Quantifying Sources of Human Fecal Contamination Loading to the San Diego River

A Conceptual Workplan developed by the Southern California Coastal Water Research Project

February 20, 2019

Workplan Summary

Genetic signatures of human fecal contamination and human-specific pathogens have been consistently detected in stormwater runoff from the San Diego River. Given that the risk of human water-contact illness is elevated by this fecal contamination and, given looming regulatory deadlines to reduce wet-weather fecal contamination loading, this conceptual workplan seeks to improve the accuracy of human fecal contamination loading by quantifying different potential human fecal sources. The specific goal of this project is to estimate human fecal contamination loads from five potential sources: public sewers through exfiltration, private laterals through exfiltration, onsite wastewater treatment systems (septics), homelessness, and illicit discharges. Also, this workplan incorporates a dry weather survey of the San Diego River to identify if human fecal sources are an issue when it is not raining, and identify when and where site-specific microbial source tracking activities can be incorporated, as necessary.

This conceptual workplan encompasses a multi-component, multi-phased study comprising seven tasks. Task 1 creates the project governance by establishing an Advisory Committee for decision making, ensuring independent external technical review, and creating project documentation such as Sampling and Analysis Plans and Quality Assurance Project Plans.

Task 2 will quantify the human fecal contamination load to the San Diego River via exfiltration from the publicly owned sanitary sewer system. The first step in this task is to confirm that sanitary sewers are a contributor to stormwater by applying microbial community profiling. Microbial community profiling is a unique marker of sanitary sewer biofilms. If sanitary sewer microbial community profiles are found in stormwater runoff, then empirical measurements of volumetric loss from discrete sections of sanitary sewers will be conducted to quantify exfiltration rates. Since this work has never been conducted previously, a benchtop exercise will be conducted to assess potential leakage rates. Exfiltration measurements only establish the volumes that exit the sanitary sewer pipe, so the final portion of this task will utilize quickly and easily measured tracers in receiving waters to confirm subsurface transport.

Task 3 will quantify the human fecal contamination load from private laterals and septic systems. For both laterals and septic systems, the initial step will assess how frequently repairs are needed. If disrepair is frequent in laterals, then additional work will focus on empirical measurements of volumetric loss (similar to public sanitary sewers). Since empirical measurements of volumetric loss are not possible for septic systems, unique tracers will be used to quantify contributions to receiving waters.

Task 4 will quantify the human fecal contamination from homeless populations. The first step is to quantify how many homeless are present in the watershed and what proportion of the homeless population defecates in the river or its tributaries, what proportion defecates near the river only to be washed into the river when it rains, and what proportion defecate offsite and doesn't enter the river. This information will provide the basic data needed for calculating loading, however, two additional pieces of information will help validate these estimates. First, experiments will be conducted to quantify the washoff of fecal material from stream banks when it rains. Second, we will verify contributions from homelessness by conducting upstream/downstream sampling near large densities of homeless populations.

Task 5 will quantify the human fecal contamination from illicit discharges originating from recreational vehicles (RVs). Similar to homelessness, the first step is to identify how large a source this could be by quantifying how many RVs are capable of discharging to the San Diego River. Unlike homelessness, we anticipate that surveys and questionnaires will likely not be capable of discerning the frequency of illegal dumping. Therefore, this task

will utilize real time sensors and automated equipment to detect and sample discharge events near the greatest RV densities to quantify human fecal inputs.

Task 6 will quantify the frequency and location of human fecal contamination in dry weather. Unlike wet weather where an entire watershed of sources can mix and flow downstream, dry weather sources are often isolated and more tractable. Regulated MS4 dischargers currently implement a routine dry weather illicit connection/illicit discharge (IC/ID) monitoring program for storm drain outfalls in dry weather, precisely to find and remove these individual sources. We intend to supplement the ongoing IC/ID monitoring with a dry weather screening of receiving waters in the San Diego River. The receiving water monitoring is intended to identify if human fecal contamination exists and, where it occurs, identify those places where site specific actions can be taken to remediate the source(s).

Task 7 will focus on reporting including data management and analysis, milestone-based oral reports, regular quarterly written updates, as well as the project Final Report. A Draft and Final Report will be written for Technical Committee review and Advisory Committee review and approval.

In total, this project will require approximately 48 months to complete. Advisory Committee and Technical Committee review will be milestone-based to ensure all information generated is applicable and actionable. Quarterly reports will be written to ensure communication is up-to-date. Draft and final reports will undergo Technical Committee review and must be approved by the Advisory Committee.

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Background

San Diego watershed managers face a vexing problem. Despite stringent efforts that have controlled the largest sources of human fecal pollution (and the greatest risk to bathers), studies have quantified human fecal genetic markers and human specific viruses in urban runoff (Steele et al 2018). Thus, the remaining human fecal sources will be much more difficult to find and control.

Finding human fecal sources of contamination is particularly difficult in wet weather runoff. Traditional methods for detecting and tracking human fecal pollution – methods that rely on spatial and temporal patterns - have been ineffective in wet weather because the sources appear to be chronic and widespread based on existing technology (Cao et al 2017, Noble et al 2003, Schiff and Stevenson 1996).

The focus on human sources has become paramount as regulatory deadlines loom, such as wet weather bacteria total maximum daily loads (TMDLs). Studies to date have identified human fecal sources as the primary vector of health risk (Arnold et al. 2017, Soller et al 2017), and that controlling human fecal sources is the most cost-effective method for reducing risk (Environmental Incentives and EcoNorthwest 2017). Targeting human fecal contributions is currently the recommended pathway for moving towards compliance.

Using Previous Work to Narrow Human Sources in the San Diego River

In 2016, a desktop exercise aimed at estimating the potential loading from various human sources to the San Diego River (Environmental Incentives and EcoNorthwest 2017). The primary conclusion was two-fold. First, homeless populations and public sewer sources were likely the largest contributors of human fecal pollution. Second, there was so much uncertainty in the fecal loading estimates that additional work to confirm the estimated contributions should precede any attempt towards remediation.

Between February and September 2018, a group of San Diego River stakeholders comprised of wastewater agencies, stormwater agencies, regulatory agencies, environmental advocacy agencies, and university academics met to review the 2016 fecal loading estimates, capture the level of uncertainty for each source, and discuss specifically which sources should be considered for further study to confirm human fecal loading. Table 1 summarizes their output.

