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APPENDIX A. MORRO BAY NATIONAL ESTUARY PROGRAM BACTERIA MONITORING PROTOCOLS

Creek Bacteria Protocol (Total Coliform and *E. coli*)

Background

- The Estuary Program uses Colilerti tests for total coliforms and *E. coli*
- These tests use the Most Probable Number (MPN) technique, which uses multiple tubes with broth that are inoculated with the sample at different dilutions. After incubation, the tubes show a positive or negative reaction for the target organism. The number of positive tubes corresponds to a statistical probability that the sample contained a certain number of bacteria The IDEXX Colilert method is based on this same principle.
- At this time, the Estuary Program only tests total coliform and *E. coli* in freshwater samples, although *E. coli* can also be detected in marine samples.

Equipment

- 250 mL autoclavable bottle per site
- Two sealed IDEXX bottles per site
- Disposable gloves (one pair per site)
- Data collection sheet
- Pencil
- Clipboard
- Sharpie pen for labeling sample containers
- Ice chest
- Ice packs
- Boots

Sites

- Chorro Creek above the Ecological Reserve (UCR)
- Chorro Creek at Chorro Creek Road (CCC)
- Chorro Creek at South Bay Boulevard Bridge (TWB)
- Pennington Creek on Cal Poly property, at the bridge (CPN)
- Pennington Creek on Rancho El Chorro property at the amphitheater (PEN)
- Dairy Creek near the dog park (DAM)
- San Luisito Creek near Adobe Road (SLU)
- San Bernardo Creek (SBC)
- Warden Creek at Turri Road (TUR)
- Los Osos Creek at Clark Valley Road (CLV)

Sample Collection

1. Fill out data collection form with date, sampler names, weather in past 24 hours, current weather and recent rainfall. In the notes section of the data collection form, note things such as presence of animals and birds, abundant leaf matter, any strange debris, any unusual smells, etc.
2. Label two of the sealed IDEXX sample bottles with the three-letter site code and the date and time the sample was collected.
3. Wear gloves to protect both you and the sample.
4. Select where you will be collecting your sample: Site should be free of algal blooms, surface debris, oil slicks and congregations of water fowl. Avoid agitating the bottom sediment. Avoid splashing water. Choose area with good mixing and no stagnant pools.

5. If entering the water to take the sample, be careful to not stir up sediment. Face upstream to take the sample, so that you do not affect the sample.
6. Uncap a 250 mL autoclavable bottle, being careful not to touch the inside of the bottle or cap.
7. Submerge bottle underwater. Try to collect the sample 12” underwater or at 1/3 of the depth below the surface.
8. Fill the sample bottle with water, with the bottle facing into the current. Recap the bottle.
9. Be sure to leave headspace in the container.
10. Split the samples among the IDEXX bottles:
    - Remove the plastic seal from each IDEXX bottle and ensure that they are labeled.
    - Shake the 250 mL sample bottle vigorously approximately 25 times in a one-foot arc, within 7 seconds or less.
    - Fill each IDEXX bottle at least to the 100 mL line. Be sure to leave airspace at the top.
    - Leave the extra sample in the 250 mL bottle, then you'll know that bottle has already been used.
    - Tightly seal the IDEXX bottles and place them in the cooler.
11. At your next site, repeat the procedure with a new sterile 250-mL bottle at each site.
12. Change gloves at each site.
13. Ideally, samples will arrive at the lab within eight hours of collection, and must be maintained at 4°C or lower. If they can’t be tested within eight hours, they must be tested within 24 hours of collection.

Procedure for Total Coliform and *E. coli* Sample Analysis

All lab analysis should be conducted at the Cuesta College bacteria lab. This lab is located in the Biological Sciences building 2200, near parking lot 5. For more information on how to access this space, please contact Fiona Litton at (805) 242-6945 or Cheyenne Winn at (805) 234-1571. It is recommended to regularly check the Colilert reagent product inserts for updates to the IDEXX protocol. Previous changes to the IDEXX methods have taken place without notice.

To see the latest Colilert product insert, scan the QR code below and enter the product number **06-12999-11**:

Equipment

- Sterile deionized water
- Sterile pipette tips
- Pipettor
- Sterile, plastic mixing bottles (two per each site)
- Disposable gloves
- Safety glasses
- Colilert reagent packet
- Quanti-Tray/2000 tray
- Incubator (set for 35°C +/- 0.5°C)
- Quanti-Tray sealer and rubber insert

Sample prep

1. Wash your hands using the anti-bacterial soap.
2. Put on gloves and safety glasses.
3. Turn on the Quanti-Tray sealer (power switch on back, right hand side, just above the power cord) so it has time to pre-heat. The amber light will go on. The sealer is ready when the green light also goes on. To ensure it is adequately ready, wait another minute after the light turns green before using. Make sure there is nothing behind the sealer that would block trays from exiting after they are sealed.
4. For each creek site, please run a sample that is undiluted (1:1) and diluted (1:10) following the instructions below. Use new, sterile equipment for each site (pipettes, plastic mixing bottles, etc.).

5. As you go through the following steps, keep an eye on the time. After the reagent and the sample are mixed, you have 30 minutes to get the sample into the incubator. If this time is exceeded for any reason, you will need to re-process the sample.

For undiluted samples (1:1 dilution)

1. Mix the sample: shake sample bottle vigorously approximately 25 times in a one-foot arc, within 7 seconds or less. You must use the mixed sample within two minutes. If more than two minutes have passed, re-mix the sample.
2. Pour out excess sample so that you have only 100 mL sample remaining. You can use a clean pipette tip with the pipettor to remove the excess sample and dispose of down the sink.
3. Add Colilert reagent packet. The reagent packet should make a snapping sound when opened, be free-flowing, and should be light in color.
4. Re-cap container after adding reagent.
5. Shake vigorously to dissolve the reagent completely. If a blue flash occurs upon addition of Colilert reagent, the sample is considered invalid due to excessive chlorine and testing should be discontinued.
6. If sample is foamy, let sit for a few minutes to allow the foam to settle.
7. Sample is ready to add to Quanti-Tray.

For diluted samples (1:10 dilution)

1. Add 90 mL of sterile DI water from squeeze bottled labeled 'sterile DI water' into graduated cylinder. Add to the plastic mixing bottle.
2. Add Colilert reagent to the mixing bottle.
3. Cap and shake vigorously to dissolve the reagent.
4. Prepare the IDEXX sample bottle. Mix the sample: shake sample bottle vigorously approximately 25 times in a one-foot arc, within 7 seconds or less. You must use the mixed sample within two minutes. If more than two minutes have passed, re-mix the sample.
5. Put sterile tip onto pipettor. Check that the pipettor reads 10 mL. Use a pipettor to remove 10 mL from the center of the sample bottle. Be careful to not tip the pipette once you have filled it – you must keep the pipette upright so that the sample does not contaminate the paper plug in the pipettor.
6. Add sample to the plastic mixing bottle, with the DI water and reagent. Shake to mix. If a blue flash occurs upon addition of Colilert reagent, the sample is considered invalid due to excessive chlorine and testing should be discontinued.
7. If sample is foamy, let sit for a few minutes to let foam settle.
8. Sample is ready to add to Quanti-Tray.

To put sample in Quanti-Tray

1. Look over the tray and make sure it has no tears or wrinkles in the seals.
2. First label the back of the Quanti-Tray with a Sharpie marker. Mark the site code, date, time of testing and dilution. Label the back of the tray as '*E. coli*' to avoid confusion with other tests.
3. Use one hand to hold the Quanti-Tray upright with the well side facing the palm.
4. Squeeze the upper part of the Quanti-Tray so that the tray bends toward the palm.

5. Gently pull foil tab to separate foil from the tray. Avoid touching the inside of the foil or tray.
6. Pour the sample directly into the tray, avoiding contact with the foil. Tap the tray against the countertop sharply 2-3 times to release any air bubbles. Allow foam to settle.

To seal the Quanti-Tray

1. Wait until both the amber and green lights are lit, indicating sealer is pre-heated.
2. Check behind the sealer to ensure that nothing is blocking the slot where trays exit the sealer.
3. Place the empty sealer rubber insert on the input shelf with the large cutout facing away from the sealer.
4. Place the tray filled with sample onto the rubber insert, making sure that the tray is properly seated on the rubber insert, with each well of the tray in its corresponding hole in the rubber insert.
5. Slide the insert into the sealer until the rollers grab it and draw it into the sealer.
6. In approximately 5 seconds, the tray will be sealed and partially ejected from the rear of the sealer. Remove the rubber insert and tray from the rear of the sealer.
7. If you wish to reverse the motor drawing the rubber tray into the sealer (if a misaligned tray is accidentally fed into the sealer), press and hold the reverse button on the front of the sealer. However, do NOT reverse the motor once the rubber insert has been drawn fully into the sealer.
8. When done sealing all trays, turn the sealer off.

NOTE: There must be water present in the top well for the results to be valid. Alert staff if no water is present in the top well after sealing.

Incubating the samples

1. Be careful to place your samples in the incubator labeled '*E. coli*'.
2. The incubator does not need to be pre-heated before the samples are incubated. Do not change any of the settings on the incubators.
3. Once all trays have been sealed, place them in the incubator on the wire shelves. They can be stacked on top of each other. Leave space between the stacks of trays to allow air circulation. Do not place anything on the bottom floor of the incubator.
4. On the data sheet, note the time the samples were put into the incubator and the incubator temperature, which should be taken off the thermometer inside the incubator (not the digital read-out on the front of the incubator). The incubator temperature should be 35°C +/- 0.5°C during the incubation period.

Cleaning up

1. Triple rinse all autoclavable mixing bottles with hot tap water. Do not use soap. Once rinsed, place them in the bin that is labeled 'DIRTY'. Screw caps lightly onto the bottles but don't tighten.
2. Rinse the pipette tips and place in the autoclavable tray and place in bin.
3. Put the graduated cylinder in the bin of dirty glassware. Cover the top with foil, located in the drawer.

4. Triple rinse the 250 mL sample collection bottles and place the caps on loosely. Place in the bin of dirty glassware.
5. Staff will sterilize the equipment before your next visit to the lab.
6. Rinse the IDEXX collection bottles and place in the recycle bin. Throw the lids in the garbage since they are not recyclable.
7. Dispose of gloves in the trash. Wash hands with anti-bacterial soap.

Reading the Sample Trays for Total Coliform and *E. coli*

The trays must incubate between 24and 28 hours at 35°C before reading. If you read the trays later than the 28-hour incubation time period, the results are not valid.

Fill out the bottom section of the data collection form with your name, the incubator temperature and the date and time the results were read.

For total coliform results

1. Pull out the color comparator from the drawer. It is located in a red bag that says "DO NOT AUTOCLAVE".
2. Hold the incubated Quanti-Tray and comparator out at arm's length and below eye level. This makes it easier to see the color change.
3. Count the number of large wells (including the large rectangular well at the top of the tray) and small wells that are as yellow or more yellow than the comparator tray. These wells as positive for total coliforms. All wells that are less yellow than the comparator are negative for total coliforms.
    a. There are a total of 49 large wells and 48 small wells per tray. The 49 large wells include a large rectangular well at the top of the tray.
4. Record how many wells are more yellow than the comparator (positive for total coliforms) on the data collection sheet.
5. Use the MPN chart and based on the number of large and small wells that changed color, record the MPN value on the datasheet.
6. If your sample was diluted, you must multiply the MPN value by the dilution factor (i.e., multiply by a factor of 10 if diluted original sample 1:10) to get the MPN value for the original, undiluted sample.

For *E. coli* results

1. Put the incubated Quanti-Tray and color comparator into the UV cabinet and push the red button to turn on the UV light.
2. Look through the viewing screen on the cabinet and count the number of large and small wells that are both fluorescing AND yellow. They must be fluorescing as much or more than the color comparator. Fluorescence of any color (as long as the well is yellow under ambient light) is considered a positive well for *E. coli*.
3. Record number of yellow and fluorescent wells on the data sheet (positive for *E. coli)*.
    a. If it is difficult to distinguish whether the well is both yellow and fluorescing when it is in the cabinet, use a Sharpie to make each of the fluorescing wells while it is in the cabinet. Then pull the tray out of the cabinet and verify that all of those fluorescing wells also have the yellow color change.

b. If the well fluoresces but did not turn yellow, this is not a positive result for *E. coli* but rather some other undetermined organism.

4. Look up the MPN value from the chart and record on the data sheet.
5. If your sample was diluted, then you must multiply the MPN value by the dilution factor (i.e., multiply by a factor of 10 if diluted original sample 1:10) to get the MPN value for the original, undiluted sample.
6. Be sure to turn off the UV light when you are done.
7. Return the color comparator to its red bag in the drawer. Do NOT autoclave the comparator!

Once the readings are complete

1. The trays must be autoclaved before they can be thrown out. Double bag the trays in two autoclave bags and place in the bin of dirty glassware to be autoclaved. Staff will autoclave and then dispose of the trays. Do not use twist ties on the bags.
2. Do NOT turn off the incubator.

Bay Bacteria Protocol (Enterococcus)

Background

- Enterococci are a group of bacteria found primarily in the intestinal tract of warm-blooded animals. Unrelated to coliforms, they are a subgroup of the fecal streptococci group. Enterococcus is less sensitive to salt than *E. coli* and is thus considered a better indicator in marine environments.
- The Estuary Program uses Enterolert tests to monitor enterococci in marine samples, although the tests may also be used for freshwater samples.
- Enterolert tests are based on the Most Probable Number (MPN) technique, which uses multiple tubes with broth that are inoculated with the sample at different dilutions. After incubation, the tubes show a positive or negative reaction for the target organism. The number of positive tubes corresponds to a statistical probability that the sample contained a certain number of bacteria.

Equipment

- One sealed IDEXX bottle per site
- Disposable gloves (one pair per site)
- Data collection sheet
- Pencil
- Clipboard
- Sharpie pen for labeling sample containers
- Ice chest
- Ice packs
- Boots
- Tide book
- Extendable sampling pole

Sites

Front Bay:

- Coleman Park (COL)
- Tidelands Park (TID)
- Windy Cove (WIN)
- State Park Marina (SPM)

Back Bay:

- Pasadena Point (PAS)
- Baywood Park (BAY)
- Cuesta Inlet (CIN)
- Sharks Inlet (SIN)
-

Creek Sites (tidally-influenced):

- Los Osos Creek near South Bay Blvd (GS1)

Sample Collection

Note: For most bay sites, sample on an outgoing tide on a high enough tide to ensure safe access (> 4ft). GS1 should be treated as bay site although they are collected near a low tide (<2ft).

1. Fill out data collection form with date, sampler names, weather in past 24 hours, current weather, time of high tide and height of high tide. There is a tide book in the lab. In the notes section, note things such as presence of animals and birds, abundant leaf matter, any strange debris, any unusual smells, etc.
2. Record the three-letter site code and the date and time the sample was collected.
3. Wear gloves to protect both you and the sample.
4. If sampling on a beach, collect it on an incoming wave.
5. Select where you will be collecting your sample: Site should be free of algal blooms, surface debris, oil slicks and congregations of water fowl. Avoid agitating the bottom sediment. Avoid splashing water. Choose site with good mixing and no stagnant pools.
6. If entering the water to take the sample, be careful to not stir up sediment.
7. Remove the plastic wrap that seals the bottle.
8. Unseal and uncap a 120 mL IDEXX bottle, being careful not to touch the inside of the bottle or cap.
9. Submerge bottle underwater. Try to collect the sample 12” underwater or at 1/3 of the depth below the surface.
10. Fill the sample bottle with water, with the bottle facing into the current. Recap the bottle.
11. Be sure to leave headspace in the container.
12. Store in the ice chest.
13. Change gloves at each site.
14. Ideally, samples will arrive at the lab within eight hours of collection, and must be maintained at 4°C or lower. If they can’t be tested within eight hours, they must be tested within 24 hours of collection.

Procedure for Enterococcus Sample Analysis

Sample analysis procedure is very similar as Colilert testing, but needs a different type of reagent and a different incubator. Only one bottle is needed per site since only 10 mL are needed for each test.

All lab analysis should be conducted at the Cuesta College bacteria lab. This lab is located in the Biological Sciences building 2200, near parking lot 5. For more information on how to access this space, please contact Nick Soares at (805) 316-4160 or Cheyenne Winn at (805) 234-1571.

Equipment

- Sterile deionized water
- Sterile pipette tips
- Pipettor
- Sterile, plastic mixing bottles
- Disposable gloves
- Safety glasses
- Enterolert reagent packet
- Quanti-Tray/2000 tray
- Incubator (set for 41 +/- 0.5°C)
- Quanti-Tray sealer and rubber insert

Sample prep

1. Wash your hands using the anti-bacterial soap.
2. Put on gloves and safety glasses.
3. Turn on the Quanti-Tray sealer (power switch on back, right hand side, just above the power cord) so it has time to pre-heat. The amber light will go on. The sealer is ready when the green light also goes on. To ensure it is adequately ready, wait another minute after the light turns green before using. Make sure there is nothing behind the sealer that would block trays from exiting after they are sealed.
4. Collect one sterile mixing bottle per site. You must use new, sterile equipment for each site and each dilution (pipettes, plastic mixing bottles, etc.).
5. As you go through the following steps, keep an eye on the time. After the reagent and the sample are mixed, you have 30 minutes to get the sample into the incubator. If this time is exceeded for any reason, you will need to re-process the sample.

For all bay samples (1:10 dilution)

1. Add 90 mL of sterile DI water from squeeze bottled labeled ‘sterile DI water’ into graduated cylinder. Add to the plastic mixing bottle.
2. Add Enterolert reagent packet to the mixing bottle. The reagent packet should make a snapping sound when opened, be free-flowing, and should be light in color.
3. Cap and shake vigorously to dissolve the reagent.
4. Prepare the IDEXX sample bottle. Mix the sample: shake sample bottle vigorously approximately 25 times in a one-foot arc, within 7 seconds or less. You must use the mixed sample within two minutes. If more than two minutes have passed, re-mix the sample.

5. Put sterile tip onto pipettor. Check that the pipettor reads 10 mL. Use a pipettor to remove 10 mL from the center of the sample bottle. Be careful to not tip the pipette once you have filled it – you must keep the pipette upright so that the sample does not contaminate the paper plug in the pipettor.
6. Add sample to the plastic mixing bottle, with the DI water and reagent.
7. If sample is foamy, let sit for a few minutes to let foam settle.
8. Sample is ready to add to Quanti-Tray.

To put sample in Quanti-Tray

1. Look over the tray and make sure it has no tears or wrinkles in the seals.
2. First label the back of the Quanti-Tray with a Sharpie marker. Mark the site code, date, time of testing and dilution. Label the back of the tray as 'Entero'.
3. Use one hand to hold the Quanti-Tray upright with the well side facing the palm.
4. Squeeze the upper part of the Quanti-Tray so that the tray bends toward the palm.
5. Gently pull foil tab to separate foil from the tray. Avoid touching the inside of the foil or tray.
6. Pour the sample directly into the tray, avoiding contact with the foil. Tap the tray against the countertop sharply 2-3 times to release any air bubbles. Allow foam to settle.

To seal the Quanti-Tray

1. Wait until both the amber and green lights are lit, indicating sealer is pre-heated.
2. Check behind the sealer to ensure that nothing is blocking the slot where trays exit the sealer.
3. Place the empty sealer rubber insert on the input shelf with the large cutout facing away from the sealer.
4. Place the tray filled with sample onto the rubber insert, making sure that the tray is properly seated on the rubber insert, with each well of the tray in its corresponding hole in the rubber insert.
5. Slide the insert into the sealer until the rollers grab it and draw it into the sealer.
6. In approximately 5 seconds, the tray will be sealed and partially ejected from the rear of the sealer. Remove the rubber insert and tray from the rear of the sealer.
7. If you wish to reverse the motor drawing the rubber tray into the sealer (if a misaligned tray is accidentally fed into the sealer), press and hold the reverse button on the front of the sealer. However, do NOT reverse the motor once the rubber insert has been drawn fully into the sealer.
8. When done sealing all trays, turn the sealer off.

NOTE: There must be water present in the top well for the results to be valid. Alert staff if no water is present in the top well after sealing.

Incubating the samples

1. Be careful to place your enterococcus samples in the incubator labeled 'Enterococcus'.

2. The incubator does not need to be pre-heated before the samples are incubated. The incubator temperature should be between 41 +/- 0.5°C during the incubation period.
3. As you finish sealing each tray, place them in the incubator on the wire shelves of the incubator. They can be stacked on top of each other. Leave space between the stacks of trays to allow air circulation. Do not place anything on the bottom floor of the incubator.
4. On the data sheet, note the time the samples were put into the incubator and the incubator temperature, which should be taken off the thermometer inside the incubator (not the digital read out).

Cleaning up

1. Triple rinse all autoclavable mixing bottles with hot tap water. Do not use soap. Once rinsed, place them in the bin that is labeled 'DIRTY'. Screw caps lightly onto the bottles but don't tighten.
2. Rinse the pipette tips and place in the autoclavable tray and place in bin.
3. Put the graduated cylinder in the bin of dirty glassware. Cover the top with foil, located in the drawer.
4. Triple rinse the 250 mL sample collection bottles and place the caps on loosely. Place in the bin of dirty glassware.
5. Staff will sterilize the equipment before your next visit to the lab.
6. Rinse the IDEXX collection bottles and place in the recycle bin. Throw the lids in the garbage since they are not recyclable.
7. Dispose of gloves in the trash. Wash hands with anti-bacterial soap.

Reading the Sample Trays for Enterococcus

The trays must incubate between 24 to 28 hours at 41 +/- 0.5°C before reading. If you read the trays later than the 28-hour incubation time period, the results are not valid.

Fill out the bottom section of the data collection form with your name, the incubator temperature and the date and time the results were read.

1. Put the tray into the UV cabinet and push the red button to turn on the UV light.
2. Look through the viewing screen on the cabinet. Count the number of large and small wells that are fluorescing blue. These wells are positive for enterococcus. Any amount of fluorescing is considered to be a positive result.
    a. There are a total of 49 large wells and 48 small wells per tray. The large, rectangular well at the top of the tray is counted as a large well.
    b. If a well is blue but not fluorescing, wait until the end of the 28-hour incubation time-frame to re-read the sample. Contact staff if this occurs.
3. Record number of blue fluorescing wells on the data sheet. If the wells fluoresce a color other than blue, do not record these as positive wells.
4. Look up the MPN value from the chart (the same chart as for the Colilert-18 results) and record on the data sheet.
5. Multiply the result by the dilution factor (i.e., multiply by a factor of 10 if diluted original sample 1:10).
6. Be sure to turn off the UV light when you are done.

Once the readings are complete

1. The trays must be autoclaved before they can be thrown out. Double bag the trays in two autoclave bags and place in the bin of dirty glassware to be autoclaved. Lab personnel will autoclave and then dispose of the trays. Do not use twist ties on the bags.
2. Do NOT turn off the incubator.

Morro Bay National Estuary Program Bacteria Monitoring

Date of Sample Collection:	
Name of Sample Collector:	
Name of Sample Analyst:	
Rainfall in past week:	

High Tide Height:	
High Tide Time:	
Weather, past 24 hours:	
Weather currently:	

Site	Collection Time	Analyte	Dilution Factor	# Large Wells	# Small Wells	MPN/100 mL	Notes
		Total coliforms					
		E.coli					
		Enterococcus					
		Total coliforms					
		E.coli					
		Enterococcus					
		Total coliforms					
		E.coli					
		Enterococcus					
		Total coliforms					
		E.coli					
		Enterococcus					

	Colilert-18	Enterolert
Date of analysis:		
Time incubation started:		
Incubator temp when started:		
Name of sample reader:		
Date samples read:		
Time samples read:		
Incubator temp when read:		

Estuary Program Office: 805-772-3834

Morro Bay Police: 805-772-6225

Morro Bay Harbor Patrol: 805-772-6254

Emergencies: 911

Confirm well count: _____

Entered by: _____ Date: _____

QA'd by: _____ Date: _____

Bacteria Quality Assurance (QA) Protocol

Overview: Each month, the Estuary Program runs a series of tests to assess our data quality. These quality assurance (QA) measures include running a frozen sample at a known concentration to test accuracy, running a blank sample of deionized water to test to check sterility, and running duplicate samples to assess precision.

