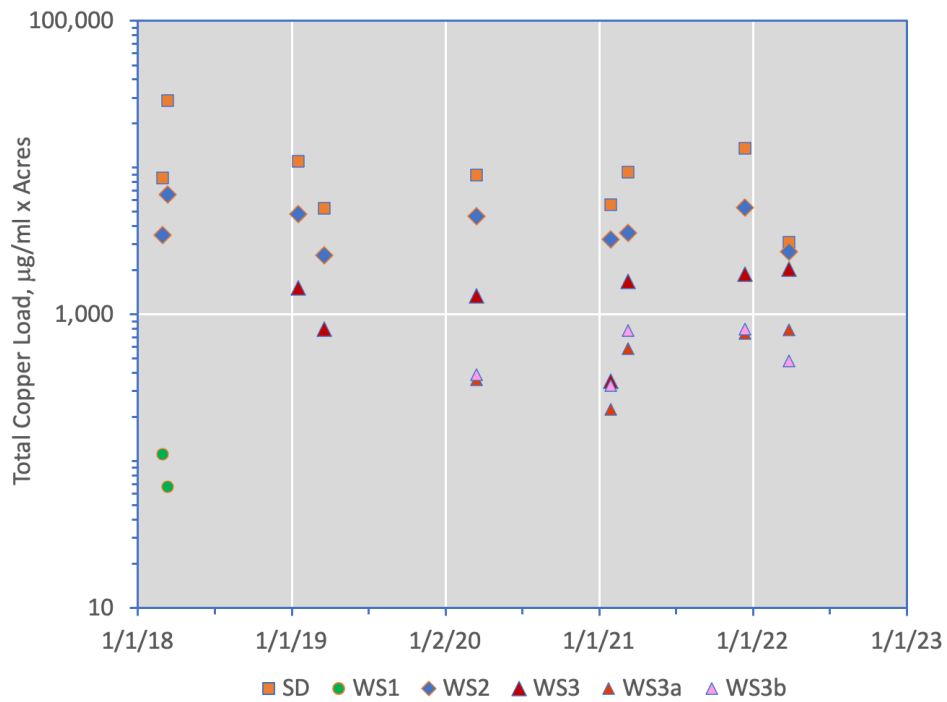


Figure 7. Loads of total copper for each site.

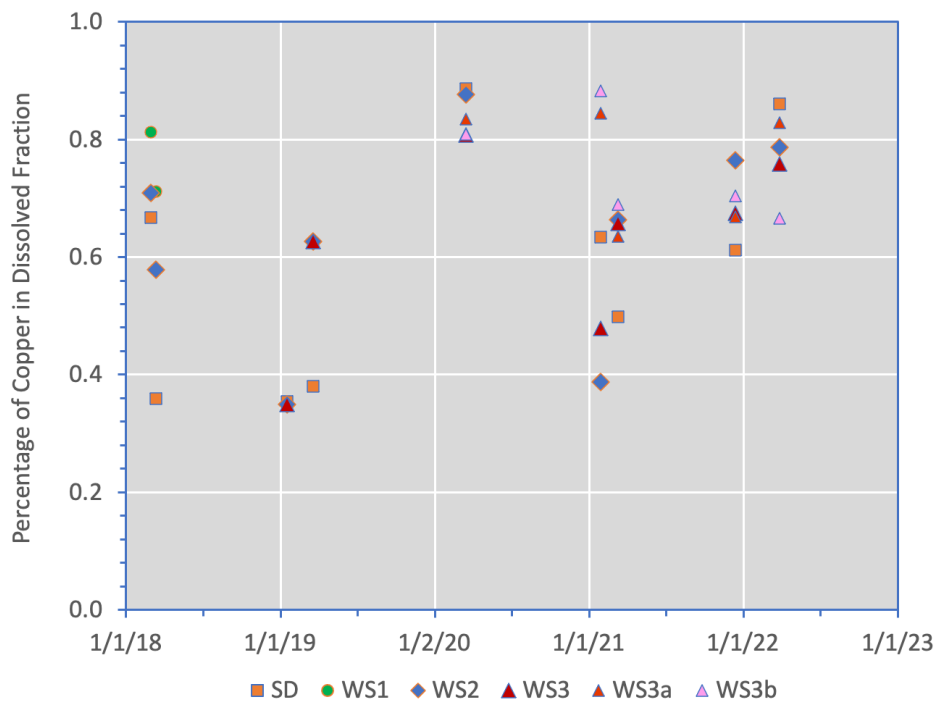


Figure 8. Percentage of copper in the dissolved fraction.

