APPENDIX G - QA/QC Plan

1. Topanga Source Identification Study Quality Assurance/Quality Control Plan (April 2013)

1. Title and Approval Page

Topanga Creek Source Identification Study

Quality Assurance/Quality Control Plan

Resource Conservation District of the Santa Monica Mountains

UCLA Jay Lab

DRAFT April 2013

APPROVED BY:

_____ ***RCDSMM***

_____ ***UCLA JAY LAB***

_____

Technical Advisory Committee

2. Table of Contents

3. Distribution List

Copies of this plan are reviewed by members of the Topanga Source ID Technical Advisory Committee.

This QA Plan will be reviewed annually and any changes and revisions will be forwarded to all appropriate parties. In the event a major revision to the QA Plan is required, each copy will be reissued to all appropriate persons. All revisions to the Plan will be clearly marked on each page with a revision number and revision date.

When modifications to the QA Plan become necessary, ***the RCDSMM Project Manager*** is responsible for ensuring that current revisions are included in the QA Plan, and that distribution of the revised Plan is made to all appropriate parties.

4. Project/Task Organization

Table 1.

Name	Responsibility	
Rosi Dagit	RCDSMM, Project Manager	Site Sampling, Flow, Water temp., Air temp., Salinity, Conductivity, pH, Nutrients, Turbidity, pictures, DO, observations, IBI
Jenna Krug	RCDSMM	
Jenny Jay	UCLA, Principle Investigator	Site Sampling, FIB analysis, Molecular marker analysis, IBI
Timothy Riedel	UCLA	
Vanessa Thulsiraj	UCLA	
Amy Zimmer-Faust	UCLA	
Robert Torres	UCLA	
Richard Sherman	Topanga Underground	Septic system analysis
Steve Braeband	BioSolutions	
Jed Furhman	USC	Viral analysis

5. Problem Definition/Background

A Quality Assurance/Quality Control (QA/QC) Plan is the basis for assessing and maintaining the quality of data collected for the Topanga Source Identification Study.

Need for Project:

In spite of the removal of houses and their accompanying septics within the Rodeo Grounds area of the lower watershed (and other coastal engineering solutions described below) Topanga State Beach continues to receive poor water quality ratings. The beach has exceeded the targets set for Fecal Indicator Bacteria based on the Ocean Standards (AB411) based on weekly samples collected by the City of Los Angeles Environmental Monitoring Division. This happened frequently enough for Topanga Beach to be identified by Heal the Bay as the 4th most polluted beach in the state for the 2010-2011 season and as the 10th most polluted in 2011-2012. No systematic sampling of the creek or adjacent up and downcoast reaches of the beach have been done since 2004. Since that time, a number of conditions have changed.

Topanga Creek has been listed by the Regional Water Quality Control Board 303(d) list for lead in the upper watershed and bacteria at Topanga Beach. No other pollutants of concern have been listed for the watershed. Topanga Creek has no storm water conveyance systems per se, but in actuality stormwater is "conveyed" and enters the creek in a variety of ways.

A variety of analytical methods now allow for connecting bacteria to a specific source - human, dog, horse, bird, etc. In collaboration with the State of California Source Identification Project (SIPP), the fecal source tracking of samples proposed will provide additional insight concerning actual sources.

Over the past few years, a number of actions have been implemented to reduce possible bacterial contamination of Topanga Beach. In 2008, Los Angeles County Department of Beaches and Harbors upgraded their septic system associated with the restrooms and lifeguard station. Since 2008, the septic systems located within the former Rodeo Grounds Road and Snake Pit area have been removed. The septic systems associated with the Topanga Ranch Motel (ranger house only), Reel Inn, Cholada's, the winery, and the Topanga Feed Bin are now being pumped weekly or as needed in compliance with CA Department of Parks and Recreation requirements.

Why then is Topanga beach still experiencing high bacterial levels?

6. Project Task/Description

Quality Assurance and Quality Control are two independent and interrelated functions. First, Quality Assurance is defined as a system of general programmatic activities implemented to ensure that Quality Control is performing adequately. Quality Control is defined as a series of specific activities performed to provide reproducible data. Consequently, quality assurance serves as a "quality control" for the quality control function.

QA/QC Plans consist of primarily two functions: (1) the QA function is the assessment of the quality of the data (accuracy and precision) and, (2) the QC functions which are the activities that maintain or improve data quality. These two functions combined form a control loop. For instance, when accuracy or precision (a QA function) is unacceptable, QC functions must increase until the quality of the data is acceptable.

Quality Assurance involves meeting programmatic requirements but on occasion requires the implementation of external checks on data quality. These external checks may include independent system audits, third party sample and analysis for accuracy and precision, comparison to know calibration standards or inter-laboratory audits.

Quality Control functions are usually a series of frequent (daily, weekly, monthly) routine internal checks, such as calibrations and routine maintenance. A complete Quality Assurance Plan encompasses both QA and QC functions and strives to identify which function is addressed by a specific activity.

7. Data Quality Objectives for Measurement Data

For the Topanga Source identification Study, we have identified the following goals and objectives as requiring implementation of a QA/QC Plan.

1. Identify the likely sources (both physical location and source, i.e., human, bird, dog, horse, etc.) of bacterial contamination at Topanga Beach by testing the creek from MM 2.02 to the beach to test the hypothesis that the creek is grade "A", and test in and around the lagoon to fine tune our understanding of when and investigate why it gets grade "F".
2. Collect all data in a reliable, consistent, accurate and precise manner.
3. Collect all data completely at each sampling event.

Detectability is the ability of the method to reliably measure a pollutant concentration above background. There are two components to define detectability: method detection limit (MDL) and practical quantification limit (PQL) or reporting limit (RL). **The MDL is the minimum value which the instrument can discern above background but no certainty to the accuracy of the**

measured value. For field measurements the manufacturer's listed instrument detection limit (IDL) can be used.

- The PQL or RL is the minimum value that can be reported with confidence (usually some multiple of the MDL).

Sample data measured below the MDL is reported as ND or non-detect. Sample data measured ≥ MDL but ≤ PQL or RL is reported as estimated data. Sample data measured above the PQL or RL is reported as reliable data unless otherwise qualified per the specific sample analysis.

Precision is the degree of agreement among repeated measurements of the same parameter, and provides information about the consistency of methods. Precision is expressed in terms of the relative percent difference between two measurements (A and B). For field measurements, precision is assessed by measuring replicate (paired) samples at the same locations and as soon as possible to limit temporal variance in sample results. Field and laboratory precision is measured by collecting blind (to the laboratory) field replicate or duplicate samples. For paired and small data sets project precision is calculated using the following formula:

$$\text{Precision} = \frac{(A-B)}{((A+B)/2)} \times 100$$

Bias (Accuracy) is a measure of confidence that describes how close a measurement is to its "true" value. Methods to determine and assess accuracy of field and laboratory measurements include, instrument calibrations, various types of QC checks (e.g., sample split measurements, sample spike recoveries, matrix spike duplicates, continuing calibration verification checks, internal standards, sample blank measurements (field and lab blanks), external standards, etc. Bias/Accuracy is usually assessed using the following formula:

$$Accuracy = \frac{MeasuredValue}{TrueValue} \times 100$$

Completeness is a measure of the percentage of valid samples collected and analyzed to yield sufficient information to make informed decisions with statistical confidence. The completeness criterion for this project is 80 percent of the compiled analytical data per each analytical parameter for each vessel participating in the program. Because of the variety of vessels and discharges sampled, and the possibility for weather or other shipping-related delays resulting in missed holding times, a completeness criterion of less than 100% is to be expected.

Project completeness is determined for each pollutant parameter using the following formula:

$$\frac{T-(I+NC)}{} \times (100\%) = \text{Completeness}$$

Where T = Total number of expected sample measurements.

I = Number of invalid sample measured results.

NC = Number of sample measurements not produced (e.g. spilled sample, etc).

Representativeness Representativeness is a measure of how well the sample reflects the typical water condition. Sample representativeness will be established by collecting samples as described in the "Sample Processing" section of the TOPANGA LAGOON AND CREEK SOURCE IDENTIFICATION WATER QUALITY STUDY version 16 November 2012 for extensive details of the sample process design.

Table 2.

Parameter	Precision (Method detection limit)	Accuracy	Range
Dissolved Oxygen – YSI 55	DO% sat – 0.1% DO mg/l – 0.01 mg/l Temp – 0.1^{0}C	~2% ~0.3mg/l 0.2^{0}C	0-200% 0-20 mg/l -5-+45^{0}C
pH – Oakton pHTestr 30	0.01	0.01	-1.00-15
Conductivity – Oakton ECT Testr low range	0.1ms	~1%	200-2000ms
Turbidity – La Mottes 2020we/wi	0.01 NTU 0.1 NTU 1 NTU	0-2.5NTU~0.05% 2.5-100 NTU~2% >100 NTU ~3%	0-4000 NTU
Smart 3 Colorimeter (LaMotte)-Ammonia- N (High range)	0.05 ppm	0.01 ppm	0.00-4.00 ppm
Smart 3 Colorimeter (LaMotte)-Nitrate- N	0.10 ppm	0.01 ppm	0.00-3.00 ppm
Smart 3 Colorimeter (LaMotte)-Nitrite- N	0.02 ppm	0.01 ppm	0.00-0.80 ppm
Smart 3 Colorimeter (LaMotte)-Orthophosphate	0.05 ppm	0.01 ppm	0.00-3.00 ppm
UCLA Equipment			
FIB Colilert	±75%	±1 count X dilution factor	(1 to 2419) X (dilution factor)
FIB Enterolert	±75%	±1 count X dilution factor	(1 to 2419) X (dilution factor)
Con6 Conductivity Probe	±5%	±1%	0 to 200 mS/cm
ABI StepOnePlus (qPCR)	TBD*	TBD*	TBD*

*TBD = the qPCR equipment and assays are undergoing rigorous testing throughout the scientific community to understand performance characteristics

8. Training Requirements

Normalization training on equipment for scientists and volunteers will be conducted by the RCDSMM and UCLA at the start of the project, and once a year at minimum. All staff and volunteers will demonstrate ability to calibrate equipment and proper use of equipment to the project supervisor. Internal audits of standard operating procedures will occur randomly, but at least once per quarter.