1. QA Procedure for Creek Sites

Periodically you will be asked to conduct additional procedures for quality assurance (QA) purposes. These include a precision test, an accuracy test, and a sterility test.

- Bring all necessary sampling gear, as outlined in the Creek Bacteria Protocol.
- Choose one of your sites to be the QA site for the month.
- For your QA site, you will need one additional IDEXX bottle (three total for QA site)
- For all other sites, bring two IDEXX bottles as usual.

At the site…

1. At your QA site, collect the 250mL Nalgene bottle completely full to the top (no headspace).
2. Shake the 250mL bottle approximately 25 times in a one-foot arc, within seven seconds or less, then dispense into the three IDEXX bottles. Fill two bottles to the 100 mL mark. The remaining sample goes in the third bottle and should be almost a 100 mL sample.

At the lab…

1. Analyze your QA sample as usual, with one bottle used for an undiluted sample and one bottle for a 1:10 sample. If one of your bottles has slightly less than 100mL in it, use that bottle for the 1:10 sample.
2. You will be conducting additional procedures to test the precision, accuracy and sterility of the equipment and methods. Record all results on a green QA datasheet.

Precision Test (Replicate)

- With the third IDEXX bottle, run as an undiluted sample. The results from the two undiluted samples will be compared to determine if they meet our precision criteria.

Accuracy Test

1. Staff will provide a 100 mL water sample of known *E. coli* concentration.
    a. See “Procedure for Preparing Frozen Sample” for instructions on how to prepare a sample of a known concentration.
2. Add a packet of Colilert-18 to the 100 mL sample.
3. Swirl to dissolve.
4. Seal in tray and incubate.
5. Record on datasheet as ‘*E. coli* Accuracy’.

Sterility Test (Blank)
Run blank sample for *E. coli*:

1. Use a clean graduated cylinder to measure 100 mL of sterile DI into a mixing bottle.
2. Add a packet of Colilert-18 to the water.
3. Swirl to dissolve.
4. Seal in tray and incubate.

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2. QA Procedure for Bay Sites

Periodically you will be asked to conduct additional procedures for quality assurance (QA) purposes. These include a precision test, an accuracy test and a sterility test.

- Bring all necessary sampling gear, as outlined in the Bay Bacteria Protocol.
- Collect one IDEXX bottle at each site as usual.
- Choose one of your sites to be the QA site for the month.

At the lab…

1. Analyze your samples as usual, running one 1:10 enterococcus test for each site.
2. You will be conducting additional procedures to test the precision, accuracy and sterility of the equipment and methods. Record all results on a green QA datasheet.

Precision Test (Replicate)

- Randomly pick one of your sites, and run a second enterococcus tray. The results from the two samples will be compared to determine if they meet our precision criteria.

Accuracy Test

1. Staff will provide a 100 mL water sample of known enterococcus concentration.
    a. See "Procedure for Preparing Frozen Sample" for instructions on how to prepare a sample of a known concentration.
2. Add a packet of Enterolert to the 100 mL sample. Do not dilute this sample!
3. Swirl to dissolve.
4. Label tray with 'Enterolert Accuracy'. Seal in tray and incubate.
5. Record on datasheet as 'Enterococcus Accuracy Check'.

Sterility Test (Blank)
Run a blank samples for enterococcus:

1. Use a clean graduated cylinder to measure 100 mL of sterile DI into a mixing bottle.
2. Add a packet of Enterolert to the mixing bottle.
3. Swirl to dissolve.
4. Label tray 'Enterolert Blank'. Seal in tray and incubate.
5. Record information on green QA datasheet.

Procedure for Preparing Frozen Sample

Equipment

- Frozen vial of known concentration (in box in freezer)

- One 100mL IDEXX bottle per vial
- QA datasheet (printed on green paper)
- Sterile deionized water

Procedure

1. Remove vial(s) from freezer. Record lot number and expiration date on log sheet on freezer door.
2. Place vial outside of freezer and start a timer for 15 minutes to allow the vial to equilibrate at room temperature.
3. Pour 100mL of sterile distilled water into a new 100mL IDEXX bottle.
4. Open the vial and aseptically transfer the colored disc, located on top of the cotton plug, to the IDEXX bottle. The disc may occasionally stick to the vial. If this occurs, use sterile tweezers to gently remove the disc.
5. Swirl the sample and tell the volunteer to allow it to stand for 10-15 minutes. Instruct them to mix by inverting the bottle 10 times and to double check that the disc has completely dissolved.
6. Record the vial lot info on the green datasheet.
7. Use within 30 minutes of hydration. Add one packet of the corresponding reagent and incubate with the rest of the samples.

To look up the range using the lot number:
https://www.idexx.com/en/water/resources/certificates-analysis-water/

A copy is printed and put in the QA binder. We record the month it was received and each month it was used, along with if the sample passed or failed.

Pipette Checks and Servicing

The Estuary Program uses adjustable single channel pipettes for dispensing water and preparing dilutions. Pipettes are checked gravimetrically at 1 mL, 5 mL, and 10 mL quarterly, or more frequently as needed. Pipettes are also serviced annually by an accredited service provider, or when they fail the criteria listed below. These criteria are consistent with manufacturer specifications and ISO 8655.

Acceptance criteria are as follows:

- For 1 mL, ± 2% RPD from desired volume
- For 5 mL, ± 1% accuracy from desired volume
- For 10 mL, ± 1% accuracy from desired volume

Supplies:

- Pipettors
- Clean pipette tip
- Deionized water at room temperature*
- 2 clean 100 mL or 250 mL beakers
- Scale

**Density of water at 20°C is 0.9982 mg/ul (0.9982g/1 mL)*

Procedure:

1) Turn on scale and wait about 15 to 20 minutes for it to warm up.
2) Use all calibration weights to verify the scale readout. Record values in scale log book.
3) Fill one beaker with approximately 100 mL of deionized water. Place the empty beaker on the scale and tare it.
4) Adjust the setting of the pipettor to 1 mL to start.
5) Collect 1 mL of water and dispense it into the beaker on the scale. Record the value.
6) Tare the scale again, then repeat the process of collecting, dispensing, and recording the sample weight at 1 mL for a total of three measurements.
7) Then, change the pipettor to 5 mL. Repeat the process above three times for the 5 mL.
8) Finally, change the pipettor to 10 mL. Repeat the process three times for the 10 mL.
9) Calculate the average volume dispersed for each, then calculate the RPD from the desired volume. If the RPD exceeds the acceptance criteria, repeat the process again. If the RPD still exceeds the acceptance criteria, send to an accredited service provider like Transcat or Pipettes.com.

Autoclave Operation and Quality Assurance Protocol

The procedures outlined below are designed to test for sterilization efficacy and ensure that the components of the autoclave are operating properly. The QA tasks are divided into two groups; those that should be conducted each run and those that should be conducted monthly. The Cuesta lab has two autoclaves: a Market Forge Sterilmatic autoclave (silver) that is used to sterilize waste before disposal, and a Fisherbrand SterilElite autoclave (white) that is used to sterilize water and reusable equipment before the next round of sampling. If parts break or are in need of service, reach out to the Cuesta laboratory staff. This protocol was written with guidance from the State Water Board's SOP, the SLO Public Health lab's Quality Assurance Manual, and excerpts from the Standard Methods for the Examination of Water or Wastewater.

Market Forge Sterilmatic	Fisherbrand SterilElite
	
• Used Quantitrays • Expired QC pellets • Positive ProSpore biological indicators	• Reusable lab supplies (bottles, graduated cylinders, pipette tips, etc) • Sterile DI water

Operating the brand SterilElite Autoclave (for supplies)

1. When preparing the autoclave for a sterilization cycle, begin by checking to see if the door is open or closed. If the door is closed, there might be items inside that the Cuesta lab staff are sterilizing. Before opening the door, check that none of the indicator lights are lit, as this means the unit is currently in use (even if you don't hear it running).
2. If the autoclave is empty, proceed to the next step. If the autoclave is filled with equipment, contact the Cuesta lab staff before removing the items.
3. Check the hose on the side of the autoclave to ensure the water line is between two blue water level markers. If the water level is below the lower marker, open the black cap on top of the autoclave and add distilled or deionized water until the

level is back between the two markers. Close the black cap before starting the sterilization cycle.

4. Place your items in the autoclave and turn the power on. Before starting the cycle, **refer to the QA steps below** and proceed accordingly depending on if you are conducting the QA procedures for every run or are running through the monthly QA procedures.
5. On the front control panel, select “121°C” and the “Scissors” icon, denoting that you are sterilizing equipment.
6. If you are sterilizing lab equipment only, select the “Steam” icon. If you are sterilizing water, select the “Flask” icon. Each icon will display a green or yellow light once selected. Double check your selections before starting the cycle.
7. Close the autoclave door and press the “Start” button.
8. After starting the cycle, the autoclave will start pressurizing and increasing the internal temperature immediately. Set the timer on the fan (located on the wall to the right of the autoclave) for roughly the same duration as the autoclave run to ensure proper ventilation.
9. The autoclave run lasts approximately 45 minutes, and you can track the progress on the control panel. You must wait until the cycle is complete to open the autoclave. DO NOT attempt to open the autoclave when it is on the “Dry” step of the cycle.

Operating the Market Forge Sterilmatic Autoclave (for waste)

1. When preparing the autoclave for a sterilization cycle, begin by checking to see if the door is open or closed. If the door is closed, there might be items inside that the Cuesta lab staff are sterilizing. Before opening the door, check that the pressure gauge has returned to 0 and the temperature gauge is reading below 100°C.
2. If the autoclave is empty, proceed to the next step. If the autoclave is filled with equipment, contact the Cuesta lab staff before removing the items.
3. Close the drain using the dial beneath the autoclave opening. Fill the autoclave with distilled or deionized water until the water level reaches the distinct water lines present in the autoclave’s interior.
4. If the red “Low Water Indicator” is on, and you have filled it with water as outlined above, then press the red “Reset” button.



5. Attach the free end of the yellow hose to the faucet at the nearest sink and turn on the cold water. This ensures there will be enough water for the full duration of the cycle (see above).
6. Double-check that the temperature dial is turned to the hottest setting (121°C/250°F).
7. Set the “Exhaust Selector” switch to “Fast”.
8. Place your items in the autoclave. Before starting the cycle, refer to the QA steps below and proceed accordingly depending on if you are conducting the QA procedures for every run or are running through the monthly QA procedures.
9. Close the autoclave door and set the timer for a 20-minute cycle.
10. After setting the timer, the autoclave will start pressurizing and increasing the internal temperature immediately. Set the timer on the fan (located on the wall to the right of the autoclave) for roughly the same duration as the autoclave run to ensure proper ventilation.
11. Upon completion of the sterilization cycle, turn the faucet off and remove the hose. Drain the water from the hose into the floor drain at the front right corner of the autoclave. If you have difficulty removing the hose from the faucet, pliers can be found in the tool chest in the main lab space.

Quality Assurance (QA) steps for each run

1. Before running the autoclave, place autoclave tape on items spaced throughout the tray. Tape is not necessary on each individual item, but should be placed on the majority of items to ensure heat and pressure conditions were met throughout the autoclave chamber.
2. Retrieve the Maximum Registering Thermometer and turn the red needle so it is directly above the black needle. The black needle measures the current temperature, but the red needle will mark the highest temperature reached during the sterilization cycle. Place the thermometer in the autoclave tray.

3. Run the sterilization cycle and wait for at least 10 minutes before opening and removing the items.
4. Upon removal, check that the autoclave tape has black stripes and record it as a "Pass" on the Autoclave QA Checklist. If the stripes are not black, rerun the autoclave. If the problem persists for a second cycle, record it as a "Fail" on the checklist and contact the Cuesta laboratory staff.
5. After it has cooled to ambient temperature, read the temperature denoted by the red needle off of the Maximum Registering Thermometer and record on the checklist. The reading should always be 121°C or higher. If this temperature has not been reached, rerun the autoclave and notify Cuesta laboratory staff if the problem persists.

Monthly Quality Assurance (QA) steps

NOTE: For monthly QA, it is most efficient to run both autoclaves simultaneously so only one positive control is necessary. This will likely be after Creek and Bay bacteria have been completed for the month.

1. Before running the autoclaves, set up the autoclave tape and Maximum Registering Thermometer as you would for a typical run.
2. In addition, retrieve three ProSpore biological indicators from the refrigerator. Place one inside glassware containing approximately 10mL of water and leave the other outside the autoclave. Use a sharpie to write a "C" for "Control" on the indicator left outside, "S" for "Supplies" on the Fisherbrand SterilElite indicator, and "W" for "Waste" on the Market Forge Sterilmatic indicator.
3. Run the sterilization cycle and ensure the cycles are complete and the units have depressurized before opening and removing the items.
4. Check and record the status of the autoclave tape and thermometer.
5. Remove the ProSpore indicator from the glassware and place all indicators (including the control that was left out of the autoclave) in an incubator set to 55°C for 48 hours. Cuesta has given us permission to use an incubator in the classroom next-door to the lab for this process. Record the date, time, and internal temperature of the incubator when you start the incubation on the QA checklist.
6. When you return to the lab after 2 days, make sure that the test ampules are negative by checking that there has not been a color or turbidity change. In addition, ensure that the control has changed from purple to yellow, indicating a positive result. As long as the test is negative and the control is positive, record the test as a "Pass" on the QA checklist. If either of these indicators are not as described, then rerun the test with new indicators. If the problem persists, reach out to Cuesta laboratory staff and record the test as a "Fail" on the QA checklist. Record the date, time, and internal temperature of the incubator when you read the indicator results on the checklist as well.

7. After reading the indicator's results, they must be disposed of properly. Negative indicators can be thrown away in the trash but any positive indicators must be autoclaved before disposal to kill off the bacterial growth.

Contact List

Name	Organization	Email	Phone #
Cheyenne Winn	Cuesta Lab Manager	cheyenne_winn@cuesta.edu	(805) 234-1571
Alejandra Garcia	Cuesta Lab Tech		(805) 863-7625
Cuesta Police	Cuesta PD (Emergency/Injury)		(805) 546-3205
BI Customer Service	Mesa Labs Contact	bicustomerservice@mesalabs.com	(406) 585-9535

Supply List

Keep all QA records, lot information, and reference materials in the Quality Assurance binder located in the drawers.

Item	Part Number	Website
ProSpore Biological Indicator (10^5 population)*	PS-5-10	ProSpore® Ampoule Biological Indicator | Mesa Labs
H-B Durac Maximum Registering Thermometer with Individual Calibration Report	1191Z86	H-B DURAC® Maximum Registering/Autoclave Bi-Metal Thermometer (thomassci.com)

** ProSpore indicators require overnight shipping. Coordinate with the Mesa Labs supplier ahead of time to ensure someone will be available to receive the shipment. If the shipment is delayed, the ampules will remain viable as long as they stay below 35°C for a maximum of 7 days. Immediately refrigerate between 2-8°C upon arrival.*

APPENDIX B. MORRO BAY NATIONAL ESTUARY PROGRAM
WATER QUALITY MONITORING PROTOCOLS AND FIELD GUIDE

Water Quality Monitoring Protocols and Field Guide



Updated May 2023

Field Hazards and Precautions

Poison Oak and Stinging Nettle are two noxious plants that grow alongside many creeks throughout the watershed. If you need to move your sampling location upstream or downstream slightly to avoid contact with these plants, please do so!

If you do brush up against poison oak, wash the affected area with Tecnu and Cala gel, which are available in our office.

If you brush up against stinging nettle, it is best to soak the affected area in cold water and apply a Benadryl gel to block the antihistamine response.



Poison Oak:
Identify by shiny 'leaves of three' and lack of thorns on branches. Leaves turn red and orange seasonally. Branches are reddish. Loses leaves in the winter.

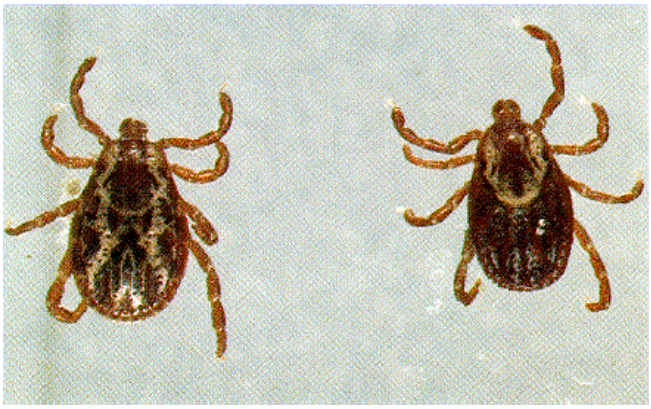


Stinging Nettle:
Leaves have jagged edges, and small grainy flowers hanging from where leaves meet the stem. These plants may be 10 feet tall.

Field Hazards and Precautions

Ticks are often found in cool riparian areas during the spring and summer. They will climb onto clothing and then onto skin where they bite and attach. They are known to transmit diseases, and should be avoided and removed immediately.

Rattlesnakes are also occasionally encountered in the watershed. They can be identified for the dark diamond pattern and characteristic rattle. If you see a rattlesnake, leave the area immediately.



Two common varieties of ticks in the Morro Bay area.



Rattlesnakes: Identify by diamond patterning and rattle at the tail.

Emergency Phone Numbers

EMERGENCY: 911

Morro Bay Police: (805) 772-6225

Morro Bay Fire: (805) 772-6242

Morro Bay Harbor Depart. (805) 772-6254

County Sheriff: (805) 781-4550

California Highway Patrol: (805) 593-3333

National Estuary Program Office: (805) 772-3834

Makenzie O'Connor Cell: (805) 776-3778
Nick Soares Cell: (805) 316-4160)
Ann Kitajima Cell: (805) 391-7457

Wildlife:

Marine Mammal Rescue Center: (415) 289-7327
Injured or sick marine mammals.

Pacific Wildlife Care: (805) 543-9453
Injured or orphaned wildlife (fawns, birds of prey, other animals)

Gate Codes for Site Access

Chorro Creek Ecological Reserve (CCER): 6012

Dairy Creek: 3834

The MBNEP has its own lock on this gate. It has 'MBNEP' etched on it. The combination is 3834.

Pennington Creek Gate: 1901

The MBNEP has access to one of CalPoly's locks. It is a combination lock that is usually chained to two other locks. Please leave this lock and gate as you found it – if it was locked, lock it after you walk through. Be sure you lock it so that all three locks can open the gate. Cars are not allowed upstream of UPN when the road is wet. If you have any questions about if you can drive, contact Cal Poly.

San Bernardo Creek Automatic Star Gate: 2595

Note – don't drive through. Park on San Bernardo Creek Road.

Walters Creek, Gun Clubs: 1920

The gun club site should only be accessed on Tuesdays when the range is shutdown.

Site Access Permission

The staff and volunteers of the Morro Bay National Estuary Program have landowner permission to monitor at sites located throughout the Morro Bay watershed. Permission has been granted at the following specific sites:

Site Code	Site Description	Landowner Name
UCR	Chorro Creek on Cal Poly property, near the vineyard operations	Cal Poly (Aaron Lazanoff)
APN	Upstream of the Beef Unit	Cal Poly (Aaron Lazanoff)
UPN	At the Beef Unit corrals near the creek	Cal Poly (Aaron Lazanoff)
CPN	Pennington Creek at the bridge on Cal Poly property	Cal Poly (Aaron Lazanoff)
PEN	At the amphitheater on Rancho El Chorro Outdoor School	Camp KEEP
CLV	Los Osos Creek, Clark Valley Road private crossing.	Dave O'Brien (Forest Nurseries)
CER	At the road crossing on the Chorro Creek Ecological Reserve	California Department of Fish and Wildlife
CAN	At the road crossing on Canet Road	Within the rightaway of the county-maintained bridge
TWB	At the S. Bay Blvd. bridge	State Parks
SLU	On Adobe Rd. at the bridge crossing	Within the rightaway of the county-maintained bridge
DAL, DAM, DAU	DAL is at the bottom of the park, DAM near the dog park, and DAU at the border of National Guard and Parks property.	San Luis Obispo County Parks
CHO	Downstream from the Highway 1 bridge	Camp San Luis Obispo (Paige Farrell)
COO	At the first bridge on the Coon Creek trail	State Parks
TUR	At the Turri Road bridge near Los Osos Valley Road	Within the right of way of the county-maintained bridge
CCC	At the Chorro Creek Road crossing	Coastal San Luis Resource Conservation District

If you have any questions regarding landowner permissions, please contact Monitoring Program staff at 805-772-3834 or monitoring@mbnep.org.

Water Quality Monitoring for the Morro Bay Volunteer Monitoring Program

Where to take measurements:

- Optimum location: center of creek at 1/3 of water depth
- Try to sample in part of creek with mixing, do not sample in stagnant pools.
- Don't stir up water when taking measurements.
- Face upstream when taking sample – stay downstream of the equipment/sample.

Dissolved Oxygen, Temperature, and Conductivity with YSI ProQuatro

The YSI ProQuatro meters can measure dissolved oxygen (DO), temperature, salinity, conductivity, specific conductance, and pH. We use the meters to measure DO (in % and mg/L), temperature (in °C), pH, and specific conductance (in µS/cm) which is a temperature adjusted conductivity value. Some meters also display salinity on them. If you are taking measurements in the creek, you can typically ignore the salinity value.

Instructions for use:

1. Turn on the meter before leaving the office, or upon arrive to the site, by holding down the green power button on the lower right. will need to hold down the power button for about 3-5 seconds for it to turn on.



Power button

2. This meter requires 10-15 minutes to stabilize before you can take readings. Turn the meter on before you leave the MBNEP office, or as soon as you reach your monitoring spot. When the Dissolved Oxygen, % saturated, is reading close to 100%, the meter is warmed up and ready to be used.
3. Remove the grey sleeve from the probe. Place the entire probe in the water. The entire probe, up to the cable, must be submerged to ensure that all necessary instrumentation in contact with the water.
4. Give the probe a quick shake to dislodge any air bubbles, and then gently move the probe wile measuring. The instrument requires a minimum of 0.5ft/sec of flow across the probe for an accurate reading to be taken. NOTE: It gives a better measurement if you hold the probe rather than letting it hang in the water.
5. All analytes are displayed on the same screen. The order of the analytes might vary from meter to meter, so check the units. The units on the meter should match the units on the datasheet.
    a. Dissolved oxygen concentration: DO mg/L
    b. Dissolved Oxygen percent saturation: DO % L
    c. Specific Conductance: spc uS/cm
    d. pH: pH units
    e. *Salinity (sal ppt) – some meters only*
    f. Temperature: °C

6. Most analytes are temperature dependent, so the temperature must be stabilized in order for the other readings to be recorded. Once the values have stabilized, record them on the datasheet.
    a. Unlike dissolved oxygen, conductivity values stabilize very well. If the numbers continue to jump around once the temperature has stabilized, check that the probe is fully submerged in water and the shake the probe to dislodge any air bubbles. If that doesn't work, turn the meter off and then back on (this should be done as a last resort, as you will need to wait for the D.O. to warm up again. NOTE: Conductivity should be a whole number (i.e., not decimals). If the number keeps jumping around or there are decimals in the value, then it is usually an indication that there is an air bubble or the probe is not fully submerged.
7. When finished monitoring
    a. Rinse the probe thoroughly with D.I. water
    b. Insert the probe into the grey sleeve
    c. Leave the meter on as you travel o the next monitoring site, or turn it off when done sampling. To turn the meter off, hold down the power button until the screen goes blank.

<u>Equipment Malfunctions and Common Mistakes</u>

DO meter won't stabilize:

- This meter can jump back and forth between a few values for a long time. Pick a mid-range value of those that are being repeatedly displayed and record on datasheet.
- Make sure that the sensor is fully submerged in the water.