**Table 5. Means and standard errors for percentages of copper loads from SD, WS2, and WS3 represented by loads from each subwatershed.**

		Dissolved Copper	Total Copper
Percent of SD	Mean	0.53	0.49
from WS2	SE	0.06	0.06
Percent of WS2	Mean	0.01	0.01
from WS1	SE	SS <sup>1</sup>	SS
Percent of WS2	Mean	0.36	0.37
from WS3	SE	0.07	0.08
Percent of WS3	Mean	0.51	0.41
from WS3a	SE	0.16	0.06
Percent of WS3	Mean	0.74	0.53
from WS3b	SE	0.33	0.14

<sup>1</sup> = Single sample

High concentrations of dissolved copper have historically been linked to architectural copper (Arnold 2005, Pennington and Webster-Brown 2008, Kelly et al. 2011). The preponderance of dissolved copper in Carmel stormwater discharges suggests architectural sources. To investigate this possibility, samples were collected during a storm that dropped 0.9 inches of rainfall on 12/23/2021 from downspouts at three locations: Sunset Center (on Mission Street between 8<sup>th</sup> Avenue and 10<sup>th</sup> Avenue), City Hall (on Monte Verde Street between Ocean Avenue and 7<sup>th</sup> Avenue), and Scout House (at Mission Street and 8<sup>th</sup> Avenue). Sunset Center and City Hall have copper gutters and downspouts, and Scout House has aluminum gutters and downspouts.

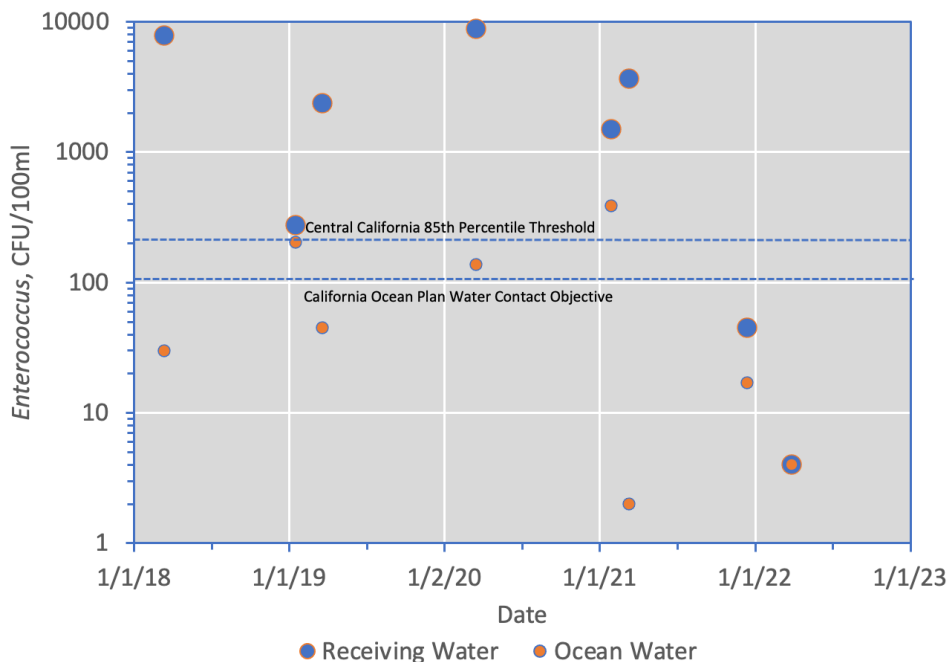
Rainwater coming from copper gutters and downspouts at City Hall and Sunset House had six to ten times the copper concentrations as compared to the sample from Scout House (Table 6). Samples collected several days after rainfall at two watershed locations had much lower copper concentrations, which were still dominated by dissolved copper at levels consistent with storm samples from all sites (Figure 8).

