

Culebra Nearshore Water Quality Monitoring Program Work Plan

*Developing a water quality monitoring program to evaluate changes in LBSP exposure in
Culebra, Puerto Rico*



1. Background

This monitoring work plan is a key component of [Culebra's LBSP Ridge to Reef Monitoring Program](#) aimed at providing standardized guidelines and methods to monitor changes in land-based pollutant loads, nearshore land-based pollutant exposure, and seagrass habitat responses across the island. These monitoring activities are spatially and temporally coordinated and represent the basis of [Culebra's Integrated LBSP Monitoring and Evaluation Framework](#) to successfully evaluate progress toward achieving NOAA's LBSP management goals and outcomes on Culebra.

2. Monitoring Objectives

This Work Plan outlines nearshore water quality monitoring to evaluate changes in land-based sources of pollution (LBSP) exposure in the coastal environment over time and is aligned with the following NOAA LBSP management goals, outcomes, and evaluation questions:

Management Goal:

- By 2030, improvements to Culebra's nearshore water quality and sedimentation.

Management Outcomes:

- Improve nearshore water quality.
- Reduce terrigenous sedimentation.

Evaluation Questions:

- Have nearshore water quality conditions improved?
- Has nearshore terrigenous sedimentation decreased?

This water quality monitoring program was also designed to:

1. Develop water quality characterization and monitoring guidelines and protocols that can be implemented throughout Puerto Rico and the greater Caribbean as a model for cost-effective water quality monitoring.
2. Establish reference-based water quality targets with which to evaluate the success of LBSP management investments on Culebra.
3. Conduct outreach activities to inform and to engage both local community, federal, and Puerto Rican government officials in ongoing natural resource management, restoration, and conservation efforts on Culebra.

3. Monitoring Location and Frequency

Monitoring Locations

The nearshore monitoring will occur at 13 fixed stations identified in Figure 1 and Table 1. These locations were selected based on a rigorous analysis of watershed hydrology and coastal hydrodynamics as well as the level of existing level LBSP exposure and anticipated changes to LBSP exposure due to management actions ([Appendix A: Identifying Priority Fixed-Site Monitoring Locations](#)). A consideration was made, to include locations where baseline (pre-restoration watershed restoration activities) seagrass and water quality monitoring surveys had been completed in 2014. These fixed monitoring stations are co-located with [Seagrass monitoring transects](#) to facilitate a ridge to reef evaluation of stressor exposure and response to coastal watershed restoration projects on nearshore habitats throughout Culebra.



Figure 1. Nearshore water quality monitoring stations on Culebra, including the location of rain gauges.

In Figure 1, LBSP Restoration sites (blue) are locations where LBSP BMPs have been implemented and are therefore locations where LBSP management is occurring. Control sites (yellow, red, and green) are locations where there is presently no LBSP management occurring. These control sites were selected to represent a range of LBSP impairment, including LBSP Control (yellow) which have no known direct discharge of LBSP but are representative of the range of external factors (currents, wave and wind energy) that may be encountered at the treatment sites; a Negative Reference site (red) of significant LBSP impairment, as well as a Positive Reference site (green) of low LBSP impairment. Tracking changes in water quality at both impaired and restored watershed points will contribute information about changes in the levels of exposure to LBSP impacts though time following the presence and implementation of installed LBSP BMPs.

Monitoring Frequency

Water quality monitoring at all 13 sites will be conducted by the Protectores de Cuencas on a monthly basis for at least one full year. This preliminary dataset will build on existing water quality monitoring data sets, establish baseline and/or post-restoration water quality characteristics at each site, identify those water quality parameters that are the strongest indicators of LBSP exposure, establish LBSP impact spatiotemporal exposure patterns, and develop reference-based targets for LBSP management. Additionally, rain gauge installations at representative control and treatment locations will be focused on documenting the occurrence, frequency, and intensity of rain events.

Table 1. Nearshore fixed water quality monitoring station locations.

Monitoring Station	Code	Watershed	LBSP Treatment Group	Latitude	Longitude
Aeropuerto	AP	Aeropuerto	Negative Control	18.3083690	-65.2979940
Cabra	CA	Cabra	LBSP Restoration	18.3059370	-65.2813820
Casa Azul	CAZ	Culebra	LBSP Control	18.3006040	-65.2899960
Cementerio	CM	Cementerio	LBSP Control	18.3070770	-65.2854440
Coronel	CO	Coronel	LBSP Restoration	18.3088810	-65.2902890
Fulladosa Bay	FDB	Fulladosa	LBSP Restoration	18.2957150	-65.2873460
Fulladosa Point	FDP	Fulladosa	LBSP Control	18.2968560	-65.2858520
Fulladosa Ramp	FDR	Culebra	LBSP Restoration	18.2995720	-65.2882960
Little Cabra	LC	Cabra	LBSP Control	18.3020900	-65.2804920
Mosquito	MQ	Mosquito	Positive Reference	18.2920310	-65.2639030