9. Documentation and Records

Data sheets and Chain of custody (COC) forms are used to document sample collection, processing, and storage. Lab notebooks are used to document PCR/qPCR analyses. Samples of data sheets and forms are found in Appendix A.

Table 3.

Document Type	Data Sheets	Primary User	Storage
Data Sheets	Flow	RCDSMM	RCDSMM
	Nutrients	RCDSMM	RCDSMM
	Calibration	RCDSMM	RCDSMM
	Lagoon/Beach in-situ variables	RCDSMM	RCDSMM
	Upper Sites in-situ variables	RCDSMM	RCDSMM
	DNA Extraction	UCLA	UCLA
Chain of Custody Reports	Molecular Bottles	UCLA	UCLA, RCDSMM
	Nutrient Bottle	RCDSMM	RCDSMM
	Lifeguard septic	BioSolutions/RCDSMM/UCLA	RCDSMM
Electronic Databases	All data	RCDSMM and UCLA	RCDSMM
Project Reports	Monthly	RCDSMM	RCDSMM

10. Sampling Process Design

During Year 1, samples will be collected monthly in the dry season (7 months) and bi-weekly during the wet season (5 months), and at first flush at seven locations: Topanga Lagoon beach outlet (City site), east wall of the Lagoon, under the PCH bridge and at the upper end of the Lagoon in the Snake Pit, on the west end of the county beach near American Apparel, and then upstream at the Rodeo Grounds and TC Blvd Bridge MM 2.02 in order to document any input coming downstream from the upper watershed. The sampling schedule is included in Appendix B.

Tests will include, but not be limited to, bacterial DNA, viral DNA/RNA, total coliforms, *E. coli,* enterococci, and human-specific markers and other host–associated markers (gull, horse, and dog), community bacterial analysis, and viruses. Based on results, sampling will be focused in Year 2 to target areas of identified concern in order to specifically locate probable sources.

Samples collected during Year 1 that are high in either FIB or Human Specific Marker (HSM) will be further evaluated using a suite of host-specific markers. Specifically, we will be using assays developed for gull, horse and dog, all of which have been included in the SIPP method evaluation study. DNA will be extracted and amplified as described above. Depending on these results, additional tests for viruses or to clarify if the FIB source is from septic systems or direct human fecal materials may be added.

A tiered approach to focus sampling on areas of highest concern will provide a cost effective effort to problem identification. Using the IMS on the ground, real time data collection will also allow us to more closely target areas of concern.

We will also incorporate standard Citizen Water Quality Monitoring parameters to the sampling effort in order to see how the condition of the creek today compares to that observed in 2004, and identify potential remedial actions. This will also provide an outreach opportunity for Stream Team volunteers, local students and members of the Watershed Stewards Program. The nutrient testing of grab samples would be done using a Smart 3 Colorimeter. Samples would be collected at the same time and schedule as that proposed above.

The Citizen Water Quality Monitoring standard parameters include:

- Physical parameters- site conditions, water temperature, flow
- Chemical parameters- salinity, dissolved oxygen, pH, conductivity, Nitrate-Nitrogen, Nitrites, Ammonia –Nitrogen, Orthophosphates, turbidity
- Biological parameters- Algae percent cover and community composition, macro-invertebrate community characterization

All sites will be sampled at the first flush rain event.

If we identify a "hot spot" for either E. coli or enterococci other than first flush, then we will follow up with two days of focused sampling to narrow the FIB source ID. This will include a total of 10 samples at up to seven sites. Additionally, the bacterial community signature, which can further distinguish a human source from septics, graywater or direct deposit, will be collected at these same sites. Samples for human specific viruses will also be collected at five "hot spots" in the wet season and possibly as many as five other locations during first flush or other rain events.

Table 4. Proposed Sampling locations for 2012-2014 (UTM 11N)

Site Name	X_ Easting	Y_ Northing	Elevation (ft)	# Samples Wet	# Samples Dry
Beach Upcoast (BU)	353726	3767515	0	2/mo + first flush	1/mo
Beach Outlet (BO)	353896	3767506	0	2/mo + first flush	1/mo
Lagoon Outlet (LO)	353872	3767529	0	2/mo + first flush	1/mo
Lifeguard Station Beach (LG)	353968	3767553	0	2/mo + first flush	1/mo
Topanga Lagoon (TL)	353887	3767573	0	2/mo + first flush	1/mo
PCH Bridge - 0m (HB)	353868	3767649	0	2/mo + first flush	1/mo
Lifeguard Station Septic (LS)	353994	3767655	0	1/mo	1/mo
Snake Pit – 300m (SP)	354015	3767841	0	2/mo + first flush	1/mo
Brookside Drive – 1700m (BR)	354075	3768713	0	2/mo + first flush	1/mo
Topanga Bridge – 3600m (TB)	353522	3770391	200	2/mo + first flush	1/mo
SIPP SITES					
Scratchy Trail – 4800m (ST)	353518	3771500	500	2/mo + first flush	1/mo
Owl Falls – 6500m (OF)	352673	3772373	700	2/mo + first flush	1/mo
Falls Drive (FD)	352535	3772259	750	occasional	
Behind Abuelitas (BA)	351570	3772891	700	occasional	

Analyses:

Below is a list of the methods used to evaluate the microbial composition of each water sample. The qPCR methods listed here are subject to change based on results of the SIPP Method Evaluation Study. The approximate volume of sample water filtered for each analysis and filter type (Polycarbonate PC or mixed cellulose esters HA) is provided in parentheses below.

At all sites

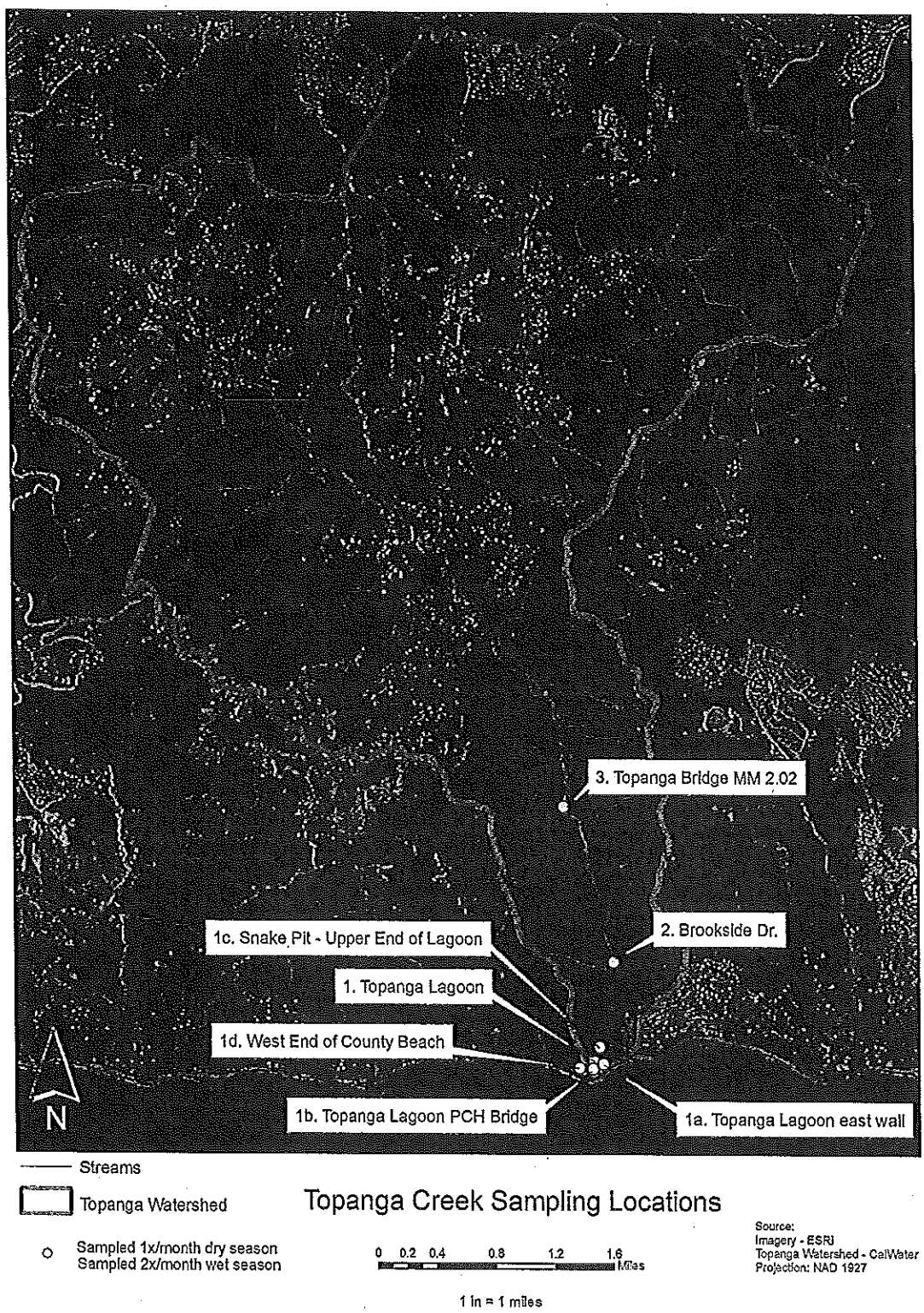
1. Culturable enterococci (IDEXX Enterolert) –2 dilutions used if needed, otherwise default dilution was 1;10 as recommended by the manufacturer (1 ml, 10 ml)
2. Culturable *E. coli* (IDEXX Colilert) – 2 dilutions used if needed, otherwise default dilution was 1;10 as recommended by the manufacturer (1 ml, 10 ml)
3. Bacterial qPCR (200 ml PC)
    a. Human-associated *Bacteroides*
        i. HF183 Taqman
        ii. BacHum
    b. Gull *Catellicoccus*
4. Human virus qPCR (500 ml HA)
    a. Enterovirus
    b. Adenovirus
    c. Polyomavirus
    d. Norovirus

Additional markers for use in hot spot analysis include:

1. Enterococci qPCR (200 ml PC) on a subset of samples
2. Dog-associated *Bacteroides* DogBac qPCR (200 ml PC)
3. Gull *Catellicoccus* qPCR (200 ml PC)
4. Horse-associated *Bacteroides* HoF597 PCR (200 ml PC)
5. Community Analysis by T-RFLP (400 ml PC)

Horse and Community analysis results will be provided in Year 2.