Displays LO BAT or will not turn on:

- You will not be able to collect data. Be sure to notify staff of the problem so that it can be corrected.

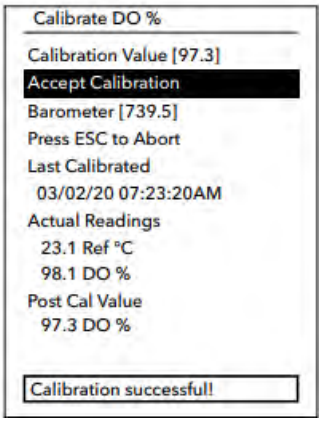
Field Calibration of YSI ProQuatro Meter for Dissolved Oxygen

The YSI Pro2030 meter should be turned on and allowed at least 10-15 minutes to warm up before using. After the meter is warmed up, the meter should stabilize at about 100% dissolved oxygen concentration (DO %) while still in the grey sleeve. After the meter has warmed up for 10-15 minutes, check to see what the reading for "DO %" is.

- If the value for DO % is between 99% – 101%, use the meter as normal.
- If the value for DO % is less than 99% or greater than 101%, **conduct a field calibration**.

To conduct a field calibration, please use the following instructions:

1. Check the conditions inside the grey sleeve. There should be a small sponge at the bottom of the sleeve, which should be **damp, but not sopping wet**. Pour/pinch out any excessive water within the sleeve, and allow the meter to re-stabilize within the sleeve.
2. If the values return to between 99% and 101% after removing excessive water, then continue to use the meter as normal. If the value is still less than 99% or greater than 101%, proceed to Step 3.
3. Allow the DO values to stabilize within the grey sleeve, if they are not already. Then press and hold the "Cal" button. From the Calibrate Menu, highlight DO and press enter. Select DO%.
4. This should prompt a screen that looks like this:



5. Observe the "Actual Readings" for stability. If there are no significant changes for approximately 40 seconds, then select "Accept Calibration."
6. A notification box will display "Calibration successful!" and the handheld will beep before you are returned to the Calibrate Menu.
7. Allow the meter to stabilize again before removing from the sleeve. Then, use the meter as normal.

Turbidity with HACH 2100Q Portable Turbidimeter

- Triple rinse the syringe with creek water or use pipette with clean tip.
- Use WhirlPak collected from sample site. Shake the Whirlpak vigorously before extracting the sample.
- Fill glass sample cell to the mark (white band/arrow), using the syringe or pipette. Handle cell by the top, not the sides.
- If outside of cell gets wet, wipe off cell with a Kim-wipe – do not use your shirt or a paper towel since it could scratch the cell.
- Place a small drop of silicone on the cell then rub in with a Kim-wipe to reduce the effect of scratches in the glass on the reading.
- Turn instrument on using the blue power button.
- Insert sample cell with diamond on cell lined up with the notch on meter.
- Close lid and press the black button below the word: “Read”.



- Record value when the number displays on the screen. Record units in NTU.
- Turn instrument off.
- Empty cell. Triple rinse cell and syringe with DI. Leave cap off so cell can air dry.

Collecting Nutrient QA Sample

- Collect equipment: sample container, cooler and ice packs. They are all available in the back of the storeroom near our refrigerator.

- Label sample container as follows:
    - Client: Morro Bay National Estuary Program
    - Location: Code for your site
    - Project: MBNEP
    - Sampler: Your name
    - Date
    - Time [NOTE: It's very important to note the time because we need to get the sample analyzed by the lab before the holding time is exceeded.]
    - Sample#: Code for site

- At site, wade into stream at sampling location, taking care to not stir up water. Stay downstream of the area where you will be collecting the sample.
- For the non-acidified bottle: Open the container underwater at 1/3 the depth and fill.
- Dump out the water. Repeat two additional times so that the bottle has been triple rinsed.
-
- For the acidified bottle: Open the container underwater at 1/3 the depth and fill. Leave air space at the top.
- Cap the container.
- Store in cooler and return to NEP office.
- Put sample bottle in the refrigerator. Return the ice packs to the freezerLet VMP staff know that you've left samples in the fridge.
- Samples will be picked up by the lab courier such that the appropriate hold time will be met.

Flow Measurement Protocol

- Choose a point on the creek with the following characteristics:
    - Wadeable (less than 3 feet deep) and not moving so swiftly as to be unsafe;
    - Lacks obstructions within 15 ft up or downstream of site;
    - Depth greater than 0.2 feet;
    - Bottom of the creek should be relatively flat and even;
    - Bottom of the creek should be solid so you won't sink;
    - Site at the beginning of a riffle, not a pool.



- Extend the tape measure or stadia rod across the section of the creek to be measured. Place the 0 end of the tape at the point where water is present on the left bank (when facing downstream). Make sure you use the side of the tape that measures feet in tenths, not inches. The tape must be held in place firmly during all measurements and not moved.
- You should move rocks/sticks/vegetation out of the way in the section upstream of your transect in order to achieve uniform and perpendicular flow. Try to avoid a transect that has eddying. However, only make these changes before you begin taking measurements.
- Measure the width of the creek bed with water. Record in the 'Total Width of Stream' field on the data collection form.
- Record the Equipment ID number for the meter.
- Divide the total length into equal segments following these guidelines:
    - o If your site is < 5 feet wide – take at least 6 readings with positive velocities o If your site is > 5 feet wide – take at least 12 readings with positive velocities o Don't measure at segments smaller than 0.25ft

    - You should have a “Zero depth, zero velocity” measurement at each end of the transect that represents the wetted edge (where the water meets the side of the channel).
- If the creek length is not easily divided into segments of even length, it is fine to have shorter or longer segments at either end, but all other segments should be of equal length.
    - For example, if the creek is 12.3’ wide, you’ll take measurements at the following points on the tape measure: 0’, 1’, 2’, 3’, 4’, 5’, 6’, 7’, 8’, 9’, 10’, 11’, 12’ and 12.3’.
- Begin by sliding the sensor onto the mounting shaft of the top-setting rod and securing by tightening the thumbscrew. Be sure to mount the sensor so that the cable is coming straight up. Turn only until snug, do not over tighten.
- Take your first depth and velocity reading at the 0 point on the tape measure, which is the start of the wetted width. Don’t skip that measurement, even if it looks like the water isn’t really moving. Place the top-setting rod in the water so that the sensor faces upstream, directly into the current. Stand downstream of the meter and tape so that you do not interfere with the flow. Do not stand directly behind the flow meter or you might create eddies – try to stand off to the side. Make sure the rod sits flat on the bottom, stands upright, and that there are no rocks, sticks, algae, etc. interfering with the sensor.
- Take the first reading at the edge of the wetted width, which is the 0 point on the measuring tape.
- Under ‘Distance from Bank’, record the distance from the point on the left bank where the water begins to the point where the flow measurement is being taken.
- Visually measure the depth of the creek using the graduation lines on the hexagonal rod.
  Lines are measured in tenths of feet. Single line = 0.1 feet, double lines = 0.5 feet, triple lines = 1.0 feet. Record the depth on the data collection sheet.
- Adjust the rod so the sensor is raised above the bottom of the channel by setting the gauge on the top of the rod to the depth of the water. To set the sensor at the correct depth, press the trigger to slide the smaller rod up or down. The smaller rod has graduations marked in feet starting with ‘0’ for depths less than 1 foot. For example, if the creek depth is 1 foot, move the small rod so that the 1 foot graduation lines up with the “0” on the large rod. If the creek depth is 1.4 feet, raise the small rod so the 1 foot graduation is aligned with the “4” on the large rod.
- Be sure the electrodes on the sensors are submerged. They are two shaded areas on the front of the sensor. If they are not submerged, there is insufficient depth to take a reading and you can record ‘0’ flow in the fps column on the datasheet. (Typically less than 0.2 feet.) Make sure the electrodes on the sensor are facing directly into the flow.

Measuring Flow with HACH FH950:

- Locate the blue handheld unit of the FH950, and attach the cable to the top of the unit. Hand tighten the cable, being careful to not overtighten.
- Use the mount on the handheld unit to attach handheld to the top-setting rod. Be cautious to not mount the unit in a place that inhibits you from moving the rod up and down.

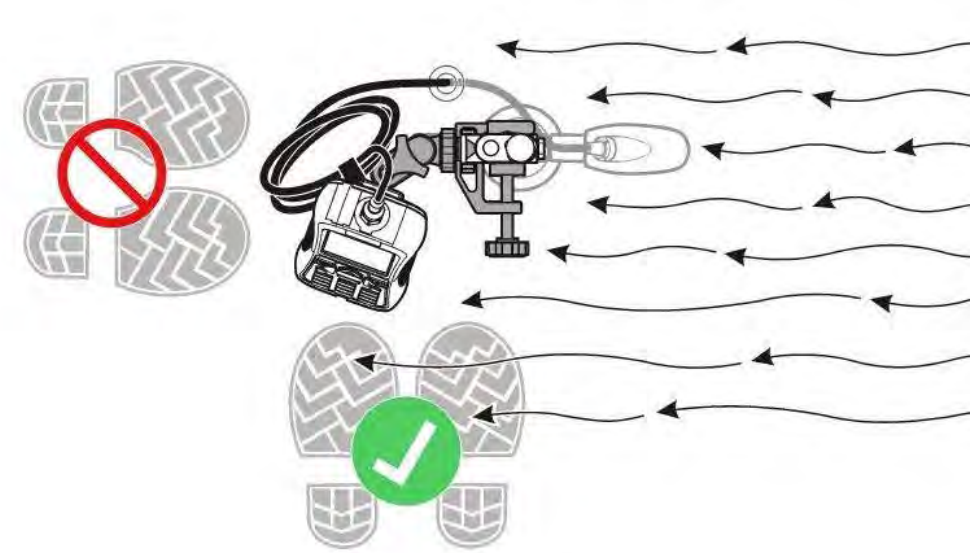


- Next, attach the sensor to the USGS top-setting rod. Turn the thumb screw clockwise to secure the sensor onto the mounting shaft. Mount the sensor so that the cable is coming straight up, not off to the side. Do not overtighten the thumb screw.
- Press the FH950 power button to turn on the unit.
- From the Main Menu screen, select *Profiler*. Then enter the evaluator's first initial and last name, and select *OK*.
- Next, select Stream and enter the stream name or reach that is being measured, then select *OK*.
- The next screen will prompt the user to enter the stage reference. The stage reference is usually an elevation value from an object that cannot be moved (e.g., a survey marker, staff gauge, bridge). Enter value if applicable. Otherwise, just select OK.

Recording Flow

NOTE: If two surveyors are present, have one person take flow measurements with the FH950 and the other record data on the datasheet. If only one person is present (i.e. a staff member), print out FH950 recorded measurements upon return from the site, and stable data to the corresponding datasheet.

1. Start on the left bank. The first station for the discharge measurement is the left bank wetted edge.
2. Place the top-setting rod in the water so that the sensor faces upstream, directly into the current. Do not stand directly behind the flow meter or you might create eddies – try to stand off to the side (see photo below). Make sure the rod sits flat on the bottom, stands upright, and that there are no rocks, sticks, algae, etc. interfering with the sensor.



3. From the Profiler Menu screen, select *Edge/Obstruction*, then select *Left*.
    a. This will automatically set the depth and velocity to zero. Check depth at zero to make sure this depth is actually zero. In some situations the 0.00 marker may not have 0.00 depth. (If depth does not = zero, jump to Step 6 and 7 as described, then return to step 4.)
4. Next, enter the distance to the station by selecting *Dist. to Vertical*; enter the distance between the zero marker of the tape/stadia rod to the current station and select *OK*.
    a. If the left wetted edge is at 0.00 ft on the stadia rod, enter 0.00. If the wetted edge lies at a different value (i.e. the left wetted edge is at 1.0 feet), enter the applicable value.
    b. Then select the *Next* at the bottom of the screen to move onto the second station.
5. Move the top setting rod/sensor to the next station.
6. Measure the depth of the creek, using the hexagonal ticks on the top-setting rod in tenths of an inch. On the handheld unit, select *Set Depth* from the Profiler Menu

and enter in the stream depth at the station being measured, then select *OK*. Adjust the vernier scale on the top-setting wading rod to the proper depth.

7. Next, select *Measure Velocity*. Ensure that the sensor is facing directly into the flow at the station, and that there is no debris or flow obstruction at that station. *NOTE: Because the sensor needs to be oriented directly into the flow at the station, it may not face directly upstream.*
    a. For velocity options, select *One-Point*, then select *0.6*. The FH950 will then display the depth at which to set the sensor. This depth can be ignored.
    b. Next, select *Capture*, and wait for the FPA time to elapse, then select *OK*. Lastly, select *Main*. The FH950 will then return to the Profiler Menu screen for that station.
    c. Select *Next* to continue to the next station.
8. Repeat steps 6 and 7 at equal increments, until the right bank wetted edge is reached.
9. At the right bank, select *Edge/Obstruction* on the Profiler Menu and select *Right*.
    a. This will automatically set the depth and velocity to zero. If depth is not equal to zero, change this value in the *Set Depth* option.
    b. Next, select *Dist. to Vertical* and enter the distance between the starting point and the right bank wetted edge. Select *OK*.
10. When all fields are complete, check the final channel summary and save data.
    a. From the Profiler Menu, select *Channel Summary*. The total cross-section discharge will be shown in a box at the top of the screen and a bar graph will be shown at the bottom of the screen.
    b. Select the *Cont.* (i.e., Continue) box at the bottom of the screen to toggle the bar graph between the depth, velocity, and percent discharge of cells along the cross section.
    c. <u>If any changes need to be made</u> (e.g., because the top-setting wading rod was not adjusted properly or a cell had a partial discharge of 10% or greater), such as adding stations, checking entries, or retaking velocity measurements, follow the directions provided in the note below.
    d. Then, select *Save Data and Exit* and name the file as desired. The FH950 only allows for eight characters in the file name. Lastly, select *OK*.

Making Changes to FH950 Data:

- If stations need to be added or deleted, options for *Prev., Next, Ins.,* and *Del.* appear at the bottom of the Profiler Menu screen.
- Use *Prev*. (i.e., Previous) and *Next* to navigate to the station where you would like to insert (i.e., *Ins*.) or delete (i.e., *Del*.) a station.
- For example, to insert a new station between Stations 3 and 4, select *Prev*. until the Profiler Menu display screen shows information for Station 3, and then select *Ins*. The new station will be named Station 4 and subsequent stations will be renamed sequentially. To delete Station 3, navigate to that station and select *Del*., then select *Yes* to confirm, and lastly select *OK* to return to the Profiler Menu.
- If the Channel Summary reports an unexpectedly large partial discharge, check the Depth and Distance to Vertical entries by navigating to the station(s) of

concern using *Prev*. and *Next* and editing as necessary by referring to the field datasheet.

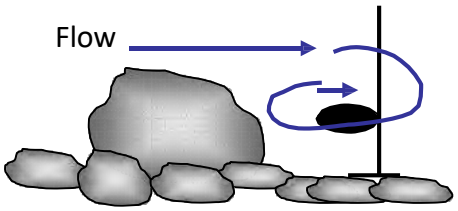
- To retake a velocity reading, select *Measure Velocity* at the station of concern.

Additional Notes on Measuring Flow

Ideally, flow is measured in an area that has a uniform shape and smooth boundary. Obviously a natural stream channel has neither. Nevertheless, you can try to increase the accuracy of your flow measurements by placing your transect at a site that does not have vegetation or rocks that sit higher in the water column than the sensor. These obstructions create swirls and eddies that disrupt a uniform flow.

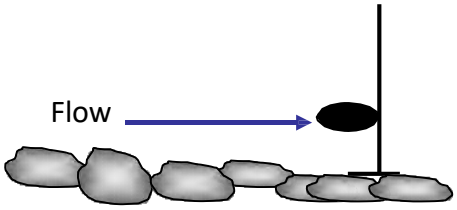
Obstructed Flow

When there are eddies from an obstruction, some of the flow is actually heading in the upstream direction, passing by the sensor from the back to the front. This is when the sensor reports a negative number.



Unobstructed Flow

You should move rocks, vegetation, or even your transect in order to minimize these disruptions and so the flow passes the sensor from the front to the back. Makes these adjustments along the transect BEFORE you start collecting measurements.



Because the flow meters clearly indicate if the flow is not coming directly from an upstream direction, we can record the flow in these areas as 0 and make an effort to relocate the transect, ultimately making our measurements more accurate.

Instructions for Monthly QA

Equipment:

- WQ Kit
- QA sample bottle (provided by staff)
- Cooler (located under the refrigerator)
- Ice packs (located in the freezer, to the right of the refrigerator)
- Extra-large WhirlPak (provided by staff)

Field Instructions:

- At your designated QA site, compete the **blue** QA datasheet in place of the typical white datasheet.
- For YSI measurements (temperature, DO, conductivity), place the meter in the creek and allow measurements to stabilize. Record first measurements under "**1st reading**" on the datasheet. Remove the meter from the creek briefly then return the meter to the same location. Allow the meter to re-stabilize, then record second measurements under "**2nd reading**."
- Collect a WhirlPak sample as usual in the extra large WhirlPak. On the bag, write the date, site, time sampled, and sampler. Store this sample in the WQ Kit.
- Additionally, fill a QA sample bottle from the same location as the WhirlPak collection. On the bottle, write the date, site, time sampled, and sampler. Store this sample in the cooler.
- On the datasheet, write in QA sample details under "QA Sample Collection".
    - You can leave the Sample ID # blank.
    - Duplicate or blank samples are run occasionally. If staff did not instruct you to collect a duplicate or blank sample, you can also leave this section blank.
- During flow measurements, only take second measurements at **THREE LOCATIONS** within your flow transect. These three second measurements should be taken at locations that have measurable flow.

Office Instructions:

- Upon returning to the office, place the sample bottle in the refrigerator.
- Process turbidity as normal, extracting from the large WhirlPak. Invert the sample and press read for a second reading.
- Process orthophosphates as PO_4 twice from two extracted samples from the WhirlPak bag. Clean the cuvette thoroughly with DI water after each analysis.
- Process a blank orthophosphates as PO_4 sample using the DI water labeled "For Nutrients Only."
- Store WhirlPak in the refrigerator when complete.

Protocol for Bimonthly Nutrient Monitoring

Monitoring to be conducted by staff only

On a bi-monthly basis, ten sites throughout the watershed are monitored for nutrients and algal cover, in a brief time period (approximately a four-hour period). Samples are collected and sent to a certified laboratory (BC Labs) for analysis. Photo monitoring for algal cover is also recorded.

NOTE: Previously, this monitoring was conducted quarterly, and thus called Quarterly Nutrient Monitoring (QNM).

Equipment:

- YSI Pro 2030 (for measuring DO/temp)
- Sample bottles, one per site
- Cooler + ice packs
- Datasheets
- Clipboard
- WQ Kit + flow equipment (if conducting WQ during site visit)
- Camera
- Whiteboard & dry erase pen (if desired)

Sites:

- Dairy Creek, middle (DAM)
- Pennington Creek, upper (UPN)
- San Luisito Creek, lower (SLU)
- San Bernardo Creek (SBC)*
- Chorro Creek, upper (CHO)*, Chorro Creek, middle (UCR), Chorro Creek, lower (TWB)
- Los Osos Creek, Clark Valley (CLV)
- Warden Creek, middle (TUR)
- Los Osos Creek, at Turi Road and Southbay BLVD (GS1) *Sample at a low tide, < 2 feet*

Preparation / Scheduling:

- Monitoring is conducted every other month
    - February, April, June, August, October and December
- Notify landowners as appropriate (UCR, CHO, SBC)
- Contact lab to schedule courier with minimum of 1-week notice. Sampling must be done on a day the courier can come (either Monday or Wednesday).
    - If done on a Wednesday and worried about time, Los Osos side can be completed on Tuesday afternoon (later the better)
    - Maximum sample hold time is 48 hours at 4°C, but assume 24 hours of those 48 hours will be dedicated to courier transportation (24 hour effective hold time at MBNEP).
- BNM sampling can be conducted on the same day as QA/Ag sampling/seeps to reduce the number of courier trips
- BNM can be conducted on the same day as regular WQ monitoring and/or creek bacteria monitoring
    - Bring all necessary supplies if conducting multiple protocols during the same site visit.

Field Protocol:

- Upon arrival at site, collect readings of dissolved oxygen in mg/L, % saturation, and water temperature. Record readings on BNM datasheet.
- If conducting regular WQ, fill out BNM datasheet and WQ datasheet. Indicate that a WQ datasheet has been filled out using the check box for "WQ datasheet filled out"
- Record observations of algal coverage, using the following metrics:
    - Estimate the percent cover of algae, including both filamentous and microalgae.
        - Select and record a percent coverage score: 0 = Absent (0%), 1 = Sparse (<10%), 2 = Moderate (10 – 40%), 3 = Heavy (40 – 75%), 4 = Very Heavy (>75%).
    - Record the size of the area assessed. Estimate the average width and total length of the observable reach and multiply these figures (i.e. 1m x 1m, 0.5 m x 2m, etc.)
    - Note the presence or absence of filamentous algae and macroalgae. Record either present (P) or absent (A).
- Collect a sample:
    - Label the bottle with date, time, site code and sampler's name.
    - Remove the cap.
    - Select a sampling spot within a riffle.
    - Fill and empty the bottle three times.
    - Fill the bottle to the top and replace cap.
    - Store bottle in cooler, and record time sample taken on the datasheet.
- Photodocument the algal conditions:
    - Write the site name, date and time on the white board. Take a picture of it. (optional)
    - Take a picture upstream of the sample collection point, at the sample collection point, and downstream of the sample collection point.
    - Take any additional photos that document algal conditions
- Upon return to the office, store samples in the fridge. Complete the Chain of Custody for the lab immediately.

Data Management:

- If a site had both BNM and WQ done, make sure it is indicated on both the BNM and WQ datasheets.
    - During data entry, make sure you enter data from both the WQ datasheet, and on the BNM datasheet.
    - When you enter the data, check the box saying that you entered the data. In Access, indicate that the entry is BNM.
    - When staff QA the data, they will check the "data QA'd" box.

- All lab data will get stapled to the completed BNM datasheet. If there was a separate WQ datasheet filled out, staff will also attach a copy of the lab data to that datasheet.

Photo File Management:

- All photos taken during BNM should be uploaded to: DATA - Documents\Monitoring\vmp\VMP Photos\Nutrient Monitoring - QNM BNM
    - Put photos in corresponding year/month folders
    - Use the following nomenclature when naming photo files: YYYYMMDD_SITE_purpose_description.jpg

        - For example, a photo from DAM during BNM taken on January 1, 2020 might be named: “20200101_DAM_BNM_algaecover.jpg”
        - This specific nomenclature allows us to easily tell the date/purpose of the photo, without opening each individual file.
    - If photos were taken for both as monthly site condition photos and BNM photos, make sure to copy files to: DATA - Documents\Monitoring\vmp\VMP Photos\Water Quality Sites and Conditions
        - Photos here are organized by site, and use the same nomenclature (YYYYMMDD_SITE_purpose_description.jpg)

QA: _____

QC: _____

Water Quality Monitoring Data for the Morro Bay Volunteer Monitoring Program

Date:	Time:	Site ID:
Team names:		
Current weather:	Rainfall in past week:	

Analyte	Equipment ID	Results
Water Temperature	DOE-MBV-	°C
Dissolved Oxygen (concentration)	DOE-MBV-	mg/L
Dissolved Oxygen (% saturation)	DOE-MBV-	%
Conductivity (temperature corrected)	DOE-MBV-	µS/cm
pH	DOE-MBV-	pH units
Turbidity*	TUN-MBV-	NTU
Photo Monitoring	Camera ID:	Photo Numbers:
Nutrient Sample	Time collected:	

Notes/Field Observations
Algae Present? **YES** or **NO**
Approx. % Algae Cover:
Trash/Litter Present? **YES** or **NO**
Oily Sheen or scum? **YES** or **NO**
Odor detected? **YES** or **NO**
Fish present? **YES** or **NO**
Other Notes? See backside for low flow

Turbidity samples processed in office

Flow Start Time: _____
Flow End Time: _____

Flow Volume Measurement

Staff Plate Reading: _____
Reading Time: _____

If your site is < 5' wide, take 6 non-zero readings. If your site is > 5' wide, take at least 12 non-zero readings.