Monitoring Station	Code	Watershed	LBSP Treatment Group	Latitude	Longitude
Puerto Manglar Control	PMC	Puerto Manglar	LBSP Control	18.3057740	-65.2566650
Puerto Manglar Reference	PMD	Puerto Manglar	Positive Reference	18.3050480	-65.2510630
Puerto Manglar Treatment	PMT	Puerto Manglar	LBSP Restoration	18.3057570	-65.2517240

4. Monitoring Parameters

Physical and biological indicators provide a measurable proxy for quantifying changes in stressors, exposures, and responses over time to evaluate management success. Water quality parameters were selected for monitoring that are known to be good indicators of nearshore water quality condition ([USCFTF Watershed Partnership Initiative Priority Ecosystem Indicators](#)). In particular, indicators that are linked to both the LBSP management that is conducted in the watershed while also being linked to potential changes in habitat response.

Changes in LBSP pollutant exposure—corresponding changes in habitat response are being quantified using [Seagrass monitoring transects](#)—will be evaluated through comparisons of site-specific measurements of indicators at LBSP Restoration sites over time, as well as with LBSP Control sites. If measures of indicators at LBSP Restoration sites are trending toward LBSP Control or Positive Reference sites this would suggest that pollutant exposures and habitat responses are ‘improving’ as a result of management actions. Therefore, the parameters selected for this monitoring work plan were: (1) indicators of water clarity and nutrient water quality threats, and (2) logistically feasible; and are outlined below:

- Indicators of water clarity: Turbidity, Chlorophyll-a—measures the light emission at a certain wave length to gain insight of phytoplankton photosynthetic efficiency; CDOM—Colored Dissolved Organic Matter that absorbs light in the blue and UV part of the electromagnetic spectrum, giving water a yellow-brown color; and PAR—Photosynthetic Active Radiation.
- Indicators of nutrients and pathogenic bacteria: Nitrogen (nitrite, nitrate, ammonia, ammonium, and total nitrogen); Phosphorus (total phosphorus and orthophosphate—phosphorus in its simplest form that is up taken by photosynthetic organisms at the base of the marine food web); silica, partly attributable to runoff; *Enterococcus* and *E. coli*; and sucralose and caffeine—pathogenic bacteria and human waste tracers.
- Water physical properties: Temperature, Conductivity/Salinity, Dissolved Oxygen, and pH.

5. Data Collection and Processing Methods

Data and/or samples for water quality parameters are collected at two depths (1 and 2 meters) and include: 1.) temperature; 2.) conductivity—to determine salinity; 3.) dissolved oxygen; 4.)

pH; 5.) turbidity; 6.) chlorophyll-a; 7.) CDOM—Colored Dissolved Organic Matter; 8.) PAR—Photosynthetic Active Radiation; 9.) fecal indicator bacteria; and 10.) nutrients and tracers. Parameters 1–7 will be collected in the field using the Eureka Manta 35 Water Quality Sonde. PAR will be assessed using a buoy-suspended light sensing system (e.g., Hobo and Apogee); and discrete water samples will be acquired to assess nutrients, tracers, and fecal indicator bacteria. Water samples for nutrients and tracers will be shipped to the NOAA National Center for Coastal Ocean Sciences, Monitoring and Assessment Branch, Stressor Detection and Impacts Division. Protectores de Cuencas Laboratory in Culebra will implement the Quanti-Tray system to detect the presence of Enterococci using the Most Probable Number (MPN) model. Finally, a [handheld digital tester](#) will also be implemented to collect an additional measurement of salinity.

Eureka Manta+35 Water Quality Sonde

Parameters to be measured with the Eureka Sonde (Figure 2) include; temperature (degrees C), pH, conductivity ($\mu\text{S}/\text{cm}$), turbidity (Formazin Nephelometric Unit FNU), dissolved oxygen (% and mg/L), and CDOM (ppb), Chlorophyll-a ($\mu\text{g}/\text{L}$). The day prior to or the morning of sampling, the Eureka should be charged or changed to fresh batteries and all probes should be calibrated using the standard calibration reagents and procedures outlined in Section D of the [Eureka Manta User Manual](#). Select calibrations standard whose value is close to the values you expect to see in the field. Note: for the nearshore water quality monitoring, confirm that conductivity/salinity is calibrated for brackish/saltwater and crosscheck with the handheld salinity tester using a standard solution of known concentration. Imprecise calibration can/will result in inaccurate instrument readings. For best results, use fresh calibration solutions and discard once they have been used. All calibration reagent waste should be disposed of or collected as indicated on the chemical safety data sheet. Visit the [Manufacturer's Website](#) to learn about the [Instrument Sensor Parameters](#) range and accuracy.