**Table 6. Total and dissolved copper concentrations in storm runoff from roof gutters and downspouts and in watershed locations several days following rain.**

Date	Weather	Location	Total Copper, µg/L	Dissolved Copper, µg/L	Percent Dissolved
12/23/2021	Wet	City Hall	139	125	89.9
12/23/2021	Wet	Sunset Center	169	119	70.4
12/23/2021	Wet	Scout House	3.65	1.3	35.6
3/31/2022	Dry	WS2	4.98	4.18	83.9
3/31/2022	Dry	4 <sup>th</sup> @Monte Verde	4.85	4.51	93.0

### Effects of SD loads on Receiving Water Concentrations

Receiving water concentrations of *Enterococcus* consistently exceeded both ASBS and Ocean Plan objectives from 2018 until March 2021, whereas neither sample collected since then exceeded either objective (Figure 9). Ocean water samples collected away from the direct influence of the storm drain discharge also exceeded one or both *Enterococcus* water quality objectives between January 2019 and January 2021, indicating background *Enterococcus* emanating from sources distant from SD.



**Figure 9. Concentrations of *Enterococcus* in Receiving Water and Ocean Water samples adjacent Carmel 4<sup>th</sup> Avenue storm drain.**

Linear regressions were performed to investigate whether SD and/or OW concentrations of *Enterococcus*, dog, gull, human, and anthropogenic fecal bacteria affected RW concentrations. SD concentrations were found to affect RW concentrations of dog, human and anthropogenic fecal bacteria (Table 7). RW concentrations of anthropogenic fecal bacteria were also significantly associated with concentrations at OW (Table 7). An absence of effects on RW *Enterococcus* concentrations by either SD or OW suggest a variety of sources not associated with storm runoff.

**Table 7. Linear regression of SD and OW on RW concentrations of *Enterococcus*, dog, human, and anthropogenic (dog + human) fecal bacteria. Bold figures indicate significant regressions ( $p < 0.10$ ).**

Indicator	Site	Equation	Adjusted R <sup>2</sup>	$p$
<i>Enterococcus</i>	SD	= 0.9329(RW) + 6383	0.0103	0.3402
	OW	= -0.0039(RW) + 115.7	-0.1551	0.8144
Dog	SD	= 0.5853(RW) + 19.37	0.5572	<b>0.0203</b>
	OW	= 0.1913(RW) + 5.491	0.0965	0.2344
Gull	SD	= 4874(SD) – 704.1	0.1091	0.5504
	OW	= 0.7000(RW) + 2.003	0.1193	0.2360
Human	SD	= 1.1215(RW) + 18.72	0.3274	<b>0.0806</b>
	OW	= 0.0194(RW) + 0.5713	0.0032	0.3509
Anthropogenic	SD	= 1199(RW) + 6283	0.3522	<b>0.0709</b>
	OW	= 0.3434(RW) + 0.8178	0.3702	<b>0.0644</b>

Estimates of the contributions of SD loads to *Enterococcus* (CFU/100ml), dog, gull, human, anthropogenic fecal bacteria (gc/ml), and copper concentrations at RW were made by calculating the dilution of SD discharges in each RW sample using dissolved copper concentrations as a conservative parameter (Table 8). Unlike *Enterococcus* and fecal bacteria, copper concentrations were orders of magnitude lower in RW and OW samples than in SD samples (Appendix A). This characteristic of copper, and primarily dissolved copper, makes it ideal for calculating the dilution of SD water in each RW sample. Five sampling events since 3/13/2018 included measurements of dissolved copper at RW (Table 8). SD dilution in these five RW samples resulted in estimated RW *Enterococcus* derived from SD ranging from 0 – 4975 CFU/100ml, with three values >3500 CFU/ml.

**Table 8. Estimates of storm drain contributions to *Enterococcus* and fecal bacteria in samples from RW. SD concentrations of *Enterococcus* and fecal bacteria data are included in Appendix A. Bold fecal indicator figures are considered to be above background concentrations. NS = Not sampled.**

Date	SD Cu dissolved, $\mu\text{g/L}$	RW Cu dissolved, $\mu\text{g/L}$	Dilution (SD $\div$ RW)	<i>Entero.</i> CFU/100ml	Dog gc/ml	Gull gc/ml	Human gc/ml	Anthro. gc/ml	Copper $\mu\text{g/L}$
3/1/18	31.4	0.406	77.34						0.61
3/13/18	57.4	17.7	3.24	3577* **	<b>42.9</b>	0.0	0.0	<b>42.9</b>	49.3**
1/17/19	21.8	NS	-	-	-	-	-	-	-
3/20/19	11.2	NS	-	-	-	-	-	-	-
3/16/20	43.8	NS	-	-	-	-	-	-	-
1/28/21	19.7	3.92	5.03	4975* **	<b>20.4</b>	0.0	0.2	<b>20.5</b>	6.19*
3/10/21	25.8	8.13	3.17	4320* **	1.1	<b>16.4</b>	7.7	8.7	16.3*
12/13/21	46.3	0.45	102.9	2	0.1	<b>13.1</b>	1.2	1.3	0.73
3/28/22	14.8	0.06	246.7	0	0.0	0.0	0.0	0.0	0.07

\*= Concentration exceeded the ASBS 85<sup>th</sup> percentile.