Total Width of Stream: _____ **Equipment ID: FLOW-MBV-** _____ ***If flow not taken, please fill out back form.***

Distance from Bank (feet)	Depth (feet)	Velocity (feet/second)
1)		
2)		
3)		
4)		
5)		
6)		
7)		
8)		
9)		
10)		
11)		
12)		
13)		
14)		
15)		
16)		
17)		

Distance from Bank (feet)	Depth (feet)	Velocity (feet/second)
18)		
19)		
20)		
21)		
22)		
23)		
24)		
25)		
26)		
27)		
28)		
29)		
30)		
31)		
32)		
33)		
34)		

Water Quality Monitoring Nutrient Analysis

Reason flow not measured (circle or write in)

1. Water level too low to measure (<0.10 ft³/s) **YES** or **NO**
2. Creek visibly disconnected **YES** or **NO**
   NOTE: Check upstream and downstream for signs of disconnect. Take photos/notes of location.
3. Other (equipment failure, time etc). **Please explain:**

Date Collected		**Date Analyzed**	
Time Collected		**Time Analyzed**	
Collected By		**Analysis Conducted By**	
Site Code		**Did you collect an extra bottle for the lab?**	**Yes / No**

Temperature at time of analysis (°C)		*

**The sample should be at room temperature (>15°C). If sample is <15°C, allow sample to acclimate to room temperature.*

Analyte	Equipment ID	Meter Reading		Dilution Factor		Result
Orthophosphate as PO_4 (mg/L)	COL-MBV-	mg/L	X		=	mg/L

Dilutions:

- If the sample reading is less than **2.50 mg/L**, the dilution factor is 1.
- If the first reading is greater than **2.50 mg/L**, re-run the analysis with a 1 pipette (5mL) of creek sample and 1 pipette (5mL of DI water). Now, the dilution factor is 2.

When analysis is complete:

- Place whirlpak in black refrigerator when done. Do NOT discard excess sample.

To be completed by staff only:					
Analyte	**Dilution Factor**	**Uncorrected Result (mg/L)**	**Reagent Correction Factor (mg/L)**	**Date of RCF Analysis**	**Corrected Nutrient Result (mg/L)**
Orthophosphates as PO_4					
Orthophosphates as P					*

** If corrected nutrient result is less than 0.11, please circle and write <0.11 below.*

☐ Check if entered into Nutrient Data spreadsheet.

Chain of Custody
Monthly (0)

82171

TEST DESCRIPTION - See Reverse side for Container, Preservative and Sampling information

Client: **Morro Bay National Estuary Program**
Address: Attn: Ann Kitajima
601 Embarcadero, Suite 11
Morro Bay, CA 93442

Phone: (805)772-3834 Ext 14 Fax:
Contact Person: Ann Kitajima
Project Name:
Purchase Order Number:
Quote Number:

Sampler(s)

Sampling Fee: _____ Pickup Fee: _____
Compositor Setup Date: ___/___/___ Time: ___/___

Lab Number: **CC** **8-1977**

Method of Sampling: Composite(C) Grab(G)
Type of Sample **SEE REVERSE SIDE**
Potable(P) Non-Potable(NP) Ag Water(AgW)
Bacti Type: Other(O) System(SYS) Source(SR) Waste(W)
Bacti Reason: Routine(ROUT) Repeat(RPT) Replace(RPL) Other(O) Special(SPL)
Wet Chemistry-Turbidity,PO4-P Diss.,pH
Field Filtered - PO4-P Diss. 16oz(P), 40ml(VFS), 8oz(P)

Samp Num	Location Description	Date Sampled	Time Sampled																

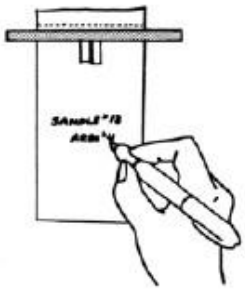
Remarks:

Relinquished Date: Time:
Received By: Date: Time:

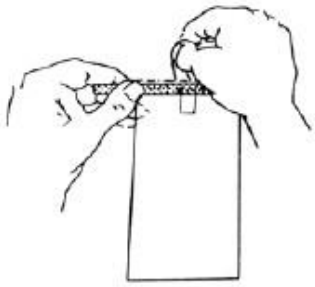
Relinquished Date: Time:
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Relinquished Date: Time:
Received By: Date: Time:

INSTRUCTION SHEET FOR WHIRL-PAK® SAMPLE BAGS



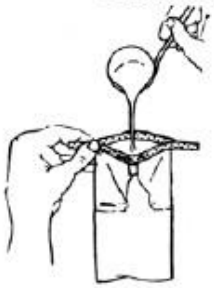
1. Label the bag with sample information if necessary.



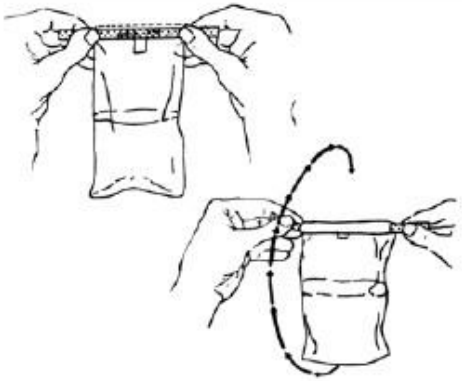
2. Tear off the top of the bag along the perforation.



3. Use pull tabs on each side to open the bag. Sometimes a little pull on the bottom of the bag helps open it completely.



4. Put sample, liquid or solid, into bag. Leave enough space at the top for closing and mixing if needed.



5. Pull the ends of the wire to close the bag. Holding the bag by the wire ends, whirl the bag three complete revolutions to form a leakproof seal. Whirling the bag will form the tightest seal. Larger bags can be closed by "folding" the tab over as tightly as possible.



6. Bend the wire ends over onto the bag to complete the closing.

In-Office Sample Analysis

Return to the office with the sample. All equipment for analysis is contained in the bin in the white cupboards. The bin is labeled “Nutrient Analysis Equipment.”

Measure Sample Temperature

1. Remove IR thermometer from its black case.
2. Point thermometer at Whirl-pak bag.
3. Press and hold grey button. You’ll see a red laser light which indicates where the thermometer is measuring.
4. Temperature should be between 18 and 25°C before conducting analysis.
5. If sample is too cold, place in incubator located under table with monitor for about five minutes and then re-measure the temperature.

Sample Analysis for Orthophosphates

Equipment:

- HACH DR/890 or DR/900 meter with glass cuvet and plastic cap
- HACH orthophosphate reagent
- Kim Wipes
- DI water
- Pipetter labeled “PHOSPHATE”
- Pipette tips
- Timer
- Scissors
- PO4 Waste Container



Sample Analysis Procedure

1. Reuse the same gloves and pipette tip from the nitrate analysis.
2. Record the meter ID# on the datasheet.
3. Triple rinse the glass cuvet and plastic cap with DI water.
4. Turn on meter by pressing the EXIT button on the upper right hand corner of keypad.
5. Upon startup, “79 PO4” should be displayed on the bottom left hand corner of

the screen.



6. If "PRGM 79" is not displayed, press the PRGM button. The display will show: "PRGM?" Then enter "79" and push the ENTER button on the bottom right hand corner of the keypad. "79 PO4" should now appear on the bottom left hand corner of the display.
7. Using the pipette labeled "PHOSPHATE", pipette 5 mL of sample water into the cuvet twice for a total of 10 mL.
8. Cap the cuvet. Clean the outside of the cuvet with a Kim Wipe.



9. Insert the cuvet into the meter with the diamond shape on the cuvet facing forward, as shown in the photo to the right.
10. Place the lid on the meter.
11. Press the ZERO button on the bottom left corner of the keypad. The display should now read "0.00 mg/L".
12. Remove the cuvet from the meter and remove the cap. Use scissors to open silver reagent packet and add powder to the cuvet.
13. Cap the cuvet.
14. Swirl vigorously for 30 seconds and re-clean with a Kim Wipe. Place the cuvet back into the meter with the diamond shape on the cuvet facing forward and place the lid back onto the meter.
15. Press TIMER and then ENTER which are both on the bottom right hand corner of the meter keypad. This starts the timer for the two minute reaction time.
16. Wait for the two minute reaction to complete.
17. After the timer finishes beeping, push the READ button on the bottom of the keypad. (Note: If the READ button is pushed before the timer finishes beeping, you will get a zero reading.)
18. The screen will then show the result.
    a. If the value is less than 2.50 mg/L: Record the value on the datasheet.
    b. If the value is greater than 2.50 mg/L: Sample requires dilution. Store sample in the fridge and notify staff.
19. Clean up:
    a. Empty the glass cuvet in the waste container labeled 'PO4 Waste'.
    b. Triple rinse the cuvet and cap with DI water and discard into PO4 Waste container.
20. Put the used pipette tip in the bin labeled 'Dirty Glassware'.
21. Put the water sample in the fridge.
22. Return equipment to bin and store on shelf.
23. Leave datasheet by sign-in sheet.

Procedure for Dilutions of Orthophosphate Samples

To be Conducted by VMP Staff Only

If orthophosphate results are greater than 2.50 mg/L, then the sample requires a dilution to obtain an accurate result. VMP staff check the sample fridge at the start of each day to determine if samples are present that require further dilutions.

Equipment:

- HACH DR/890 or DR/900 meter with glass cuvets, black plastic caps and meter cap
- Orthophosphate reagent made by HACH (Phos Ver 3)
- Kim Wipes
- DI water in a bottle
- Adjustable pipetter
- Pipette tip
- Timer
- Scissors
- PO4 Waste Container

Sample Analysis Procedure:

1. Put on gloves.
2. Record the date and time of analysis on the datasheet.
3. Triple rinse the glass cuvet and plastic cap with DI water.
4. Triple rinse the pipette tip, inside and out.
5. Place a pipette tip on the pipetter.
6. Dial the pipetter to 5 mL.
7. Dispense 5 mL of DI water from the bottle into the glass cuvet.
8. Dispense 5 mL of sample water into the glass cuvet.
9. Clean the outside of the cuvet with a Kim Wipe.
10. Process sample as usual.

APPENDIX C. MORRO BAY NATIONAL ESTUARY PROGRAM
YSI EXO3 SONDE CALIBRATION AND DEPLOYMENT PROTOCOLS

Morro Bay National Estuary Program
YSI EXO3 Sonde Protocol

Sensor Maintenance

Sensors and calibration fluids need to be replaced at regular intervals to assure data quality. Maintenance tasks need to be performed regularly as well. EXO3 sensors should always be calibrated prior to longer continuous deployments.

Item	Action	Interval
Calibration fluids	Replace with new standard	6 months after opening or 12 months after manufacture. Check bottle label
pH sensor	Replace sensor module (SKU: 577603-02)	After 12-18 months
DO sensor	Replace sensor cap (SKU: 599110)	After 12 months
DO sensor	Clean with lint-free cloth or soft brush (see manual for specs)	Before each calibration
Conductivity/temperature sensor	Clean with lint-free cloth or soft brush (see manual for specs)	Before each calibration
Conductivity/temperature sensor	Clean electrode channels with mild solution of clean water and dish soap.	Between long-term deployments, or as needed based on condition
Total algae sensor	Clean with lint-free cloth (see manual for specs)	Before each calibration
6-pin connectors, sensor connectors, rubber gaskets, cable connectors	Clean and apply krytox grease	As needed, at least every 6 months
Sonde batteries	Replace	Dependent on use. Replace when battery life falls below 20% between field visits. Or below 40% for continuous deployments.
EXO central wiper	Replace	Annually, or between long-term deployments

Antifouling

Long-term deployment intervals and highly productive waters can result in biofouling of equipment. If the EXO3 shows signs of biofouling, clean the sonde guard thoroughly to reduce the potential for sensor drift.

- Clean any minimal biofouling from the sensor guard with a cloth soaked in dish soap.
- If fouling still remains, soak the guard in soapy water or gently scrub heavy biofouling with a small plastic scrub brush.
- NOTE: Do not sand or polish the inside of the guard bottom! This may affect readings.

Sensor Calibration

EXO3 sensors should always be calibrated prior to continuous deployments using the Kor software. This process can be done on the handheld unit, or with a computer with Kor installed (via Bluetooth connection or with USB connector). All calibration records are stored within the Kor software.

SmartQC scores indicate what kind of attention each sensor needs:

- **Green**: means the sensor is calibrated properly and all parameters are within factory-defined limits.
- **Yellow**: means that the sensor will still perform within factory-defined limits, but that during calibration enough of an adjustment was required to suggest that the sensor is drifting from those limits. This might mean that the equipment will need additional attention in the future, like a new DO cap. A yellow score is likely okay for discrete measurements, but should not be used for longer term deployments.
- **Red**: means that the sensor is not performing within factory-specified limits. Some component of the sensor may need to be replaced, or the sensor may be in need of a calibration.

Quarterly Calibration Strategy: Multi-Sensor Calibrations

Other than the built-in depth sensor, all removable sensors should be calibrated according to the Multi-Sensor Calibration strategy ("multi-cal") to minimize variability between sensors and the excessive use of calibration fluids. Follow the general steps outlined below to conduct a multi-cal:

- Before removing any sensors, **ensure all replacement dates (particularly for DO sensor caps and pH sensor modules) are up to date on the Kor software**. It is good practice to return the sensors to the same sonde where they had been installed prior to calibration, however this can be challenging to track in the midst of the calibration process. As long as the replacement dates are correct, the sensor will retain this information regardless of where it is installed (it just makes replacement tracking more complicated when sensors are shuffled around). If needed, refer to the Replacement Sensor Tracking spreadsheet to match sensor serial numbers with the appropriate sonde and update if sensors are moved.
- As of April 2025, we have six active sondes that are calibrated every three months. Typically, all sondes and sensors are updated at one time in a quarterly calibration process. To start the process, begin by calibrating the conductivity/temperature sensors. Many of the subsequent sensors rely on accurate temperature readings during their calibrations.
- Remove the temperature/conductivity sensors from four of the sondes, and remove all "other" sensors from the remaining two sondes. The central wiper should remain installed on all sondes.
- Distribute the temperature/conductivity sensors between the two sondes. If some sensor ports are empty, plug the exposed port with the included plugs (located in the EXO toolbox). Follow the specific instructions for conductivity/temperature sensors described in the section below and calibrate each sensor in sequence as outlined in the multi-cal procedure (video linked above).
- Following successful calibration, redistribute the temperature/conductivity sensors so each sonde has one installed. Delegate the sondes so two will be used for each sensor type (DO, pH, and Total Algae). Remove and reinstall the sensors so that, aside from the port containing the temperature/conductivity sensor, the three remaining ports contain the same type of sensor.
- Follow the sensor-specific instructions outlined below, and calibrate each sensor in sequence as outlined in the multi-cal video.
- Once all sensors have been calibrated, return them to their respective sondes.

Depth

- Make sure that the depth sensor is not immersed or suspended in any solution.
- In the Calibrate menu, select Depth and then select Calibrate. 0 is the only acceptable calibration value for depth.
- Observe the Pre Calibration Value readings and the Data Stability, and when they are stable, click Apply to accept this calibration point. This process zeros the sensor with regard to

current barometric pressure. You can cross-check current barometric pressure using the blue EXO handheld unit.

NOTE: If the SmartQC score for depth comes back as Yellow, clean the sensor thoroughly and ensure that the sensor is completely dry before calibration. If needed, use a can of compressed air to dry off the sensor to perform a better calibration. (Do NOT stick any tools or utensils inside the pressure sensor vent hole. The sensor membrane is extremely thin and easily punctured.) If issues persist,

Conductivity / Temperature

- Clean the conductivity cell with the supplied soft or lint free cloth brush before calibrating.
- Use 1413 µS/cm conductivity standard for creek deployments. Pour conductivity standard into a clean and dry or pre-rinsed EXO calibration cup, filled to the second marked line.
- Immerse the probe end of the sonde into the solution, gently rotate and/or move the sonde up and down to remove any bubbles from the conductivity cell. Allow at least one minute for temperature equilibration before proceeding.
- In the Calibrate menu, select the *Conductivity* sensor and then select *Specific Conductance*, and then select *1413 standard*.
- Observe the Pre Calibration Value readings and the Data Stability, and when they are stable, click *Apply* to accept this calibration point. Click *Complete* when done.

NOTE: If the data do not stabilize after 40 seconds, gently rotate the sonde or remove/reinstall the cal cup to make sure there are no air bubbles in the conductivity cell.

Dissolved Oxygen

- Clean the probe with the supplied soft or lint free cloth brush before calibrating.
- Place the sensor in either a continuously oxygenated environment, such as a large beaker on a spinning plate (between setting 6 -8), or a continuously sparged aquarium with an air pump.
- Wait approximately 5 minutes before proceeding to allow the temperature and oxygen pressure to equilibrate.
- In the Calibrate menu, select *ODO*, then select *ODO % sat* or *ODO % local*. (NOTE: Calibrating in ODO % sat automatically calibrates ODO mg/L and ODO % local and vice versa.)
- Observe the Pre Calibration Value readings and the Data Stability, and when they are stable, click *Apply* to accept this calibration point.
- Click *Complete* when done. View the Calibration Summary screen and QC Score. Click *Exit* to return to the sensor calibration menu.
- Dissolved oxygen readings from the EXO3 can be compared to YSI Pro2030 meters and/or to Winkler titration results.
    - Place the sensor in either a continuously oxygenated environment, such as a large beaker on a spinning plate, or a continuously sparged aquarium with an air pump.
    - Wait approximately 5-10 minutes to allow the temperature and oxygen pressure to equilibrate.
    - Confirm that DO readings from the EXO resemble YSI readings and/or are +/- 1 mg/L from Winkler results.

NOTE: If the SmartQC score for DO comes back as Yellow, clean the sensor and ensure that all debris is removed from the surface. Ensure that proper calibration procedures were followed. Typical errors include not allowing enough time for the calibration chamber to come to equilibrium (10-15 minutes). Check the lens cap for scratches, or if sensor has been left in dry air longer than 8-hr (if so it needs to be rehydrated for 24-hr).

pH

- Select the 3-point option to calibrate the pH probe using three calibration standards: buffers with a pH of 4, 7, and 10.

- Start with the pH 7 buffer. Rise the sensor with **tap water** then pour fresh pH 7 standard into a clean and dry or pre-rinsed EXO calibration cup, to the second marked line. In a separate pre-rinsed EXO calibration cup, pour the contents of the pH 7 solution from the previous calibration to use as a rinse.
    - NOTE: the process takes approximately 500mL (about 1 bottle of each standard solution). Be sure you have adequate solution before starting this process.
- Rinse the probe end of the sonde by placing it in the calibration cup containing the old pH 7 solution and removing it after 30 seconds.
- Immerse the probe end of the sonde into the fresh solution, gently rotate and/or move the sonde up and down to **remove any bubbles from the conductivity cell**.
    - Allow at least 1 minute for temperature equilibration before proceeding. This is important, as the actual pH value of all buffers varies with temperature.
- In the Calibrate menu, select *pH*, then select *Calibrate*.
- Observe the Pre Calibration Value readings and the Data Stability, and when they are stable, click *Apply* to accept this calibration point.
- Click *Add Another Cal Point* in the software for pH 10.
- Thoroughly rinse the sensor and calibration cup with **tap water**, then repeat this procedure with the old pH 10 buffer rinse and fresh pH 10 buffer.
- Click *Add Another Cal Point* in the software for pH 4.
- Thoroughly rinse the sensor and calibration cup with **tap water**, then repeat this procedure with the old pH 4 buffer rinse and fresh pH 4 buffer.
- Click *Complete* when done. View the Calibration Summary screen and QC Score. Click Exit to return to the sensor calibration menu.

NOTE: If the SmartQC score for pH comes back as Yellow, ensure that the sensor is clean and free of any air bubbles. Check that there are no cracks or visual damage to the glass bulb.

Total Algae (Chlorophyll and Phycocyanin)

- Clean the sensor face with the supplied lint free cloth before calibrating.
- Take the 125mg/L Rhodamine WT solution out of the fridge and transfer 5mL into a volumetric flask or measuring cup that can hold over 1000mL.
    - NOTE: If the 125mg/L Rhodamine WT solution is expired (2 years past the date marked on the bottle) you must prepare a new solution.
- To make a new calibration solution, transfer 5mL of Rhodamine WT dye (Kingscote Chemicals item #106023) into a volumetric flask or measuring cup and add deionized water until it reaches the 1000mL volumetric mark. Mix well and transfer into a storage bottle with a label to record the date the solution was made. Store the 125mg/L Rhodamine WT solution in the equipment room fridge. Only perform this step if the current batch is expired.
- Add deionized or distilled water to the flask/cup until it reaches the 1000mL volumetric mark. For extra accuracy, use a graduated cylinder to measure out 955mL of DI water.
- The new solution will be 0.625mg/L Rhodamine WT. Mix well and make sure to use within 24 hours.
- In the Calibrate menu, select the TAL-PC sensor and then select the Chlorophyll channel. The sensor should be calibrated in the default RFU units.
    - NOTE: The chlorophyll and phycocyanin channels need to be calibrated separately. Calibrating one channel does not automatically calibrate the other.
- Place the sonde into the calibration cup with clean water to calibrate at the zero point. DI water is not required but is preferred.
- Observe the Pre Calibration Value readings and the Data Stability, and when they are stable, click Apply to accept this calibration point. Click Add Another Cal Point when done.
- Empty the calibration cup and add your freshly prepared 0.625mg/L Rhodamine WT solution. Place the sonde into the cup and observe the data stability.

    - NOTE: A conductivity/temperature sensor must also be installed to perform the full calibration for the TAL-PC sensor.
- As the sensor is stabilizing, observe the temperature displayed and refer to the chart below to set the standard value. You will have to manually change the standard, it does not self-correct. For the chlorophyll channel, use the Chl RFU column.

	Chlorophyll 0.625 mg/L Rhodamine		Phycocyanin 0.625 mg/L Rhodamine	
Solution Temperature (˚C)	Chl RFU	µg/L chlorophyll	PC RFU	µg/L phycocyanin
30	14.0	56.5	11.4	11.4
28	14.6	58.7	13.1	13.1
26	15.2	61.3	14.1	14.1
24	15.8	63.5	15.0	15.0
22	16.4	66	16.0	16.0
20	17.0	68.4	17.1	17.1
18	17.6	70.8	17.5	17.5
16	18.3	73.5	19.1	19.1
14	18.9	76	20.1	20.1
12	19.5	78.6	21.2	21.2
10	20.2	81.2	22.2	22.2
8	20.8	83.8	22.6	22.6

- Once you have checked the standard value and the reading has stabilized, click Apply and then click Complete.
- After performing the 2-point calibration for the chlorophyll channel in RFU, perform the same steps as described above for the phycocyanin channel in RFU. Calibrate the phycocyanin channel using both clean water and using the 0.625mg/L Rhodamine WT solution. When setting the standard value for the Rhodamine solution, refer to the PC RFU column of the table above.
- After calibrating the phycocyanin channel in RFU, the TAL-PC sensor is ready for deployment.

Calibration Data Management

The YSI EXO software generates logs for calibrations when they are performed with a computer or the handheld unit. Calibration logs should be stored in this folder:

DATA-Documents\Monitoring\vmp\vmp admin\Equipment and Supplies\Calibration\EXO3 Records

By default, these records will be stored on a local drive for whatever computer you are using to calibrate the sensors. Either copy and paste the calibration logs into the folder above or, if you have a connection to the Shared drive, designate the above folder path as the default saving location. Calibration logs can be accessed within the KOR EXO software by going to the Calibration tab, clicking on “Find Calibration Records”, and downloading the resulting list. Results can be filtered by date or

parameter.