Figure 2. Eureka Manta+35 water quality sonde.

To make measurements:

1. Water physical and quality parameters are measured at two depths (i.e., surface ~1 m and deep ~bottom 2 m) for all monitoring—the depth at which the instrument is reading depth should be consistent.
2. Submerge the sonde probe in the water at the designated sampling location to 1 m deep.
3. Allow the sensors to stabilize before recording readings on the handheld tablet and data sheet.
4. Save the measurements to the data log including sample ID and date in the filename.
5. Submerge the sonde probe to ~2 m deep and repeat steps 1–4.

6. Rinse the probes with purified water after sampling.

Maintenance:

1. Clean the instrument periodically with warm soapy water. Liquid dishwashing soap is fine. Do not use abrasives. Do not use acetone. Do not clean with gasoline, kerosene, or industrial cleaners. Mild household cleaners work well. Clean sensor stems with a soft brush.
2. Replace any o-rings with visible cracks. Keep o-rings greased with silicon grease (found in your Maintenance Kit).
3. Always remove batteries and clean your Manta prior to storing it for prolonged periods.
4. Store probes in the provided calibration cup, preferably vertically, if possible, with at least 1/8th inch of purified water in the bottom of the cup to keep probe membranes moist. Probes should be replaced regularly on the schedule outlined in the [Eureka Manta User Manual](#).

HOBO (Onset) and Apogee PAR Sensors

In aquatic optics, the downwelling irradiance attenuation coefficient (E_d) of Photosynthetically Active Radiation (PAR, 400–700 nm) can be used as a broad indicator of the availability of light for photosynthesis and a proxy for water quality. Low-cost calibrated sensors will be used to obtain PAR and supplement water quality measurements. Also, both PAR and water quality measurements, like chlorophyll concentrations and total suspended matter, can be used to validate satellite derived water quality parameters.

HOBO (Onset) Sensor Standard Operating Procedure

Two Onset HOBO Pendant Temperature/Light Loggers ([MX2202](#)) will be deployed at known depths structured in a buoy system (Figure 3) to collect light measurements at two depths (1 and ~1.5–2 meters) and approximate PAR attenuation (K_d PAR). Higher K_d PAR values indicate more turbid waters, whereas low K_d PAR values indicate more transparent waters.

Initial Setup:

1. Download the HOBO mobile app from App Store (iOS) or Google Play (Android).
2. Open the HOBO app and press the center button on the top HOBO sensors to connect via Bluetooth. In Devices, the HOBO sensor should appear In Range. Verify and match the serial number of the sensor.
3. Select the HOBO and go to Configure, add Top to the name, before serial number and set the Logging Interval to 1 second.
4. Repeat these steps for the bottom HOBO sensor, adding Bottom before the serial number.

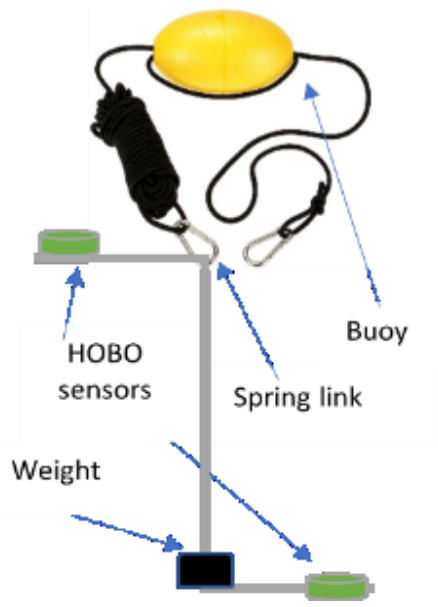


Figure 3. Onset Hobo pendant Temperature /Light data logger.

To take measurements:

1. Select an area to deploy the sensor from a boat or dock. Make sure the area is safe and that the sensor can drift away to avoid clouds from the boat or dock.
2. Setup sensors to start/end simultaneously. Connect to each of your HOBO sensors and select Configure and go to Start Logging and select Date/Time. Select today's date and set time to 10 minutes from current time (e.g., 10:15AM, set start time to 10:25AM). Press Done.
3. Go to the Stop Logging, Stop Logging (Time Options) select After, select 15 minutes and press Done. Make sure both sensors (Top/Bottom) are set up. Secure tie or hold rope tow line.
4. Deploy sensors in water and allow them to drift away from shadows. Records observations in a field datasheet of time of deployment, weather (wind and cloud cover) and sea (clam, sight chop, etc..) conditions.
5. After the selected time for deployment has passed (e.g., 15 minutes), recover sensors from water. If possible, rinse sensors with fresh water. Repeat steps 1–5 at other sites.