Table 1. Best professional judgement estimates of human fecal contamination from different sources and the relative uncertainty in these BPJ estimates. Based on these factors and if existing monitoring was occurring, the different sources were prioritized for further study.

Potential Source	Relative proportion of Contamination[1]	Level of Uncertainty	Ongoing Monitoring?	Prioritized for Study?
Homeless	High	High	Yes	No
Public Sewer Exfiltration	Medium	High	No	Yes
Private sewer laterals	Medium-Low	High	No	Yes
Septic systems	Low	Medium	No	Yes
Sanitary Sewer overflows	Low	Low	Yes	No
Illicit connections/ Illegal Discharges	Low	Low	Yes	No

[1] from Environmental Incentives and EcoNorthwest 2017

The highest priority sources to investigate for this set of stakeholders was homeless populations, public sewer exfiltration, private sewer laterals, and septic systems. This was due to the modest to high levels of potential contributions, a high level of uncertainty, and lack of any new data coming forward to refine the current estimates of human fecal contamination. The stakeholders present also agreed that sanitary sewer overflows, illicit connections and illicit discharges (IC/ID) were potential sources, but were reasonably certain they comprised a low proportion of the contamination because mandatory monitoring and reporting programs indicated a low frequency of occurrence for these sources. The exception was illicit discharges from recreational vehicles (RVs), which appear to be random events and not well-suited to the current monitoring for IC/ID. Therefore, RVs were added to the list of priority sources for evaluation.

Goal of This Study

The goal of this study is to produce estimates of human fecal loading in the San Diego River during wet weather from five potential sources: public sewer exfiltration, private lateral exfiltration, homeless populations, illicit discharges from RVs and septic systems failures. The ultimate objective is to create a figure similar to Figure 1, which quantifies not only the loading from each source, but the confidence in the loading from each source sufficient to make future management decisions about the need for management actions. Secondary, but equally important, goals include identifying locations and times where and when the greatest risk of loading occurs, so these places and occasions can be most effectively targeted for remediation.

Figure 1. Keystone graphic for this study estimating total human fecal loading during wet weather for each source, plus the relative contribution among all sources, for remediation decision making.

Scope of Work

This study has a multi-phased, multi-task approach to tackling this scope of work. The first task establishes a governance for this project since the outcomes have both technical, political, and potentially large fiscal implications. The second thru fifth tasks tackle the individual sources by implementing traditional and cutting-edge sampling designs, followed by traditional and research-grade laboratory analysis required to get answers to managers' difficult questions. The sixth task focuses on dry weather monitoring throughout the watershed because little data exists to assess if human fecal contamination is widespread like it is during wet weather. The seventh task focuses on data analysis and reporting, bringing together existing knowledge and the knowledge gained through the sampling and analysis during this study to provide positive steps towards improved public health and cost-efficient strategies for compliance.

This workplan covers nearly all human fecal sources within the San Diego River watershed. Recognizing not all stakeholders have jurisdictional responsibility for all sources, the workplan was designed modularly so stakeholders can focus only on pertinent sources within their control or add sources not addressed (i.e., recycled water, sanitary sewer overflows, etc.). Finally, this workplan targets the San Diego River watershed because it is the primary target of current regulatory efforts, but expanding to additional watersheds can be accommodated.

Task 1. Project Governance and Planning

Task 1A. Establish an Advisory Committee

This project will include an Advisory Committee to guide project participants and stakeholders in interpreting findings and making management decisions throughout the course of the project. The committee will be able to weigh in on how the project should be staged and in what order the stages should occur, as well as help evaluate and select study design risk factors. The committee also will provide recommendations on whether the data collected during the project are compelling enough to merit management action, and/or whether additional follow-up confirmation studies are needed. Finally, the committee will lend strategic authority and rigor to the process of approaching private-property owners.

The makeup of the advisory committee should incorporate the multi-faceted and directly affected decision makers of the San Diego River watershed including:

- San Diego Regional Water Quality Control Board
- City of San Diego Public Utilities Department
- City of San Diego Stormwater Department
- County of San Diego Department of Environmental Health
- County of San Diego Public Works Department
- San Diego CoastKeeper
- San Diego River Park Foundation

Additional members of the Advisory Committee may include other potentially affected decision makers including:

- Other regulated stakeholders in the San Diego River Watershed
- US EPA, Region IX

SCCWRP recommends a Technical Committee, either as a second committee or imbedded within the Advisory Committee, to provide technical advice and oversight. Selection of the Technical Committee members will follow a two-step process. First, SCCWRP will recommend a series of technical skill areas that should comprise the committee, which will be reviewed and approved by the Advisory Committee. Second, SCCWRP will provide at least three nominees for each skill category; the Advisory Committee will make the final selection. To ensure fairness, any member of the Advisory Committee can veto any Technical Committee nominee or can provide additional nominees for Committee consideration within the skill category.

Task 1B. Create Sampling and Quality Assurance Plans

For each of the five human fecal sources – public sewer exfiltration, private laterals, septic systems, homeless populations, and illicit discharges from RVs, – a Conceptual Model and a Sampling and Analysis Plan will be prepared. The Sampling and Analysis Plan will contain the specific actions for measuring human fecal contaminant loading including number of sampling sites, locations, timing, replication, and what will be measured, including but not limited to precipitation, flow, traditional fecal indicator bacteria, source tracking microbes or chemicals, and human pathogens.

A Quality Assurance Project Plan (QAPP) will also be prepared to ensure a technically rigorous project. The QAPP will provide the necessary documentation for any third party to assess the quality of the data produced including accuracy, precision, bias, and representativeness.

The Conceptual Model, Sampling and Analysis Plan and the QAPP will be reviewed and approved by the Advisory Committee and Technical Committee.