The default report format provided by Kor makes it challenging to quickly look back at calibration records and review the status of individual sensors. As an alternative, the Master EXO Calibration tracker is where the most important calibration information will be stored for quick access. In order to format data quickly for adding to the master spreadsheet, the clean EXOcalibration R script can be downloaded and initiated to properly filter and format the Kor report file. Once the script has run, the summarized information can be copy/pasted into the Master spreadsheet.

After downloading the script, place it in the same folder along with the Kor calibration reports and run the following code in RStudio (any blue text within <<>> must be edited by the user):

```
setwd("<<INSERT FILE PATH WHERE YOU HAVE DOWNLOADED THE CALIBRATION REPORT AND SCRIPT>>")

install.packages("tidyverse")

cal_file<-read.csv("<<INSERT NAME OF CALIBRATION REPORT FILE>>.csv", header=F)

source("cleanEXOcalibration.r")

cal_sum<-cal.file.sum(cal_file)

write.csv(cal_sum, "<<NAME THE FILE SOMETHING YOU WILL REMEMBER>>.csv")
```

The resulting csv file will populate within the working directory folder. The data contained inside can then be copy/pasted into the Master spreadsheet on SharePoint.

Deployments

Deployment intervals can be set between 15 minutes and 1 hour intervals. For short-term deployments (days-weeks) that include pH and DO measurements, a logging interval of 15 minutes is recommended. To maximize battery and sensor life, it is recommended to start/activate/enable internal logging file immediately prior to deployment with a delayed start. For long-term deployments (weeks-months), a logging interval of 1 hour is recommended to conserve battery life.

The EXO sondes allow for an averaging duration to be specified. If pH is being collected for the study, it is important to set an averaging duration other than zero. Two to 3 minutes is suggested. The reason for this is that if the average duration is set to zero (default), only an instantaneous measurement will be collected at each measurement interval. The pH probe will likely not have adequate warmup time to equilibrate, and biased pH data could result. Note that after deployment, it is important to set the averaging duration back to zero for calibration post-checks, to get quick response to each standard/buffer. The averaging duration can be edited in the Advanced deployment settings.

It is important to recalibrate depth before each deployment, after having arrived at the deployment site. Calibrations conducted at the office (roughly at sea level) can result in negative depth measurements when deployed at higher altitudes.

Sensor Storage

Short Term: For interim storage, users should keep sensors moist, but not submerged. Store the sonde upright, with either a moist sponge, or approximately 0.5 inches of deionized water in the bottom of the calibration cup. Close the calibration cup tightly to prevent evaporation. To protect the cable connector, either leave the cable installed on the connector or install a port plug.

Long Term: For longer term storage, store sensors according to the directions below or the corresponding EXO University video. Plug any open port plugs.

Conductivity/Temperature Sensor

- Clean electrode channels before storage with a soft cleaning brush (included in the sonde maintenance kit). Sweep channels about 15-20 times. If deposits have formed use a mild solution of dish soap and water to brush the channels.
- Store the sensors either dry or wet.

Depth Sensor

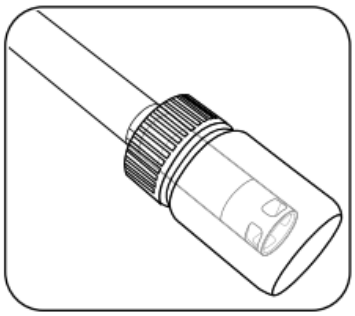
- Depth sensors can be stored dry, in water-saturated air, or submerged in clean water.

Dissolved Oxygen Sensor

- Leave the sensor installed in the sonde, and submerge it in clean water in the calibration cup. Screw the cup on tightly to prevent evaporation.
- The DO sensor should remain in a moist or wet environment. If the dissolved oxygen sensor remains dry for more than 8-hr, the sensor must be rehydrated. To rehydrate, soak the DO sensor cap in warm (room temperature) tap water for approximately 24 hours. Following the soak, calibrate the sensor and store it in a moist environment.

pH Sensor

- Remove the sensor from the sonde and insert its sensing end into the small bottle containing pH 4 buffer, as shown in the photo below. If this is unavailable for any reason, pH sensor can by stored in **tap water** (not DI water).



- Like DO, pH sensors should remain moist/wet at all times. If the pH sensor has been allowed to dry, soak the sensor for several hours (preferably overnight) in a 2 molar solution of potassium chloride (KCl). If KCl is unavailable, a tap water or pH 4 buffer soak may restore function. If the sensor is irreparably damaged, users must replace the sensor module.

Total Algae Sensor

- Optical sensors like the Total Algae sensor require very little maintenance. Wipe the optical surface at the tip of the sensor with a non-abrasive, lint-free cloth. Sensors may be removed or left in the sonde for long-term storage. If removed, store in dry air with the shipping cap covering the sensor surface to protect against physical damage.

Sensor Drift

After calibration, all sensors have a tendency to drift out of calibration over time. Some sensors have more severe drift than others. Factors such as the age of the sensor, concentration of different substances in the water, and the sensor technology play a role in drift. This concern is most important when doing continuous monitoring deployments that can last several days. It is possible to quantify drift by taking two measurements: one measurement immediately after calibration, and a second measurement at the end of the monitoring period.

For example, the drift of the optical DO sensor can be calculated by taking an initial measurement of 100% saturated DO after calibration and then taking another measurement in a 100% saturated environment at the end of the sampling period and subtracting the first measurement from the second.

In this example, we could say that the first measurement showed 100% saturation and the final measurement showed 105% saturation. It is best to take three measurements at each time to confirm the average. With a difference of +5%, we can divide 5% over the elapsed time since the calibration and estimate a drift rate:

$$\frac{Total\ Drift}{(T_2 - T_1)} = Drift\ Rate$$

105% - 100% = 5%
$Time_1$ = 9:00
$Time_2$ = 17:00

$$\frac{5\%}{17{:}00 - 9{:}00} = 0.63\ {}^{\%}/_{hour}$$

This drift should be calculated regularly for dissolved oxygen because it can be done quickly and easily in the field. It is important, however, that the DO sensor cap be gently wiped before each 100% saturation measurement so that droplets do not affect the final result. It is also possible that the first post-calibration value is slightly more or less than 100% because the local air pressure may be higher or lower than the standard atmospheric pressure.

The EXO sonde automatically calculates a gain for ODO sensor. The gain value can drift over time, and the YSI system's SmartQC can determine if the gain drift is exceeding factory specifications. If the gain drift is excessive and causing frequent errors, the sensor cap may need to be replaced. SmartQC results may be sufficient to determine if the drift is substantial. It may not be necessary to carry out the above calculation unless the SmartQC drift is substantial and you need to confirm the magnitude of the error.

APPENDIX D. MORRO BAY NATIONAL ESTUARY PROGRAM DISSOLVED OXYGEN IN THE BAY (“DAWN PATROL”) PROTOCOL

Morro Bay Volunteer Monitoring Program Dissolved Oxygen in the Bay ("Dawn Patrol") Protocol

MBNEP Provided Equipment:

- YSI Pro2030 (DO/Temp/Salinity) meter with safety leash Clipboard
- Laminated data sheet and pencil
- DI water to rinse probes
- Headlamp
- Lifejacket

Recommended Personal Equipment:

Heavy nylon or water repellant jacket and pants (rubberized clothing or wetsuits can get hot) under layers of fleece. Use neoprene or rubber gloves and booties and wool cap or bandana to cover head.

Preferred optimal tides for monitoring lowest level of oxygen in the bay would be at minimum tides. The tricky part is to account for sufficient tides to avoid getting stuck! General amount of time necessary for collection is 30 minutes.

MINIMUM TIDES NECESSARY TO GET IN AND OUT:

At least 3ft+ for an incoming tide

At least 4ft + for an outgoing tide

Procedure:

1. Choose a day that has an early morning high tide. You'll need three feet or more of water when you're taking your measurements. Field work should be conducted within two hours after sunrise. Choose a tide that allows enough water to get back safely without getting stuck in the mud (see above).
2. The most efficient route for upper bay sites (ATP, STP, LO2 and PSP), is to check ATP (Tidelands Park boat launch) from dock then drive to STP (State Marina) and launch there to get other three sites. The three backbay sites (CSI, SHI, and CHI) can be accessed by launching from Cuesta Inlet.
3. Before setting out, turn on the YSI meter to give it time to warm up. It's a good idea to turn on the meter as you start out so it has adequate time to stabilize before you start taking measurements.
4. Use the safety leashes to clip the meters to either yourself or your kayak. We've lost many meters at the bottom of the bay!
5. For safety, and to help fill out the data sheets, use the headlamps. Don't forget to wear a lifejacket.
6. On the data sheet record the date, time, dawn time, sunrise time, height of tide, whether it is in or out going, and the current weather (especially wind, clear, foggy or rain).

7. Paddle to the site. Approximate your location based on landmarks.
8. Measure the DO at the site.
9. Remove the probe from the chamber.
10. Hit the Mode button so that the units shown for the reading is '%'.
11. Place probe in the water just below the surface and move the probe slowly. You want to move the probe at an even pace, about 2 feet per second, either back and forth or in a circle. Alternatively, you could use faster and shorter strokes in an up and down motion. You'll know when you're moving the probe correctly because the meter reading will stabilize.
12. Wait until the meter reading stabilizes.
13. Record DO value at the surface (or a depth value of '0') in %.
14. Hit the Mode button to switch the units to mg/L. Wait for the reading to stabilize and record the value.
15. Record the temperature.
16. Measure salinity at the site.
17. Insert the probe just below the surface of the water and bob it up and down so that all air bubbles are forced out of the holes in the probe. The probe must be submerged in the water beneath the vent holes on the top of the probe. If not adequately submerged, the water won't come in contact with the probe and the reading will be inaccurate.
18. Hit the Mode button until the units for the reading are 'ppt.'
19. Wait for the reading to stabilize. (The range message must complete.)
20. Record the salinity value on the datasheet.
21. Rinse the probe with DI water and return to the storage chamber on the side of the meter.
22. Paddle to the next site and continue monitoring.
23. Upon completion of data collection, return equipment and datasheet to the MBNEP office. Let MBNEP staff know if there are any problems with the equipment.

Dawn Patrol	Date:	Time:	Sunrise:
Samplers:		Weather:	
Tide (height & time): Incoming / Outgoing (circle)			Equipment ID:

		Readings at the Surface				
Site	Time	Salinity (ppt)	Temp (C)	DO (mg/L)	DO, %	Description
CSI						1/2 way between sign board on shore at boat launch & the other side of inlet
SHI						mouth of shark's inlet - end of golf course and across from last houses
CHI						next to 'no hunting' sign, water stick marker, south of private pier, north of cuesta inlet mouth
PSP						across from big brown house up on point, water marker to the west
LO2						midway between red tube bouy and black water marker 100 yds apart
SPM						directly out from museum of natural history
ATP						just off boat tie-up at tidelands

APPENDIX E. MORRO BAY MONITORING PROGRAM STREAM PROFILING PROTOCOL

Morro Bay Volunteer Monitoring Program Stream Profiling Protocol

Equipment List

- Tripod
- Level
- Stadia Rod
- Survey Tapes (100' and 200')
- Clipboard, datasheet, pencil
- Camera
- GPS Unit
- Machete, loppers
- Rebar, PVC pipe, flagging (if needed)
- Hammer (if needed)
- Stakes for measuring tape
- Tecnu, wipes, gloves, tyvek suit (if needed)
- Long sleeves, pants, boots, water.
- Compass
- Hand level

Main Steps

1. Locate site, take pre-survey photos
2. Define cross section
3. Measure required channel dimensions
4. Set up level
5. Survey the cross section
6. Measure water surface slope
7. Take post-survey photos and clean up.

Procedure

1. **Locate Site, Take Photos**

Before heading out into the field, download available avenza maps and bring a printed copy of the most recent site description document. Sites should be marked with flagging and PVC pipes that are about 2 feet tall. The pipes sit over rebar stakes. If only one pipe/rebar can be found, focus your search for the other stake at an angle that is perpendicular to the channel, and use a plot of the cross section from previous surveys (included in site description document) to measure the distance from the known stake to where the unknown stake should be located.

Once you have located the site markers, take initial photos to document conditions prior to the survey. Take at least one photo at each of the following locations: from left bank marker looking toward right bank, from right bank marker looking toward left bank, from downstream of center of cross section looking upstream, from upstream of center of

cross section looking downstream, from center of cross section looking at right bank, and from center of cross section looking at left bank. It is also helpful to take photos of the markers themselves if not visible at the listed photo orientations. Be sure to photograph any other notable conditions such as eroding banks, woody debris jams, or downed trees.

Once pre-survey photographs are taken, utilize machetes and loppers as needed to clear any vegetation that obstructs your ability to string the tape and survey the cross section.

2. **Define Cross Section**

Remove PVC pipes to reveal rebar benchmarks, then attach the measuring tape's metal hook to the rebar benchmark on the left bank (determine left bank by looking downstream). Stretch the tape tight across to the rebar benchmark on the right bank and fasten by wrapping the tape around the rebar then knotting.

3. **Measure Required Channel Dimensions**

a) Bankfull Width: To determine the bankfull width of the channel at the cross section, find the bankfull edge of the channel on each bank. Use stakes or have crew members hold the tape to string it tightly across the channel from one bankfull edge to the other. This is the **Bankfull Width**, record it on the datasheet. Ensure that the bankfull tape is strung directly below the established cross section tape. The bankfull edge is the point that water reaches during high flow events that occur approximately every 1.5 years. Because flows reach this line regularly and exceed this line more rarely, the vegetation, substrate/soil, and bank slope often change at this point. Look for indicators of flow like scour lines, stain-lines on rocks, changes in vegetation, presence of perennial and long-lived vegetation, changes in bank slope, point bars and undercuts, and presence of soils or leaf litter.

Be sure to consider your local knowledge or consult someone with local knowledge hydrology, climate, vegetation, and recent flow events. Always consider more than one indicator. If it is difficult to identify bankfull indicators at the transect location, look both upstream and downstream of the site for indicators and extrapolate to where the bankfull line would fall at the transect. If reliable indicators can only be determined on one side of the stream channel, extend the tape on a level plane from that point to the other side of the channel. Use a hand level on the tape or have another crew member stand upstream or downstream to spot it to make sure the tape is level. It may be helpful to consider stream channel morphology and evolution. Stream channels change over time and channels adjust to be able to contain regularly occurring flows. Think about what level of flows might "fit" in the channel before it begins to overflow its usual banks. In incised stream channels, the point at which banks overflow can be more difficult to conceptualize and identify. Instead, think about the level at which flows

might start to cause substantial erosion of banks or significant changes in channel morphology. The more irregularities there are in climate/precipitation in recent years, the more difficult it will be to determine the bankfull edge.

For more details, see guidance from the Water Board included at the end of this protocol.

b) Max Bankfull Depth (previously referred to as bankfull depth): While keeping the tape stretched across the bankfull channel, now imagine there was only a thin trickle of water in the stream. It would follow a path that wound through the deepest part of the channel bottom; this path is called the thalweg.

To find the thalweg, use the stadia rod to locate the deepest point in the channel. Once this is established, measure the distance from the channel bottom up to the tape that represents a bankfull flow (Figure 1). This is your **Max Bankfull Depth,** record it on the data sheet. Once you have recorded this measurement, you can remove the stakes from the bankfull tape, you'll need it to measure flood-prone width.

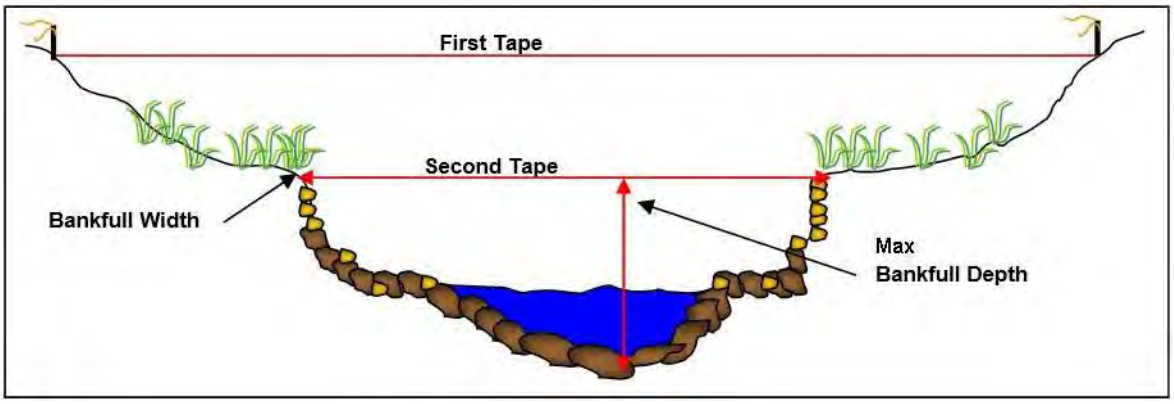


Figure 1. Locating bankfull width and bankfull depth.

c) Flood-Prone Depth and Width: First, multiply the measured max bankfull depth by 2, this is the **Flood-Prone Depth**, record it on the datasheet. The flood-prone width is measured at a height above the thalweg equal to 2 times the max bankfull depth.

To take this measurement, have the crew member that located the thalweg with the stadia rod stay put. Station other crew members on each side of the bank holding the tape. While holding the stadia rod vertically on the streambed at the thalweg, the rod holder should hold the tape with their finger to the flood-prone depth, the point on the stadia rod equal to 2 times the max bankfull depth. Next, the crew members holding the tape should raise the ends of the tape to that level and extend it in both directions to the point at which the tape intersects the banks.

Often, it takes a few minutes for the tape holders to adjust their positions until the tape is level. Once you are certain the tape is level at the flood-prone depth and has been extended to the banks on both sides, this is your **Flood-Prone width**, record it on the datasheet. While it is easiest to have 3 crewmembers present to perform this measurement, it can be done with 2 people (Figure 2). For measuring long flood-prone widths with 2 people, it is better to have one person stake the tape in on one bank at the correct level, then extend it to the other side while the rod holder stays in the thalweg and ensures that the tape stays at the flood-prone depth level.

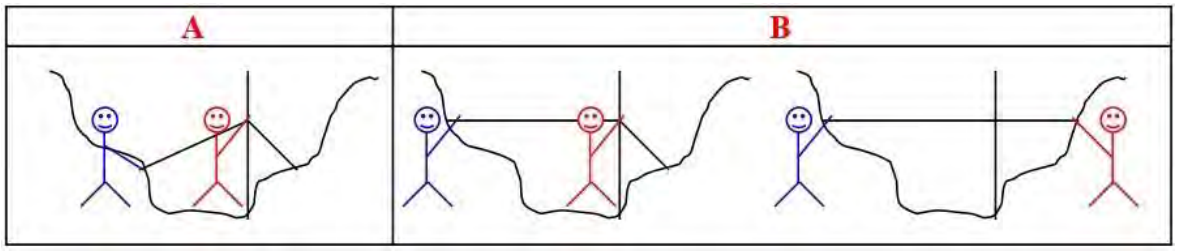


Figure 2. Measuring flood-prone width with 2 people.

d) Entrenchment Ratio: Divide the flood-prone width by the bankfull width, this is the **Entrenchment Ratio**, record it on the datasheet.

4. **Set up the Level**

Ideally, you want to be able to survey the cross section without moving the level, so try to locate the level so that it is ***above*** the entire line of measuring tape, although not above the extent the rod will extend, and not somewhere blocked by trees or other vegetation.

When setting up the tripod, set it up in sturdy ground with the legs of the tripod spread sufficiently to keep it steady. Attach the level to the screw in the middle of the base on top of the tripod, tighten, and then adjust level until the bubble is centered in the middle of the circle. Be sure that there is sufficient space for the level-reader to stand and take readings without accidentally touching the tripod and shifting the bubble.

5. **Survey the Cross Section**

First, record a compass bearing from the left bank benchmark to the right bank benchmark. Then, place the stadia rod directly on top of the left bank rebar benchmark. Use a hand level to ensure that the stadia rod is held vertically. Use the level to view where the cross hairs intersect the rod. This will be the benchmark (BM) station (STA) on the datasheet. Record the reading in the backsight (BS) column for this station.

Next, place the stadia rod at the base of the left bank rebar. Record the level reading in the foresight (FS) column for station 0 on the datasheet.

Continue reading and recording this stadia reading in the foresight column at each horizontal foot across the transect until you reach the right bank benchmark. Utilize the description section of the datasheet and the codes listed at the top of the datasheet to note channel features as you go. At the least, record the locations of and take stadia readings at the bankfull edges, wetted edges, and thalweg. Take these readings exactly where they occur along the transect tape, not just at the nearest foot mark. Hold the base of the stadia rod gently to the ground surface as you go. Do not let the rod sink into any areas of fine sediment or mud.

When you reach the right bank benchmark, take the final station's foresight reading at the base of the rebar and record a benchmark backsight reading with the rod placed on top of the rebar. It is important that the person reading the level communicates with the person holding the rod to ensure that the rod was level at the time the reading was taken. Keep an eye on the bubble throughout this process to make sure that the tripod position has not adjusted.

For transects that are particularly long, deep, or obscured by brush, you may not be able to view the stadia rod along the whole transect from the original level location. If the obstructions cannot be cleared, you will need to move the level. Before moving the level, take a reading at the last visible station and select a new location that offers the most visibility. The rod holder should keep the rod in the exact same position except for rotating the numbered side to be visible at the new level location. Once the level is set up in its new position, record the stadia reading to the last station in the backsight column of the datasheet and note that you moved the level. Then, move to the next station and proceed with recording stadia readings at each horizontal foot in the foresight column.
Similarly, if the level or tripod gets bumped during the survey and the bubble becomes off-centered, re-center the bubble and take a backsight reading to the last measured station. Ensure that the stadia rod is placed in the exact same location when taking this backsight reading. Be sure to make a note of instances like this on the datasheet.

6. **Measure Water Surface Slope**

To measure slope, you'll need the level to be positioned so that the line of sight upstream and downstream of the transect includes two riffle pool sequences. If this cannot be achieved from your level location(s) during the survey, relocate the level at this time. The best location for this is often in or near the channel itself rather than on the banks. If it is not possible to capture two riffle pool sequences, place the level in the location that offers the furthest visibility upstream and downstream of the transect.

Once the level is in a suitable position, locate the furthest riffle upstream of the cross section that is within your line of site. Measure, along the centerline of the bankfull channel (Figure 3), the distance from the cross section to the beginning of that riffle (Figure 4). This is the **Upstream Distance**, record it on the datasheet.

At the beginning of that riffle, have a crew member hold the stadia rod at the water's surface while another crew member takes the stadia reading with the level. This is the

Upstream Reading, record it on the datasheet.
Similarly, locate the furthest riffle downstream of the cross section that is within your line of sight, and repeat the same steps to measure your **Downstream Distance** and **Downstream Reading** (Figure 4) — record those on the data sheet.

Draw a simple map in the space provided to illustrate the reach from which the slope measurement was taken.

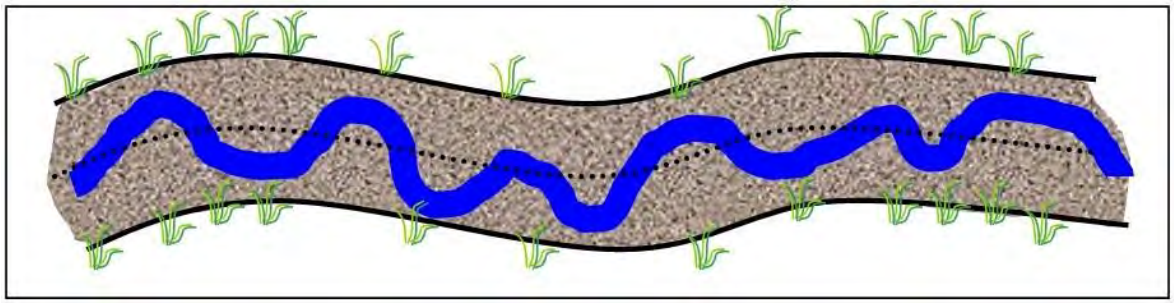


Figure 3. Dotted line represents centerline of the bankfull channel; blue line represents a summer low flow channel.