\*\*= Concentration exceeded the Ocean Plan objective.



To determine whether the estimated contributions of SD *Enterococcus* to RW could be sufficient to cause RW exceedances, the SD contributions were subtracted from measured RW concentrations. This revealed that on 3/13/18 a reduction of RW *Enterococcus* concentrations by 3577 would have left 4217 CFU/100ml which exceeds the Ocean Plan by an order of magnitude, suggesting potent sources in OW. On 1/28/21 and 3/10/21, subtraction of the estimated SD contributions to RW *Enterococcus* concentrations would have resulted in values of -3475 and -670, respectively suggesting the RW exceedances on these dates could have been due largely to SD contributions.

Measuring the contribution to *Enterococcus* concentrations by fecal bacteria quantified with qPCR would be necessary to unequivocally determine the anthropogenic component in *Enterococcus* Ocean Plan and ASBS exceedances. Because there is no direct correspondence between *Enterococcus* CFU/100ml and dog, gull, or human fecal bacteria measured by qPCR methods, diluted SD gc/ml values estimated in RW samples were screened for those data that represented values clearly above background, which was arbitrarily set at 10 gc/ml.

RW dog fecal bacteria had the two highest estimated contributions from SD (i.e., Table 11; 43 gc/ml on 3/13/2018 and 20 gc/ml on 1/28/2021). Gull fecal bacteria at RW had the 3<sup>rd</sup> and 4<sup>th</sup>-highest contributions from SD (i.e., 16 gc/ml on 3/10/2021 and 13 gc/ml on 12/13/2021). There were no estimated contributions to RW concentrations of human fecal bacteria from SD above background concentrations. This observation is especially notable for the sample from 3/13/2018, when WS2 had a concentration of human fecal bacteria of 3779 gc/ml, whereas WS1 and SD both had no human fecal bacteria detected (Appendix A). The above-background contributions of SD anthropogenic fecal bacteria to RW concentrations were both due to dog fecal bacteria. While SD discharges delivered diluted concentrations of *Enterococcus* to RW that exceeded Ocean Plan and ASBS objectives, the only anthropogenic fecal bacteria above background were derived from dogs. Moreover, the broad distribution of *Enterococcus* sources in the SD watershed and the low amount of variation in SD *Enterococcus* loads due to significant associations with WS2 dog fecal bacteria suggest that reducing WS2 dog fecal bacteria would be unlikely to make a large difference in SD *Enterococcus* loads.

RW concentrations of total copper due to SD discharges exceeded either the Ocean Plan or the ASBS objective in 50% of samples since 3/1/2018, but not since 3/10/2021 (Table 16). The only Ocean Plan exceedance occurred on 3/18/2018 and the ASBS 85<sup>th</sup> percentile was exceeded on 1/28/2021 and 3/10/2021 (Table 16).

## Conclusions

### Watershed Loads and Sources

Contributions of loads from WS2 to SD differed among fecal indicators. On average, WS2 contributed 46% of *Enterococcus* loads from SD, indicating broadly distributed sources across the entire SD watershed. Loads of dog, gull, human, and anthropogenic fecal bacteria from WS2 averaged 4 – 51,000 times greater than loads from SD, indicating potent sources for these fecal bacteria within WS2. WS1 loads of dog fecal bacteria averaged 72% of WS2 loads and none of the other fecal bacteria loads from WS1 exceeded 27% of WS2 loads, suggesting the

WS1 could be an important source of dog fecal bacteria. Contributions of *Enterococcus*, gull, and human fecal bacteria loads from WS3 to WS2 averaged 84%, 13%, and 48%, respectively. Loads of dog and anthropogenic fecal indicators from subwatershed WS3 were 5.1 and 2.4 times greater than from WS2, identifying WS3 as a major contributor of these indicators into WS2 and SD. Loads of dog and human fecal bacteria from WS3a averaged 2 – 24 times greater than those from WS3. Dog and gull fecal bacteria were not measured in WS3b, but loads of human fecal bacteria from this subwatershed averaged 45 times greater than from WS3. These results identify WS3a, and WS3b as important subwatersheds for management actions to reduce loads of dog, human, and anthropogenic fecal bacteria into SD.