Data Download:

1. Open the HOBO app and press the center button on the top HOBO sensors to connect via Bluetooth. In Devices, the HOBO sensor should appear *In Range*.
2. Select the sensor and press *Readout*. After the readout is complete, go to *Data Files* at the bottom of the app. A graph should appear with the sensor name. Repeat steps for the bottom sensor.
3. Once all data has been read out, go to *Data Files*, select graphs and press Share. Select Excel file (.xlsx) and send the generated files to your email.
4. Check email and download the files to your computer, tablet, or upload to a shared folder.

5. Training will be provided to the user for processing the data.

APOGEE PAR Sensor Standard Operating Procedure

The Apogee PAR sensor setup consists of [2 PAR Quantum sensors](#) and a Bluetooth data logger. One sensor is used for reference above water measurements and the other is used for taking profile measurements of PAR underwater (Figure 4). Follow instructions carefully to collect the most reliable PAR data possible since optical PAR data is greatly affected by shadows and sensors not aligned correctly.



Figure 4. APOGEE Photosynthetic Active Radiation (PAR) above-water (upper panel) and below-water (lower panel) sensors.

Hardware Setup:

1. Remove equipment from the bag and make sure all connections are tightened. Connectors are designed to be firmly finger-tightened only. When fully tightened, 1–2 threads may still be visible. **WARNING:** Do not tighten the connector by twisting the black cable or sensor head, only twist the metal connector (blue arrows).
2. Remove sensor cap cover and store in bag.
3. To accurately measure incoming radiation, the sensor must be level. Place the above-water sensor on a flat surface and check the level bubble for additional alignment. Use a tripod when possible.
4. When measuring from a boat, place the above-water reference sensor on the boat canopy or stern at the most level location possible.
5. When selecting the sensor placement, avoid shadows from trees, posts, the boat, and yourself while collecting the data. Consider for boat drift and sensor location.
6. Avoid collecting PAR data from the boat on rough seas or windy days.

7. The cable for the underwater sensor is 10m, untangle the cables if necessary to avoid twisting or moving the sensor while sampling.

Instrument Setup:

1. Download Apogee Connect from the App store or Google Play store.
<https://apps.apple.com/us/app/apogee-connect/id1398029402>
https://play.google.com/store/apps/details?id=com.apogeeinstruments.apogeeconnect&hl=en_US.
2. Open App and hit the “+”.
3. Press the green button on the μ Cache unit and hold for 3 seconds.
4. When μ Cache is recognized in the app, click on its name “uc###”.
5. Select the sensor model you are connecting.
6. Calibration: Do not change this number.
7. Click “Add”. Add the 2 sensors option, so now both sensors are collecting simultaneously. For the multiplication factor, make sure Channel **A (above water)** is 100, and Channel **B (underwater)** is 125. Your sensor is now added and reading in real-time.
8. Lower instrument with underwater sensor to the 1 m marking on cable. Make sure no shadows from the dock, boat or from yourself are over the instrument.
9. Collect data using logging for 5 minutes at 1 m depth. See section **Begin Logging** for details. Take notes when conditions change while sampling (ex. waves, intermittent clouds, etc.). Repeat sample if conditions change dramatically.
10. Once the instrument has stabilized (2–3 minutes), take a screenshot of the real-time reading screen in the *Apogee Connect App* and write down data values for channel A and channel B in the field data sheet. This number is preliminary as the final value will be calculated from the downloaded data. Final value will be the average of the values recorded during the 5-minute logging.
11. Turn off logging and click save. Lower instrument to the 2 m marking (if possible); **wait for 1 minute** and repeat sampling for 5 minutes (**Begin Logging**).
12. While waiting for the 5 minutes, collect the weather conditions while sampling, (presence of clouds, watercolor, presence of debris, sargassum, high waves/wind, etc.). Turn off logging.
13. Once sampling is completed, remove the sensor from water and if no further sampling is expected, wash the sensor with fresh water. Follow steps in section **Collect Logs** to download and share data.

Begin Logging:

1. Click on the “Settings” gear icon.
2. Scroll down to the bottom and hit Match Current Time.
3. Scroll down and toggle on the “Logging Enabled” button.
4. Set the Logging interval (this determines how often a data point is recorded).
5. Set the Sampling interval (this determines how many readings are averaged to create the data point referred to in step 3). Make sure the sampling is set to 1 second and logging interval to 10 seconds.
6. Click the green Save button.

