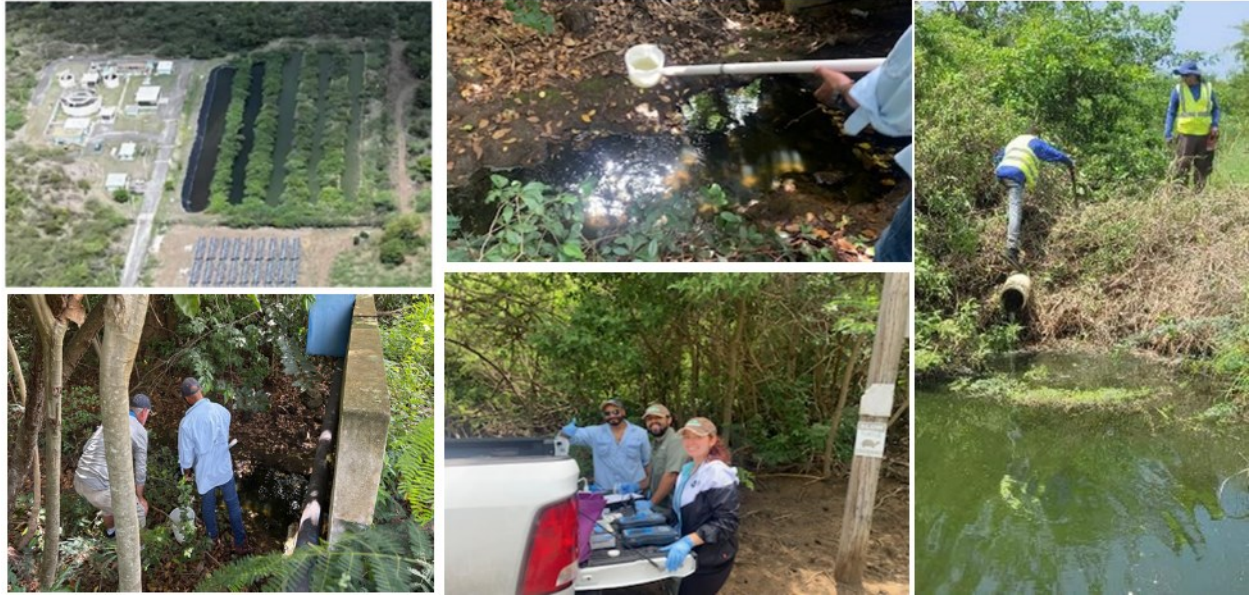


Culebra Cabra Watershed Water Quality Monitoring Work Plan

A watershed water quality monitoring program to evaluate changes in land-based sources of pollution (LBSP) stressors in Culebra, Puerto Rico



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.

1. Monitoring Objectives

On September 8, 2016, the United States Environmental Protection Agency (EPA) issued a final National Pollutant Discharge Elimination System (NPDES) permit for the Culebra Wastewater Treatment Plant (WWTP) owned by the Puerto Rico Aqueduct and Sewer Authority (PRASA) ([Permit No: PR0026549; US EPA, 2017](#)). Effluent limitations for a host of parameters are specified as part of permit conditions. Founded in the ridge to reef principles, this work plan outlines watershed water quality monitoring to evaluate change in LBSP pollutant loads from stormwater and undertreated sewage over time. The monitoring is aligned with the following NOAA LBSP management goals, outcomes, and evaluation questions:

Management Goal:

- By 2030, improvements to Culebra's watershed water quality including fecal pathogens

Management Outcomes:

- Improve watershed water quality.

Evaluation Question:

- Have watershed water quality conditions improved?

This the water quality monitoring program was also designed to:

1. Implement the Culebra Community Watershed Action Plan for Coral Reefs and Water Quality (Sturm et al. 2014) to support the management and mitigation of LBSPs on Culebra.
2. Build off/develop a water quality baselines for Culebra's impaired watersheds to identify and quantify sources of increased human-derived nutrients inputs into Ensenada Honda.
 - a. Provide the basis to assess the performance of implementing a [Floating Treatment Wetland \(FTW\)](#) to reduce Culebra's WWTP effluent nutrient loads prior to discharge to Ensenada Honda.
 - b. Develop a better understanding of bacterial pathogen loads delivered to Ensenada Honda to inform additional wastewater treatment needs to improve water quality within the Bay.
 - c. 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, Frequency

Monitoring Locations

To complete these objectives, water quality monitoring will be conducted at nine locations identified in Figure 1 and Table 1. These locations were selected in keeping with prior water quality monitoring efforts in 2018 and 2021 following the criteria below.

1. Four points within Culebra's Wastewater Treatment Plant (WWTP). The first location is the effluent point from the WWTP, just beyond the chlorination contactor, to establish a baseline of the inflow entering the tertiary treatment system. The second sample will be taken at the end of Pond 3 (i.e., P3_out), prior to discharge into Pond 4 where additional nutrient management actions are planned. The third sample will be collected from Pond 4 discharge pipe into Pond 5 (i.e., P4_out), to assess the performance of implementing the floating treatment wetland to reduce nutrient loads. Finally, the fourth sample point is at the end of Pond 5 (i.e., P5_out), to assess the final nutrient load exiting the tertiary treatment system.
2. Two WWTP downstream sampling locations include the bridge over PR-250, as well as the stream crossing point over the lower road, prior to discharge to Ensenada Honda.
3. Three sites in the Coronel and Aeropuerto watersheds to determine water quality at additional point and nonpoint sources of excess nutrients entering Ensenada Honda.