Figure 1. Map of the proposed 2012-2014 Topanga Creek Watershed sampling locations



11. Sampling Methods Requirements

Table 5.

Sample	Equipment	Container	Preservative	Holding Time	Cleaning
FIB/Molecular	Pole sampler, Bridge sampler, IDEXX kits, filters	Polypropylene Bottle	ice	<6 hours	Wash then store with small amount of 10% HCl
Nutrients		½ liter Nalgene bottle	ice	<8 hours	Wash
IBI	D-frame Kick net-0.5mm mesh	Sterile whirlpac or ziploc	Ice and then freezer	1 year	Thoroughly clean net after each site
Diatom and soft-bodied algae	Equipment depends on substrate and could include: Syringe Scrubber, 4 cm diameter PVC delimiter	Wash bucket to composite samples	Diatoms- 40 ml composite water and 5 ml lugol preservative Algae – 25 ml of composite sample filtered using hand pump and stored in petri dish on ice	1 year	All equipment cleaned thoroughly after each site. New filters and syringes for each sample.

12. Sample Handling and Custody Requirements

Although this data is not being used in a regulatory setting, documenting who has physical control of each sample at ALL TIMES is a standard operating procedure that we MUST follow. Please be sure that the name and times are correct for each step of the process.

Time and Name of person who collected the sample

Time and Name of the person who transports the sample to the lab/RCDSMM

Time and Name of the person who fixes or manipulates the sample for testing

Time and Name of the person who reads the results

Time and Name of the person who enters the data

Time and temperature of ice chest where samples being stored prior to fixing.

13. Analytical Methods Requirements

13.1 Equipment preparation and calibration prior to EACH sampling event

ALL FIB SAMPLES NEED TO BE COLLECTED BEFORE DIRECT SUNLIGHT HITS THE WATER.

13.1.1 Prepare the FIB sample bottles (2 liter acid washed polypropylene) as follows:

1) Shake acid already in bottle vigorously -- enough to cover all surfaces (for tea bottles make sure handle gets some acid too).
2) Pour out acid from sample bottle into waste bottle.

13.2 Standard Operating Procedures for sampling events

Before leaving the cars, make sure all equipment is in hand.

It is really important that data collection be done in the same sequence and at consistent locations. Please follow the steps below at each site.

It is really important to sample at the same location consistently!

When walking to the site, avoid stirring up sediments!

Walk along the bank to the sampling location, or cross the creek at least 5 meters away from the sampling location to avoid stirring up the sediments.

Take a photo of the site from the photo documentation point.

It is important to stand in the same place, face the same direction and frame the photos consistently month to month so we will have a photo sheet to refer to.

Feel free to take any additional photos to illustrate flow conditions or other things of interest.

Getting a group shot of the sampling team is also great if there is time!

Change gloves between each site.

IMPORTANT NOTE FOR LAGOON TEAM- Stop by the Lifeguard Station and upload the photos and get calendar notes on lagoon-ocean condition.

13.2.1 Procedure for Collecting FIB/Molecular, Virus and Nutrient samples

Walk along the bank to the sampling location, or cross the creek at least 5 meters away from the sampling location to avoid stirring up the sediments.

If it is not possible to reach without stepping into the creek, then use the pole sampler to gather the water sample and disturb the sediment as little as possible.

Wear gloves. Remove the cap, submerge the bottle at the site and fill at least half way. Put cap back on, shake well and discard the water on the bank, not into the creek. Dump the water in the same spot to further dilute any acid washed out.

Repeat this three (3) times.

Creek: Sample upstream of all other measurements. Point mouth of bottle upstream and ahead of your body. Do not use your hand or lid to strain water. Collect as little of bottom sediment or surface scum as possible. Ideally the bottle will collect water 4-6 inches below the surface, but if creek is too shallow just get what you can. Fill bottle at least 75% full, but if creek is too shallow just get what you can.

Ocean: Collect on incoming waves with mouth of bottle seaward and ahead of your body in approximately knee deep water. Fill bottle at least 75% full.

Place bottle in ice chest within 15 minutes of sampling. Be sure that samples are not exposed to direct sunlight.

Note the sample collection time on the data sheet and on the bottle.

Repeat with the Nutrient Grab Sample bottle after recording the bottle number on the data sheet.

If VIRUS samples are collected, repeat this process and fill the virus sample bottle.

Change gloves between each site.

13.2.2 Procedure for Collecting In –Situ Water Quality Data

Upon arrival at the sampling location, set down tote in the shade on the bank.

Place the air thermometer on the tote in the shade. Allow at least 3 minutes before reading.

(If using the hydrolab, be sure the propeller is turned on! Probe can rest on its side on the substrate. Record all data.)

Measure salinity using the refractometer. Lift the cover. Place several drops of water on the glass plate, drop the cover and hold the instrument towards a light source. Salinity is read by looking at where the blue area meets the white area. Numbers are on the right side of the center line.

For the YSI 55 DO meter, enter the altitude (refer to data sheet for each site) and the salinity with the probe in the chamber. Gently swirl the probe with the silver probe section just below the surface of the water until the DO reading stabilizes. Record the DO both in mg/l and in % saturation. Rinse the probe with distilled water and re-insert into the side housing chamber.

Use the water temperature reading from the YSI 55. Record.

Measure pH by submerging the probe into the grab sample bottle. Record

Measure conductivity by submerging the probe into the grab sample bottle. Record

Turn over data sheet and record the weather conditions and ambient observations.

Before leaving the site, make sure all probes are rinsed with distilled water and placed into the tote.

Review the data sheet and make sure all data has been properly recorded.

NOTE: Turbidity, Nutrient tests and FIB tests will be completed back at the lab.

13.2.3 Procedures for Measuring FLOW and ALGAE 2 people needed

Each site requires a unique data sheet, so be sure you have one for each location where flow will be measured.

Place the 0m end of the meter tape on the west bank of the channel (left side looking upstream) and pull straight across the channel and hold tightly using a rock and your feet.

Note the wetted channel width on data sheet. Data recorder also can hold one end of the tape.

Flow recorder stands on the downstream side of the tape.

At each distance, record the depth and flow, using the Marsh-McBirney Flo-Mate 2000. Be sure the flow head is pointing directly upstream and is 1/3 of the depth up from the bottom. You can either just hold it in place or adjust the head on the pole.

Wait until the flow reading stabilizes, then record.

Note if there is algae under the point generated by the random number table, and if so, what type.

If no algae is present, note substrate type or other vegetation type as shown on the back of the flow data sheet.

13.2.4 HOLDING TIME INFORMATION

The holding time for the FIB and VIRUS samples is no more than 6 hours from the time collected to processing in the lab. Samples should be on ice at all times during transport.

Holding time for Nutrient sampling is no more than 24 hours, although within 8 hours is preferable. Samples should be on ice at all times during transport.

13.2.5 Post-sampling Procedures

All gear and waders will be rinsed and returned to either the RCD shed or back to UCLA.

If any equipment needs repair or maintenance, this will be done asap.

UCLA team will head back to the lab to process the samples.

RCDSMM Team will conduct nutrient analysis.

RCD team will head to RCD office to input data, download and label photos.and do nutrient testing.

All data sheets will return to the RCD office for entering and be archived there.

As soon as the FIB and DNA data is available, it will be provided to the RCD and included into the central database.

13.3 FIB and Marker Lab Processes

Processing includes analysis for fecal indicator bacteria (FIB) using IDEXX Colilert-18™ and Enterolert™ methods for enumeration of total coliform, *E.coli*, and enterococci, respectively. For PCR analyses, samples will be filtered onto 0.45-µm pore size polycarbonate filters and stored in 2ml beaded microcentrifuge tubes at -80C. Later, filters will go through DNA extraction (DNA-EZ protocol, Generite, Inc) and extract will be analyzed in triplicate using a suite of endpoint and qPCR protocols for specific hosts, including (but not limited to) human, dog, gull, and horse. Extracts will also be tested for inhibition using spiked serial dilutions.

13.3.1 FIB

Use autoclaved nalgene 100ml bottles that have been filled with 90 or 99 ml of diH_2O.

Feed bottles with Colilert-18 or Enterolert-24 powder.
Tap on table to settle the powder.
Snap open away from face.
Pour powder into bottle.
Work plastic powder holder to get out last bits of powder. Lots of dust will remain.
Close Bottle and shake.

Inoculate 90 ml bottles with 10 ml sample and 99 ml bottles with 1 ml sample.

Shake and pour into IDEXX plates.

Squeeze plates with plastic on top and pull foil tab a little to open up pocket.
Pour into pocket.

Hold up plate and tap out any major bubbles.
Set plate into metal tray and let sit for a while so remaining foam is reduced.

Seal plate in seal-o-meal thingy.
Turn on sealer and wait for green light to turn on before using
Place plate in rubber mask and load into the machine.
Typically needs a little lift to begin feeding in

Put sealed plates into incubators.
Don't stack more than 6 high
Check notes on incubator doors.
Coliert -18 = 35 °C
Enterolert-24 = 41 °C

Read plates.
Coliert -18 = 18 hours
TC = Yellow wells scored
EC= Yellow wells AND UV fluorescent scored
Enterolert-24 = 24 hours
ENT = UV fluorescent – score ANY wells fluorescing a blue color above the general background fluorescence. If you notice a diffuse glow across all wells, please note this (but do not score those wells). Green fluorescent wells are not scored.

Write the score on the back of each plate. On one plate per group note the time and date plate read and the person reading.

13.3.2 Molecular Biology

All molecular marker SOPs come directly from the SIPP Core Lab SOP repository. These are followed exactly and any deviations from these protocols must be strictly documented.

13.3.2.i Filtering Sample

Sterile disposable filter holders are loaded with 0.4um HTTP white filters using flame sterilized tweezers. 200 ml of sample is filtered and washed with <20ml of PBS. The filter is folded, placed in a screw-cap tube preloaded with acid washed beads. The tube is screwed shut and placed on ice and kept shaded. The time at which the tube is placed on ice is the end of the holding time. Once all filters are completed the tubes are transferred to a -80C freezer until needed for DNA extraction.