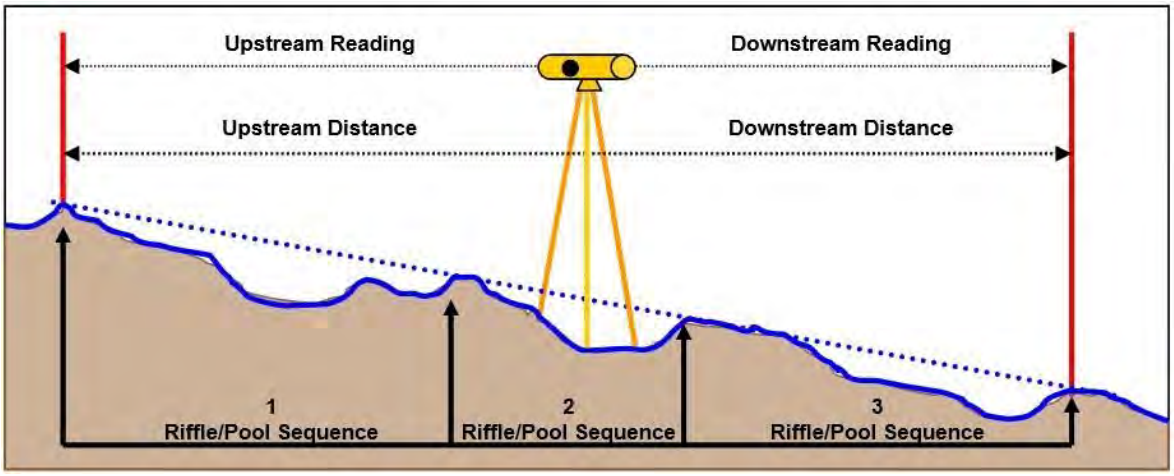


Figure 4. Measuring Slope. Capturing an even number of riffles and pools in the slope reach helps produce a representative measurement.

7. Take Post-Survey Photos and Clean Up

Before taking the cross-section tape down, take at least one photo at each of the following locations: from left bank marker looking toward right bank, from right bank marker looking toward left bank, from downstream of center of cross section looking upstream, from

upstream of center of cross section looking downstream, from center of cross section looking at right bank, and from center of cross section looking at left bank. If possible, try to capture the entire length of the tape in your upstream and downstream photos. Supplement with additional photo points as necessary so that the entire length of the cross section has been documented.

Remove measuring tape from rebar, replace PVC pipes over rebar, and apply fresh flagging if needed to assist in relocating site for subsequent surveys.
Use dawn soap, tecnu, and IvyX wipes to clean yourself and any equipment that has come into contact with poison oak.

Stream Profiling Datasheet

Creek:		Site ID:		Date:		VMP Staff:
Volunteers:						
Foot #	Stadia Reading	Foot #	Stadia Reading	Foot #	Stadia Reading	Bankfull and Floodplain Dimensions
0		35		70		Bankfull Width:
1		36		71		Bankfull Depth:
2		37		72		Flood-Prone Width:
3		38		73		Slope Measurements
4		39		74		Upstream Distance:
5		40		75		Upstream Reading:
6		41		76		Downstream Distance:
7		42		77		Downstream Reading:
8		43		78		Notes
9		44		79		
10		45		80		
11		46		81		
12		47		82		
13		48		83		
14		49		84		
15		50		85		
16		51		86		
17		52		87		
18		53		88		
19		54		89		
20		55		90		
21		56		91		
22		57		92		
23		58		93		
24		59		94		
25		60		95		
26		61		96		
27		62		97		
28		63		98		
29		64		99		
30		65		100		
31		66		101		
32		67		102		
33		68		103		
34		69		104		

APPENDIX F. MORRO BAY NATIONAL ESTUARY PROGRAM SURFACE ELEVATION TABLE MONITORING

Morro Bay National Estuary Program
Surface Elevation Table Monitoring
for
Measuring Sediment Accretion in Morro Bay Estuary

Purpose and Methods

The surface elevation table (SET) –also referred to as a sedimentation erosion table - is used to measure the sediment accumulation on the surface of a marsh or mudflat over time. In conjunction, a thin layer of feldspar can be placed around the SET as a marker horizon to measure shallow subsidence of the surface caused by compaction, desiccation, or other processes (Figure 1).

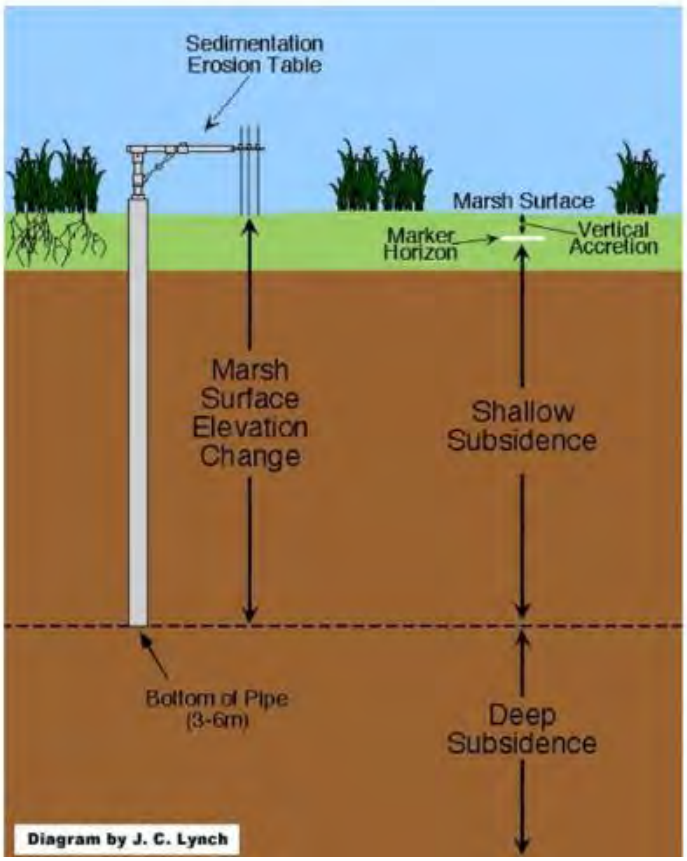
The SET is attached to a stable benchmark on the marsh surface (Figure 1). The benchmark is a 3'' aluminum pipe driven into the sediment as far as possible (until refusal). The benchmarks are driven by hand, and extend 6 to 8 inches above the sediment surface. In order to take measurements, the SET is placed over the benchmark and the surface elevation is measured relative to the benchmark at radial points around the station. Since a footprint in the measurement area can contaminate the readings for years, a platform or walkway, which serves as a working surface can also be constructed (Figure 2). We anticipate using these platforms in the salt marsh as needed, but not in the mud flats.

The plan for Morro Bay is to use the SET devices mainly in and adjacent to the salt marsh, and to use marker horizons and some PVC benchmarks elsewhere in the estuary. The final site locations, will be determined based on field conditions, site access, and the expertise of the Battelle consultants. A final map showing exact locations will be made available to you upon installation. Both salt marsh and intertidal mudflats will be represented.

Recording measurements after the initial installation consists of walking to the benchmark, attaching the SET device, and measuring the elevation. The precision of measurements at the 95% confidence interval for the SET technique ranges from ±0.4 mm to ±1.5 mm, depending on substrate characteristics. For the horizon markers, a small core is taken and the amount of sediment that has accumulated above the horizon marker is measures from the core. The direct measurements of elevation change can be used to measure overall sediment deposition or erosion, to compare rates of sediment deposition in different regions of the estuary, and to provide data to 'ground-truth' the numerous sediment models of the estuary. Elevation change can be compared to the local rate of sea-level rise to determine the potential for submergence or elevation of the wetland.

We plan to take measurements pre and post wet season in the first year, and annually thereafter. We will adjust the sampling frequency depending on results, and in response to extreme events such as major storms. The time required to collect data, once on site, is relatively short (less than an hour for an experienced operator) and so man-hours depend on ease of access to the site. Our data collection program should extend for at least five years; probably longer assuming the method proves effective. Additional information on the SET methodology can be found at: http://www.pwrc.usgs.gov/resshow/cahoon/

Layout of Surface Elevation Tables:



Surface Elevation Tables Data Collection Form

SET DATASHEET

Date _____

Location Morro Bay

Transect A B C

Station low high

direction	pin	reading (mm)	notes
_____	1	_____	_____
	2	_____	_____
	3	_____	_____
	4	_____	_____
	5	_____	_____
	6	_____	_____
	7	_____	_____
	8	_____	_____
	9	_____	_____
_____	1	_____	_____
	2	_____	_____
	3	_____	_____
	4	_____	_____
	5	_____	_____
	6	_____	_____
	7	_____	_____
	8	_____	_____
	9	_____	_____
_____	1	_____	_____
	2	_____	_____
	3	_____	_____
	4	_____	_____
	5	_____	_____
	6	_____	_____
	7	_____	_____
	8	_____	_____
	9	_____	_____
_____	1	_____	_____
	2	_____	_____
	3	_____	_____
	4	_____	_____
	5	_____	_____
	6	_____	_____
	7	_____	_____
	8	_____	_____
	9	_____	_____

MARKER HORIZON DATASHEET: SALT MARSH SAMPLING LOCATIONS

Date: _____
Observers: _____
Location: Morro Bay
Transect: A B C
Station: low high

plot location	repl.	random x	random y	readings (3 per core)	notes
_____	1	29	29	_____ _____ _____	_____
	2	16	19	_____ _____ _____	_____
	___	18	14	_____ _____ _____	_____
	___	38	18	_____ _____ _____	_____
_____	1	20	12	_____ _____ _____	_____
	2	36	18	_____ _____ _____	_____
	___	14	38	_____ _____ _____	_____
	___	8	11	_____ _____ _____	_____
_____	1	44	10	_____ _____ _____	_____
	2	27	11	_____ _____ _____	_____
	___	18	36	_____ _____ _____	_____
	___	40	28	_____ _____ _____	_____
_____	1	15	17	_____ _____ _____	_____
	2	45	29	_____ _____ _____	_____
	___	43	12	_____ _____ _____	_____
	___	19	14	_____ _____ _____	_____

APPENDIX G. MORRO BAY NATIONAL ESTUARY PROGRAM BIOASSESSMENT AND ALGAE MONITORING PROTOCOL

Morro Bay National Estuary Program Bioassessment Monitoring Protocol

Refer to the "*Standard Operating Procedures for the Collection of Field Data for Bioassessments of California Wadeable Streams: Benthic Macroinvertebrates, Algae, and Physical Habitat*" March 2025. Prepared by the California State Water Resources Control Board Surface Water Ambient Monitoring Program (SWAMP) SOP-SB-2025-0001.

NOTE: Sections 4.3, 4.4, and 5.2 of the protocol, involving collecting and preparing algal samples, and are not conducted by our organization due to lack of lab facilities and inadequate budget.

While conducting bioassessment monitoring, staff and volunteers must follow the California Department of Fish and Wildlife Aquatic Invasive Species (AIS) Decontamination Protocols, as follows:

California Department of Fish and Wildlife
Aquatic Invasive Species Decontamination Protocol

Purpose

California Department of Fish and Wildlife (CDFW) is committed to protecting the state's diverse fish, wildlife, and plant resources, and the habitats upon which they depend. Aquatic Invasive Species (AIS) are non-native aquatic organisms that negatively impact our state's environment, economy, or human health. Preventing the spread of AIS and disease-causing pathogens in CDFW activities, CDFW-permitted activities, and all activities outside the purview of CDFW's authority protects the natural resources of the state.

Application of this protocol is intended to prevent the spread of AIS and pathogens by removing biological agents by physical or chemical means, henceforth referred to as 'decontamination.' This protocol was developed based on the best available science and identifies the decontamination methods empirically tested with the highest efficacy across the greatest number of AIS and pathogens of greatest concern in California.

Many AIS and pathogens are difficult, if not impossible, to see in the environment, and can be unknowingly transported in entrapped water and on equipment. Therefore, assume that any AIS or pathogen is or could be present, and decontaminate between watersheds (or locations within watersheds if site-specific concerns warrant), to prevent the spread of AIS.

All equipment, including but not limited to, wading, dive, and sampling equipment (e.g., water quality probes, nets, buckets, substrate samples, etc.); fishing gear; and watercraft, must be decontaminated using one or more of the protocols listed below. Use your best judgment and field sampling needs to select the method(s) appropriate for your location, equipment, and schedule.

This document is formatted to provide concise decontamination protocols (page 3-5), along with the supporting information for these recommendations (Appendix A. Summary of Decontamination Methods Considered and Their Efficacy by Species, and Appendix B. Aquatic Invasive Species and Pathogen Profiles). Any brand names used in this document do not represent endorsement by CDFW, but rather are referenced relative to their active ingredients and demonstrated efficacy in peer-reviewed publications for the purpose of AIS decontamination.

For more information about invasive species in California visit the California Department of Fish and Wildlife's Invasive Species Program webpage. For questions about AIS, implementing these protocols, or to report an invasive species sighting, email invasives@wildlife.ca.gov or call (866) 440-9530.

Best Practices for Working in Aquatic Systems

Along with decontamination, the following best practices should be implemented when practical to ensure activities minimize the potential to spread AIS:

- When working in flowing water begin upstream and work downstream to avoid transporting AIS upstream to uninfested areas.
- Only work in one waterbody per day and decontaminate your equipment at the end of the day.
- If you must work in multiple waterbodies, use separate equipment for each site and decontaminate it at the end of the day. Bag used equipment and keep separate from unused equipment to prevent cross-contamination.
- If you must work in multiple waterbodies in a single day and cannot use separate equipment, decontaminate it at the site you are leaving, prior to traveling to the next site. When feasible, conduct work in waters believed to be AIS-free first, and sites known or suspected to have AIS last.
- Felt soled boots are more difficult to decontaminate than rubber soled boots because they can retain moisture for more than a month. If possible, choose boots with rubber soles.
- Clean all equipment before decontaminating. Debris reduces the efficacy of all decontamination methods by sheltering organisms from exposure and/or neutralizing chemicals.

Equipment Decontamination for Aquatic Invasive Species and Pathogens

Not all decontamination methods are appropriate for all circumstances. Choose the decontamination method(s) appropriate for the equipment being decontaminated and site-specific considerations. When possible, opt for the method effective for the most AIS and pathogens as possible. If multiple methods must be used, perform them sequentially, and never mix chemicals.

Hot Water Immersion[1]: 50°C (122°F) for 30 minutes

CAUTION: *Wear appropriate personal protective equipment to avoid burns, such as heat resistant gloves and/or a splash apron. Hot water can damage heat-sensitive equipment. Do not use hot water to decontaminate equipment that cannot tolerate temperatures at or above 50°C (122°F).*

Step 1. Scrub equipment with a stiff bristled brush to remove all organisms, mud, debris, etc. Thoroughly brush small crevices such as boot laces, seams, net corners, etc. and rinse.

Step 2. Immerse equipment in 50°C (122°F) or hotter water for 30 minutes. Weigh equipment down if necessary. Maintain the water temperature at 50°C (122°F) for the duration of the 30-minute soak.

Drying[2]: 14°C (57°F) for 8 days OR 35°C (95°F) for 30 hours OR 70°C (158°F) for 15 minutes

Step 1. Scrub equipment with a stiff bristled brush to remove all organisms, mud, debris, etc. Thoroughly brush small crevices such as boot laces, seams, net corners, etc. and rinse.

Step 2. Allow equipment to dry completely. Once completely dry, maintain equipment completely dry at 14°C (57°F) for 8 days OR 35°C (95°F) for 30 hours OR 70°C (158°F) for 15 minutes.

NOTE: New Zealand mudsnails, quagga/zebra mussels, and other mollusks can take several days to lethally desiccate due to their protective shells. The post-dry clock begins following internal tissue desiccation of mollusks. Removing mollusks before drying reduces the time needed to achieve effective results for all other species.

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[1] Tested effective for quagga/zebra mussels, didymo, chytrid fungus, whirling disease, sudden oak death, and white-nose syndrome. See Appendix A for more information.

[2] Tested effective for quagga/zebra mussels, New Zealand mudsnails, didymo, chytrid fungus, whirling disease, sudden oak death, and white-nose syndrome. See Appendix A for more information.

Virkon® S (21.41% potassium peroxymonosulfate + 1.5% sodium chloride) Immersion[3]: 2% solution for 20 minutes

CAUTION: *Virkon® S is a corrosive chemical known to degrade metals and materials over time. Avoid exposure to yourself and others, and prevent spills or contamination to the environment. Always read and follow all the product's label and Safety Data Sheet (SDS) procedures. Virkon® S must be mixed and used in a well-ventilated area, preferably outdoors. Wear appropriate personal protective equipment such as tightly fitting safety goggles (EN 166), chemical resistant gloves, and a splash apron to avoid contact with eyes, skin, and clothing. This chemical is known to cause serious damage to eyes if exposed; when working in the field bring a travel eye wash station.*

Step 1. Scrub equipment with a stiff bristled brush to remove all organisms, mud, debris, etc. Thoroughly brush small crevices such as boot laces, seams, net corners, etc. and rinse.

Step 2. Following the product label instructions mix a 2% solution.

Step 3. Immerse equipment in the 2% solution and soak for 20 minutes. Weigh equipment down if necessary.

Step 4. Rinse equipment and dispose of solution and rinse water in a municipal sewer system. Never dispose of solution or rinse water where it can drain into a waterbody.

APPENDIX H. MORRO BAY NATIONAL ESTUARY PROGRAM AGRICULTURAL MONITORING PROTOCOL

Agricultural Monitoring Protocol

Monitoring to be conducted by staff only

Three sites throughout the watershed are monitored each month for nitrates as nitrogen, total nitrogen, organic nitrogen, total Kjehldal nitrogen (TKN), ammonia, nitrite, total phosphorus, orthophosphates as P, and total suspended solids (TSS). Samples are collected and sent to a contract laboratory for analysis within the specified hold time.

Equipment:

- Calibrated EXO3 sonde with handheld
- WQ Kit (should include flow equipment and camera)
- Laboratory bottles (see below)
- Cooler with ice packs
- Ag datasheets
- Clipboard
- Camera

Lab Bottles:

- 1 TSS bottle (1000mL plastic bottle)
- 2 H2SO4 bottles (16oz acidified bottle DO NOT RINSE!)
- 1 NO2/NO3 bottle (16oz plastic bottle)
- 1 HNO3 Nalgene (250 mL acidified Nalgene DO NOT RINSE!)
- 2 PO4 field filtration kits (contains 40 mL glass vial, syringe, and filter)



Sites:

Site Code	Creek	Lat	Long
310CAN	Chorro Creek	35.35314883	-120.7886531
310SLU	San Luisito Creek	35.35594843	-120.7861122
310TUR	Warden Creek	35.30230822	-120.7764337

Preparation / Scheduling:

- Contact the laboratory to schedule sample drop off with minimum of 1-week notice.
- Samples can be dropped Monday to Thursday before 3pm.
- Samples should be dropped off at Fruit Growers Laboratory in San Luis Obispo (3442 Empresa Drive, San Luis Obispo).
- Samples must be kept at 4°C throughout travel and arrive at the lab on the same day as sampling.

Field Protocol:

- Upon arrival at site, collect EXO and record readings on Ag datasheet.
- Collect a sample:
- Select a sampling spot within a riffle.
    - Fill and empty the large TSS bottle three times.
    - Fill the bottle to the top and replace cap.
    - Homogenize the sample using the EPA method.
    - Fill each bottle using the homogenized sample.
- Locate the appropriate FGL label. Write in date, time, and sampler's name. Label the clean, dry bottle with the appropriate label.
- Store bottle in cooler, and record time sample taken on the datasheet.
- Upon return to the office, store samples in the fridge. Complete the Chain of Custody for the lab immediately.

APPENDIX I. MORRO BAY NATIONAL ESTUARY PROGRAM EELGRASS PERMANENT TRANSECT MONITORING PROTOCOL

Morro Bay National Estuary Program Eelgrass & Algae Field Transect Protocol
("Permanent Transect Monitoring")

Monitoring takes place at six permanent stations located throughout Morro Bay. Each station has one to three permanent transect locations for monitoring. All transects are 50 meters long. At each site there is one 'Long' transect and two 'Depth' transects. Long transects are 50 meter transects that are monitored at 20 distinct quadrat locations. Depth transects are 50 meter transects that are parallel to the Long transect and are monitored at only 12 distinct quadrat locations.

Table 1. Monitoring Locations

Site	Type of Transect	Latitude	Longitude
Coleman	Long	35.37208	-120.860604
Reference	Long	35.35753	-120.853
Reference	Depth	35.35742	-120.853
Embarcadero	Long	35.35943	-120.85174
Marina	Depth – A	35.34579	-120.846
Marina	Depth – B	35.34587	-120.846
Marina	Depth – C	35.34567	-120.84653
Chorro	Long	35.33774	-120.847
Pasadena	Long	35.33323	-120.849

Coleman and Marina stations are located on the East side of the navigation channel. Tidelands, Pasadena, Embarcadero, Reference and Chorro stations are located on the West side of the navigation channel.

NOTE: The procedure differs depending on the density of eelgrass present at the transect. In areas with dense eelgrass coverage, 30 shoots can be collected from each site for biomass analysis. For areas with sparse eelgrass coverage, adequate shoots may not be present. In this case, five shoots are collected from the monitoring site. The protocol is divided into steps for Dense Sites and Sparse Sites.

<u>Supplies Checklist</u>

- o waterproof datasheets (up to 3 per site)
- o (2) 300ft standard and metric Keson Transect Tapes
- o (1) 15 meter metric Keson Transect Tape
- o (1) photo whiteboard
- o Dry erase markers
- o (2) Plastic rulers (metric)

- (4) temporary transect end stakes
- GPS unit
- Plastic clipboards & pencils
- Ziploc Bags (1 gallon)
- Waterproof biomass sample labels
- Waterproof camera
- PVC 0.25 m^2 meter quadrat

Setting up Transects and Quadrats

1. Review site maps for the site and determine which transect is being monitored (A, B or C).
2. Select the appropriate datasheet for which quadrats must be counted.
3. Fasten the Keson tape with the 0 end at the northern stake. (Reference site maps if needed.)
4. Take photos of the transect from each end of the transect looking in towards the area to be assessed.
5. All quadrat measurements are completed on the shallow side of the transect tape.
6. Place a sampling quadrat (using the distances provided on the datasheet) on the shallow side of the transect. Position the quadrat so that the center mark of the quadrat is on the specified Keson tape distance mark.

Photo Documenting Quadrats

1. Take photos before disturbing any of the vegetation in the quadrat. Fill out the whiteboard with the transect and quadrat information for the quadrat picture:
    a. Site Name
    b. Transect Name (A,B or C)
    c. Quadrat Number (location on tape)
    d. Date
    e. Time
2. Place the whiteboard outside the quadrat frame, within the area captured in the photo.
3. Take a photograph of the quadrat. Take the photograph as vertical as possible, including the entire quadrat frame, the whiteboard photo label, and the Keson tape measure. Try to avoid shadows and reflections in the photograph.

Measuring Quadrat Percent Coverage and Canopy Height

1. Identify the seagrass and algae species present in the quadrat.

2. Estimate % cover of each species (seagrass and algae) in the quadrat. Minimum % cover increments are 5%. Record the percent coverage for each vegetation type in the *Coverage %* columns on the datasheet.
3. Randomly select a clump of eelgrass blades from within the quadrat. Extend the leaves sideways to their maximum length without uprooting.
4. Use the 15-meter Keson tape to measure the length *in centimeters* from the substrate to the leaf tips. Ignore the longest 25% of the eelgrass leaves in the clump.
5. If grass density is very low and a clump of blades cannot be grouped, make measurements on 5 individual shoots within the quadrat. Record the 5 separate measurements in the *Canopy Height (cm)* column.
6. If the quadrat contains algae but no eelgrass, use a ruler to measure the depth of the algae mat (in centimeters) from the surface of the mud.
7. Record the measurements in the *Canopy Height (cm)* column on the datasheet.
8. Note whether the canopy height measurements were taken for algae or eelgrass.