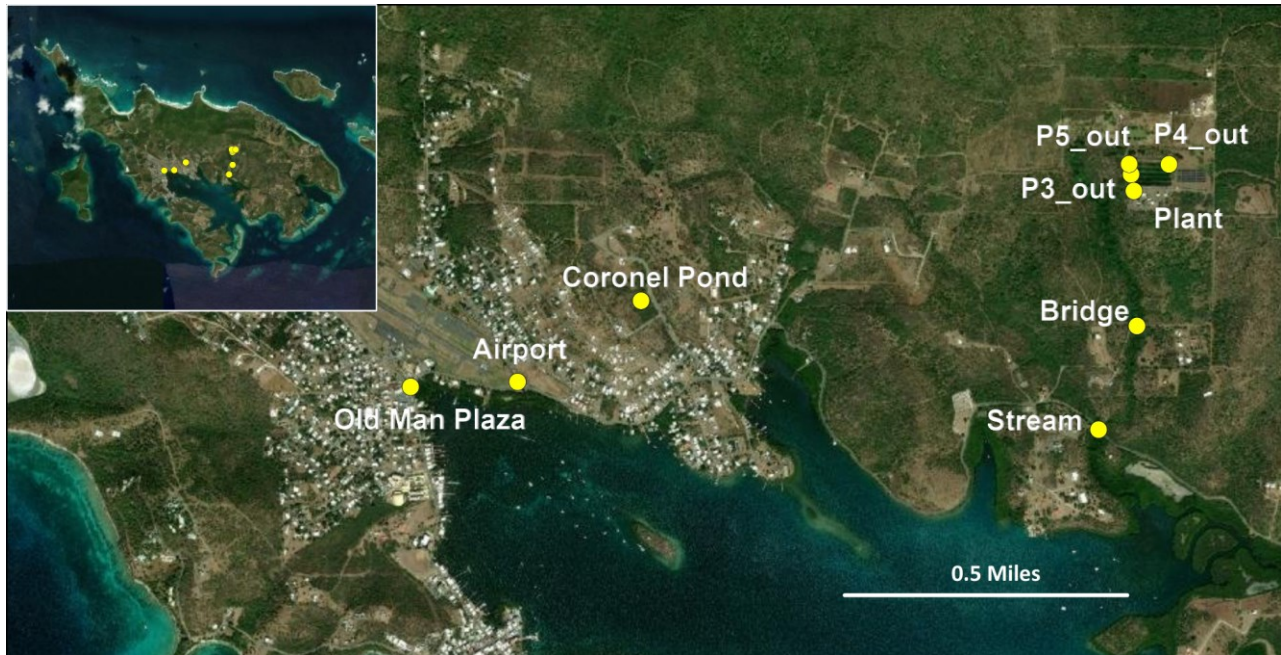


Figure 1. Watershed water quality fixed monitoring stations on Culebra.

Table 1. Watershed water quality fixed monitoring station location and descriptions.

| Monitoring Station | Watershed | Location Description | Latitude | Longitude |
|--------------------|------------|---|-----------|------------|
| Old Man Plaza | Aeropuerto | Urban and stormwater drainage | 18.310608 | -65.303091 |
| Aeropuerto | Aeropuerto | Urban and stormwater drainage | 18.310753 | -65.299732 |
| Coronel Pond | Coronel | Sediment retention pond | 18.313164 | -65.295858 |
| Stream | Cabra | Stream crossing point over lower PR-250 | 18.309336 | -65.281485 |
| Bridge | Cabra | Bridge where stream passes under PR-250 | 18.312416 | -65.280278 |
| WWTP Plant | Cabra | PRASA sample point WWTP effluent | 18.316432 | -65.280371 |
| Pond 3_out | Cabra | WWTP end of Pond 3 prior to discharge | 18.316927 | -65.280462 |
| Pond 4_out | Cabra | WWTP Pond 4 effluent | 18.317220 | -65.279270 |
| Pond 5_out | Cabra | End of Pond 5 prior to exiting the WWTP | 18.317229 | -65.280526 |

Monitoring Frequency

Water quality monitoring at all nine stations will be conducted by the Protectores de Cuencas on a monthly basis for at least one full year. This preliminary dataset will build on the 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.

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 robust indicators of watershed water quality conditions ([USCFTF Watershed Partnership Initiative Priority Ecosystem Indicators](#)). In particular, indicators of cultural eutrophication that are linked to wastewater management practices conducted in Culebra's urban area and Wastewater Treatment Plant, while also linked to potential changes in habitat response.

Changes in pollutant exposure will be evaluated through comparisons of site-specific measurements of indicators before and after the installation of floating treatment wetlands at the WWTP. If measures of indicators at monitoring sites demonstrate the reduction of nutrient fluxes from wastewater at the treatment plant and nonpoint sources in the Aeropuerto and Coronel watersheds, this would suggest that pollutant exposure—and ostensibly 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, nutrient, and pathogenic bacteria water quality threats; and (2) logistically feasible; and are outlined below.