Task 2. Quantifying Human Fecal Loading from Public Sewer Exfiltration

Task 2A. Exploiting biofilms to verify the potential for sanitary sewer exfiltration

This subtask adapts microbial community profiling of sanitary sewer biofilms to track exfiltration into storm drain conveyances or receiving water. Originally developed and tested at the University of Wisconsin, Milwaukee, researchers have taken advantage of advances in DNA genetic sequencing and bioinformatics to quantify the unique microbial community in the biofilm of sanitary collection systems (McLellan et al. 2015). These researchers have successfully used this technology to detect and track combined sewer overflows into Lake Michigan receiving waters. Preliminary attempts to replicate this approach in San Diego have been very promising. As shown in Figure 2, microbial community profiles in three samples from storm drains and sanitary sewers from the San Diego River watershed are distinctly different from one another. In fact, a subset of the microbial community is distinctly different between separate sanitary sewers, providing hope that this technique can help pinpoint specific sewer system segments.

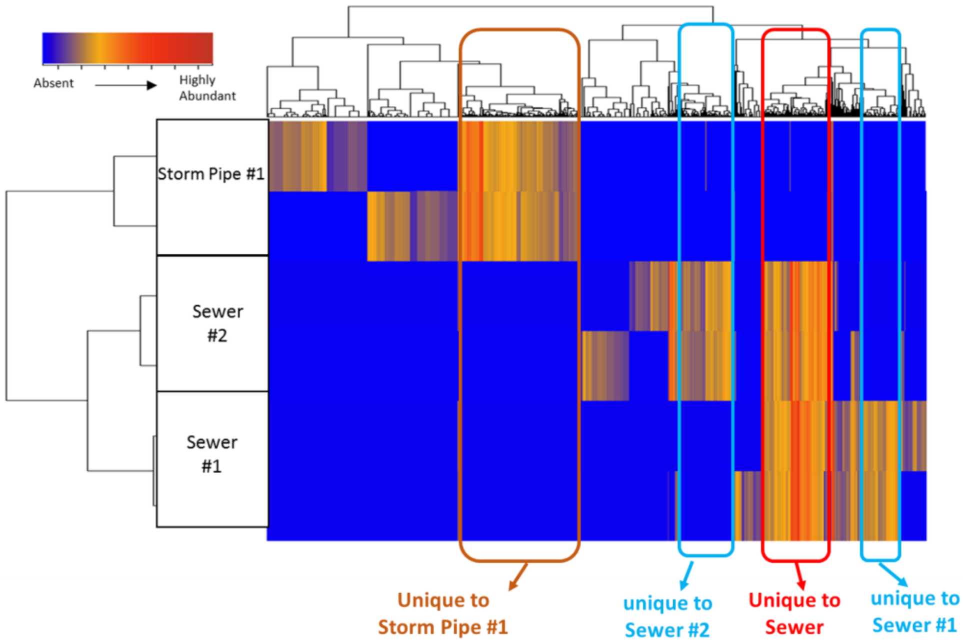

Figure 2. Microbial community profiling of storm drains and sanitary sewer samples from the San Diego River Watershed. More than 2,000 different microbes were identified, and their relative abundance shown by color scale. Note the uniquely abundant microbial communities in sanitary sewers, but not in storm drains, which makes this a potentially powerful tool for sanitary sewer source tracking.

Three steps will be required to complete this subtask. The first step is to profile microbial communities in biofilm and water samples from sanitary sewers and storm drain conveyances. For this method to work, microbial communities from sewage and storm drains must be distinctly and consistently different so that exfiltrated sewage can be detected in storm drains. Therefore, up to 100 samples will be collected, half from storm drains and half from sanitary sewers, all during dry weather. Roughly 60 of the 100 samples will be collected from different locations. The goal of this sampling will be to determine if microbial communities are comparable from site-to-site. The remaining 40 samples will be repeated collections from a subset of locations verifying that biofilm microbial communities are comparable from time-to-time.

The second step is final method refinement, which must be completed to ensure the technique is sensitive, as well as resistant to inhibition, confounding and degradation. A description of the microbial community profiling is provided in Appendix A. However, DNA extraction and analysis are often difficult during wet weather when suspended solids, humic substances, and potential toxins can all cause interferences. Refining the method to reduce these interferences will be critical to ensure adequate sensitivity for human fecal source tracking.

The third step is to use a factorial-based study design to sample storm drain conveyances in San Diego River watershed areas of concern during multiple storm events. The factorial design will address issues such as sanitary sewer age, materials of construction, and geology, among others. This step can be conducted in phases or tiers, starting with the times and/or locations with the greatest potential for sanitary sewer biofilm signals in storm drain conveyances. If successful, this method can easily and inexpensively be used systemwide.

The products from this subtask include:

A) a refined protocol for microbial community profiling of sanitary sewer biofilms for future use by others
B) a survey of storm drain conveyances for the presence of unique sanitary sewer biofilm microbial community profiles in the San Diego River watershed

Task 2B. Empirical measurements of volumetric loss

If the biofilm microbial community profiling in Task 2A indicates that sanitary sewers are contributing to stormwater runoff, then (assuming sanitary sewer overflows did not occur) measuring how much sewage exfiltrates from the sanitary sewer collection system is the next step.

Public utility agencies all have inspection and repair programs for the approximately 3,500-mile sewage collection system in the San Diego River Watershed to prevent catastrophic failures and ensure routine maintenance. However, microscopic cracks and gaps not discernable by closed circuit TV or video could be large enough for microbes to escape. Provided there is a subsurface transport mechanism during wet weather, exfiltration is a plausible human fecal source to receiving waters.

Part of the uncertainty associated with exfiltration is how little data exists on this phenomenon. While there are a multitude of sanitary sewer infiltration studies, we have identified only a single study measuring sanitary sewer exfiltration (Brown and Caldwell 2005). Therefore, empirical measurements of sewer exfiltration for this project will require a two-step process. First is a pilot study to refine measurement techniques, followed by full implementation to estimate volumetric loss watershed-wide.

From an engineering perspective, direct measurement of exfiltration from sewer line segments is the most straightforward approach to determining potential fecal contamination loading from these structures. We will begin the Pilot Study by conducting a benchtop exercise, allowing for the development of the sampling apparatus, and testing different types of pipe and joint materials in the laboratory. Once we can demonstrate measurements under laboratory conditions, and baseline exfiltration rates are measured under controlled conditions, the Pilot Study will move to the field. For field deployments, the sampling apparatus will need to seal the ends of a defined section of sewer collection pipe using inflatable bladders and partially fill the pipe with recirculated water. Exfiltration rates will be empirically measured by calculating volume losses over time using changes in head height. This task can only be completed with the assistance of the collection system owners, who will need to temporarily divert or pump sewage flows around the pipe test section. The cost of this bypass structure, if needed, is not included in the costs of this workplan.