Collect Logs:

1. If disconnected, reconnect the μ Cache by pressing the green button for 3 seconds.
2. Click on the “Collect Logs” icon.
3. Select “Append to existing” to add to an existing dataset (Station) already on your phone, or “Create New” to start creating a new data set.
4. Confirm Start and End date match the range of data you want to download.
5. Click “Collect Logs”.
6. Once all the Logs are collected, the graphs will automatically fill in on the dashboard and the name of the station code will appear on the heading.
7. Select “Export Dataset” to export file from phone or tablet via email or text.
8. Download emailed data and upload to the project's google shared folder.

Processing Logs:

Contact scientist [William Hernandez](#) at [Environmental Mapping Consultants](#) for guidance on processing the APOGEE PAR logs.

Nutrient and Tracers

Discrete water samples will be collected at two depths (surface and ~ 2 meters depth) for the analysis of nutrients including nitrate (NO_3^- mg/L), nitrite (NO_2^- mg/L), orthophosphate (HPO_4^{3-} mg/L), ammonium (NH_4^+ mg/L), total nitrogen (TN mg/L), total phosphorus (TP mg/L), and silica (HSiO_3^- mg/L); and the tracers of caffeine and sucralose. These last two analytes are exclusively of human origin and enter watersheds and the coastal marine environment via wastewater inputs.

Before you leave for the field make sure you have:

1. Pre-labelled sample bottles.
 - a. 125 ml HDPE for nutrients—two bottles for each nearshore monitoring station, one each surface and deep (~ 2 m).
 - b. 250 ml HDPE for tracers—one bottle for each nearshore monitoring station collected at depth (~ 2 m).
2. Nitrile gloves (one pair per person per site).
3. Cooler $\frac{1}{4}$ full of wet ice – for samples.
4. Distilled or deionized water (1 gallon)
5. Blue ice (leave in freezer, for shipping).

Sample collection:

1. Put on fresh gloves and change gloves between sites.
2. At each site, do the surface sampling first. If other sampling/measurements are being done, recommend doing nutrient/tracer sampling first to avoid stirring up sediments.
3. Fill the sample bottle full and drain completely (rinse) three times including the cap at least once.
4. Fill the sample bottle with site water a fourth time leaving 10% head space to avoid damage and sample loss when freezing.

5. Cap bottle tightly and place in the cooler.
6. Do this for one 125 ml bottle (nutrients) for each nearshore site surface sample.
7. For a bottom water sample use the Niskin bottle. Before deployment, verify that the bottle is securely fastened to rope. Refer to the [Wildco® Alpha Horizontal Water Bottle User Manual](#) for operational details. Also, verify that rope is labeled appropriately to mark the appropriate bottle submersion depth (~2 m).
8. Retrieve Niskin and collect a water sample in a 125 ml bottle for nutrients following steps 3 through 5—do not forget to rinse.
9. Additionally, from the Niskin bottle collect one sample in a 250 ml bottle for tracer analysis (using the same protocol above). The Niskin bottle has a 2.2-liter capacity so it should provide plenty of water to appropriately rinse bottles as well to collect an additional ~250 ml sample for fecal indicator bacteria analysis (see below).
10. Rinsing the Niskin bottle with distilled/purified water between stations is not necessary (but is a good practice) as long as you rinse it thoroughly at each monitoring station.
11. Multiple Niskin casts per site can be done if needed but **strongly discouraged**. If multiple casts are necessary, the Niskin does not need to be cleaned between casts.
12. **Remember:** At one nearshore site per sampling mission, fill one each bottle type—one 125 ml, one 250 ml—with distilled/purified water. These should be labeled “Field Blank”, and should be rotated around to different sites on each sampling trip, with two nearshore field blanks per trip—nutrients and tracers.

Sample Storage and Shipment:

1. Samples should remain in the cooler **on ice** with the lid closed while in the field and promptly transferred to the freezer upon returning to shore.
2. Samples should be frozen solid (at least 12 hours, preferably 24 hours) prior to shipment.
3. Shipping coolers should be packed with sample bottles on the bottom and blue ice on the top (with at least one layer of blue ice)
4. Try to minimize the amount of air space in the cooler. This keeps things colder and reduces movement/potential damage to bottles during shipment. Cardboard or recycled paper or other filler can be used.
5. Use strapping tape to fasten the cooler closed.
6. David Whitall (NOAA NCCOS) will provide UPS shipping labels (via pdf) for overnight shipping. Affix the label to the cooler with label (clear) shipping tape.
7. Preferentially ship samples between Monday and Wednesday. Shipping on a Friday is **strongly discouraged** as it may result in weekend delivery time delays.
8. Ship bottles with a complete packing list and sample metadata that includes the exact alphanumeric bottle labels, the sites visited, the bottle number, volume and type of analyses, as well as the corresponding site salinities from the Eureka and handheld instruments.