- Indicators of water clarity: Turbidity; Optical Brighteners; Chlorophyll-a—measures the light emission at a certain wavelength to gain insight of phytoplankton photosynthetic efficiency; and CDOM—Colored Dissolved Organic Matter that absorbs light in the blue and UV part of the electromagnetic spectrum, giving water a yellow-brown color.
- Indicators of nutrients and pathogenic bacteria: Nitrogen (nitrite, nitrate, ammonia, ammonium, and total nitrogen), Phosphorus (total phosphorus, and orthophosphate—the simplest form of phosphorus that is up taken by photosynthetic organisms at the base of the marine food web), monochloramine—disinfectant used in municipal water treatment, and *Enterococcus* and *E. coli*.
- Water physical properties: Temperature, Conductivity / Salinity, Dissolved Oxygen, and pH.

5. Data Collection and Processing

Data and/or samples for water quality parameters are collected at surface and include: 1.) temperature; 2.) conductivity—to determine salinity; 3.) dissolved oxygen; 4.) pH; 5.) turbidity; 6.) chlorophyll-a; 7.) CDOM; 8.) nutrients and tracers; 9.) flow; and 10.) fecal indicator bacteria. Parameters 1–7 will be collected onsite using a Eureka Manta 35 Water Quality Sonde; a [handheld digital tester](#) will also be implemented to collect an additional measurement of salinity. Nutrients and fecal indicator bacteria will be assessed based on discrete water samples; three are collected at each monitoring station. Two water samples will be processed and analyzed at the Protectores de Cuencas Wet Laboratory on Culebra. One water sample is for nutrient analysis implementing the HACH instrumentation (SL100 Analyzer, DRB200 reactor, and DR3900 spectrophotometer) and reagent kits; the HANNA HI 96715 Portable Photometer and reagents; and the TUNNER AquaFluor Fluorometer. The second water sample is to detect the presence of Enterococci using the IDEXX Quanti-Tray system and the Most Probable Number (MPN) model. The third water sample will be shipped to the NOAA National Center for Coastal and Ocean Studies

(NCCOS) contract laboratory [Geochemical and Environmental Research Group](#) at Texas A&M University for analytical grade nutrient testing. Lastly, flow will be monitored at strategic WWTP locations during each sampling event by recording the time taken to fill a specific volume of water.

Due to the shallow nature of the system, water bodies are assumed to be well-mixed, therefore, no pond or stream profiles will be collected, and all water samples will be taken from 4–6 inches below the surface using a 500 ml capacity High Density Polypropylene graduated dipper. When collecting a sample, to the extent possible, avoid disturbing the water and sediment to prevent resuspending contaminants and artificially influencing parameter measurements and sensor readings. Use a purified water control for each measurement as appropriate and rinse all equipment three times with purified water and three times with water from the collected sample prior to taking discrete samples. Consistently disinfect hands between samples to avoid contaminating samples and note the surroundings at each sample location, detailing observations such as oil in the water, unexplained bubbles, rain events, or debris nearby in sample datasheets.

Eureka Manta+35 Water Quality Sonde



Figure 2. 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), CDOM (Colored Dissolved Organic Matter, ppb), and 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 calibration standards whose value is close to the values you expect to see in the field. Note: for the watershed water quality monitoring, confirm that conductivity/salinity is calibrated for freshwater and crosscheck with the handheld salinity tester using a 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.

To make measurements:

1. Submerge the sonde probe in the water at the designated sampling location making sure all probes are submerged. Alternatively fill up the cup.
2. Allow the sensors to stabilize before recording readings on the handheld and data sheet.
3. Save the measurements to the data log including sample ID and date in the filename.
4. Rinse the probes with purified water after sampling.

Maintenance:

5. 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.

6. Replace any o-rings with visible cracks. Keep o-rings greased with silicon grease (found in your Maintenance Kit).
7. Always remove batteries and clean your Manta prior to storing it for prolonged periods.
8. 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](#).

Discrete Water Samples

Three discrete water samples will be collected at each monitoring station for the analysis of nutrients and fecal indicator bacteria. Two samples (~250 ml) will be collected in [Low Density Polyethylene \(LDPE\) bags](#) (Figure 3) and an additional sample in a 125 ml HDPE bottle. Samples will be transported back to the Protectores de Cuenas Wet Laboratory on Culebra. Bagged samples will be analyzed for nutrients and fecal bacteria. Bottled samples will be frozen solid and shipped for additional EPA-certified analytical grade nutrient testing.



Figure 3. Sample bags for nutrients and fecal indicator bacteria.

Before you leave for the field make sure you have:

1. Pre-labelled sample bags and bottles.
 - a. LPDE bags—two per monitoring location; one for nutrients and one for fecal indicator bacteria.
 - b. 125 ml HDPE bottles—one per monitoring location for analytical-grade nutrient testing.
2. Nitrile gloves (one pair per person per site).
3. Extension pole with dipper pitcher.
4. Cooler ¼ full of wet ice for samples.
5. Distilled or deionized water for rinsing collection pitcher and bottles.
6. Blue ice (leave in freezer, for shipping).