For the full Implementation Study, we will use a factorial design to examine differences in exfiltration rates from pipes of various ages, materials of construction, pipe condition rating, pipe bed materials, and geology, among other potential factors. A factorial design involves testing replicates of each factor (or combinations of factors), enabling watershed managers to determine where and when other pipe sections are most at risk. Table 3 outlines the minimum sampling effort recommended for this study based upon current input from local public utility agencies, but the Advisory Committee will assist in the final selection of factors for this task.

Once the risk factors are defined and tested, the empirical leak rates will be extrapolated to estimate potential fecal loading from all pipes with similar risk factors (i.e., similar age and materials of construction). Using GIS, this information can be used to map the proximity to surface waters, determine the order in which sections of sanitary pipe are inspected, or be utilized as another factor to prioritize sanitary sewer rehabilitation projects.

Table 3. Example of sampling effort for a factorial design based on 4 types of construction materials and 3 age ranges. Every combination is tested in triplicate. Note: not all combinations may exist (i.e., clay pipe <10 years old).

Material of Construction	Age			
	<10 years	10-25 years	>25 years	Total No. Sampling Events
Clay	3	3	3	9
Concrete	3	3	3	9
PVC	3	3	3	9
CIP	3	3	3	9
Total No. Sampling Events	12	12	12	**36**

The products for this subtask include:

A) a quantitative measurement of fecal loading from publicly owned sewer lines based on their age, materials of construction, geological setting or other factors

B) a standardized procedure to test segments of sewer line for exfiltration in the future

C) a list of criteria that can be used to prioritize aging sewer lines for inspection, rehabilitation, or replacement

Task 2C. Verifying sewer exfiltration transport to receiving waters

A major concern expressed by stakeholders is that transport needs to occur following exfiltration to move any fecal contamination from outside the pipe to receiving waters. Previous studies have attempted to measure subsurface transport using groundwater wells. Not only is this method expensive, but it is limited in its ability to quantify total loadings because borings are rarely in the exactly right location.

Biofilm microbial community profiling (a unique source marker for sanitary sewers) was recommended in Task 2B to establish that exfiltration could be transported to receiving waters. However, empirical measurements of volumetric loss present a perfect opportunity to verify subsurface transport. A unique tracer, such as potassium bromide, can be added to the water used to fill the pipe during the exfiltration measurements in Task 2B. This unique tracer can then be used simultaneously to determine if the exfiltrated water is making its way into stormwater conveyances. Water in nearby storm drain conveyances or creeks can be quickly and cheaply measured at numerous locations in near-real time for the presence of potassium bromide using conductivity probes and confirmed by collecting water samples for chemical analysis in the laboratory. Concentrations of potassium bromide also can be used for estimating dilution.

The products for this subtask include:

A) quantitative measurement of a unique tracer to definitively estimate transport from sections of test pipe to nearby receiving waters

Task 3. Quantifying Human Fecal Loading from Lateral Lines and Septic Systems

Task 3A. Assessing the frequency of leaking laterals

There are thousands of homes and businesses in the San Diego River watershed. Each of these has a lateral that connects them to the public sanitary sewer system. Unlike the public sewer system, however, there is no routine inspection or maintenance program for private sewer laterals. Repairs typically occur only after catastrophic failure, at which time a lateral may have been leaking for many years. To get a sense of how frequently private laterals may have an unknown structural defect, we analyzed data from the City of El Cajon, who is the only city in the San Diego River watershed requiring lateral inspections upon major renovation of a specific size. Figure 2 shows that out of the 548 mandatory inspections by certified plumbers between 2009-2018, 131 homes (24%) required repairs and 43 homes (8%) had a major structural defect such as a broken pipe or pipe offset that could be exfiltrating.

At least 100 homes will have their laterals inspected during this project. The number of inspections can be increased or decreased as a function of cost versus confidence in the results. Inspections will be conducted by certified home inspectors. Inspections may include visual inspections via closed circuit television (CCTV) or video, pressure testing, dye testing, or smoke testing. Addresses for private laterals will be selected at random based on likelihood of leakage. Likelihood of leakage will be based on factors such as age, materials of construction, geology, history of lateral spills, and distance to receiving water, amongst others.

Homeowners will be contacted prior to inspection and all inspections will be conducted free of charge to the homeowner. We anticipate as much as 50% of homeowners may decline the inspection, but the study design can accommodate high rejection rates by simply selecting additional addresses within the same combination of leakage factors.

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Type of Repair	% of Laterals That Need
Root intrusion	
Build Up	
Broken Pipe	
Offset	
Sag	
Corrosion	
Outdated Plumbing	

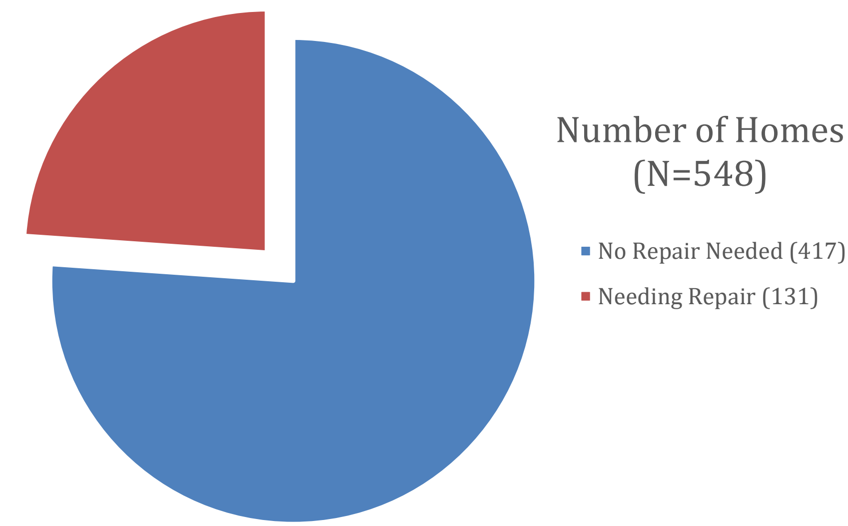

Figure 2. Results from the City of El Cajon's mandatory private lateral inspections and subsequent repairs between 2009-2018.