Counting Eelgrass Shoots, Measuring Water Depth

1. Carefully separate eelgrass shoots around the outside of the transect frame to determine which shoots are rooted within the frame. Shoots that are lying down into the frame should be folded back out of the frame to avoid confusion.

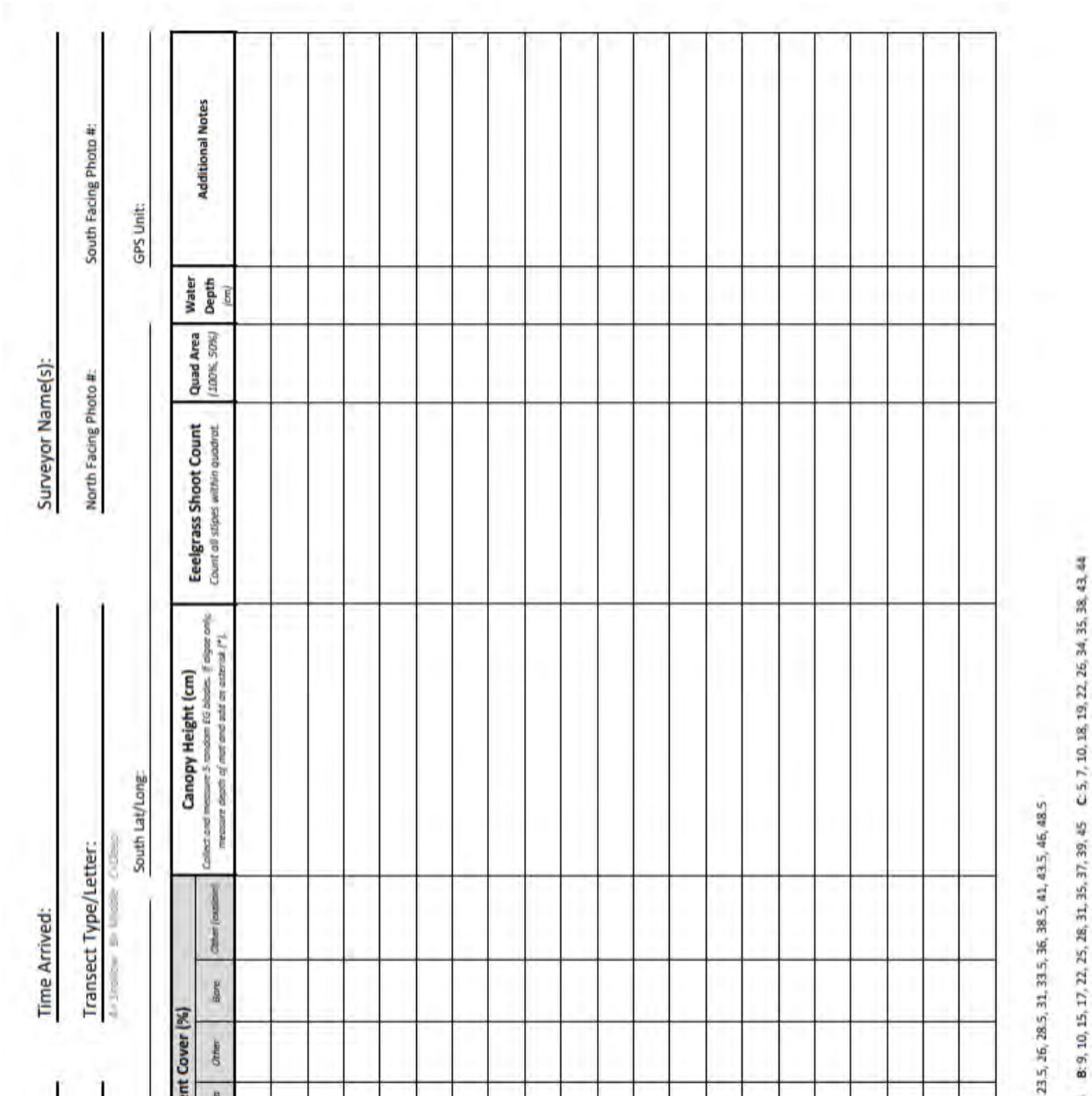


2. If the frame is very dense with evenly distributed shoots, count 25% or 50% of the quadrat. Use the small white quarter squares to mark off which portions of the quadrat will be counted.
3. Fill in the % area counted (25%, 50%, 100%) in the *Quad Area* column on the datasheet.
4. Count shoots individually, feeling down to the base of the shoot at the sediment. Where possible, count all shoots in the quadrat (100% of the quadrat).
5. Record the total number of shoots for the area counted in the *EG Shoot Count*

column.

6. If the majority of the quadrat is submerged in water, use the plastic ruler to measure water depth in centimeters. Record the water depth in the *Water Depth (cm)* column on the datasheet.
7. Do not record water depth if less than 50% of the quadrat is submerged. Record zero if water depth is less than 1cm.

Morro Bay National Estuary Program
Permanent Transect Monitoring Datasheet



APPENDIX J. MORRO BAY NATIONAL ESTUARY PROGRAM EELGRASS INTERTIDAL BED CONDITION MONITORING PROTOCOLS

Morro Bay Intertidal Eelgrass Bed Condition Monitoring Protocol

Intertidal eelgrass monitoring will take place in 5 of the most significant beds throughout Morro Bay. The goal of monitoring is to determine eelgrass bed conditions through density, patchiness, and observational data. Typically, a 150 meter, 7 quadrat survey, can be completed in one tide window with 2-3 people (3 ideal). Ideal tides for intertidal monitoring are below -0.5 feet. Beds are considered separate if they are 50 meters or more apart.

Eelgrass monitoring should be conducted once per year in the spring (April – May). Intertidal monitoring will be conducted at eelgrass beds at Coleman Beach, North Sandspit, Reference Bed, Osyter Bed, and Windy Cove. Refer to the attached map for eelgrass bed locations.

Supplies:

- 0.50m x 0.50m quadrat
- 50m or longer Keson Transect Tape
- (1) photo whiteboard
- Dry erase board markers
- Meter stick or metric ruler
- GPS unit
- Waterproof camera
- Plastic clipboard & pencils
- Rite-in-the-rain datasheets (one per quadrat)
- Squirt bottle for cleaning eelgrass
- Field notebook
- Personal Gear: wading boots or wetsuit, depending on how bed is accessed.
- Kayak, paddleboard or surfboard depending on bed locations

Protocol:

Complete a quadrat every 25 meters along the transect. Begin at transect start point, which is defined by landmarks and lat/long coordinates. Record transect orientation on datasheet (e.g. Survey direction from North to South)

1. Fill out the site name (not site code), date, time, surveyors, GPS point and transect orientation on datasheet.
2. Record the quadrat meter mark on the datasheet. (the first quadrat is quadrat 0).
3. Anchor the meter tape at the start of the transect.
4. Place the quadrat at the starting point along the landward edge of the bed, but slightly water ward so that the quadrat is not at the very edge, if possible. Placement should be in eelgrass. Place along the landward edge of the bed to prevent double sampling during subtidal surveying.

Note: If eelgrass is not present at the zero mark, move forward up to 12 meters to

place the quadrat. If there is no eelgrass within 12 meters, then circle “No Eelgrass” next to stipe counts and move on to the 25 m mark on the transect. For example, if there is no eelgrass present at meter 0, but there is eelgrass at meter 10, the quadrat would be done at meter 10. Quadrat meter mark on datasheet would be recorded as ‘10’, and GPS location would be recorded for the 10 m mark. Regardless of where the quadrat ends up, the next one should be attempted at the 25 m mark (and if no eelgrass there, that point can be shifted +/- 12 meters to find eelgrass).Take a GPS point for the quadrat and record lat/long on datasheet.

5. Record site name, date and quadrat number on the white board. Take a photo of the white board and then a photo of the quadrat.
6. Quadrat: Using the point intercept method, count the total number of corners or intersection points (25 total) that have the following directly under the intersection or at the inside corners or edges of quadrat. Within each category below there can be a maximum of 25 counts, but there can be multiple categories present at each point. Use finger to check if material is directly under the point (only include in count if directly under) and check for layering (e.g. gracilaria may be under or over eelgrass, but either way it should be counted if under that point).
    a. Eelgrass (live blade or stipe)
    b. Gracilaria: red and stringy
    c. Ulva: green and leafy
    d. Fauna (indicate type: e.g. sand dollar, small brown snail)
7. Stipe Count: Count the number of stipes in each of the 4 center squares (see Figure 1) of the quadrat to measure density. Keep counts separate for each square, though order of counts doesn’t matter.

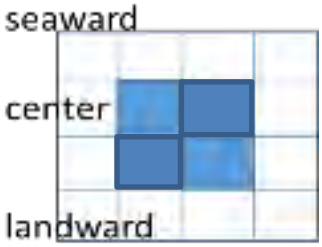


Figure 2: Count the number of stipes in each of the 4 center squares

8. Blade Condition: At the nine points on the quadrat (as shown in Picture 2), repeat the following steps.
    a. At the first of the nine points, select the nearest stipe that is within a half square distance of the point in all directions (including outside of the quadrat). For this stipe, measure the length of the longest blade from the sediment to the tip of the blade. Record to nearest milimeter.

Record if the longest blade is broken or not by putting an astrick next to blade length.

b. Wash the whiteboard off and then label the whiteboard with date, quadrat number and time. Spread the same stipe on the whiteboard so that all blades are visible if possible. Use a bottle of water/spray bottle to wash off the blades and board before taking the photo.

c. Take one photo that includes the label and one photo close up to the eelgrass with the blade spread out. These photos will be used later for image classification.

d. Count the total number of blades connected to the stipe (E.g.: 5 blades in Figure 3)

Out of the total blade count, record how many of the blades have dark spots, breakage at the tip, epiphytes/bryozoans, or epifuana, respectively. If epifuana or epiphytes are present, be sure to label what type of species they are (E.g.: 4/5 have dark spots, 3/5 have turf algae and bryzoan, 5/5 have small brown snails)

If no stipe within ½ a squares distance in all directions, skip (leave cells on datasheet blank).

e. Repeat with eight remaining intersection points.

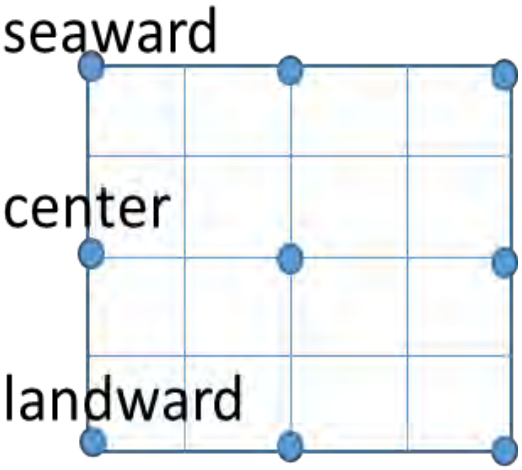


Figure 3: Photo location details



Figure 4: Example photo for blade condition

Intervals between quadrats (mapping)

9. Anchor the meter tape or have one person hold the end. Continue from the quadrat for 25 meters using the meter tape tape. Lay the meter tape out following the margin of the water's edge. At each meter between quadrats, score each meter as yes (1) or no (0) for the presence of eelgrass above (exposed) and below (submerged water).
10. E.g. Eelgrass present above and below at meter 1 would be recorded as 1 1. No eelgrass above or below would be recoded as 0 0.
    - Note: that its actually hard to keep at the margin's edge, so we usually just go in a straight line and look toward the water edge.
11. Every 25 meters, complete steps 2-9 until 7 quadrats (150m) have been completed. If there is no eelgrass at the next measurement point on the transect, move to the nearest eelgrass within 12 meters (forward or back), which will not necessarily be located in the most dense eelgrass. If there is no eelgrass within that distance in either direction, move to the next 25m point

and circle "no eelgrass" next to "Stipe Counts" on the datasheet.

Datasheets and Record Keeping

1. Check that all fields of the datasheets were completed, site info was filled out correctly and writing is legible.
2. Scan data sheets. Email datasheets out if necessary. Save a scanned copy of each datasheet on SharePoint:
    a. Save all files related to the effort on SharePoint under **DATA – Documents/Projects/Restoration/Eelgrass/Monitoring/Bed Condition Monitoring (Cal Poly)**.
    b. File original datasheets in Eelgrass datasheet folder. Make a photocopy to bring into the field when necessary.
3. Record field notes in the field notes Word document:
    a. Include: date, site, survey type (intertidal/subtidal), crew, tide and a brief description of what was done. Include comments such as if that was a good tide for that site or additional gear to bring for future surveys.
4. Download photos from camera. File photos in appropriate MBNEP file, and rename using appropriate nomenclature..
5. Rinse and put away gear (including GPS and field camera)

Intertidal Bed Condition Monitoring Site Descriptions

Coleman Beach: (MBNEP Site code: COL)

- Direction of survey: South to north
- Lat/Long of start: 35.371670, -120.859150 (by building)
- Lat/Long of end: 35.372140, -120.860630 (by bend in beach, closer to bathrooms)
- Survey type: intertidal and subtidal
- Launch point: Coleman Beach Parking lot
- Best tide window for subtidal surveys: -0.5' tide or lower

This bed is the longest, most continuous bed and consistently has the best visibility. It seems to have an even amount of subtidal and intertidal eelgrass.



Figure 5: Start of Coleman surveys. Start point is at the edge of the building and extends north 150 m, ending at the elbow in Coleman Beach.

North Sand Spit: (MBNEP Site Code: NSS)

This bed has also been referred to as Tidelands on past datasheets.

- Direction of surveys: North to south
- Lat/long start: 35.368839, -120.858842
- Lat/long end: 35.36817, -120.85741
- Survey type: intertidal and subtidal

- Launch Point: Target Rock for north end. This site can also be accessed by launching from the NEP office and walking down the sandspit.

This bed starts across from the T-Pier and continues south. This is a patchy bed. There are side channels throughout the bed that punch holes in the bed. There is a steep drop off in depth, so the bed is narrow, with more intertidal than subtidal eelgrass. The 150m mark is across from the South T-pier.

Reference Bed: (MBNEP Site Code: REF)

- Direction of surveys: N S (subtidal), *S N (intertidal)* unfortunately the subtidal and intertidal were completed in opposite directions)
- Lat/long start:
- Lat/long end:
- Survey type: intertidal and subtidal
- Launch Point: easiest from Tidelands Park steps, could also be done from MBNEP office.

This site has mid-range visibility. Eelgrass becomes pretty patchy in this section of the bay. This is the shortest bed. Intertidal and subtidal both extend for 65 meters. There are patches that continue south, but they are farther than 50m apart and subtidal portions are difficult to find under most conditions. On very calm days with good visibility or on very low tides they are easier to find.

Windy Point (MBNEP Site Code: EMB)

- Direction of surveys: North to south
- Lat/long start: 35.351251, -120.845271
- Survey type: intertidal and subtidal
- Launch Point: easiest from Tidelands Park steps, could also be done from MBNEP office.
- Description of start point: Start point is just north of the concrete stairs, but the nesting point.

State Park Marina (MBNEP Site Code: MAR)

- Direction of surveys: North to south
- Lat/long start: 35.34782, -120.84490
- Survey type: subtidal only
- Launch Point: State Park Marina or Windy Cove.
- Description of start point: 50m north of the north red buoy.

This bed is subtidal only. It is best surveyed on a low tide (around -0.5ft). Most of the bed lines the navigation channel and visibility is very poor, so diving is not recommended. We surveyed this at a - 0.5ft tide and weren't able to reach the bottom of the eelgrass when just floating on the surface. This is not a good bed to survey on windy days because it lines the navigation channel. It is hard to keep kayaks out of the navigation channel. The 0 quadrat is 50m north of the red buoy.

Oyster Bed

- Direction of surveys: North to south
- Lat/long start: 35.3414658, -120.8485381
- Lat/long end: 35.3401456, -120.8481862
- Launch Point: Windy Cove or State Park Marina

This transect was established in 2023 after Permanent Plot surveys were discontinued. The north end of the transect is roughly co-located with the Permanent Plot designated “O1”.

APPENDIX K. MORRO BAY NATIONAL ESTUARY PROGRAM EELGRASS MAPPING GROUNDTRUTHING METHOD

Eelgrass Mapping Effort: Ground Truth Methodology

Goals: To support the multi-spectral eelgrass mapping effort to create a bay-wide map of intertidal submerged aquatic vegetation, the Estuary Program works with a contractor to collect the imagery using multispectral imagery and/or drone imagery. To support this effort, Estuary Program staff collect data on the ground to verify the vegetation classification scheme. Data is collected prior to the flight to provide groundtruthing data to support create of the classification. Data is also collected following the flight to provide confirmation on areas that the contractor is having trouble classifying.

Equipment: Data is collected on a EOS Arrow using ESRI Collector software.

Locations: These vary by year and are dictated by the contractor's needs.

Methodology:

- All data points are collected on foot, though a paddleboard or kayak can be to access different locations within the bay. All points are collect on foot to ensure adequate time for the GPS unit to stabilize and collect as accurate of location points as possible. Because of this, low tide windows and areas shallow enough to stand in are targeted.

- Areas that needed groundtruthing for the previous aerial flight are targeted, as well as additional locations requested by the contractor. Areas with the following characteristics are targeted: Areas with mixed vegetation; areas with discrete substrate or vegetation; and areas with different densities of eelgrass (dense, medium, patchy).

- At each location, the GPS unit is held in place until it stabilized to submeter accuracy (typically around 20 cm).
  Dominant vegetation and secondary vegetation types are recorded. If the point is not mixed, the dominant vegetation is also entered as the second dominant vegetation. Georeferenced photos are also taken in some locations.

- Additionally, ground truthing at some locations consist of landscape photos with written descriptions of the area rather than specific coordinates, as requested by the contractor.

APPENDIX L. MORRO BAY NATIONAL ESTUARY PROGRAM SHOREBIRD SURVEY PROTOCOL

Morro Bay Volunteer Monitoring Program Shorebird Survey Protocol

(from Point Reyes Bird Observatory – Pacific Flyway Project)

Shorebirds can be counted more easily than many birds due to their use of tidal flats and other open habitats. Further, shorebirds generally move shoreward as the tide rises, affording land-based observers an improving view until the flood tide forces the birds to alternative high tide habitat. However, due to their habits of aggregating, sometimes in very large, mixed-species flocks, and flying (often unexpectedly during a count!), skill and experience are necessary to obtain accurate counts.

COUNTING METHODS

Counting methods vary, depending on the number of birds, their edginess, and census conditions. The number of birds in a flock can be obtained by:

1. Counting birds individually.
2. Counting birds in groups of some number
3. Estimating the number of birds in a flock without actually counting them.

The first two methods are always more accurate and preferred over the third, which should be used only for preliminary observations of flocks or to salvage an interrupted count.

Counting birds individually: If there are only a few birds in an area, (less than 100 total), they should be counted individually.

Counting birds in groups of some number: If you are counting larger flocks, say 200 birds, begin at one end of the flock - count the first 10 birds, then the second 10 birds, continuing until you can easily see the birds in groups of 10. At that point, start counting by 10-bird groups. This method can be generalized to counting by 20-bird, 25-bird, 50-bird, or even larger groups, for larger flock sizes. This method can also be used for counting birds in flight.

Estimating the number of birds: It is wise to initially estimate numbers of all the birds quickly, to determine the relative proportions of the most common species, to roughly determine the numbers of the less common species, and to determine whether the species are segregated or homogeneously mixed throughout the flock. Then, if the actual count is interrupted, some reasonable estimates of numbers will be possible. Making a rapid estimate of the number of birds may be the only way to salvage a count in which birds fly away before they are counted.

SPECIES IDENTIFICATION

1. Try to identify all the birds (except dowitchers) to species.

2. Whenever possible, allocate unidentified shorebirds to species groups (such as Least Sandpiper or Western Sandpiper) rather than just listing them as unidentified large or small shorebirds.On the census form we have provided species categories that might commonly be used. Four groupings: yellowlegs spp., dowitcher spp., phalarope spp., and Least or Western sandpiper are self-explanatory. The plover species would include Semipalmated Plover, Snowy Plover, and Killdeer. The large sandpiper grouping could include any combination of Willet, Marbled Godwit,

Greater Yellowlegs, Long-billed Curlew, or Whimbrel. If you use any of these categories please indicate which species you believe could have been involved. Write in any species not on the census sheet in one of the empty columns at the bottom of the census form and provide details on the sightings.

APPORTIONING THE COUNT OVER SEVERAL SPECIES

1. Count out each species separately.
2. Count all species together.
3. Count severa1 species together.

Counting each species separately: Counting each species separately is time consuming if there are many species to count but can be the least confusing method if there are few abundant species per counter.

Counting all species together: If the group of shorebirds comprises primarily one species, with a few individuals of other species, it is often easiest to count the total number of birds, then to count out the rarer species and subtract their numbers from the total, to get the number of the common species. An assistant, recording for the counter, can aid in this process by either counting the rarer species in the flock or by making the subtractions on the recording form. In cases where there are tens of thousands of shorebirds and several species are very abundant, it may be necessary to count all species together and to sample species composition at many places in the flock in order to determine the proportion of the total made up by each species.

Counting severa1 species simultaneously: In a mixed flock, it is often fastest to make a combined count of similarly sized species. For example, say there are six species present: Dunlin, Western Sandpiper, Least Sandpiper, Willet, Marbled Godwit, and Long-billed Curlew. It might be easiest to count the first three species together, by writing down the number of the less common species as they are encountered and keeping a running tally of the most common species in your head until the flock has been scanned completely. Similarly, the Willet, Marbled Godwit, and Long-billed Curlew could be counted together. Counting several species simultaneously can often be done most effectively if there is both a counter and a recorder. The counter can give the recorder the counts of the less common species as they are seen but accumulate the count for the most common species until the flock is completely counted.

WHAT TO DO WHEN BIRDS FLY DURING A CENSUS

Movement of birds during a census creates some particularly troublesome problems. Our basic strategy is to count the birds before they move from tidal flat areas. Counts at shoreline segments should not take longer than about 1 hour so that the overall amount of bird movement is minimized. It is also important that counters in the same general area of the Bay are counting at the same time. Using the guidelines below, we hope that you will be able to best judge whether or not you should count certain birds.

1. If you are covering a shoreline segment: Count birds that fly into and stay in your area during the first hour of your count. You should count birds that fly out of your area only if they are leaving for non-tidal, high tide roosts (such as salt ponds). If a bird (or flock) moves to another tidal flat area and it is less than an hour since your starting time for that area and there is still a counter visible to you in the area into which the bird(s) moved, you should not count the bird(s) for your area. Alternatively, if a bird (or flock) moves into your area in the first hour of your count, you should count them, unless it is apparent to you that they have been counted by an observer in an adjacent area and the observer did not see them move to your area.

2. If you are covering a non-tida1 area at the same time that shoreline segments are being covered: Only count flying birds in your area that leave for other tidal areas. If birds leave your area for the tidal flats (this shouldn't' happen too often) do not count them if it is less than an hour since tidal flat counts have begun. Do not count birds that fly into your area once the shoreline segment counts have begun.

3. For both tidal and non-tidal sites: If you do not know where departing birds go, note them separately on your form with the time they leave and general direction that they go. Make similar notes on arriving birds. If birds fly by your area without stopping, note them separately with the time and direction of flight, if possible. Do not include any of the birds noted separately in the totals for your area; we will decide after the census if they have been counted by any other team. However, the best decisions on whether or not a flock shou1d be counted are often most easily made in the field.

FOR FIRST TIME CENSUSERS: WHAT YOU 'LL WANT TO REMEMBER TO BRING

- Binoculars
- Telescope (if you have one)
- Wrist watch
- Letter or card indicating permission to restricted land (if applicable)
- Recording pad (use census form for final tally only)
- Pencils (mechanical pencils don't need to be sharpened if they break)
- Rubber boots (your letter will indicate if needed for your area)
- Warm layers of clothes, water, sunscreen
- These forms and instructions
- A field guide (although there usually isn't time to refer to it during the census)

Morro Bay Volunteer Monitoring Program Shorebird Census Location Information

See Map for reference. All boat sections concentrate the on the main body of the bay excluding the salt marsh and Chorro/Los Osos creek outlets. Those areas will be covered from the shore.