To collect discrete water samples:

1. Put on fresh gloves and change gloves between sites.
2. Rinse the graduated dipper pitcher three times with purified water and three times with sample water, then collect the sample.
3. 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.
4. Fill each bag at least halfway or more with the sample.
5. Holding the bag by the wire ends, whirl the bag three complete revolutions to form a leak proof seal (Figure 3). Tie a knot with the wire ends to secure and test if sealed by flipping the bag upside down and squeezing lightly; place bags in the cooler.

6. For the additional 125 ml sample, rinse HDPE bottle by filling and draining completely three times including the cap at least once.
7. Fill the sample bottle with site water a fourth time leaving 10% head space to avoid damage and sample loss when freezing.
8. Cap bottle tightly and place in the cooler.

HACH SL1000 Nutrient Analysis

The HACH SL1000 Portable Parallel Analyzer (PPA) (Figure 4) will be used on the first ~250 ml bagged water sample to measure, nitrite, free and total ammonia, monochloramine, total nitrogen, orthophosphate, and nitrate, in parallel using ChemKey reagents and probes. The day prior to or the morning of sampling, the PPA should be charged or changed to fresh batteries and the nitrate probe should be calibrated (to measure nitrate with the probe see section below) using the standard calibration reagents and procedures outlined on page 14 of the [Hach SL1000 Portable Parallel Analyzer \(PPA\) User Manual](#). Also, visit the [manufacturer's website](#) to learn about the [Instrument Sensor Parameters detection ranges](#).



Figure 4. HACH SL1000 Analyzer with Chem Keys.

To make measurements with ChemKeys:

1. Turn on the analyzer.
2. Use a bag or fresh pair of gloves to hold Chem Keys, do not touch them with bare fingers.
3. Quickly slide the ChemKey chips into slots in the bottom of the analyzer in one movement, pressing all the way to the back. The order in which the Keys are installed does not matter.
 - a. If you receive an error message, the barcode was likely not read correctly; take the Chem Key out with a bag or glove covering your hand and slide the key back in.
4. Rinse the sample cup with the sample.
5. Fill the meter sample cup to the fill-line with the sample.
6. Put the meter into the meter sample cup.
7. Wait for the sound alert and/or the meter removal animation (within 1 to 2 seconds), then immediately remove the meter from the sample cup.
8. Lay the meter flat and wait until the measurement is completed. The parameters have different reaction times. The display shows a progress bar with the time that remains until the measurement is completed.
9. The measurement values are shown on the display.
10. Save the ChemKey measurements to the data log including sample ID and date in the filename.
11. See Page 12 of [HACH SL1000 \(PPA\) User Manual](#) for illustrations on how to operate PPA ChemKeys.

12. For some errors and warnings, it is a requirement to clean the Chemkey slots, the optical windows, the barcode window, the sample detector or to replace the trays.

Maintenance:

1. Refer to the [HACH SL1000 \(PPA\) User Manual](#) for the detailed cleaning procedures.
2. Clean the exterior of the instrument with a moist cloth and a mild soap solution and then wipe the instrument dry as necessary.
3. Use isopropyl alcohol to clean the parts—ChemKey slots, optical and barcode windows, sample detector, and trays.
4. Use water and a mild detergent to clean the light shield.

To measure nitrate with the probe:

Before you start:

1. Read the [Nitrate ISE Probe Full User Manual](#) and the direct ISE nitrate measurement [Method 8358 Document](#) for reference.
2. To condition the probe, soak the probe in approximately 25 ml of the lowest concentration standard solution used for calibration for 30 to 60 minutes. Do not add the nitrate ionic-strength adjuster (ISA) to the standard solution.
3. Calibrate the probe according to the instructions on page 5 in the [Nitrate ISE Probe Full User Manual](#). Also, refer to Section 9 *Advanced Operation nitrate options* in the [HACH SL1000 \(PPA\) User Manual](#).
4. During calibration, measure the standard solutions from lowest to highest concentration for best results.
5. Make sure that the calibration solutions and the samples are at the same temperature (± 2 °C/ ± 3.6 °F) for best results.

To make measurement:

1. Rinse the sample flasks with sample water.
2. Fill sample flask with 25 ml of sample.
3. Dissolve one packet of nitrate (ISA) reagent in the sample flask, agitate the sample until no more clumps of salt remain in the bottom of the sample.
4. Submerge probe tip into sample and press “Read” on analyzer. Hold the probe in the sample until the reading stabilizes.
5. Record the stabilized probe value shown on the display on the data sheet and on the analyzer.

Note: The sensing element in the probe will measure other ions that are known to interfere, including perchlorate, iodide, bromide, and chloride. The probe response to other ions usually causes a positive error. To remove most of the interferences, make certain that the nitrate ionic-strength adjuster (ISA) is added, which adjusts the pH to between 3 and 5. The measurements are usually made at this pH range to remove carbonate and bicarbonate interference and to decrease the organic acid interference.