For those laterals that are inspected and found to be leaking, homeowners will be incentivized to repair or replace their lateral. Although every repair will be unique, the most common repair is likely to be relining using cured-in-place-pipe (CIPP) resins. With CIPP technology, costly and destructive excavation and replacement is unnecessary. CIPP can be completed in less than a day while the homeowner is not at home (home access not necessarily required). Moreover, CIPP can also secure joints where the lateral connects to the public sewer system, eliminating another potential location of leakage. Incentives will be standard amounts paid directly to contractors (not homeowners) upon photo-documented completion of repairs. Pre-selected licensed contractors at the standard rate will be available to homeowners, but homeowners can select whichever contractor they wish (standard incentive amounts will not change, but homeowners can pay the difference).

While onsite at a selected subset of sites, empirical measurements of volumetric loss from private laterals will be conducted. We will use the same methods as described for public sewers in Task 2B – that is, plugging the line at the street, filling the line with water, and measuring the amount of water lost over time. Theoretically, the time required for this analysis should be much less because the pipe segment volume is much smaller.

The products from this subtask includes:

A) *percent (with specified levels of confidence) of households with leaking or failing private laterals in the San Diego River Watershed based on inspections*
B) *risk profiles for the San Diego River watershed based upon inspections and GIS maps of risk factors*

Task 3B. Assessing the frequency of underperforming septic systems

Like private laterals, the first step for this project is to quantify the frequency of failing septic systems in the San Diego River watershed. To accomplish this, we will use a similar incentivized, stratified random-based design to septic inspection and repair. Septic systems are notably less abundant in this watershed compared to laterals.

At least 100 homes will have their septic systems inspected during this project. The number of inspections can be increased or decreased as a function of cost versus confidence in the results. Addresses for septic system inspections will be selected at random based on likelihood of leakage. Likelihood of leakage will be based on factors such as age, materials of construction, geology, frequency of emergency pump-outs, and distance to receiving water, amongst others.

Inspections of septic systems typically first require pump out; then, they can be inspected visually through access ports or by video camera. Because septic systems are not amenable to volumetric loss techniques, the project will adopt two other measurement techniques that also are being used for public sewers: (1) leaving behind chemical tracers such as potassium bromide, and (2) profiling the microbial community of septic systems. If the potassium bromide technique is used, researchers will place conductivity probes in the nearby stormwater conveyance. If tracer signatures are observed, then samples for analysis of potassium bromide and microbial community profiling will be collected from the storm drain conveyance. Concentrations will be used to estimate dilution and calculate mass contribution.

The products from this subtask include:

A) percent (with specified levels of confidence) of households with underperforming or failing septic systems in the San Diego River Watershed based on inspections
B) risk profiles for the San Diego River Watershed based on inspections and GIS maps of risk factors

Task 4: Quantifying Direct Inputs from Homeless Encampments

Subtask 4A: Conducting a census and survey of homeless populations

This subtask will attempt to determine how many homeless live within the watershed, including in or near waterbodies of concern. Researchers will identify encampments and count every homeless person using a variety of techniques including, but not limited to, aerial surveys using manned or unmanned aircraft with high-resolution infrared sensors. For locations where advanced aerial imaging techniques don't reach (i.e., under bridges), follow-up on-the-ground census work will take place. The survey will be repeated at least three times during the wet season to assess variability over time.

Next, researchers will assess the load of fecal material entering stormwater conveyances from outdoor defecation by homeless persons. This will require collaboration with social scientists to conduct face-to-face interviews with homeless persons living in or near stormwater conveyances, including a survey to determine their sanitation habits. Survey questionnaires will include how many times and how often individuals defecate in or near storm drain conveyances or streams, what proportion defecate outside of the stream only to be washed off during the next rainstorm, and what proportion defecate offsite and will not wash into the stream.

The products for this subtask will include:

A) an estimate of the homeless population living in and around storm water conveyances
B) an estimate of the percentage of homeless individuals that defecate in or near storm water conveyances, such that their feces would be expected to be carried into the conveyance
C) an estimate of human fecal loading in storm water attributable to the homeless population

Subtask 4B: Conducting fecal washoff experiments

After determining how many homeless live in watersheds of concern during time periods of interest, researchers will estimate fecal loading to receiving waters (i.e., how much of the fecal material will end up the waterbody of concern). This will be particularly important if the surveys indicate a large fraction of homeless defecate not directly into the water, but rather on the stream banks that come into direct contact with water when flood waters rise.

To conduct fecal washoff experiments, known quantities of fecal material will be placed on stream banks or storm drain conveyances, then measured to assess what remains after a variety of storm events. Because discharging human fecal material is illegal, canine or equine fecal material will be used as a surrogate. To supplement the washoff rates, researchers will track flood heights relative to experimental plots and measure canine or equine fecal markers in the stream. An alternative to stream-side experimental manipulations is to use mesocosms in SCCWRP's outdoor experimental laboratory.

The product for this subtask include:
A) experimental evidence about how much human fecal material washes into stormwater conveyances during rain events

Subtask 4C: Confirming homeless fecal contributions

The third step is to confirm contributions from homeless populations. This step will use an upstream-downstream study design. Using data from Subtask 4A, we will identify as many locations as possible with relatively dense homeless populations that defecate in or near the waterbody. Researchers will then sample immediately upstream and downstream for fecal indicator bacteria, human fecal markers, and viruses. If homelessness is a major source of fecal pollution, concentrations are expected to be higher downstream than upstream.

The biggest challenge to the upstream-downstream approach is sample size. The upstream-downstream approach works well if the homeless population is dense enough to be a significant source of fecal loading and if the upstream signal is small and relatively stable. If there is substantial variation in concentrations from sample to sample, or if the background concentrations are large relative to the homeless inputs, then the upstream-downstream approach may not show any differences, indicating this source remains small relative to other human fecal sources.