13.3.2.ii Extracting DNA

Use UCLA DNA Extraction Chain-of-Custody form.

Source : DNA EZ (RW03) extraction SOP for SIPP

Filename: DNA-EZ_extraction_SOP_for_SIPP_final.doc

EXCEPTIONS: The SPC spiked lysis buffer step is not done. All steps labeled as optional are done. Nanodropping is not mandatory.

Ordering:

GeneRite, LLC Phone number: 732-419-2488 Email: info@generite.com

Website: http://generite.com/

Pricing: Yiping worked out a deal with GeneRite (All reagents will be from the same lot)

Product Name: DNA-EZ ST1 (Part no: K200-01C-50) (individual components will also be available for purchase) Columns: DNAsure 30C columns S5111-30C-50

Beads: S0205-50 glass beads

Prep:

1. Spray and wipe bench surface with 10% bleach, leave for ~15min; spray and wipe with 70% ethanol to remove residual bleach
2. Wipe pipets with DNAway, gather clean racks and microtubes
3. Setup extraction tubes and label as necessary

Get racks and set up microtubes accordingly: (back to front)

1.5mL microtube (i.e. extract tube 1)

1.5mL microtube (i.e. extract tube 2)

Collection Tube

Collection Tube

Collection Tube

Collection Tube with spin column

1.5 or 2.0mL microtube (May be more comfortable to use a 2.0mL tube)

1.5mL microtube

Bead Tube

Make SPC-Lysis buffer

Unfreeze salmon and let thaw completely. Vortex thoroughly before use (after use store at 4°C).

Calculate salmon stock needed for lysis buffer:

#samples for extraction + 4extra = S

500uL(volume lysis buffer added to each sample) x S = Total Volume

(Total Volume x 0.2ng/uL) / 10ng/uL = ul Salmon Stock needed

Total Volume - Volume Salmon Stock = uL DNEasy lysis Buffer needed

Once SPC lysis buffer is made, vortex to mix, Let sit before use to reduce foaming from vortexing

Extraction for filters:

1) Add 500uL spc-lysis buffer to each filter tube (i.e. a filter placed into a tube preloaded with beads during filtration process). One should not transfer a frozen filter from one tube to another. Pipet buffer slowly, so excessive foaming is not a problem.
2) Tighten caps and bead beat samples at maximum setting on Bead Beater (biospec) for 2minutes.
3) Centrifuge tubes at 12,000 rcf for 1min.
4) Pipette maximum volume of supernatant out of the bead beating tube, and add to a new 1.5ml microfuge tube. It is useful to tilt the tube at an angle and avoid any type of pellet. There should be enough volume available that it is not a problem to retrieve this volume. *Throughout this extraction do not put tubes next to each other and only have one tube open at a time to minimize the chance of contamination.*
5) Centrifuge at 12000 rcf for 1min
6) Pipette out 350ul supernatant (take care not to disturb the pellet) and add it to 1000ul binding buffer in a new, labeled 1.5mL (or 2.0mL) microcentrifuge tube. **If 350uL is not a feasible number, please record the actual number added to the binding buffer.** Pipette up and down to mix and gently vortex. (Note: Everyone should be able to get 400uL out very easily, but if there are new lab techs I usually set up test samples for them ahead of time and let them practice pipeting the supernatant out). It is necessary to thoroughly mix the sample and binding buffer either by gently vortexing or flicking the tube. Also, after mixing the tube should be centrifuged for a couple seconds to get all the liquid off the cap and minimize risk of contamination. 1.5mL tubes should work fine for this but if people are new at extracting it is a good idea to use 2.0mL tubes.
7) Add 675uL of the DNA/binding buffer mixture (from step 6) to spin column and centrifuge for 1 minute at 10,000 rcf (discard flow through). *(Optional: change the collection tube to minimize chance of contamination)* Repeat step 7 once. Adding 700uL should be fine, but if inexperienced people are extracting it is better to start at a lower volume (600uL) and do this step an additional time. The collection tubes should be changed each time because contamination will become a problem. However, if collection tubes are not changed each time be sure to put kimwipes down and blot the tubes between each step.
8) Place the spin column in a new collection tube.
9) Add 500uL EZ wash buffer and centrifuge for 1 minute at 10,000rcf (discard flow through). *(Optional: change the collection tube to minimize chance of contamination)* Repeat step 9.
10) After the second wash, place spin column in another new collection tube and centrifuge at 10,000 rcf for 1min (to get rid of any residual ethanol in the wash buffer).
11) Put column into final 1.5mL microcentrifuge tube. Add 50uL warmed elution buffer (warmed to 60 deg C), let sit for 1 minute, centrifuge for 1 minute at 10,000 rcf (keep flow through). Repeat. Pipet directly into the middle of the column.

12) Vortex the final 100uL elution buffer/DNA solution and aliquot 50uL or less to other lo-bind microcentrifuge tubes to save for later analysis. How many aliquots will depend on how many assays will be run with that DNA.
13) Store extracted DNA in -20°C freezer.

*Nanodrop each sample and record both A260 ratios for each sample.

13.3.2.iii qPCR/PCR

13.3.2.iii.a HF183taqman qPCR Assay

Target: Human

Protocol: Standard Operating Procedure for HF183, HumM2, BuniF2, and BsteriF1, QPCR Assays

File : SOP_HF183_HumM2_BsteriF1_BuniF2.pdf

13.3.2.iii.b BacHum qPCR Assay

Target: Human

Protocol: SIPP QPCR SOP for Human Specific Marker in Bacteroidales ("Kildare method")

File : SIPP_SOP_BacHum_QPCR.pdf

13.3.2.iii.c Gull2Taqman qPCR Assay

Target: Gull

Protocol: SIPP SOP for QPCR Gull-2 assay

File : SIPP SOP Gull-2-QPCR.pdf

13.3.2.iii.d DogBact qPCR Assay

Target: Dog

Protocol: SIPP SOP for QPCR DogBact assay

File : SIPP SOP NOAA DogBact QPCR.doc

13.3.2.iii.e HoF597 PCR Assay

Target: Horse

Protocol: SIPP Conventional PCR SOP for horse-specific Bacteroidales marker

File : SIPP_SOP_convPCR_horse_specific_marker_bacteroidales.doc

13.4 NUTRIENT Testing Processes

Once back at the shed, remove all nutrient water samples from cooler and record cooler temperature on nutrient data sheet. Water samples should be at ambient temperature for testing. Fill 5 clean vials for each sample, 3 vials with 10 ml (one is the SAMPLE BLANK, one is for Ammonia-N, and one is for Phosphate) and 2 vials with 5 ml of sample water (one each for Nitrate-N and Nitrite-N). Line up sample vials from each site in front of the corresponding sample bottle, and organize samples in order from lagoon to upstream samples. The LaMotte SMART3 Colorimeter is used to test for nutrients and the LaMotte Turbidimeter 2020we is used to test for turbidity. The following tests are completed: Turbidity, Ammonia-Nitrogen High, Phosphates Low, Nitrates-N, and Nitrites-N. The instructions listed below are taken from the LaMotte SMART3 Colorimeter Operator's Manual (nutrients) and the LaMotte Turbidimeter 2020we Operator's Manual (turbidity). Procedures from the manuals have been modified slightly to reflect actual test procedures. Once testing is complete and all data is recorded onto the Nutrients data sheet, discard test samples into a bottle labeled Poison or Toxic, and discard water samples. Rinse all vials and sample bottles well with tap water and let dry.

13.4.1 Turbidity:

Calibrating the turbidimeter (every time it is used):

- Turn the turbidimeter ON
- Select Measure
- Select Turbidity – With Blank
- Fill one clean vial with 10 ml of the 0 NTU standard and one vial with 10 ml of the 1 NTU standard. Make sure there are no air bubbles in the standard sample and clean the vials with a lint free cloth (e.g., kimwipes).
- Place the 0 NTU standard into the turbidimeter and Select Scan Blank. Remove 0 NTU standard.
- Place the 1 NTU standard into the turbidimeter and Select Scan Sample.
- Scroll down and Select Calibrate. Use up or down arrows to calibrate reading to 1.0 and press Enter. Select Set Calibration. Remove standard.

Test turbidity at each site sample using the sample blank as the sample, and the 0 NTU standard as the blank. Record readings onto Nutrients data sheet.

13.4.2 Ammonia-Nitrogen High Range

PROCEDURE

1. Press and hold until colorimeter turns on.
2. Press to select **TESTING MENU.**
3. Scroll to and select **ALL TESTS** (or another sequence containing **005 Ammonia-N HR**) from **TESTING MENU.**
4. Scroll to and select **005 Ammonia-N HR** from menu.
5. Rinse a clean tube (0290) with sample water. Fill to the 10 mL line with Sample (SAMPLE BLANK).
6. Insert tube into chamber, close lid and select **SCAN BLANK.** (See Note)
7. To one of the 10 ml sample vials, Add 8 drops of Ammonia Nitrogen Reagent #1 (V-4797). Cap and mix. Wait 1 minute.
8. Use the 1.0 mL pipet (0354) to add 1.0 mL of *Ammonia Nitrogen Reagent #2 (V-4798). Cap and mix. Allow 5 minutes for maximum color development.
9. At end of the 5 minute waiting period, immediately mix, insert tube into chamber, close lid and select **SCAN SAMPLE.** Record result.
10. Press to turn the colorimeter off or press the exit to a previous menu or make another menu selection.

13.4.3 Phosphate-Low Range

PROCEDURE

1. Press and hold until colorimeter turns on.
2. Press to select **TESTING MENU.**
3. Select **ALL TESTS** (or another sequence containing **078 Phosphate LR**) from **TESTING MENU.**
4. Scroll to and select **078 Phosphate LR** from menu.
5. Rinse a clean tube (0290) with sample water. Fill to the 10 mL line with sample (SAMPLE BLANK).
6. Insert tube into chamber, close lid and select **SCAN BLANK.**
7. Remove tube from colorimeter. To a 10 mL sample vial, use 1.0 mL pipet (0354) to add 1.0 mL of *Phosphate Acid Reagent (V-6282). Cap and mix.
8. Use the 0.1 g spoon (0699) to add one measure of *Phosphate Reducing Reagent (V-6283). Cap and mix until powder dissolves. Wait 5 minutes for full color development. Solution will turn blue if phosphates are present.
9. At end of 5 minute waiting period, mix, insert tube into chamber, close lid and select **SCAN SAMPLE.** Record result.
10. Press to turn colorimeter off or press to exit to a previous menu or make another menu selection.