Maintenance:

1. Regular maintenance is necessary. Refer to the [Nitrate ISE Probe Full User Manual](#) for product overview.
2. For the best accuracy, stabilization time and life of the probe, fully rinse the probe with deionized water between measurements to keep the probe clean.
3. Signs of probe contamination include:
 - a. Incorrect or irregular readings.
 - b. Slow stabilization times.

- c. Calibration errors.
4. To remove contamination:
 - a. Rinse the probe with deionized water regularly to remove mineral or sample buildup on the sensing element.
 - b. Blot dry with a lint-free cloth. Do not touch the tip of the probe.
 - c. Polish the white reference junction on the probe tip with a soft cloth or cotton swab. Do not polish the PVC sensing element in the center of the probe tip. Do not use cleaning solutions.
 - d. Soak the probe for 30 minutes in a 100-mg/L nitrate-nitrogen (NO_3^- -N) standard solution, and fully rinse with deionized water.
5. Storage
 - a. Rinse probe with deionized water.
 - b. Make sure that the probe sensor is dry, then install the sensor protection cap.
 - c. Keep the sensor cap on to prevent damage to the sensing element.
 - d. Make sure to condition the probe after long-term storage; refer to page 5 in the [Nitrate ISE Probe Full User Manual](#).

HANNA Ammonia Medium Range Photometer

The HANNA HI 96715 Portable Photometer (Figure 5) will be used on the first ~250 ml bagged water sample to measure the total concentration of ammonia nitrogen ($\text{NH}_3\text{-N}$ mg/L) in the sample.



Figure 5: HANNA HI 96715 Ammonia Medium Range photometer.

Before you start:

Read the [Hanna Instruments HI 96715C Instruction Manual](#) for reference.

To make measurement:

1. Turn the meter on by pressing ON/OFF. The display briefly shows all tags on.
2. When the beeper sounds briefly and the LCD displays dashes, the meter is ready. The blinking "ZERO" indicates that the instrument needs to be zeroed first.
3. Fill the cuvette with 10 ml of unreacted sample, up to the mark, and replace the cap.
4. Place the cuvette into the cuvette holder and ensure that the notch on the cap is positioned securely into the groove.
5. Press ZERO/CFM and the lamp, cuvette and detector icons will appear on the display, depending on the measurement phase.
6. After a few seconds, the display will show "-0.0-". The meter is now zeroed and ready for measurement.

7. Remove the cuvette, open it and add 4 drops of HI 93715A-0 First Reagent (6 drops in case of seawater analysis). Swirl the cuvette gently to mix (do not shake; this can produce bubbles and interfere with the measurement).
8. Add 4 drops of HI 93715B-0 Second Reagent to the cuvette (10 drops in case of seawater analysis). Replace the cap and swirl the cuvette to mix.
9. Replace the cuvette into the cuvette holder and ensure that the notch on the cap is positioned securely into the groove.
10. Press and hold READ/ /TIMER for three seconds. The display will show the countdown prior to measurement. The beeper is playing a beep at the end of countdown period.
11. Alternatively, wait for 3 minutes and 30 seconds and just press READ/ /TIMER. In both cases, the lamp, cuvette and detector icons will appear on the display, depending on the measurement phase.
12. At the end of measurement, the instrument directly displays concentration in mg/L of ammonia nitrogen (NH₃-N) on the LCD.

Interferences:

Organic compounds like: chloramines, aldehydes, alcohols or acetone (negative error); Sulfide, color or suspended matter in large amounts may also cause interference. Read manual for reference

Turner AquaFluor Handheld Fluorometer

The AquaFluor handheld fluorometer (Figure 6) will be used on first ~250 ml bagged water sample to measure Chlorophyll-a (µg/L), and optical brighteners (ppm). Read the [AquaFluor Instruction Manual](#) for channel configuration and calibration procedures.



Figure 6: Turner AquaFluor Fluorometer.

To make measurement:

1. Install the 4 - AAA batteries and verify the batteries are positioned correctly.
2. The cuvette **MUST BE DRY** on the outside when taking readings. Any moisture or condensation on the outside of the cuvette can affect the reading.
3. To begin, turn the AquaFluor on by pressing <ON/OFF>. Wait 5 seconds for AquaFluor to warm up.
4. Fill the cuvette with at least 3 ml solution volume or at least 3/4 full. Significant error in the readings can result if the cuvette contains less than this minimum volume.
5. The AquaFluor is very sensitive and even small amounts of material from a previous sample may contaminate the sample and result in errors. Use a clean cuvette for all readings. If you are using the same cuvette for your samples, it is very important that you thoroughly rinse the cuvette between samples. A good way to confirm the cuvette cleanliness is to read a blank solution. If the

reading is higher than the normal blank reading, the cuvette is not clean. Any bubbles in the sample will affect the readings. Take care not to introduce bubbles into samples. Remove any bubbles by lightly tapping with your finger on the outside cuvette wall or cover the top of the cuvette and tilt the sample to help dissipate bubbles.