Following high concentrations of human fecal bacteria in WS2 on 3/13/18, the sewer line under Junipero Street between 1<sup>st</sup> Avenue and 2<sup>nd</sup> Avenue was relined in mid 2020. Concentrations of human fecal bacteria before (T1) and after (T2) the sewer was relined were not statistically different, likely due to small numbers of samples, but concentrations in T2 remained several orders of magnitude below the value seen on 3/13/18. Robust concentrations of human fecal bacteria in WS3, WS3a, and WS3b suggest continuing sources of human fecal bacteria in those subwatersheds.

Copper loads from WS2 averaged 50% of those from SD, indicating broadly distributed sources within the entire SD watershed. Copper loads from WS3 averaged <40% of WS2 loads, which is also consistent with broadly distributed sources.

Total copper concentrations were significantly higher in watershed and SD samples than in ocean samples (RW and OW), indicating the high copper concentrations in RW samples originate from sources on land. Samples collected from copper roof gutters and downspouts revealed higher concentrations of copper than measured in watershed or SD samples. The downspout samples also were dominated by dissolved copper suggesting the high dissolved copper loads in watershed and SD samples arise from rainfall flowing across architectural copper (i.e., derived from buildings and infrastructure).

### Stormwater Effects on Receiving Water

Receiving water concentrations of dog, human, and anthropogenic fecal bacteria were significantly associated with SD loads, although other unmeasured factors also were evident. OW also significantly affected RW concentrations of anthropogenic fecal bacteria indicating sources other than the 4<sup>th</sup> Avenue storm drain.

Estimates of the contributions by SD loads to RW concentrations suggested that stormwater discharges contributed loads of *Enterococcus* that often exceeded both Ocean Plan and ASBS objectives. While there is no direct comparison between CFU/100ml and gc/ml data, it was found that dog, gull, and anthropogenic fecal bacteria from SD exceeded background (10 gc/ml) in several RW samples. Three of the five samples that could be analyzed for SD effects on RW concentrations exceeded *Enterococcus* Ocean Plan and ASBS 85<sup>th</sup> percentile benchmarks. These three samples had low calculated dilution factors for SD discharges that ranged from 3.2 to 5. Two of the exceeding samples were associated with high SD loads of anthropogenic fecal bacteria and both were due to dog fecal bacteria. The third exceeding RW *Enterococcus*

concentration was associated with high gull fecal bacteria. The same three samples that exceeded *Enterococcus* Ocean Plan or ASBS 85<sup>th</sup> percentile benchmarks also exceeded the benchmarks for copper concentrations. These results suggest that management actions aimed at decreasing loads of dog fecal bacteria from the watershed, and in particular subwatershed WS3, should be prioritized to reduce anthropogenic (dog) fecal bacteria. Additionally, watershed-wide management actions are needed to reduce copper exceedances in receiving water. Nevertheless, the broad distribution of *Enterococcus* sources in the SD watershed and the low amount of variation in SD *Enterococcus* loads due to significant associations with WS2 dog fecal bacteria suggest that reducing WS2 dog fecal bacteria would be unlikely to make a large difference in SD *Enterococcus* loads.

## Recommendations

- Consideration should be given to reinstating sampling at WS1 to document whether it remains a source of dog and human fecal bacteria to WS2.
- Management actions should be developed to reduce loads of dog fecal bacteria in the watershed .
- Integrity of sewer laterals in WS3 should be investigated to determine the sources and remedies for high human fecal bacteria coming from WS3a and WS3b.
- Management actions should be implemented to reduce the use of architectural copper.
- Discussions should be held with stormwater regulators to apprise them of these management actions and obtain assurances that reductions in anthropogenic fecal bacteria will relieve any regulatory burden in the absence of reductions in SD *Enterococcus* loads.