To assess sample size, statistical power analysis was conducted using human fecal markers during wet weather in the San Diego River (Figure 3). This analysis shows that as many as 30 sample events during dry weather, and up to 60 sample events (storms) during wet weather will be necessary to detect upstream-downstream differences of roughly 40%. Thus, multiple sites will need to be sampled in order to obtain sufficient data in a reasonable amount within a three-year time period.

The products for this subtask will include:
A) an estimate of human fecal loading in stormwater attributable to the homeless population

B) empirical evidence about how much human fecal material washes into storm water conveyances during rain events

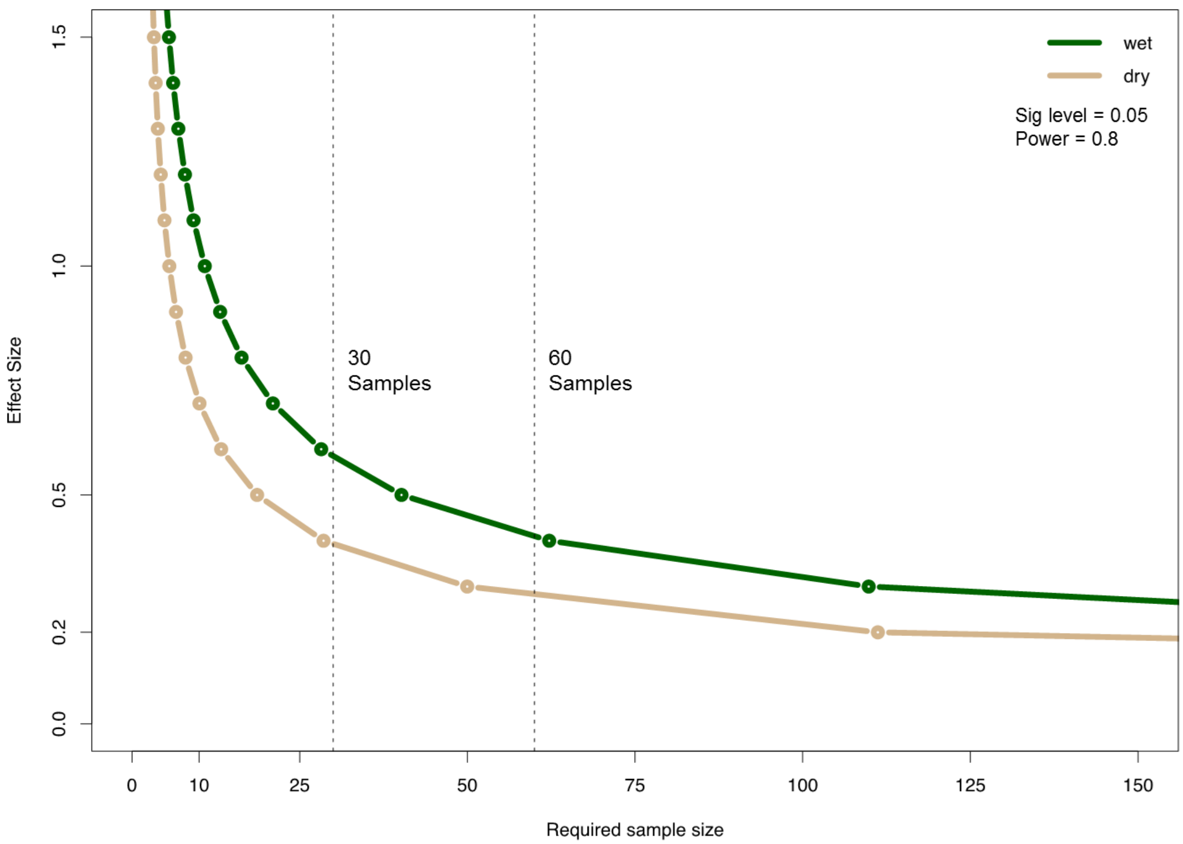

Figure 3. Statistical power analysis for detecting differences in human fecal marker (HF183) concentrations between upstream and downstream sites based on historical data from the San Diego River. More samples result in more statistical power and the ability to detect smaller and smaller differences (or "effects").

Task 5: Quantifying Direct Inputs from Recreational Vehicles (RVs)

Subtask 5A: Conducting a count of RV campers in urban areas

This subtask will attempt to determine how many RV campers live within the watershed, including in or near waterbodies of concern. This task is in some ways similar to homeless census in task 4A. Counting RVs will require a variety of techniques including on-the-ground census, parking enforcement records, or aerial surveys using manned or unmanned aircraft. Differentiation of RVs that are being lived in versus just parked will require on-the-ground confirmation. The survey will be repeated at least three times during the wet season to assess variability over time.

The products for this subtask will include:

A) an estimate of the RVs living in and around the San Diego River watershed

Subtask 5B: Quantifying illegal discharges from RV campers in urban areas

Unlike the homeless population approach used in Task 4, surveys of RV campers in urban areas is unlikely to produce reliable information about illegal discharges. Therefore, a rapid response monitoring approach will be required. This approach follows a series of steps including: a) select one or more MS4 locations where RV campers are in relatively dense aggregation(s), b) install *in-situ* sensors within the MS4 for flow, conductivity, ammonia, and potentially *E. coli* or *Enterococcus.* When one of these real-time sensors is above target values, an automated sampler will take a confirmation sample for HF183 quantification. Estimates of flow and concentration over time can be used to calculate cumulative loading per RV. This estimate can then be applied to the number of RVs quantified in Task 5A.

The products for this subtask will include:

A) *an estimate of the human fecal loading from RVs in and around the San Diego River watershed*
B) *a group of RVs to investigate for enforcement actions*

Task 6: Dry Weather Survey of Receiving Waters

Subtask 6A: Developing a Study Design

Unlike wet weather where there are repeated measurements of HF183 along the mainstem and major tributaries of the San Diego River during storm events, there have been no comprehensive surveys of HF183 in receiving waters during dry weather. In fact, dry weather sampling of storm drain outfalls in this watershed indicate very infrequent detections of HF183. Therefore, before estimates of human fecal loading in dry weather can occur, the presence of human fecal pollution must first be established. This task will aim to survey the San Diego River mainstem and major tributaries for the presence of human fecal pollution by measuring HF183. There are several strategies for conducting this type of a survey, so the output of this task is to develop a robust and mutually agreed upon monitoring plan that takes into consideration what to measure, when and where to sample, what benchmarks indicate too much pollution, and what actions will be taken if the benchmark is exceeded.