6. Insert your sample. The AquaFluor is designed with “ambient light rejection”. The black sample compartment cover does NOT need to be closed when reading samples. The orientation and cleanliness of the cuvettes can have an impact on the accuracy of your results. Refer to Section 4 in the [AquaFluor Instruction Manual](#) for details.
7. Press the button. The instrument will measure and average the fluorescence signal for 5 seconds.
8. The reading result will be displayed on the top line of the “Home” screen.
9. The top left corner will then display “WAIT” for 5 seconds.
10. Once “WAIT” disappears, another sample reading can be performed.

HACH Spectrophotometer Analysis for TN and TKN

Total nitrogen is measured spectrophotometrically on the first ~250 ml bagged water sample using the HACH equipment and reagents to digest the samples and react with indicator dyes. Required equipment includes the HACH [DRB200 reactor](#) (Figure 7), HACH [DR3900 Spectrophotometer](#) and light shield (Figure 8), and the HACH TNTplus® Reagent Sets, calibrated pipette of 1.0–5.0 ml and 0.1–1.0 ml, pipet tips, and a test tube rack (Figure 9). Note: All HACH vial tests are calibrated at 68 °F, therefore the sample temperature must be 59–77 °F before measuring for accurate results.



Figure 7. HACH DRB200 Reactor.



Figure 8. HACH DR3900 Spectrophotometer.



Figure 9. HACH TKN (s-TKN) TNT plus Vial Test

To make TKN and TN measurements:

1. Set the DRB200 reactor power to on. Set the temperature to 120 °C (248 °F).
2. Add 1.3 ml of sample, 1.3 ml of Solution A and 1 Reagent B tablet in quick succession to a dry 20-mm reaction tube. Close the reaction tube immediately. Do not invert.
3. Insert the reaction tube in the preheated DRB200 reactor. Close the lid.
4. Keep the reaction tube in the reactor for 30 minutes.
5. When the timer expires, carefully remove the reaction tube from the reactor. Let the temperature of the reaction tube decrease to room temperature.
6. Invert the reaction tube 2–3 times.
7. Use a pipet to slowly add 0.5 ml of the digested sample from the 20 ml reaction tube into a test vial 1 (red label).
8. Use a pipet to slowly add 0.2 ml of Solution D to test vial 1 (red label).
9. Quickly tighten the cap on the vial and invert until completely mixed. Immediately continue to the next step.
10. Use a pipet to slowly add 1.0 ml of undigested sample to a test vial 2 (green label).
11. Use a pipet to slowly add 0.2 ml of Solution D to test vial 2 (green label).
12. Quickly tighten the cap on the vial and invert until completely mixed.
13. Start the reaction time of 15 minutes.
14. When the timer expires, clean the vials.
15. Insert the test vial 1 (red label) into the cell holder.
16. Insert the test vial 2 (green label) into the cell holder.
17. Results show in mg/L for Total N, NO₃-N + NO₂-N, and mgL TKN.
18. Save the measurements to the data log including sample ID and date in the filename.

Remember: Total Nitrogen (TN) = nitrate + nitrite + TKN (ammonium + organic nitrogen). This relationship serves as a quick way to check your data. For specific method details and illustrations, please reference the [HACH TNTplus 880 Total Kjeldahl Nitrogen Method 10242](#) document.

Analytical Grade Nutrient Testing

Analytical grade nutrient testing will be conducted on the 125 ml water sample by a NOAA contract lab—[Geochemical and Environmental Research Group](#) at Texas A&M University—processed for nitrate (NO₃⁻ mg/L), nitrite (NO₂⁻ mg/L), orthophosphate (HPO₄⁻³ mg/L), ammonium (NH₄⁺ mg/L), total nitrogen (TN mg/L), total phosphorus (TP mg/L), and silica (HSiO₃⁻ mg/L). Newly cut grass can be a source of ammonium contamination to nutrient samples (USEPA, 2000) therefore, care should be taken to avoid nutrient sample collection during/shortly after grass cutting around the tertiary treatment system ponds.

Sample collection—remember:

1. Put on fresh gloves and change gloves between sites.
2. At each site avoid stirring up sediments when collecting water samples.
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. Repeat steps 1–5 at each watershed monitoring site.
7. At one site per sampling mission, fill one 125 ml bottle with distilled/purified water. These should be labeled “Field Blank”, and should be rotated around to different sites on each sampling trip.

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 date, sample time, exact alphanumeric bottle labels, the sites visited, the bottle number, volume and type of analyses, as well as the corresponding site salinities from the handheld salinity tester—this can be accessed from the corresponding field datasheets (see below) or the Cabra Water Quality Monitoring Data Repository.

Fecal Indicator Bacteria

The [INDEXX Quanti-Tray system](#) (Figure 10) will be used on the second ~250 ml bagged sample 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 10. 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 a sample in a 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 a permanent marker to write on the back of the 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. Fill out Fbig, Fsmall, etc. on the back of the tray.
16. Use the [Most Probable Number \(MPN\) model Table](#) to determine the number of *Enterococcus* and *E. coli* s in the water.

Flow

Flow within the WWTP's tertiary treatment system will be quantified upon each visit using a bucket and a stopwatch at the outfall of Ponds 3 and 4 (or the closest possible) quantify the flow of effluent into Ponds 4 and 5. Flow measurements together with nutrient concentrations are used to estimate the mass of nitrogen moving through and exiting the WWTP system, providing a means to evaluate the efficacy of the wastewater treatment system in removing noxious nitrogen compounds that impair terrestrial and marine wildlife.