Sample processing:

Nutrient analytical testing will be performed by a NOAA contract lab—[Geochemical and Environmental Research Group](#) at Texas A&M University—and processed for nitrate (NO_3^- mg/L), nitrite (NO_2^- mg/L), orthophosphate (HPO_4^{3-} mg/L), ammonium (NH_4^+ mg/L), total nitrogen (TN

mg/L), total phosphorus (TP mg/L) and silica (HSiO_3^- mg/L). Tracers (caffeine and sucralose) will be performed by [TDI-Brooks](#) Laboratory. The following link provides an overview of the [nutrient and tracer analytical methods](#) implemented.

Fecal Indicator Bacteria

An additional deep (2 m) water sample of approximately 250 ml will be collected from the Niskin bottle and stored in [Low Density Polyethylene \(LDPE\) bag](#) (Figure 5). Bags will be transported and taken back to the Protectores de Cuenas Laboratory for sample preparation and analysis for fecal indicators.

Sample collection:

1. Be sure to label each bag with the sampling location and date of sample collection (one bag per site).
2. Open the sample bag by tearing the top perforated part of the plastic off, then pulling the pull tabs apart, being careful not to touch the inside of the sample bag.
3. Fill the bag at least halfway or more.
4. Holding the bag by the wire ends, whirl the bag three complete revolutions to form a leak-proof seal. Tie a knot with the wire ends to secure and test if sealed by flipping the bag upside down and squeezing lightly (Figure 5).
5. Place water samples in the cooler with ice.



Figure 5. Sample bag for fecal indicator bacteria.

The [INDEXX Quanti-Tray system](#) (Figure 6) will be used to detect the presence of Enterococci, a fecal-indicator bacteria, in water samples using the [Most Probable Number \(MPN\) model](#). Enterococci bacteria live in the intestines of mammals, including humans, and their presence in water samples can indicate fecal waste contamination. The presence of *Enterococci* can indicate the presence of disease-causing constituents that can sicken those who recreate or consume raw shellfish or fish from the sample water.



Figure 6. Illustration of the IDEXX Quanti-Tray system: Sealer (left panel) and Quanti-Tray Legiolert (right panel).

To make measurements:

1. Turn on the Quanti-tray sealer.
2. Pipette: use clean gloves while placing the green pump on top of the pipette.
3. Use a pipette to take out 1 ml of sample.
4. Place the sample in the graduated container.
5. Add potable water up to 100 ml line marked on the side of the sample bottle.
6. Add one Enterolert reagent powder package to the sample bottle, close sample, and shake until it is dissolved, trying to avoid bubbles.
 - a. The [Enterolert Test](#) is a proprietary Defined Substrate Technology (DST) nutrient indicator which fluoresces when metabolized by Enterococci.
7. Let the sample sit for a few minutes to dissipate bubbles.
8. Use permanent marker to write on the back of Legiolert (tray) and indicate.
 - a. Sample location
 - b. Date
 - c. Time in
 - d. Time out
 - e. Temperature in/out
 - f. Number of fluorescent wells in top (large wells)
 - g. Number of fluorescent wells in bottom (small wells)
9. Open the Legiolert (tray) top by pulling back from the plastic front. Do not touch inside.
10. Pour sample/reagent mixture into the tray once there are no bubbles.
11. Tap or flick tray to remove air bubbles from the wells.
12. Place Quanti-Tray Legiolert into rubber insert immediately.
13. With the rubber insert on the bottom, feed the tray into the sealer small wells first, with the largest well facing out (sealed last) and push slightly to feed into the sealer.
14. Incubate tray at $41^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 24 hours.
15. After the wait time, shine UV light on the tray in a dark room and count the number of fluorescent cells, making each with an X.
 - a. Fill out F_{big} , F_{small} , etc. on the back of the tray.
 - b. Transcribe these numbers to the field datasheet

16. Use the [Most Probable Number \(MPN\) model Table](#) to determine the number of *Enterococcus* and *E. coli* in the water.

6. Data Management

Data management is the practice of collecting, storing, documenting, and using data efficiently and cost-effectively. The goal of data management is to ensure data access, quality, and integrity. To prevent the accidental data deletion/loss, redundant data storage will be utilized to ensure data resides in multiple places and in multiple media. A Nearshore Water Quality Data Google drive folder was created to collate and store all field data.