The products for this subtask will include:

A) *Sampling and Analysis Plan for a dry weather survey of the San Diego River mainstem and major tributaries*

Subtask 6B: Monitoring and Analysis

This sub-task will implement the monitoring design from task 6A. We presume this survey will sample multiple locations across multiple sampling events. Undoubtedly, samples will be analyzed for fecal indicator bacteria, HF183, but other indicators may be selected including unique sewage markers such as biofilm genetic signatures, chemical markers, or human pathogens, amongst others.

The products for this subtask will include:

A) *Dry weather monitoring survey results of the San Diego River mainstem and major tributaries.*

Subtask 6C: Working with IC/ID Programs to identify sources for remediation

Ultimately, the goal of a dry weather survey is to have no human fecal contributions. This sub-task will support the action items that follow the results of the dry weather survey. Where consistent human fecal pollutant inputs are identified in receiving waters, site-specific source investigations will need to occur for finding, confirming, and removing these site-specific inputs. While SCCWRP has no regulatory or enforcement authority, we recognize the need to work with those agencies that do. These follow up activities could be targeted upstream sampling, additional analytical parameters for further confirmation, and continuous or near real-time monitoring equipment.

The products for this subtask will include:

A) Site-specific source tracking support

Task 7. Data Management, Analysis and Reporting

Task 7A. Data Management and Analysis

All data from this project will be compiled in the project database. The database will become publicly accessible at the conclusion of the project, based on approval from the Advisory Committee. Personal information will be excluded to protect homeowner anonymity.

The products for this subtask will include:

A) Project database, including QA/QC data checkers, available for use by the participating agencies

Task 7B. Quarterly, Draft and Final Reports

The project Team will compile Quarterly Reports for the Advisory and Technical Committees to ensure regular communication and track project progress.

In addition, oral reports to Advisory and Technical Committees will occur: A) following development of study designs to ensure that Sampling and Analysis Plans are appropriately structured to support management needs, and B) following sampling campaigns and prior to written documentation to ensure that the data has been thoroughly analyzed and the proper context from all sectors is provided.

Project completion will require a final report. The draft final report will need to be reviewed by both the Technical and Advisory Committees. Comments and suggestions will be adapted in the Project Final Report, which will require Advisory Committee approval. Portions of the data analysis and Final Report may be adapted for peer-reviewed publications and/or presentations.

The products for this subtask will include:

A) Quarterly reports

B) Draft and Final Report

Summary of Completion Dates for Key Product Deliverables

The following table summarizes the recommended 48-month schedule in which key products will be completed and delivered. This schedule assumes work will begin in winter 2018-19. Contract origination past winter 2019-

20 could delay the project up to one year, as wet-weather sampling in out-years can only be conducted in winter.

Cost Schedule

The following table summarizes the estimated lump-sum costs by Subtask. Total cost is $4,140,500. A year-by-year budget is also provided. Notes are also provided where in-kind support from participants will be desired.

Schedule of Tasks and Subtasks for Tracking Human Fecal Sources

Tasks	FY Quarter 2019-20				FY Quarter 2020-21				FY Quarter 2021-22				FY Quarter 2022-23			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Task 1: Project Governance and Administration																
Subtask 1A: Establish an Advisory Committee	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Subtask 1B: Create Sampling and Quality Assurance Plans	■				■				■							
Task 2: Quantifying exfiltration from Publicly-Owned Sanitary Sewer Collection Systems																
Subtask 2A: Exploiting biofilms to verify the potential for sanitary sewer exfiltration	■	■	■	■	■	■										
Subtask 2B: Empirical measurements of volumetric loss				■	■	■	■	■	■	■	■	■				
Subtask 2C: Verifying sewer exfiltration transport to receiving waters							■	■	■	■						
Task 3: Quantifying Contributions from Lateral Lines and Septic Systems on Private Property																
Subtask 3A: Assessing the frequency of leaking laterals							■	■	■	■	■	■				
Subtask 3B: Assessing the frequency of leaking septic systems									■	■	■	■	■			
Task 4: Quantifying Fecal Inputs from Homeless Encampments																
Subtask 4A: Conducting a census and survey of homeless populations		■	■			■	■									
Subtask 4B: Conducting fecal washoff experiments						■	■	■	■							
Subtask 4C: Confirming homeless fecal contributions		■	■			■	■			■	■		■			
Task 5: Quantifying Direct Inputs from RVs																
Subtask 5A: Conducting a count of RV campers in urban areas										■	■	■	■	■		
Subtask 5B: Quantifying illegal discharges from RV campers in urban areas											■	■	■	■		
Task 6: Dry Weather Survey of Receiving Waters																
Subtask 6A: Developing a Study Design			■				■									
Subtask 6B: Monitoring and Analysis				■	■			■	■							
Subtask 6C: Working with IC/ID Programs to identify sources for remediation				■	■			■	■							
Task 7: Data Management, Analysis and Reporting																
Subtask 7A: Data Management and Analysis	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Subtask 7B: Quarterly, Draft and Final Reports	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