To take flow measurements:

1. Place the bucket underneath the effluent pipe.
2. Press **Start** on a stopwatch and immediately begin filling the bucket by allowing effluent to pour from the effluent pipe into the bucket.
3. Press **Stop** on the stopwatch at the moment the bucket fills to the 5-gallon line.
4. Repeat fill and timing procedure three times.
5. Find the average fill time (in seconds) of the three measurements.
6. Divide 5-gallons by the average number of seconds taken to fill and record this flow measurement as X gal/sec on the sample data sheet.

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 is stored in multiple places and in multiple media. A Culebra Watershed 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 2 of the following three places at all times:
 - a. Durable Excel or paper field data sheets.
 - b. Online Google Forms
 - c. Sampling instrumentation data logs if storage is enabled—keep in mind this is not a permanent or secure storage.
2. Durable excel or paper field data sheets (section 8) must be filled for the field and lab data collection.
3. Google Forms are used to transcribe the field and lab data into digital media for cloud storage and can be filled out and submitted on cell phones or tablets in the field and in the lab. There are three google forms which must be filled out and submitted on a site-by-site basis, i.e., one form submitted per monitoring site.
 - a. [In-Field Data sheet](#) (Eureka Sonde).
 - b. [Nutrient Data sheet](#) (Lab HACH parallel analyzer and spectrophotometer).
 - c. [Fecal Indicator Datasheet](#) (Lab Enterolert Test).
4. Submitted data will be uploaded by the Data Manager to the corresponding data folder Culebra Watershed Water Quality Data Google drive folder.
5. Populated excel/paper field datasets must be signed by the sampling personnel and scanned.
 - a. Hardcopies will be stored in a binder in the Protectores de Cuencas Laboratory. This binder is not to be taken to the field for sampling to prevent previous records from being damaged.
 - b. Scanned field datasheets will be uploaded to the shared Watershed Water Quality Datasheets folder, located in the Culebra Watershed Water Quality Data Google drive folder.
 - c. It is also good practice to store pre- and post-sampling checklists (see below) in the data binder.
6. Once the Google Forms and excel/paper datasheets for each monitoring site per episode have been submitted, scanned, and uploaded the Data Manager will upload the corresponding data to the Culebra Watershed Water Quality data repositories located in the Culebra Water Quality Data Google drive folder.
7. Together with the Data Manager the Monitoring Coordinator will check the uploaded watershed [field](#) and [lab](#) data for quality and integrity against the field datasheets, and make modifications, corrections, and updates as appropriate. Any data changes should be communicated and discussed with the Data Manager prior to implementation.

7. Checklists

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
 - Blue-green Algae

Sampling Equipment:

- Eureka Sonde
- Cooler with ice for sample storage
- Purified water for rinsing
- GPS or phone with FieldMaps App
- Sampling bags, bottles, lids, fasteners
- Sharpie to mark samples
- Graduated dipper and sample bucket with handle
- 5-gallon bucket
- Stopwatch or cellphone with stopwatch app
- Gloves
- Disinfectant for hands
- 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
- Hach SL1000 Portable Parallel Analyzer
- Hach ChemKeys (nitrite, total ammonia, orthophosphate) and reagents (nitrate, TKN, TN)
- Hach Digital Reactor Block, light shield, and Spectrophotometer
- Calibrated pipettes and tips for Hach Spec. analysis
- Test tubes and racks

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
- Oven turned off, Quanti-tray sealer and UV light unplugged
- Hach SL1000 Portable Parallel Analyzer turned off, all used ChemKeys removed and disposed of
- Hach Spectrophotometer turned off, and samples disposed of

Sampling Equipment:

- Sampling bags, bottles, lids, fasteners cleaned or appropriately disposed of
- 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
- Nutrient samples shipped to collaborating laboratory

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

Signature: _____

Date: _____

8. Field Datasheets

Watershed Water Quality Monitoring Field Sheet:

Site: _____ Date: _____ Time: _____

Recorded by: _____ Crew: _____

| Parameter | Units | Value |
|---|--------------|-------|
| Temperature (Eureka) | Celcius | |
| pH (Eureka) | SU | |
| Conductivity (Eureka) | us/cm | |
| Dissolved Oxygen (Eureka) | mg/L | |
| Dissolved Oxygen (Eureka) | % Saturation | |
| Chlorophyll a (Blue) (Eureka) | µg/L | |
| CDOM (Eureka) | µg/L | |
| Chlorophyll a (Red) (Eureka) | µg/L | |
| Turbidity (Eureka) | NTU | |
| Salinity (Eureka) | PSU | |
| Salinity (Handheld sensor) | ppt | |
| Ammonia (Hanna field sensor) | mg/L | |
| Chlorophyll a (Aquafluor field sensor) | µg/L | |
| Optical Brightness (Aquafluor field sensor) | µg/L | |
| Turbidity (Hach field sensor) | NTU | |