13.4.4 Nitrate-Nitrogen – Low Range

PROCEDURE

NOTE: Place Dispenser Cap (0692) on *Mixed Acid Reagent (V-6278). Save this cap for refill reagents.

1. Press and hold until colorimeter turns on.
2. Press to select **TESTING MENU.**
3. Select **ALL TESTS** (or another sequence containing **064 Nitrate-N LR**) from **TESTING MENU.**
4. Scroll to and select **064 Nitrate-N LR** from menu.
5. Rinse a clean tube (0290) with sample water. Fill to 10 mL line with sample (SAMPLE BLANK).
6. Insert tube into chamber, close lid and select **SCAN BLANK.**
7. Remove tube from colorimeter.
8. To a 5 ml sample vial, use the graduated cylinder or similar to measure 5 mL of *Mixed Acid Reagent
(V-6278) and add to tube. Cap and mix. Wait 2 minutes before proceeding to Step 10.
9. Use the 0.1 g spoon (0699) to add two measures of *Nitrate Reducing Reagent (V-6279). Cap.
10. Hold tube by index finger and thumb and mix by inverting approximately 60 times a minute for four minutes. Wait 10 minutes for maximum color development.

NOTE: At end of waiting period an undissolved portion of Nitrate Reducing
Reagent may remain in bottom of the tube without affecting results.

11. At the end of the 10 minute waiting period, mix, insert tube into chamber, close lid and select **SCAN SAMPLE**. Record result.
12. Press to turn colorimeter off or press EXIT to exit to a previous menu or make another menu selection.

NOTE: For best possible results, a reagent blank should be determined to account for any contribution to the test result by the reagent system. To determine the reagent blank, follow the above test procedure to scan a distilled or deionized water blank. Then follow the above procedure to perform the test on a distilled or deionized water sample. This test result is the reagent blank. Subtract the reagent blank from all subsequent test results of unknown samples. It is necessary to determine the reagent blank only when a new lot number of reagents are obtained.

To convert Nitrate Nitrogen (NO3–N) results to ppm Nitrate (NO3 –), multiply by 4.4.

13.4.5 Nitrite-Nitrogen – Low Range

PROCEDURE

NOTE: Place Dispenser Cap (0692) on *Mixed Acid Reagent (V-6278). Save this cap for refill reagents.

1. Press and hold until colorimeter turns on.
2. Press to select **TESTING MENU.**
3. Select **ALL TESTS** (or another sequence containing **067 Nitrite-N LR**) from **TESTING MENU.**
4. Scroll to and select **067 Nitrite-N LR** from menu.
5. Rinse a clean tube (0290) with sample water. Fill to the 10 mL line with

Sample (SAMPLE BLANK).
6. Insert tube into chamber, close lid and select **SCAN BLANK**.
7. Remove tube from colorimeter.
8. To the 5ml sample vial, use graduated cylinder or similar to measure 5 mL of *Mixed Acid Reagent
(V-6278) and add to tube. Cap and mix.
9. Use the 0.1 g spoon (0699) to add two measures of *Color Developing Reagent (V-6281). Cap and mix by gently inverting for 1 minute. Wait 5 minutes for maximum color development.
10. At the end of the 5 minute waiting period, mix, insert tube into chamber, close lid and select **SCAN SAMPLE**. Record result.
11. Press to turn colorimeter off or press button to exit to a previous menu or make another menu selection.
NOTE: To convert nitrite-nitrogen (NO2–N) results to ppm nitrite (NO2–), multiply results by 3.3.

14. Quality Control Requirements

14.1 Field QC checks

14.1.A In-situ Measurements

All probes are calibrated the day before sampling. Data sheets are used to ensure all necessary information is collected. If any parameter appears off, second or third measurements are taken in the field.

14.1.B Sample Collection

Samples are collected before direct sunlight hits the water. Field blanks are collected and then measured in the lab to ensure that samples remain cool throughout transport. Chain-of-Custody sheets are used to ensure all samples are handled correctly and tracked.

14.2 Laboratory QC checks

14.2.A Nutrient Processing

Samples are organized by site and nutrient test. Field blanks are prepared for each site and used to calibrate the Smart 3 colorimeter. Testing for each parameter is done in accordance with appropriate protocol.

14.2.B FIB/Molecular Processing

General microbiology sterile technique is used throughout the FIB/Molecular processing. Sample bottles are mixed by inversion before any subsampling occurs. When not in use, bottles are placed back on ice.

14.2.C Molecular Filtering/Extracting/Analysis

Filter blanks are created during the molecular filtering process to test for any contamination.

Extraction blanks are created during the DNA extraction process to ensure that no contamination occurs during this process. Three non-spiked blanks are run in for every PCR/qPCR 96 well plate assay.

To ensure that the DNA extraction process is working we occasionally spike a blank sample with a known amount of previously extracted DNA. This spiked extraction can then be analyzed with PCR/qPCR and the extraction efficiency estimated. Spiked samples are typically used every third or fourth extraction event.

qPCR amplification efficiency is measured by creating a standard curve with known amounts of DNA diluted 1:10 over 5 orders of magnitude. The assay's performance is considered acceptable if the amplification efficiency falls between 90 and 100%. Once a good standard curve is produced, a single point standard is used in triplicate on every 96 well plate in substitution for the standard curve. The run is considered acceptable if the single point Cq value is within ±1 cycle from the expected value.

14.3 Data Analysis QC checks

14.3.1 In situ Data

Results are compared to previous readings on a per site basis. Any site showing an extreme change in typical values will be revisited within two days and re-measured. Data entered into spreadsheet is double checked against values written on the hard copy data sheets.

14.3.2 Nutrient Data

Results are compared to previous readings on a per site basis. Any site showing an extreme change in typical values will be revisited within two days and re-measured. Data entered into spreadsheet is double checked against values written on the hard copy data sheets.

14.3.3 FIB Data

Results are compared to previous readings on a per site basis. Any site showing an extreme change in typical values will be revisited within two days and re-measured. Data entered into spreadsheet is double checked against values written on the IDEXX trays.

14.3.4 Molecular Marker

The qPCR molecular marker analysis is so new that QC checks are still currently being developed. Sites are run in triplicate and any "outlier" data will be rerun.

15. Instrument / Equipment Testing, Inspection , and Maintenance Requirements

Equipment Type	Inspection Frequency	Type of Inspection
Con6 Conductivity Probe (UCLA)	every sample event	Calibration performance
IDEXX Tray Sealer	each plate	confirm plate is sealed properly
ABI StepOnePlus qPCR	Annual	RnaseP performance verification

15.1 YSI 55 DO Meter

Service and Repair: Equipco in Concord, CA ($220 labor + parts); YSI in Ohio ($125 labor + parts)
YSI Customer Service: 1-800-765-4974 or 1-937-767-7241

Storage: Make sure storage sponge is clean and moist. Can use distilled, deionized, or tap water.

Maintenance

- Replace membrane and KCl solution in probe. There should not be any air bubbles present underneath the membrane.
- Frequency - every 2 - 4 weeks or as needed.

To check probe accuracy (as needed):

1) Check that all membranes are okay, no air bubbles
2) Calibrate all using tap water at zero altitude
3) Take readings while in storage chamber – if testing several probes, they should all be within specs of one another (+/- 2% saturation or +/- 0.3 mg/L)
4) Once readings have stabilized with probe in chamber, remove probe from chamber and place in a bucket of water
5) Determine if any are reading temperature accurately by comparing to 2 thermometers in same bucket of water (David from YSI said if they are reading temperature accurately, they should be reading DO within specs)
6) If probes are not accurate or within specs, clean the cathodes and anodes of those probes
7) Once all are cleaned and ready, complete step 1 through 6 again

Other notes about probe accuracy:

- Should be able to hold calibration readings for at least a day (if not, something is wrong)
- When testing, make sure all equipment is calibrated using correct altitude and salinity readings
- Routinely check that cathodes and anodes are clean (pg. 7 manual) for best results

Testing

1) Make sure that the silver part of the probe is submerged in the test water

2) Gently swirl the probe in the water until the DO reading stabilizes
3) Once reading has stabilized, Record the DO reading in both % saturation and mg/L (use the MODE button to switch from % to mg/L)

15.2 Other Meters

15.2.1 Salinity - Analog REFRACTOMETER

Storage: Before and after each use, the refractometer should be thoroughly cleaned with deionized or distilled water and dried. Refractometer should always be dry when stored.

Maintenance

- Clean with deionized or distilled water as needed. Make sure there are no cracks or scratches.
- Frequency - every time you use the refractometer.

Testing

1) Add water sample to prism surface (make sure to thoroughly rinse hands in test water first, use gloves, or a pipette so to reduce contamination from hands)
2) Close the cover, making sure there are no air bubbles
3) Record the number where the "contrast" line is located using the scale on the right side (0-100, ppt)

15.2.2 pH - OAKTON pHTESTR 2

Oakton Customer Service: 1-888-4OAKTON (1-888-462-5866)

Storage

- NEVER store a pH probe in deionized or distilled water. It will leach out the solution in the probe and the probe will have to be replaced. When finished using it for the day, rinse with tap water or buffer pH 4.00. Never store fully submerged in water. Should be stored long-term in buffer pH 4.00.
- If possible, place a small sponge or piece of paper in the bottom of the storage cap and moisten with clean tap water or electrode storage solution for long-term storage.

Maintenance

- Clean the probe with tap water or electrode storage solution after each measurement.
- Frequency - every time you use the pHTestr2.
- Periodic soaks in warm pH 4 buffer to help remove potential contaminants.

Testing

- Remove cap from electrode and press ON/OFF button to turn Testr ON
- Dip electrode 1/2" to 1" into test water. Stir once and let reading stabilize.
- Record pH reading. You can press HOLD/CON button to freeze the reading, and press it again to release the reading.