1. For this monitoring effort, data should be stored in at least two permanent repositories at all times:
 - a. Durable Waterproof paper datasheets (see Section 8).
 - b. Online [Nearshore WQ Monitoring Datasheet Google Form](#)
 - c. Eureka handheld data log, however this is not a permanent or secure storage.
2. Durable Waterproof paper datasheet need to be filled out in the field
3. Online [Nearshore WQ Monitoring Datasheet Google Form](#) can be filled out on cell phones or tablets in the field or in the lab to automatically upload data to the Nearshore Water Quality Data Google drive folder.
4. Data logged on the Eureka Sonde with the date of sampling listed in the file name can be downloaded, uploaded to the shared Nearshore Water Quality Data Google drive folder, and used as a data reference if needed.
5. The [Nearshore WQ Monitoring Datasheet Google Form](#) must be filled out and submitted on a site-by-site basis, i.e. one form submitted per sample site.
6. The Excel or waterproof paper datasheet must be signed by the sampling technician, and scanned.
 - a. Hardcopies will be stored in a binder in the Protectores de Cuencas Laboratory. This binder is not taken to the field for sampling to prevent previous records from being damaged.
 - b. Scanned field datasheets need to be uploaded to the shared Nearshore Water Quality Datasheets folder, located in the Nearshore Water Quality Data Google drive folder.
7. Once the Google Forms and datasheets for each monitoring episode have been submitted the Data Manager will upload the corresponding data to a Nearshore Water Quality data repository located in the Nearshore Water Quality Data Google drive folder.
8. Together with the Data Manager, the Monitoring Coordinator will [check](#) the uploaded data for quality and integrity against the field datasheets, and make any modifications, corrections, and updates as necessary. Any data changes should be communicated and discussed with the Data Manager prior to implementation.

7. Check Lists

Before Sampling Checklist

Name: _____ Date: _____

Sensor Calibration:

- Fresh batteries installed/all batteries checked for full charge
- Eureka Probes: Date of calibration: _____
- Conductivity
- Temperature
- Dissolved oxygen
- pH
- Turbidity
- CDOM
- Chl-a

Sampling Equipment:

- Eureka Sonde
- PAR sensors
- Niskin bottle and deployment line
- Cooler with wet ice for sample storage
- Purified water for rinsing
- GPS or phone with FieldMaps App
- Sampling bags, bottles, lids, fasteners
- Tape and Sharpie to label samples
- Gloves
- Binder with datasheets

Wet Lab Equipment:

- Purified water for rinsing
- Pipet and tips for fecal indicator samples
- Enterolert reagent powder package
- Quanti-tray sealer
- Legiolert (trays)
- Oven
- UV light
- Sunscreen / hat
- Water
- Towel

Notes:

Signature:

Date:

After Sampling Checklist

Name: _____

Date: _____

Sensor Care and Storage:

- Eureka probes rinsed with purified water
- Eureka probes stored vertically in calibration cup
- Sufficient water (1/8th inch) in bottom of sample cup to keep probe membranes moist
- Incubator turned off, Quanti-tray sealer and UV light unplugged
- PAR sensing system rinsed with freshwater, all optical lenses covered, and appropriately stored

Sampling Equipment:

- Sampling bags, bottles, lids, fasteners cleaned or appropriately disposed of
- Niskin bottle and deployment line appropriately rinsed and dry
- All reagents and calibration standards sealed and stored according to chemical safety plan
- Pipet and tips cleaned or appropriately disposed of
- Cooler for sample storage emptied and stored to dry
- All samples appropriately processed, filtered and/or frozen

Datasheets and Data Management:

- All fieldnotes dictated and datasheets signed/dated
- All completed datasheets photocopied to database (CamScanner)
- All datasheets transferred to dry/secure binder
- Data inputted to appropriate data portals

Analytical grade nutrient testing water sample shipping

- All water quality sample bottles frozen solid and appropriately labeled
- Blue Ice
- Shipping cooler
- Shipping label

Notes:

Signature:

Date:

8. Field Datasheets

Culebra Nearshore Water Quality Sampling Field Sheet

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Date: _____ Time: _____ Site: _____ Recorded by: _____

Tasks Completed? Sonde Bacteria Light Attenuation Lab Nutrients

Site Conditions:

Sea State (4 point): _____ Wind Direction (8 point): _____ Wind Speed: _____ Cloud Cover (0-100): _____

Water Quality:

Eureka Sonde					
Parameter	Units	Surface		Bottom	
Sample Time	Time				
Temperature	Celsius				
pH	SU				
Conductivity	µs/cm				
Dissolved Oxygen	mg/L				
Dissolved Oxygen	% Saturation				
Chlorophyll a (Blue)	µg/L				
CDOM	µg/L				
Chlorophyll a (Red)	µg/L				
Turbidity	NTU				
Salinity	PSU				
Light Attenuation		Surface		Bottom	
Sample Depth:					
Sampling Time:					
APOGEE: 5 min	PAR	Upper sensor (A)	Lower sensor (B)	Upper sensor (A)	Lower sensor (B)
HOBO: 10 min	Optical Brightness	Sampling Time:			

Lab water quality samples (list number & volume of bottles)

Surface Nutrients: _____ Deep (~2 m) Nutrients: _____ Tracers: _____

Surface salinity: _____

Bacteria:

Large wells: _____ Small wells: _____ Total: _____

Enterococci (MPN/100ml [account for dilution correctly]): _____

Notes:

9. Estimated Cost

Category	Item Description	Cost per unit	Unit	Units / day	Days / trip	# Persons	Per Year	Annual Total	Notes
Staff	Nearshore field biologists	\$35.00	hour	10	3	2	12	\$25,200.00	Estimating 2 scientists; 12 trips a year (monthly sampling) and 2, 10-hr day and 2-half days to calibrate sonde, collect the water quality measurements and discrete samples, analyze for fecal indicators, and pack nutrient water samples for shipment.
Travel	Ferry	\$75.00	roundtrip / truck	1	1	1	12	\$900.00	This accounts for costs of bringing one staff + one pick-up truck with equipment.
Travel	Ferry	\$10.00	roundtrip / person	1	1	1	12	\$120.00	1 staff.
Travel	Lodging	\$159.00	day	1	3	2	12	\$11,448.00	Estimating that nearshore field biologists will arrive in Culebra the night before sampling, stay the night prior to the sampling and the nights of sampling, and then depart on the morning of the third work day.
Travel	Gas	\$150.00	roundtrip	1	1	1	12	\$1,800.00	Estimating one pick-up truck fuel consumption from Yauco to Culebra roundtrip and within Culebra.
Travel	Per diem (full days)	\$105.00	day	1	2	2	12	\$5,040.00	Estimating that nearshore field biologists will arrive in Culebra the night before sampling, stay the night prior to the sampling and the nights of sampling, and then depart on the morning of the third work day.
Travel	Per diem (travel day)	\$78.75	day	1	2	2	12	\$3,780.00	Estimating the nearshore field biologists arrive in Culebra the night before sampling and depart on the morning of the third work day.
Services	Boat Captain	\$30.00	hour	10	1	1	12	\$3,600.00	Assuming 1 boat day needed to sample all 13 sites.
Services	Analytical nutrient testing*	\$109.50	sample	41	1	1	12	\$53,874.00	Estimated average cost per sample (nutrients or tracers). This includes supplies, instruments / equipment shipping, and analyses.
Services	Boat Costs	\$700.00	day	1	1	1	12	\$8,400.00	Assuming 1 boat day needed to sample all 13 sites.
Equipment	Equipment rental fee*	\$600.00	trip	1	1	1	12	\$7,200.00	Estimated as total cost for purchasing 1/2 Eureka sonde (\$ 13000.00); 2/3 IDEXX system for fecal indicators (\$ 8600.00) = \$ 21600.00; recovered over a 3-year time horizon in monthly rental fee installments (~ \$600.00).
Total								\$121,362.00	

* An itemized list of equipment, instruments, and supplies needed for each monitoring component can be accessed by clicking on this link ([Appendix 1: Culebra LBSP Ridge to Reef Monitoring equipment, instrument, and supply budget list](#)).

10. Links and References

- Eureka Manta Website: <https://www.waterprobes.com/>
- Eureka Manta Manual: https://b3a3d385-13c7-4581-b536-17695817f1a5.filesusr.com/ugd/7f6545_5070d8ef8e194d888b39684c72fced62.pdf
- Eureka Manta Instrument Sensor Parameters: <https://www.waterprobes.com/sensor-parameters-water-quality-monitoring>
- Onset HOBO Pendant Temperature/Light Loggers: <https://www.onsetcomp.com/products/data-loggers/mx2202#features>
- Apogee PAR sensor: <https://www.apogeeinstruments.com/pg-510-package-microcache-and-full-spectrum-quantum-with-2-meter-cable/>
- IDEXX Enterolert 24-hour detection of enterococci: <https://www.idexx.com/en/water/water-products-services/enterolert/>
- Geochemical and Environmental Research Group: <https://gerg.tamu.edu/>
- TDI Brooks: <https://www.tdi-bi.com/>