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## APPENDIX A

Concentrations of *Enterococcus*, dog fecal bacteria, gull fecal bacteria, human fecal bacteria, anthropogenic fecal bacteria, dissolved copper and total copper in each sample

Date	Sites	Entero MF	Dog	Gull	Human	Dog+Human	Dissolved Cu	Total Cu
3/1/18	WS1						9.1	11.2
3/13/18	WS1	11600	33		0	33	4.79	6.73
1/17/19	WS1	3780	2	23	14	16		
3/20/19	WS1	8200	2	0	410	412		
3/16/20	WS1							
1/28/21	WS1							
3/10/21	WS1							
12/13/21	WS1							
3/28/22	WS1							
3/1/18	WS2						25.8	36.4
3/13/18	WS2	7100	1349		3779	5128	39.8	68.8
1/17/19	WS2	3100	5	33	3	8	17.7	50.6
3/20/19	WS2	4800	0.1	0.1	132	132.1	16.6	26.5
3/16/20	WS2	6600	0.1	0.1	0.1	0.2	43.3	49.4
1/28/21	WS2	9600	906.6	24.2	11.7	918.3	13.3	34.3
3/10/21	WS2	5353	3.7	90.2	13.4	17.1	25.2	38
12/13/21	WS2	200	3	423	67	70	43.3	56.6
3/28/22	WS2	20	0	0	0	0	22.1	28.1
3/1/18	WS3							
3/13/18	WS3							
1/17/19	WS3	3780	2	23	14	16	17.7	50.6
3/20/19	WS3	8200	2	0	410	412	16.6	26.5
3/16/20	WS3	12000	9	0.1	0.1	9.1	36.2	44.8
1/28/21	WS3	2150	54.5	0.1	0.1	54.6	5.6	11.7
3/10/21	WS3	5800	9.2	120.1	26.4	35.6	36.9	56.1
12/13/21	WS3	200	2	385	63	65	42.2	62.5
3/28/22	WS3	230	0	6	2	2	51.5	67.9
3/1/18	WS3a							
3/13/18	WS3a							
1/17/19	WS3a							
3/20/19	WS3a							
3/16/20	WS3a	10200			0	0	29.9	35.8
1/28/21	WS3a	7200			7	7	19.0	22.5
3/10/21	WS3a	10800			25	25	37.3	58.7
12/13/21	WS3a	200	13	638	136	149	49.5	74.0
3/28/22	WS3a	1350			0	0	64.9	78.3

Date	Sites	Entero MF	Dog	Gull	Human	Dog+Human	Dissolved Cu	Total Cu
3/13/18	WS3b							
1/17/19	WS3b							
3/20/19	WS3b							
3/16/20	WS3b	17400			0	0	26.3	32.5
1/28/21	WS3b	7600			11	11	24.1	27.3
3/10/21	WS3b	7400			25	25	44.6	64.7
12/13/21	WS3b	200	7	920	262	269	46.9	66.6
3/28/22	WS3b	35			0	0	26.7	40.1
3/1/18	SD						31.4	47.1
3/13/18	SD	11600	139		0	139	57.4	160
1/17/19	SD	3340	0.1	280	26	26.1	21.8	61.6
3/20/19	SD	7800	3	4	157	160	11.2	29.5
3/16/20	SD	12200	128	0.1	0	128	43.8	49.4
1/28/21	SD	25000	102.4	0.1	0.8	103.2	19.7	31.1
3/10/21	SD	13710	3.4	51.9	24.3	27.7	25.8	51.7
12/13/21	SD	200	7	1345	123	130	46.3	75.6
3/28/22	SD	8	0	0	0	0	14.8	17.2
3/1/18	RW						0.406	0.64
3/13/18	RW	7794	130		31	161	17.7	43
1/17/19	RW	276	0	64	0	0		
3/20/19	RW	2360	7	37	105	112		
3/16/20	RW	8800	229	0	0	229		51.2
1/28/21	RW	1500	7.9	179.1	4	11.9	3.92	8.59
3/10/21	RW	3650	1.4	242.5	21.7	23.1	8.13	11.3
12/13/21	RW	45	5	145	0	5	0.45	1.01
3/28/22	RW	4	9	51	0	9	0.06	0.09
3/1/18	OW							
3/13/18	OW	30	99		0	99		
1/17/19	OW	204	1	4	0	1		
3/20/19	OW	45	0	16	3	3		
3/16/20	OW	138	11	37	0	11		
1/28/21	OW	390	6.4	85.9	4.7	11.1		
3/10/21	OW	2	0	113.9	0	0		
12/13/21	OW	17	1	493	0	1	0.21	0.30
3/28/22	OW	4	0	13	0	0	0.0705	0.176

Shaded cells = No samples collected