- Press ON/OFF button to turn Testr OFF
- Rinse with clean tap water

15.2.3 pH - OAKTON pHTESTR 30

Oakton Customer Service: 1-888-4OAKTON (1-888-462-5866)

Storage

- NEVER store a pH probe in deionized or distilled water. It will leach out the solution in the probe and the probe will have to be replaced. When finished using it for the day, rinse with tap water or buffer pH 4.00. Never store fully submerged in water. Should be stored long-term in buffer pH 4.00.
- If possible, place a small sponge or piece of paper in the bottom of the storage cap and moisten with clean tap water or electrode storage solution for long-term storage.

Maintenance

- Clean the probe with tap water or electrode storage solution after each measurement.
- Frequency - every time you use the pHTestr2.
- Periodic soaks in warm pH 4 buffer to help remove potential contaminants.

Testing

- Remove cap from electrode and press ON/OFF button to turn Testr ON
- Dip electrode 1/2" to 1" into test water. Stir once and let reading stabilize.
- Record pH reading. You can press HOLD/CON button to freeze the reading, and press it again to release the reading.
- Press ON/OFF button to turn Testr OFF
- Rinse with clean tap water

15.2.4 Conductivity - OAKTON ECTESTR

Oakton Customer Service: 1-888-4OAKTON (1-888-462-5866)

Storage

- Can be stored in a humid environment. If you experience drift readings, periodically let electrode fully dry.

Maintenance

- Periodically clean the electrodes by rinsing them in alcohol for 10-15 minutes. Remove white plastic cup insert to clean viscous solutions.
- Frequency - as needed; periodically

Testing

- Remove cap from electrode and press ON/OFF button to turn Testr ON. Do not remove white plastic cup insert.
- Take reading either by dip-style or cup-style
- Dip electrode into test solution, making sure the electrode is fully covered.

- Wait for reading to stabilize
- Press HOLD key to freeze reading. Press HOLD again to release reading.
- Record value
- Press ON/OFF button to turn Testr OFF
- Rinse with deionized water, replace Cap

15.2.5 Conductivity - OAKTON ECTESTR+

Oakton Customer Service: 1-888-4OAKTON (1-888-462-5866)

Storage

- Can be stored in a humid environment. If you experience drift readings, periodically let electrode fully dry.

Maintenance

- Periodically clean the electrodes by rinsing them in alcohol for 10-15 minutes. Remove white plastic cup insert to clean viscous solutions.
- Frequency - as needed; periodically

Testing

- Remove cap from electrode and press ON/OFF button to turn Testr ON. Do not remove white plastic cup insert.
- Take reading either by dip-style or cup-style
- Dip-style: dip electrode into test solution, making sure the electrode is fully covered.
- Cup-style: fill electrode cup with sample of test solution.
- Wait for reading to stabilize
- Press HOLD key to freeze reading. Press HOLD again to release reading.
- Record value.
- Press ON/OFF button to turn Testr OFF
- Rinse with deionized water, replace Cap

15.2.6 FLOW METER – Marsh-McBirney Flo-Mate 2000

Maintenance

- Routine maintenance involves cleaning the sensor with soap and water (if problem persists, clean the electrodes with very fine grit sandpaper) and changing the batteries.

16. Instrument Calibration and Frequency

All in-situ probes are calibrated using industry standard calibration solutions and procedures within 24 hours of use.

16.1 YSI 55 DO Meter

Calibration

- Frequency - every time you use the YSI 55 DO Meter

1) Turn ON
2) Press the up and down arrows at the same time
3) Enter the altitude of the study site using the up and down arrows, press Enter
4) Let the DO reading stabilize
5) Once the DO reading has stabilized, press Enter
6) Enter the salinity of the water at the study site using the up and down arrows, press Enter
7) You are now ready to test the dissolved oxygen level of the water at your study site.

16.2 Salinity - Analog REFRACTOMETER

Calibration

- Frequency - every time you use the refractometer.

1) Add a few drops of deionized or distilled water to the prism surface
2) Close the cover, making sure there are no bubbles on the surface
3) Hold it up towards the light, and look through the eyepiece (can adjust the eyepiece to focus)
4) The white on the bottom of the screen should meet the blue on the top of the screen (the "contrast" line) at zero
5) If it is not directly at zero, use the small screw on the top of the refractometer to adjust the reading to zero

16.3 pH - OAKTON pHTESTR 2

Calibration (2 or 3 points; pH 4,7, 10)

- Frequency - every time you use the pHTestr2
- Condition the Testr first, by submersing it in tap water or electrode storage solution for 30 minutes before use.

1) Press ON/OFF button to turn ON.
2) Dip electrode 1/2" to 1" into chosen buffer (pH 4,7 or 10)
3) Press CAL button - you are now in Calibrate Mode; 'CA' flashes on the display
4) A pH value close to the pH buffer value will flash repeatedly on the screen
5) After at least 30 seconds, press the HOLD/CON button to confirm calibration; 'CO' will display and then switch to the buffer value reading

6) Repeat with other buffers if necessary
- Rinse electrode in clean tap water before dipping into next buffer

16.4 pH - OAKTON pHTESTR 30

Calibration (2 or 3 points; pH 4,7, 10)

- Frequency - every time you use the pHTestr
- Condition the Testr first, by submersing it in tap water or electrode storage solution for 30 minutes before use.

1) Press ON/OFF button to turn ON.
2) Dip electrode 1/2" to 1" into chosen buffer (pH 4,7 or 10)
3) Press CAL button - you are now in Calibrate Mode; 'CAL' indicator will appear on the display (upper value is the measured reading based on the last calibration, lower value indicates pH buffer solution value)
4) Allow about 2 minutes for the Testr reading to stabilize.
5) Press HOLD/ENT button to confirm the first calibration point (upper value should now be buffer solution value, and lower value is toggling in between readings of the next PH standard buffer solution)
6) Repeat with other buffers if necessary
- Rinse electrode in clean tap water before dipping into next buffer

16.5 Conductivity - OAKTON ECTESTR

Calibration (use method used in testing - cup style or dip style)

- Frequency - every time you use the ECTestr
- Condition the Testr first, by submersing it in alcohol for a few minutes to remove oils.

1) Open battery compartment lid. The two small white keys are Increment (INC) and Decrement (DEC) calibration keys.
2) Rinse electrode in deionized water, then rinse it in calibration standard, then dip it into a container of calibration standard.
3) Press ON/OFF button to turn ON.
4) Wait several minutes for display to stabilize
5) Press the INC/DEC keys to adjust reading to match the calibration standard value.
6) After 3 seconds without a key press, the display will flash 3 times, then show "ENT." The Testr accepts the calibration value and returns to measurement mode.
7) Replace battery cap

16.6 Conductivity - OAKTON ECTESTR+

Calibration (use method used in testing - cup style or dip style)

- Frequency - every time you use the ECTestr

- Condition the Testr first, by submersing it in alcohol for a few minutes to remove oils.
1) Open battery compartment lid. The two small white keys are Increment (INC) and Decrement (DEC) calibration keys.
2) Rinse electrode in deionized water, then rinse it in calibration standard, then dip it into a container of calibration standard.
3) Press ON/OFF button to turn ON.
4) Press MODE to select Conductivity or Temperature (C)
5) Wait several minutes for display to stabilize
6) Press the INC/DEC keys to adjust reading to match the calibration standard value.
7) After 3 seconds without a key press, the display will flash 3 times, then show "ENT." The Testr accepts the calibration value and returns to measurement mode.
8) Replace battery cap

17. Inspection and Acceptance Requirements for Supplies

Supplies and reagents have specified expiration dates. Upon receipt of any new reagents or supplies, the use by date is noted, and the date received is written on the box. All bottles are checked to ensure that they are properly sealed. Reagents for nutrient testing are used up chronologically to avoid exceeding the expiration date.

18. Data Acquisition Requirements

Data is recorded in the field on hard copy sheets. Prior to leaving a field site, the data collector reviews the sheet to make sure all entries are recorded and nothing is missing.

Nutrient data is also initially logged on to hard copy data sheets, with the same requirement that all fields are filled and no data is missing.

Entries noted on the hard copies are then transcribed into an electronic database. To ensure correct and complete entry, one person reads the data from the hard copy, another person types it in and reads it back to make sure that all is correctly transcribed.

An additional review is conducted to look for missing or irregular data entries, check for outliers and perform initial graphing to check for irregularities. Any irregularities will be checked against the initial field data to identify if it was a mistake from some level of data contamination (faulty instruments, calibration errors, sample contamination or mis-labeling, holding time exceedance, etc.)

19. Data Management

The success of a monitoring project relies on data and their interpretation. It is critical that these data are:

- Of known quality,
- Reliable,
- Aggregated in a manner consistent with their prime use, and
- Accessible to TAC members and other interested parties.

Data Management includes documenting calibration, accurate and complete field notebook entries, completed Chain-of-Custody forms and laboratory data management documents.

Initially, the RCDSMM is the central data repository for all in-situ measurements, nutrient sample results and FIB results. Data is compiled in two excel spreadsheets, one by each individual site, and the other by event.

The FIB and marker data are entered and managed by the UCLA team on a separate excel workbook.

An ACCESS database is under construction and will become the complete repository for all data.

Metadata is being compiled as data is collected.

Data files will be stored on a secure computer and backed up by a remote removable hard drive. Project records and data will be retained by the RCDSMM for a minimum of five years.

20. Assessments and Response Actions

Field Assessments

RCDSMM and/or UCLA may perform a field sampling audit on randomly chosen sampling events during the season in order to evaluate the performance of the samplers. Follow-up field audits may be necessary pending audit findings. Audits will concentrate on sampling technique, sample handling, field records, field testing methods, and adherence to specific sampling protocols. These reports will identify corrective actions, if necessary.

Laboratory Assessments

Laboratories are subject to periodic and extensive audits as part of their certification. Reports of these audits will be made available to the Project Manager/Principal Investigator.