Flow

| Site ID | Sample Replicate | Bucket Size (gal) | Time to Fill (sec) | Average Fill Time | Flow (gal/sec) |
|---------|------------------|-------------------|--------------------|-------------------|----------------|
| | Sample 1 | | | | |
| | Sample 2 | | | | |
| | Sample 3 | | | | |
| | Sample 1 | | | | |
| | Sample 2 | | | | |
| | Sample 3 | | | | |

Notes:

Watershed Water Quality Monitoring Lab Nutrients and Bacteria Data Entry Sheet:

Site: _____ Date: _____ Time: _____

Recorded by: _____ Crew: _____

| Parameter | Units | Value |
|------------------------------------|-------|-------|
| Nitrite (NO ₂) | mg/L | |
| Monochlorine | mg/L | |
| Free Ammonia (NH ₃) | mg/L | |
| Total Ammonia (NH ₄) | mg/L | |
| Orthophosphate (OPO ₄) | mg/L | |
| Nitrate (NO ₃) | mg/L | |
| Total Kjeldahl Nitrogen (TKN) | mg/L | |
| Total Nitrogen (TN) | mg/L | |

Analytical Grade Nutrient Testing Samples—List number & volume of bottle(s)

Surface Nutrients: _____

Bacteria:

Large wells: _____ Small wells: _____ Total: _____

| Bacteria | | |
|------------------|-----------|--|
| Fecal Coliforms | MPN/100ml | |
| Escherichia Coli | MPN/100ml | |

Notes:

9. Estimated Cost

| Category | Item Description | Cost per unit | Unit | Units / Day | Days / Trips | # Persons | Per Year | Annual Total | Notes |
|--------------|------------------------------|---------------|--------------------|-------------|--------------|-----------|----------|--------------------|--|
| Staff | Watershed field biologists | \$35.00 | hour | 10 | 2.5 | 2 | 12 | \$21,000.00 | Estimating 2 scientists; 12 trips a year (monthly sampling) for 2, 10-hr days and 1-half day to collect the sonde measurements and discrete samples, analyze for nutrients and fecal indicators, and pack water samples for shipment. |
| Travel | Ferry | \$75.00 | roundtrip / truck | 1 | 1 | 1 | 12 | \$900.00 | This accounts for costs of bringing one PDC staff + one pick-up truck with equipment. |
| Travel | Ferry | \$10.00 | roundtrip / person | 1 | 1 | 1 | 12 | \$120.00 | 1 staff. |
| Travel | Gas | \$150.00 | truck / trip | 1 | 1 | 1 | 12 | \$1,800.00 | Estimated one pick-up truck fuel consumption from Yauco to Culebra roundtrip and within Culebra. |
| Travel | Lodging | \$159.00 | day | 1 | 2 | 2 | 12 | \$7,632.00 | Estimating that field biologists will arrive in Culebra the night before sampling, stay the night prior to the sampling and the night of sampling, and then depart on the evening of the second work day. |
| Travel | Per Diem (full days) | \$105.00 | day | 1 | 2 | 2 | 12 | \$5,040.00 | Estimating that field biologists will arrive in Culebra the night before sampling, stay the night prior to the sampling and the night of sampling, and then depart on the evening of the second work day. |
| Travel | Per Diem (travel days) | \$78.75 | day | 1 | 1 | 2 | 12 | \$1,890.00 | Estimating that field biologists will arrive in Culebra the night before sampling. |
| Services | Analytical nutrient testing* | \$109.50 | sample | 10 | 1 | 1 | 12 | \$13,140.00 | Estimated average cost per sample (nutrients or tracers) including: supplies, instruments / equipment, shipping, and analyses. |
| Equipment | Equipment rental fee* | \$1,145.00 | trip | 1 | 1 | 1 | 12 | \$13,740.00 | Estimated as total cost for purchasing 1/2 Eureka sonde (\$ 13000.00), 1/3 IDEXX system for fecal indicators (\$ 4300.00) and HACH Lab Equipment and supplies for nutrients (\$ 23,600.00) = \$40,800.00, recovered over a 3-year time horizon in monthly rental fee installments (~ \$1143.00). |
| Total | | | | | | | | \$65,262.00 | |

* 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. References

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USEPA. (2021) Indicators: Enterococci [Overviews and Factsheets]. <https://www.epa.gov/national-aquatic-resource-surveys/indicators-enterococci>

Relevant Links

Culebra WWTP EPA Permit: https://www.epa.gov/sites/default/files/2017-07/documents/final_culebra_wwtp.pdf

Eureka Manta Manual: https://b3a3d385-13c7-4581-b536-17695817f1a5.filesusr.com/ugd/7f6545_5070d8ef8e194d888b39684c72fced62.pdf

HACH SL1000 User Manual: <https://www.hach.com/asset-get.download-en.jsa?id=24364820991>

HACH TKN Method: <https://www.hach.com/asset-get.download-en.jsa?id=7639982525>

HACH TN Method: <https://www.hach.com/asset-get.download-en.jsa?id=7639983805>

Whirl-Pak Bags: <https://www.uline.com/PDF/IS-16551-SPFR.PDF>

IDEXX Enterolert 24-hour detection of enterococci: <https://www.idexx.com/en/water/water-products-services/enterolert/>