Cost Schedule for Tracking Human Fecal Sources

Task	Outcome	Cost	FY 2019-20	FY 2020-21	FY 2021-22	FY2022-23	Notes
Task 1: Project Governance and Planning							
Subtask 1A: Establish an Advisory Committee	Major project input and decision authority	40,000	10,000	10,000	10,000	10,000	
Subtask 1B: Create Sampling and Quality Assurance Plans	Project design documentation	30,000	10,000	10,000	10,000		
Task 2: Quantify Human Fecal Loading from Public Sewer Exfiltration							
Subtask 2A: Exploiting biofilms to verify the potential for sanitary sewer exfiltration	Estimate of sewage concentrations in stormwater samples from watersheds of concern throughout the San Diego region	630,000	240,000	390,000			Requires in-kind support from sewer agency
Subtask 2B: Empirical measurements of volumetric loss	Fecal loading estimate from publicly owned sewer lines, List of criteria for prioritizing aging sewer lines for inspection, rehabilitation, or replacement	280,000	90,000	100,000	90,000		Requires in-kind support from sewer agency
Subtask 2C: Verifying sewer exfiltration transport to receiving waters	Quantitative measurement of a unique tracer to definitively estimate transport from sections of test pipe to nearby receiving waters	50,000		25,000	25,000		Requires in-kind support from sewer agency
Task 3: Quantify Human Fecal Loading from Lateral Lines and Septic Systems							
Subtask 3A: Assessing the frequency of leaking laterals	Estimate of potential fecal loading from faulty private laterals in the San Diego River watershed	350,000		100,000	250,000		Includes homeowner incentives
Subtask 3B: Assessing the frequency of underperforming septic systems	Estimate of potential fecal loading from faulty septic systems in the San Diego River watershed	300,000			210,000	90,000	Includes homeowner incentives
Task 4: Quantifying Direct Inputs from Homeless Encampments							
Subtask 4A: Conducting a census and survey of homeless populations	Esitimates of human fecal loading based on number of homeless and their sanitary habits	300,000	160,000	140,000			
Subtask 4B: Conducting fecal washoff experiments	Experimental evidence of fecal material washing off stream banks	100,000		75,000	25,000		
Subtask 4C: Confirming homeless fecal contributions	Empirical estimates of human fecal contributions from homeless populations	1,485,000	475,000	475,000	475,000	60,000	Includes all wet weather sampling requirements
Task 5: Quantifying Direct Inputs from Recreational Vehicles (RVs)							
Subtask 5A: Conducting a count of RV campers in urban areas	Estimate of the number of RVs living in and around the San Diego River watershed	150,000			75,000	75,000	
Subtask 5B: Quantifying illegal discharges from RV campers in urban areas	Estimate of human fecal loading from RVs in and around the San Diego River watershed; RV(s) to investigate for enforcement actions	110,000			55,000	55,000	
Task 6: Dry Weather Survey of Receiving Waters							
Subtask 6A: Developing a Study Design	Sampling and Analysis Plan for a dry weather survey of the San Diego River mainstem and major tributaries	25,500	17,500	8,000			
Subtask 6B: Monitoring and Analysis	Dry weather monitoring survey results of the San Diego River mainstem and major tributaries	160,000	40,000	80,000	40,000		
Subtask 6C: Working with IC/ID Programs to identify sources for remediation	Site-specific source tracking support	-					No cost estimate; site-specific, as-needed pricing
Task 7. Data Management, Analysis and Reporting							
Task 7A. Data Management and Analysis	Project database, including QA/QC data checkers, available for use by the participating agencies	50,000	15,000	10,000	10,000	15,000	
Task 7B. Quarterly, Draft and Final Reports	Oral Reports, Quarterly reports, Draft and Final Report reviewed and approved by the Advisory Committee	80,000	5,000	5,000	5,000	65,000	
TOTAL		**4,140,500**	**1,062,500**	**1,428,000**	**1,280,000**	**370,000**	

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APPENDIX A. Microbial Community Profiling Laboratory Method Description

Once delivered to the laboratory, 100 mL portions of the water samples and the biofilm slurry will be filtered through 0.2 micron or 0.4 micron polycarbonate membranes to collect bacteria, then flash frozen in liquid nitrogen and stored with biofilm samples at -80°C prior to DNA extraction. Filtration controls will be processed during sample filtrations

Frozen filters and biofilm samples will be processed in batches using commercially available DNA extraction kits, such as the DNeasy Power Soil Kit (Qiagen, MD). Methods developed by Cao et al (2015) and used for the Surfer Health Study (Steele et al. 2018) will be used for DNA extraction. Additional DNA extract will be archived for analysis of *Enterococcus* and HF183. Negative Extraction Controls (NEC) containing only lysis buffer and lysis buffer and control DNA (e.g. halophile or salmon testes DNA) will be processed for every extraction in the same manner as the samples.

Roughly 100 - 350 base pair segments from highly conserved 16S ribosomal RNA (rRNA) genes in extracted DNA will be amplified using polymerase chain reaction (PCR). Potentially millions of different gene sequences will be used to elucidate the bacterial community diversity, including those intended to be diagnostic of particular sources (e.g. *Bacteroidales, Lachnospriaciae* – sewer pipes, Methanogens – septics), or potentially from individual sites. Amplified DNA fragments will be sequenced by the SCCWRP on-call contract laboratory using a state-of-the-art next-generation sequencer such as Illumina Miseq™ or Hiseq™ technology generating 20 million to 100 million sequences per run.

A mock community consisting of known amounts of microbial genomes will be analyzed in parallel with the samples and included, along with a no-template control on every run. These controls will allow for quality control including assessment of assay bias, quantification bias, sequencer error, and bioinformatic pipeline QC.

The millions of resulting sequences amplified from the samples will be processed through a customized bioinformatics software pipeline. This automated pipeline will combine the sequence reads into contiguous sequences, perform quality analysis and remove low quality sequences, correct for known systemic errors. Once the low-quality sequences are removed, any sample replicates with fewer than 10,000 sequences will be rejected and re-sequenced.

The bioinformatic pipeline will organize sequences into related groups and facilitate sample comparison using advanced hierarchical mapping statistical methods. The sequences will also be compared to public databases (e.g. Genbank, Silva) to identify the bacteria at the finest taxonomic resolution possible. Relative abundance of bacterial amplified sequence variants (ASVs) will be examined to further resolve bacterial communities.

Although particular groups of bacteria will be examined, the entire set of quality controlled amplified 16S rRNA genes will be examined and compared. We will compare sequences at the finest resolution available based on sequence quality, to increase the sensitivity of the comparison among the bacterial communities from different sources. By not relying on taxonomic identification, we will be able to use finer distinctions among the bacterial communities from different sources. Genes from the stormwater biofilms or sewer biofilms will be investigated to enable identification of new markers for southern California sewer, storm drain, or septic infrastructure.

In addition to the sequencing, Human-associated Bacteroidales (HF183) and sewer pipe-associated Lachnospiraciae (Lachno3) will be measured using a digital PCR assay following previously published protocols (Cao et al. 2015, Steele et al. 2018, Feng et al. 2018). Samples will be measured in replicate using at least 20,000 droplets for an absolute quantification of the human and sewer pipe-associated marker gene copies. This will provide an absolute measure of the gene copies compared to the relative measure obtained from the sequencing effort.