Replicates

One blind conventional and priority replicate sample will be collected each monitoring season. The purpose of the blind sample replicate is to assess sampling and laboratory error and to assess overall method variability. Duplicates will be analyzed for FIB and nutrients. Precision between the primary sample and its replicate will be determined by calculating the relative percent difference between the two samples, in the same way that precision is measured between two laboratory-fortified blanks or a matrix spike/matrix spike duplicate. The use of replicate samples extends the test of precision to the sampling method itself. The samples will be analyzed by the same lab for the same parameters. Results of the replicate analysis will be monitored by the Project Manager/Principal Investigator.

Corrective Action

The laboratory or sampling manager will notify the Project Manager/Principal Investigator if errors are noted by the laboratory or sampling personnel. The responsible party will then immediately correct the problem and will send those corrections via email to the Project Manager/Principal Investigator.

REVISIONS TO QA/QC Plan

The QA/QC Plan will be reviewed and revised as needed.

21. Reports

Monthly reports are prepared by the RCDSMM summarizing data collection, results and activities and provided to all TAC members.
Summary reports are provided to the Technical Advisory Committee twice/year.
Yearly reports are prepared at the end of each water year (October 31).

22. Data Review, Validation and Verification Requirements

Data review is the process that evaluates the overall data produced to ensure that procedures were correctly followed and that the reported data is reasonable and consistent with QA/QC results.

Data verification documents that applicable method, procedural and contractual requirements were met in field sampling and laboratory analysis. Verification checks to see if the data were complete, if sampling and analysis matched QA/QC requirements, and if Standard Operating Procedures (SOPs) were followed. Verification of data is the responsibility of the Project Manager/Principal Investigator, who should verify at least 10% of generated project data.

Data validation determines whether the data sets meet the requirements of the project-specific intended use as described in the QAPP. That is, were the data results of the right type,

quality, and quantity to support their intended use? Data validation also attempts to give reasons for sampling and analysis anomalies, and the effect that these anomalies have on the overall value of the data.

23. Reconciliation with Data Quality Objectives

The Project Manager /Principal Investigator will review and validate data against the Project's defined objectives and standard operating procedures at least annually, and prior to preparing the final report. If there are any problems with sampling and analysis, these issues will be addressed immediately and methods will be modified to ensure that data quality objectives are being met. Modifications to monitoring will require edits to the approved QA/QC Plan.

Only data that have been validated and qualified, as necessary, shall be entered into the applicable database.

APPENDIX A

DATA SHEETS AND CHAIN OF CUSTODY FORMS

TOPANGA SOURCE ID WATER QUALITY EQUIPMENT CALIBRATION SHEET

Calibration Date_____ Calibrators_____
Sampling Date_____

YSI DO Meter 55 Meter # _____ Time: Membrane changed? Yes no
% Saturation at 0 alititude, 0 salinity (probe in chamber)_____

Meter	Meter #	Time	Standard Value	Initial reading	Meter adjusted to
PH 4			4.01		
PH 7 (required)			7.0		
PH 10			10.01		
Refractometer			0		
Conductivity			1413		

NOTES:

YSI DO Meter 55 Meter # _____ Time: Membrane changed? Yes no
% Saturation at 0 alititude, 0 salinity (probe in chamber)_____

Meter	Meter #	Time	Standard Value	Initial reading	Meter adjusted to
PH 4			4.01		
PH 7 (required)			7.0		
PH 10			10.01		
Refractometer			0		
Conductivity			1413		

NOTES:

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TOPANGA SOURCE ID GEAR STAGING CHECKLIST

- Pink tote with:
  - conductivity meter
  - pH meter
  - refractometer
  - DO meter
  - thermometer
  - camera
  - gps
  - gloves
  - rinse bottle with DI or distilled water
  - clipboard for data sheets
  - pencils
  - flashlight

- Green tote with:
  - conductivity meter
  - pH meter
  - refractometer
  - DO meter
  - thermometer
  - camera
  - gps
  - gloves
  - rinse bottle with DI or distilled water
  - clipboard for data sheets
  - pencils
  - flashlight

- Cooler with thermometer
- Nutrient sample bottles (TL, SP, BR, ST, OF, plus any additional)
- 2 meter sticks
- 2 flow meters (including metal rod for one of them)
- Waders
- Ice
- Sampling pole
- Tide book
- Bug supplies, if applicable (D-frame nets, labelled jars, seive)
- Data sheets
  - RCD sites data sheet
  - UCLA sites data sheet
  - Flow data sheets
  - Nutrient Chain of Custody data sheet

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RCD SITES TOPANGA DATA SHEET

Topanga Creek Source ID Study 2012-2014

Observers:_____ **Date:**_____ **TAKE PHOTO OF EACH SITE!**
Moon phase: New 1st Half 3rd Full **Date of last rain:**_____
SUNRISE Time_____

Lagoon Entrance condition: Open Closed Overwash **Tidal stage:**_____ **Tide board info:** hi tide____ ht____ Low tide____ ht____
FLOW AT LAGOON OUTLET: none trickle garden hose fire hose

WEATHER CONDITIONS: Clear Overcast Foggy Showers Rain **WIND speed**____**Direction**____

BIRDS OBSERVED: in lagoon on beach berm in swash zone **Estimate total number:** 1-10 10-25 25-50 50-100 100+
BIRD TYPES: Gulls Willets Coots Night Herons Ducks Egrets Other:

Sample LABEL:	**LO**	**TL**	**SP**	**BR**	**TB**
Parameter	Lagoon outlet is variable and sample collection should be at point just before lagoon water hits the ocean	Lagoon on the east bank by white wall	Snake Pit – hike behind Ranch motel to sycamore tree trail (0' elevation)	Brookside Dr or Rodeo Grounds (0' elevation)	TBridge MM 2.02 ds of bridge (200' elevation)
LIGHT CONDITION					
Photo taken?					
Depth at station (cm)					
Nutrient sample	Time:	Time:	Time:	Time:	Time:
UCLA Bacteria sample	Time :	Time :	Time:	Time:	Time:
Sampling Tool used	grab	grab	grab	grab	grab
Salinity (ppt)					
Water Temp. (°C)					
Dissolved Oxygen (mg/L)					
DO (%sat)					
Conductivity (ms or µs)					
pH					
Air Temp. (°C)					

Time first sample added:_____ **Ice chest temp:** ____ **Time last sample added:**_____ **Ice chest temp:** _____

RESOURCE CONSERVATION DISTRICT OF THE SANTA MONICA MOUNTAINS

RCD SITES TOPANGA DATA SHEET

Sample LABEL:	LO	TL	SP	BR	TB
OBSERVATIONS	Lagoon outlet is variable and sample collection should be at point just before lagoon water hits the ocean	Lagoon on the east bank by white wall	Snake Pit – hike behind Ranch motel to sycamore tree trail (0' elevation)	Brookside Dr or Rodeo Grounds (0' elevation)	TBridge MM 2.02 ds of bridge (200' elevation)
Transient activity					
Human feces observed					
Other feces observed					
Water clarity					
Water color					
Water odor					
Water surface					
Algae density					
Algae Type					
Vegetation in channel?					
Vegetation type					
Debris/Trash density					
Trash type					
BMI SAMPLE? (Y/N)					

NOTES:

Use the following descriptions:
Transient activity: campsite active campsite inactive people present
Human feces observed : yes no
Other feces observed: yes no
Water Clarity: Clear Cloudy Milky Muddy OTHER
Water Color: Clear Red Yellow Green Brown Gray Other
Water Odor: None Rotten eggs Sewage Chlorine Ammonia Musty Other
Water Surface: None Oily sheen Garbage Sewage Foam Biological Floatables Other
(Biological floatables include leaves, sticks, algae, duckweed, pollen, mosquito larvae, etc)
Algae density: % cover of sampling area
Algae Type: Bull kelp Macrocystis Kelp Surf grass Eel grass Other
Debris/Trash density: None Light (0-10 items) Moderate (10-50 items) High (50+ items)
Trash type: bottles cans plastics paint cans other

I reviewed this data for accuracy (initials):_____ **Date:**_____
Data entered: Initials_____ **Date**_____ **Comments:**

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UCLA SITES TOPANGA DATA SHEET

Topanga Creek Source ID Study 2012-2014

Observers:_____ **Date:**_____ **TAKE PHOTO OF EACH SITE!**

Sample LABEL:	**BU**	**BO**	**LG**	**HB**	**ST**	**OF**
Parameter	Beach on west end just past the metal groin in front of blue house steps	Beach Outlet – in ocean knee deep towards east side of lagoon	Ocean in front of Lifegaurd station	North side of PCH bridge at 6th stanchion	Scratchy Trail - hike down to creek ds to sycalmore-alder-oak (500' elevation)	Owl Falls – end of driveway hike ds to below the confluence 2 m (700'elevation)
LIGHT CONDITION						
Photo taken?						
Depth at station (cm)						
Nutrient sample					Time:	Time:
UCLA Bacteria sample	Time:	Time:	Time:	Time:	Time:	Time:
Sampling Tool used	grab	grab-	grab	bridge	grab	grab
Salinity (ppt)						
Water Temp. (°C)						
Dissolved Oxygen (mg/L)						
DO (% sat)						
Conductivity (ms or µs)						
pH						
Air Temp. (°C)						

Time first sample added:_____ **Ice chest temp:** ____ **Time last sample added:**_____ **Ice chest temp:** ____

PLEASE TAKE SITE PHOTO & COMPLETE SITE CONDITIONS DATA ON BACK → ☺

RESOURCE CONSERVATION DISTRICT OF THE SANTA MONICA MOUNTAINS

UCLA SITES TOPANGA DATA SHEET

Sample LABEL:	BU	BO	LG	HB	ST	OF
OBSERVATIONS	Beach on west end just past the metal groin in front of blue house steps	Beach Outlet – in ocean knee deep towards east side of lagoon	Ocean in front of Lifegaurd station	North side of PCH bridge at 6th stanchion	Scratchy Trail - hike down to creek ds to sycalmore-alder-oak (500' elev.)	Owl Falls – end of driveway hike ds to below the confluence 2 m (700'elevation)
Transient activity						
Human feces observed						
Other feces observed						
Water clarity						
Water color						
Water odor						
Water surface						
Algae density						
Algae Type						
Vegetation in channel?						
Vegetation type						
Debris/Trash density						
Trash type						
BMI SAMPLE? (Y/N)						

NOTES:

Use the following descriptions:
Transient activity: campsite active campsite inactive people present
Human feces observed : yes no
Other feces observed: yes no
Water Clarity: Clear Cloudy Milky Muddy OTHER
Water Color: Clear Red Yellow Green Brown Gray Other
Water Odor: None Rotten eggs Sewage Chlorine Ammonia Musty Other
Water Surface: None Oily sheen Garbage Sewage Foam Biological Floatables Other
(Biological floatables include leaves, sticks, algae, duckweed, pollen, mosquito larvae, etc)
Algae density: % cover of sampling area
Algae Type: Bull kelp Macrocystis Kelp Surf grass Eel grass Other
Debris/Trash density: None Light (0-10 items) Moderate (10-50 items) High (50+ items)
Trash type: bottles cans plastics paint cans other

I reviewed this data for accuracy (initials):_____ Date:_____
Data entered: Initials_____ Date_____ Comments:

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FLOW DATA SHEET

Topanga Creek Source ID Study 2012-2014

Observers:_____ **Date:**_____ **TIME:**_____

Instructions: 0m is always on the west side of the channel (left side loooking upstream!) Make sure tape is tightly pulled from one edge of the water to the other. Stand on the downstream side of the tape looking upstream and start at 0m. Stand very still to avoid disturbing the water and changing flow reading.
On the upstream side of the tape, measure depth then place flow head facing directly upstream 1/3 up from bottom. Note if algae present at regular intervals and at the random number interval (see instructions below). Note type.

SITE (circle one): TB BR SP ST OF **Wetted width =** _____ **m**

Distance (m)	Depth (in)	Flow (Ft/s)	Algae (Yes/No)	RPC (m)	Algae type or other (diatoms?) see back
0				0.16	
0.25				0.39	
0.5				0.58	
0.75				0.99	
1				1.09	
1.25				1.28	
1.5				1.68	
1.75				1.78	
2				2.06	
2.25				2.29	
2.5				2.57	
2.75				2.97	
3				3.05	
3.25				3.38	
3.5				3.63	
3.75				3.87	
4				4.20	
4.25				4.43	
4.5				4.74	
4.75				4.76	
5				5.14	
5.25				5.39	
5.5				5.59	
5.75				5.96	
6				6.19	
6.25				6.48	
6.5				6.51	
6.75				6.99	
7				7.17	
7.25				7.37	
7.5				7.69	
7.75				7.90	
8				8.18	

Flow Data Sheet

SAMPLING LOCATIONS FOR FLOW:

TB Topanga Bridge MM2.02 - Measure flow on upstream side of bridge.

SF Shale Falls - Scramble down bank and cross the creek going downstream of the falls. Sampling location at base of the barrier.

RG Rodeo Grounds - Hike down the dirt road to the creek and sample at the downstream edge of the road.

SP Snake Pit - Hike behind the Ranch Motel and follow trail to the sycammore tree that leads to the creek. Sample at base of trail.

Instructions for RPC (Random Point Contact) Algae Survey:
To be completed every Wednesday sampling event
Complete after collecting flow data using the transect tape that was used for flow

1. Using a pre-generated list of random numbers, the lead will fill in the RPC locations for each sampling event.

2. Cover type data will be collected at each RPC location within every 0.25 m segment along the transect tape (i.e., one data point for 0-0.25m segment, one data point for 0.25-0.5m segment, etc.). The total number of data points will depend on the wetted width of the location (i.e., if the width is 2 m, there will be a total of 8 data points for algae for that location).

3. Find your random point on the trasect tape and record the type of algae found directly underneath that point on the tape. If there is no algae in that spot, record what is found there (see list of "other possibililities").

4. You can calculate percent cover of algae by dividing the number of times an algal species was observed by the total number of data points. For example, if you had 8 total data points and you observed *Enteromorpha* at 2 of those points, the percent cover of *Enteromorpha* would be 2/8 or 25%.

Common algae and aquatic plants:
Enteromorpha
Azolla
Duckweed
Chara
Hair Algae
Mat Diatoms
Spyrogyra
Cladophora
Rhizoclonium

Other possibilities:
Mint
Watercress
Arundo
Grass
Silt
Sand
Gravel
Cobble
Boulder
Bedrock

CHAIN OF CUSTODY – NUTRIENTS (RCD)

TESTERS: _____ **DATE**_____

Sample LABEL:

Time sample collected										
Depth at station (cm)										
Nitrate-N (ppm)										
Nitrate-N Reagant ID/ppm										
Nitrite-N (ppm)										
Nitrite-N Reagant ID/ppm										
Ammonia-N (ppm)										
Ammonia-N Reagant ID/ppm										
Phosphate (ppm)										
Phophate Reagant ID/ppm										
Turbidity (NTU)										

Time first sample tested: _____ **Ice chest temp:** _____

Nutrient data checked and entered by: _____ **Date:** _____

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UCLA ENGINEERING
Civil and Environmental Engineering
Engineering Sustainable Infrastructure for the Future

323-698-6898, Jay Lab, Boelter Hall 7678
Department of Civil & Environmental Engineering
University of California, Los Angeles
Los Angeles, CA 90095

CHAIN OF CUSTODY DATA SHEET UCLA

DATE OF SAMPLING EVENT:_____

Parameter	BU			BO			LO			TL			HB		
Samp. collector, # bot.															
Time of sampling															
Time received UCLA															
Person receiving															
Temp of field blank															
	TC:EC	ENT	qPCR	TC:EC	ENT	qPCR	TC:EC	ENT	qPCR	TC:EC	ENT	qPCR	TC:EC	ENT	qPCR
Person(s) processing															
Dilution/Volume filt.															
Time in incub/freezer															
Inc./Freezer Temp.															
Date/time read results															
Person reading															
Date/time data entered															
Person entering data															
SUPERVISOR REV.															

Parameter	SP			BR or RG			TB			ST			OF		
Sample collector															
Time of sampling															
Time received UCLA															
Person receiving															
Temp of field blank															
	TC:EC	ENT	qPCR	TC:EC	ENT	qPCR	TC:EC	ENT	qPCR	TC:EC	ENT	qPCR	TC:EC	ENT	qPCR
Person(s) processing															
Dilution/Volume filt.															
Time in incub/freezer															
Inc./Freezer Temp.															
Date/time read results															
Person reading															
Date/time data entered															
Person entering data															
SUPERVISOR REV.															

Initial	Printed Name

CHAIN OF CUSTODY DATA SHEET TOPANGA LIFEGUARD STATION DATE OF SAMPLING EVENT:_____

BIOSOLUTIONS LAB TESTS Collected by:_____

Time sample collected:_____Time samples left for UCLA/RCD:_____ Time samples handed off to PatChem:___

UCLA SAMPLE Received by: TIME: PAT CHEM Samples Received by: Time:

Sample collector									BOD			
Time of sampling									TSS			
Temp of ice chest									Alkalinity			
Time received UCLA									pH			
Person receiving									Alkalinity			
Temp of field blank									DO			
	TC:EC	ENT	qPCR						Turbidity			
Person(s) processing												
Dilution/Volume filt.												
Time in incub/freezer												
Inc./Freezer Temp.												
Date/time read results												
Person reading												
Date/time data entered												
Person entering data												
SUPERVISOR REV.												

RCD Samples Received by: **Time received:**

Sample LABEL	
Time of sampling	
Temp of ice/samples	cm
Nutrient Sample	Bottle #
Nitrates as Nitrogen	ppm
Nitrites	ppm
Ammonia -Nitrogen	ppm
Phosphates	ppm
Turbidity	NTU

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DRIFT NET DATA SHEET

DATE nets set: _____ Time nets set:_____ Setters:_____

Location nets set:_____
GPS coordinates: N 34._____ W=118. _____
WQ data

TIME	Air T	Water T	Salinity	DO	pH	Conductivity	Avg depth

Habitat characteristics: Habitat type:_____ length_____ m

Substrate	%canopy/type	%instream cover/type	% algae/type	%shading by canyon walls

Flow data – wetted width= OR time needed to fill a graduated cylinder 3 times

Dist (ft)	Depth " Flow ft3/sec
0	
0.25	
0.5	
0.75	
1	
1.25	
1.5	
1.75	
2	
2.25	
2.5	
2.75	
3	
3.25	
3.5	
3.75	
4	
4.25	
4.5	
4.75	

Net	Center depth	Center flow
1		
2		
3		
4		

Time to	Fill:	seconds
	1	
	2	
	3	

Samples collected by: 1200:_____ 1400:_____
0000:_____ 0600:_____ 1200:_____

APPENDIX B

SAMPLING SCHEDULE

Feb 2014-September 2014 Schedule for Topanga Source ID
Feb 10-11 24 hour multi-sampling effort (IMS, dye, regular sampling,sand samples inoculated?) bug id at the ranch motel in-between times
FEb 12-14 bug analysis at RCD

Feb 20 WQ sampling with microcosm collection

Mar 10 WQ sampling

Mar 25-26 24 hour drift nets set at Topanga Bridge 1200, 1800, 0000, 0600, 1200

April 10-11 prep TAC presentations and coordinate
April 16 10-noon TAC meeting Topanga Library
April 24 WQ sampling and bug collection

May 5-7 SWAMP stream survey (frogs, diatoms, bugs, etc)

School visits:
Mar 28 10:45-11:45 TMS, 12:30 - 1:30 TESin class visit from WSP interns for Topanga Watershed presentation, introduce community poster contest (impacts of dogs on beach water quality) and dog/gull poop study
April 25 12:30- 1:30 in class visit from UCLA students to introduce molecular bio study
May 2 9- 12:30 FIELD TRIP to Topanga Beach to work with UCLA students and collect samples, etc.
May 9 12:30-1:30 In class visit from UCLA students to review data and organize poster presentations
May 16 9 - 12:30 UCLA FIELD TRIP to visit the Jay lab, and present their science poster results, community poster contest judging

May 27 6-8pm Community Meeting to present science posters and community posters.

June 11-13 bug id at RCD
June 19 WQ sampling and bug collection
June 23-27 bugs at RCD

July 28-Aug 1 TSID final report preparation

Aug 19 WQ and bug collection
Aug 20-22 bug sample sort and analysis

Aug 25-29 TSID final report prep

Sept 16 WQ sampling