1. Section 1: Land Section.Use scope near Morro Rock, T-Piers, and several street-end viewing docks along the Embarcadero.

2. Section 2 & 3: Boat Section. Depart from embarcadero. Sections 2 & 3 are usually completed together. Concentrate on Grassy Island & the shore of sand spit. The boundary of Section 4 starts at the southern tip of Grassy Island and runs from the sandspit east to Fairbanks Point.

3. Section 4: Boat Section. Depart from State Park Marina. South of Grassy Island. Concentrate on shore of sandspit and any exposed mudflats. The southern boundary of Section 4 runs east to west from just north of the Marina to the sandspit.

4. Section 5: Boat Section. Depart from State Park Marina. Concentrate on sand spit and any exposed mudflats south of the Marina to approximately the northern tip of Pasadena Point. The southern boundary of Section 10 runs east to west from Cuesta Inlet to the sandspit.

5. Section 6: Land Section. Use scope around State Park Marina (to the south side) and east along the Salicornia (pickle weed) as far as necessary.

6. Section 7. Land Section. Can be done with Section 6 from the same vantage points. Boundary between Sections 6 & 7 is approximately along the Chorro Creek channel.

7. Section 8. Land Section. Use scope from Bush Lupine Point in Elfin Forest. Park at north end of 13th Street in Los Osos. If vantage point further east is necessary, go along boardwalk to Siena's View, the next point at which the boardwalk affords a bay view.

8. Section 9. Land Section. Use scope (if needed) from pullout on Pasadena Drive, from Baywood Pier, Sweet Springs Nature Preserve (enter at Broderson St.), & further west at public access near intersections of Mitchell Dr. & Doris St.

9. Section10: Boat Section. Depart from Cuesta Inlet. Concentrate on sand spit and any exposed mudflats south to approximately Cuesta Inlet. The southern boundary of Section 10 runs east to west from Cuesta Inlet to the sandspit.

10. Section 11. Land Section. Starting from west end of Butte Dr. checking near shore, then when closer to Shark's Inlet (back corner of bay), look across to shore of sand spit. Boundary with Section 10 is north end of a large bare dune.
    Note: If sand spit side of Section 10 is done on foot, the Section 11 team can do

the portion of Section of 10 that is Cuesta by the sea shoreline.

11. Section 12. Land Section. Use scope from bay side of South Bay Blvd. across from Turri Rd. Further north access is available just south of Chorro Cr. Bridge.
12. Section 13. Land Section. Ocean side of sandspit. Use Montana de Oro access. Census the beach while walking north, then walk back.
    Note: Can arrange pick up with boat on north sandspit rather than walking back.

13. Sections 14/15. Land Section. Walk from Morro Rock to North Point (or vice versa). To avoid wading Morro Creek, walk north from Morro Rock to the creek, walk back and drive to north side of creek (Highway 41 west to the end). Park and walk from north side of Morro Creek to North Point. With two people & a desire to walk less, a car can be dropped off at North Point, return to Morro Rock to begin survey, census and finish at North Point and awaiting car.

We need at least 11 bird survey leaders.

Morro Bay Volunteer Monitoring Program
Shorebird Monitoring Datasheet

Location _____ **Date** _____

Team Members (first, last names) _____

Area _____

Start Time _____ **End Time** _____

Black Bellied Plover		Western Sandpiper		Great Blue Heron	
Snowy Plover		Least Sandpiper		Great Egret	
Semipalmated Plover		Least/Western sandpip. (*) Ratio >>		Snowy Egret	
Killdeer				Cattle Egret	
Am. Black Oystercatcher		Dunlin		Green Heron	
American Avocet		Least/West/ Dunlin		Blck-cr. Night Heron	
Greater Yellowlegs		(*) Ratio >>		Virginia Rail	
Lesser Yellowlegs		Baird's Sandpiper		White Pelican	
yellowlegs spp.		Short-bill. Dowitcher		Brown Pelican	
Willet		Long-bill. Dowitcher		Turkey Vulture	
Wanderling Tattler		dowitcher spp.		Osprey	
Spotted Sandpiper		Wilson's (common) Snipe		White-tailed Kite	
Whimbrel		Wilson's Phalarope (u)		Northern Harrier	
Long-billed Curlew		Red-necked Phalarope		Red-tailed Hawk	
Marbled Godwit		Red Phalarope (u)		American Kestrel	
Ruddy Turnstone		phalarope spp.		Merlin	
Black Turnstone		Brant		Peregrine Falcon	
Surfbird		Other species (d)		Red-shouldered Hawk	
Red Knot					
Sanderling					

(u) indicates species we suspect will be very uncommon or local
Dowitchers--We suggest the censusers count all dowitchers as dowitcher spp. If dowitchers are identified to species we would like some information on how the determination was made.
Mixed Species Groups -- Indicate the possible species and number. For example, if you saw a mixed flock of 155 Willet and Marbled Godwit, you would write Willet/Marbled Godwit and 155. Whenever possible, indicate proportions of mixed species groups.
(*) Use these spaces only to record numbers of sandpipers that you are able to separate into Least or Western sandpipers. Count the species together and record the total number. If possible, list the ratio of the unseparated species.

APPENDIX M. MORRO BAY NATIONAL ESTUARY PROGRAM MACROALGAE AND BIOMASS MONITORING PROTOCOL

Morro Bay National Estuary Program
Bay Macroalgae Monitoring Protocol

Overview

This project was developed to track changes in macroalgae cover and biomass both spatially and temporally. Sites have been established to examine variations in macroalgae cover throughout the estuary as well as across differing elevations. In addition, co-location with eelgrass monitoring sites will aid in developing a better understanding of how the density of macroalgae affects eelgrass beds.
Sampling is conducted biannually in the Spring (April – June) and Winter (November – January) during low tides that fall below –0.5ft MLLW. Estuary Program and Tenera Environmental staff will work together on the fieldwork component of the project. Drying and weighing algae in the lab will be conducted by Estuary Program staff.

Sites

Table 1. Monitoring site coordinates. Each set of coordinates refers specifically to the center quadrat along the middle transect (Quadrat C2). Placement for the rest of the quadrats can be determined with a transect tape.

Site Name	Longitude	Latitude
North Sandspit	-120.858842	35.368839
Embarcadero	-120.85192	35.35948
Reference	-120.853	35.35753
Windy Point	-120.844983	35.35095
Marina	-120.846306	35.34604
Chorro	-120.846776	35.337116
Pasadena	-120.84939	35.333411
Mitchells	-120.8504833	35.3218667

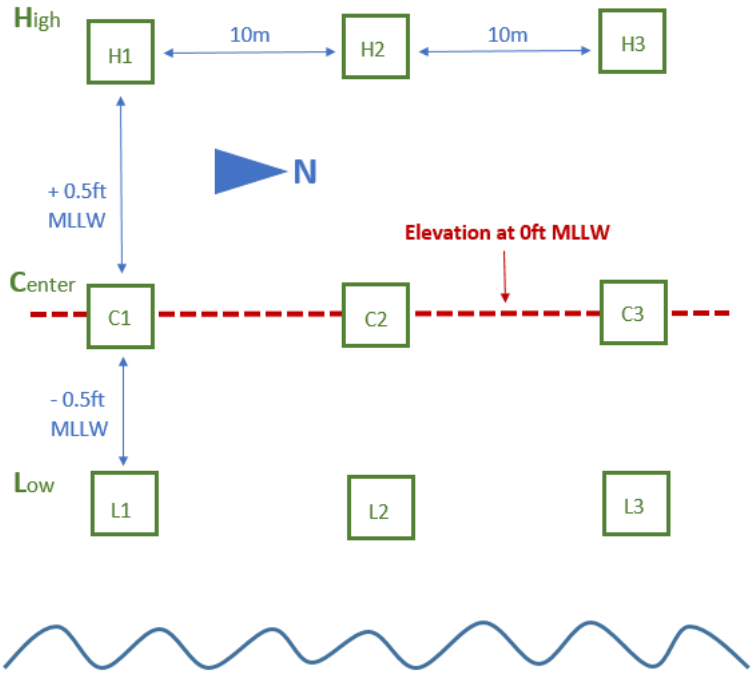


Figure 2. Simplified schematic displaying quadrat placement at each site. The center transect (Transect C) is established at MLLW, with the upper and lower transects spaced +/- 0.5ft MLLW from the middle. Horizontal distances between transects will differ between sites. Quadrats are numbered from 1-3 counting from South to North. GPS coordinates displayed in Table 1 refer to the placement of the center quadrat in the middle transect (Quadrat C2).

Methods

Field Sampling

- Check the weather, tides, and sunrise/sunset times before sampling.
- Before leaving the office, make sure to gather up the following supplies:
    - 1 EOS Arrow or Bad Elf GPS unit with sub-meter accuracy
    - 50-meter transect tape
    - 15-meter transect tape
    - 4 transect tape stakes
    - Ziploc freezer bags
    - Scissors
    - 3 1m x 1m quadrats
- Navigate to the sample site and use the GPS unit to find the location of the central quadrat (Quadrat C2), using the coordinates from Table 1.
- Use a transect tape to measure 10m in each direction, running parallel to the channel, and place the next two quadrats. Typically, each transect will be oriented roughly North to South.
- At each quadrat along the transect, complete the following:
- Take a photo of the quadrat. Include a photo of the datasheet showing which quadrat you are sampling so the photo can be traced back to its proper placement back in the office.
- Assess percent cover of macroalgae and eelgrass within the quadrat using two separate methods. If possible, estimate to the genus level. If unknown algae types are present,

determine whether it is green, red, or brown and take a small subsample from outside the quadrat for later identification.

- First, use visual estimation to obtain percent cover, rounding to the nearest 5%. Assess percent cover for all algae types, eelgrass, and bare ground.
- Second, use the point-intercept method. At each intersection point (25 total) determine what is immediately beneath the point. If more than one type of algae is stacked at a single point, only count what is on top.
- Collect a biomass sample from the center 4 squares of the quadrat (an area of 0.25m^2). If necessary, use scissors to trim around the 4 squares so you're only collecting the pieces of algae inside the center squares. Collect algae only, do not collect eelgrass samples. Rinse the algae of sediment and invertebrates as best as you can. Place the sample in a Ziploc bag and immediately label it with the Site, Date, Sampler Name, Transect Letter, and Quadrat #.
- NOTE: If algae is especially dense and you determine it will not fit in the Ziploc bag before starting your collection, you may take a subsample from 1 or 2 squares. You must record any subsampling on the datasheet.
- Once the center transect (Transect C) has been completed, use the site notes to determine how far the low transect (Transect L) is from Transect C. Use the transect tape to measure the distance, and place the quadrats 10m apart. Conduct the quadrat sampling as described above.
- Upon completing Transect L, use the site notes to measure from Transect C to the high transect (Transect H). Conduct the quadrat sampling as you did before.
- Once all 9 quadrats have been fully assessed, return to the office and place all bags of algae in the equipment room fridge.

Dry Weight Sample Processing

- Place algae sample bags in a cooler and transport to Cuesta Lab. Bring the field datasheets with you.
- Sort through algae bags one at a time. If multiple types of algae are present in a bag, separate them out by genus. If unknown algae were collected in the field, use the algae ID key and additional reference materials to determine an ID. For particularly difficult samples, reach out to Laurie McConnico (laurie_mcconnico@cuesta.edu).
- If necessary, perform a secondary rinse to remove sediment and attached invertebrates. Gently pat dry with a paper towel.
- Place each algae sample into its own foil envelope and label it with the Site, Date, Transect Letter, Quadrat #, and genus name.
- Repeat the process of separating out algae samples for each Ziploc bag taken in the field.
- Place all foil envelopes in the convection dryer and dry at 60°C for 24-48 hours, depending on the amount of algal material collected in the field. Check how the drying has progressed after 24 hours before determining whether to let it dry for a longer period. Do not dry any batch of samples longer than 48 hours.
- Remove the samples from the foil wrapping one at a time and weigh on the fine scale, recording weight to the nearest 0.01 gram.
- Record the type of algae and its dry weight on the same datasheet used in the field.
- After all samples have been dried, weighed, and recorded, dispose of the samples in an outdoor trash can.

Oven and Lab Protocols

Oven Temperature

1. Flip the middle switch to 'Power' to turn on the oven.
2. Flip the lower switch upwards to 'Heat'.
3. Set the dial thermostat to 60° F.
4. The red (Power) and orange (Heating) indicator lights on the front of the oven should illuminate.
5. When the orange indicator light has gone off, open the oven door and record the temperature on the internal thermometer.
6. Adjust thermostat dial as needed.

Scales:

The scales need to be warmed up before it is used, the longer the better. One hour is the minimum and two hours is ideal. If the scales are being used frequently (weekly) it is better to leave it on all the time. If it is used less frequently (monthly) then it is better to turn them off in between uses. During times of intensive use, they should be left on.

Temperature fluctuations: fluctuations in temperature affect drift on the scale (even 2-3°C) can affect it.

To use the scale, put an item on the scale and wait for it to stabilize. Count to five, then record that value. If the item sits on the scale for too long, the weight will continue to change due to the mechanics of the scale. Don't leave anything on the pan for too long as it will throw off the calibration of the unit. Place items on the scale gently.

High-Capacity Balance
The high-capacity balance should be checked for accuracy before weighing in each round of samples. The calibration weights are kept in three separate black plastic boxes in the drawer underneath the scales.

1. Make sure the bubble level in the back left corner of the scale is centered. Adjust the front feet of the scale to relevel if needed.
2. Turn the scale on. The scale display should fill in completely to indicate a self-check.
3. Make sure the surface of the scale is clean and that there is no water on the metal plate.
4. Tare the balance by pressing the 'ZERO' button. The scale will beep when zeroing is complete.
5. Remove the Gross Scale Weights from the drawer. There are separate black boxes for calibration weights of 200.00g, 300.00g and 500.00 g.
6. Wear gloves for moving the calibration weights from the storage boxes to the scale.
7. When the scale is stable, press the CAL button to begin calibration.
8. Place the indicated calibration weight on the center of the scale (500.00 grams)
9. After calibration, the calibration weight is displayed with weight units.
10. Remove the calibration weight.

11. Perform weight checks with the smaller weights in the calibration weight set. Perform weight checks with the 200.00- and 300.00-gram weights. Do not exceed the capacity of the scale: 810grams.
12. When testing with calibration weights, record the time and date of the test, weight of the calibration weight, and scale reading in the scale logbook.

If the scale is left on for an extended period or bumped off balance, it may drift. If the scale drifts by 0.02 or more, clean and recalibrate the scale.

Fine Scale Balance

The scale capacity is 70.0000 grams, do not exceed this capacity when performing weight checks.

The fine scale balance should be checked daily for accuracy before weighing in any filters (new or used). The scale should be calibrated when the temperature in the room has stabilized. If the oven will be used concurrent with the scale, allow the oven to warm the room for 15 minutes before calibrating the scale. It is also important to watch the scale for signs of drift when weighing for an extended period. If the weight of the scale drifts more than 0.0002 grams when closed and stabilized, recalibration may be necessary. Calibrate weekly during intensive times of use, or every time during low times of use. Make sure the scale has been on for 1-2 hours first.

1. Position the scale so that the glass doors will open fully to enable weighing the samples.
2. Make sure the bubble level in the back left corner of the scale is centered. If it isn't, use the dials located on the front sides of the scale to level the scale.
3. Turn the scale on. The scale display should fill in completely to indicate a self-check.
4. Make sure the surface of the scale is clean and all of the glass sides are closed.
5. Tare the balance by pressing the 'ZERO' button. The scale will beep when zeroing is complete.
6. Remove the 'Fine Balance Weights' (in the grey box) and scale calibration logbook from the drawer underneath the scales.
7. Scale calibration weights should only be handled with gloves. It is preferable to use plastic tongs to transfer the fine calibration weights from the box to the scale.
8. When the scale has zeroed and stabilized, press the CAL button to begin calibration.
9. Place the indicated calibration weight in the center of the scale and gently close the glass doors (50.0000 grams).
10. When the scale has stabilized it will beep and display the calibration weight with weight units.
11. Remove the calibration weight and replace it in the grey box. Perform several weight checks with the smaller weights in the calibration weight set. Perform weight checks with the 30.0000, 20.0000, 5.0000, 2.0000-gram weights in the set.
12. While testing with calibration weights, record the time and date of the test, weight of the calibration weight, and scale reading in the scale logbook.

Annual Scale Maintenance and Calibration: The High-Capacity Balance and Fine Scale balance are checked and calibrated annually by an independent calibration firm.

Desiccator Cabinet

At the beginning of each lab session, check the desiccant in each desiccator. Desiccant may need to be re-charged in the oven if the ambient humidity exceeds 15%. (Alternatively, additional fresh desiccant can be added to the cabinet for a short-term fix.)

Check the humidity indicator card in both desiccators at the beginning of each lab session. The indicator circles will change from blue to pink if the humidity of the cabinet increases. To recharge Silica Gel Desiccant, bake at 105-110°C until beads are deep orange. Beads are green when they are “wet.” To bake Drie-Rite desiccant crystals, spread the desiccant into baking pans and heat to 230°C (450° F) for one to two hours and crystals are blue.

Upon removal from the oven, desiccant should be placed immediately into the cabinets or storage containers. Loosely cap storage containers until desiccant has cooled (15-minutes) then screw the cap onto the container.

The desiccator cabinet is very sensitive to being opened on humid days. Even opening it for a few seconds might impact the color of the indicator circles. When possible, minimize how long the door is open and keep a close eye on the indicator cards. Add more desiccant if necessary.

There are two cabinets – one for prepped, clean filters and one for used filters (filters with sediment on them). Do not cross contaminate the cabinets and never put “wet” items into the cabinets. Only put items that have properly dried in the oven first.

Data Management

Data recorded on paper datasheets will be entered into an Excel spreadsheet by Estuary Program staff. Any field data collected by Tenera Environmental will be shared with the Estuary Program prior to processing at the Cuesta lab.

APPENDIX N. MORRO BAY NATIONAL ESTUARY PROGRAM EQUIPMENT CALIBRATION PROTOCOLS

Morro Bay Volunteer Monitoring Program Equipment Calibration Protocols

Turbidity Calibration Protocol

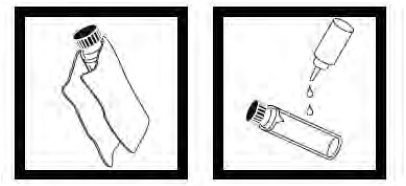
Hach 2100Q

This instrument is designed for long-term stability and minimal need for recalibration. It is recommended that recalibration be performed at least once every three months, or more often if experience indicates a need. To guarantee accurate calibration, use only the formazin standards issued by Hach.

The formazin calibration standards have a long shelf life, but must be stored properly. Standards should be stored in their respective kit with cover in place, out of direct sunlight. For long term storage, refrigeration at 5° C is recommended, do not store above 25° C.

Calibration:

- Shake the 10 NTU, 20 NTU, 100 NTU and the 800 NTU standards vigorously for 2-3 minutes to resuspend particles.
- Allow standard to stand undisturbed for 5 minutes.
- Gently invert the bottle 5 to 7 times.
- Prepare the sample cell for measurement using traditional preparation techniques. (Oil the sample cell and wipe with velvet cloth or KimTech wipe).



- Let the cell stand for one minute after oiling.
- Turn on the meter using the power button.
- Insert the 20 ntu standard cell, and align the orientation marks on the cell and chamber.



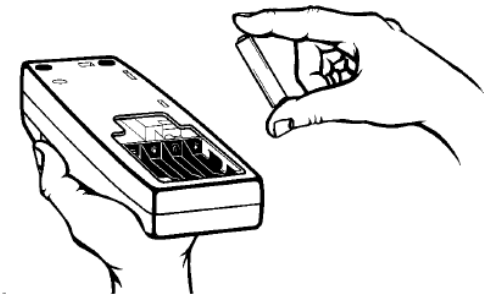
- Close the lid and press the CAL button, that looks like a small graph: ⍁
- Press READ and the meter will stabilize to read the sample and calculate

the correction factor. The display will show the 20.00 ntu value on the screen. Insert the 20.00 ntu standard with correct alignment and press the READ button.

- Repeat these procedures sequentially for the remaining standards as the meter prompts.
- After reading the final standard (800 ntu), the meter will re-display the S0 screen from the beginning of the calibration.
- Remove the 800 ntu standard and press CAL to accept the calibration.

Battery Replacement:

- This meter works on 4 AA-size alkaline batteries. Brand name 'digital' batteries are recommended for best performance.



Conductivity Calibration Protocol

YSI Pro2030 and YSI ProQuatro

Calibration:

The YSI Pro2030 and YSI ProQuatro meters require only a single point calibration to adjust the meter for all parameters. For freshwater meters, use 1413 us/cm conductivity standard for calibration. For saline or brackish wares, use 50,000 us/cm conductivity standard.

- Turn the instrument on and allow it to complete its self test procedure.
- Select a calibration solution most similar to the sample the meter will be measuring.
- Pour at least 3 inches of solution into a clean glass calibration jar.
- Insert the probe into the beaker deep enough to completely submerge the oval shaped hole on the side of the probe. Do not rest the probe on the bottom of the container, suspend it above the bottom by at least ¼ inch. Allow at least 60 seconds for the temperature reading to become stable. Move the probe vigorously from side to side to dislodge any air bubbles from the electrodes.
- Press and hold the CAL button until a new window pops up, then select specific conductance, and uS.
    - Use the UP ARROW or DOWN ARROW key to adjust the reading on the display until it matches the value of the calibration solution you are using.

- Once the display reads the exact value of the calibration solution being used (the meter will make the compensation for temperature variation) press the ENTER key.

Battery Installation

YSI meters require two alkaline C-cell batteries. Under normal conditions, the average battery life is 425 hours at room temperature without using the back light. A battery symbol will blink in the lower, left corner of the display to indicate low batteries when approximately 1 hour of battery life remains.

To install or replace the batteries:

1. Turn the instrument off and flip over to view the battery cover on the back.
2. Unscrew the four captive battery cover screws.
3. Remove the battery cover and remove the old batteries if necessary.
4. Install the new batteries, ensuring correct polarity alignment (figure 1).
5. Place the battery cover on the back of the instrument and tighten the four screws. Do not over- tighten.

The waterproof instrument case is sealed at the factory and is not to be opened, except by authorized service technicians. Do not attempt to separate the two halves of the instrument case as this may damage the instrument, break the waterproof seal, and will void the warranty.

SENSOR MAINTENANCE: CONDUCTIVITY

The openings that allow sample access to the conductivity electrodes should be cleaned regularly. The small cleaning brush included in the Maintenance Kit is intended for this purpose. Dip the brush in clean water and insert it into each hole 10 to 12 times. In the event that deposits have formed on the electrodes, it may be necessary to use a mild detergent (laboratory grade soap or bathroom foaming tile cleaner) with the brush. Rinse thoroughly with clean water, then check the response and accuracy of the conductivity cell with a calibration solution.

Dissolved Oxygen Calibration Protocol

YSI Pro2030 and YSI ProQuatro

Before calibrating, determine if meter is being calibrated for fresh water (creeks) or salt water (bay) monitoring. Determine the approximate altitude of the area where the meter will be used.

Calibration:

- Ensure that the sponge inside the instrument's calibration chamber is saturated with de-ionized water.
- Rinse the probe with de-ionized water.
- Insert the probe into the calibration chamber.
- Turn the instrument on by pressing the ON/OFF button.

- Wait for the dissolved oxygen and temperature readings to stabilize, approximately 15 minutes.
- Press and hold the Calibrate key for 3 seconds. Using the up or down arrow key, highlight Dissolved Oxygen and press enter. The Pro2030 will indicate 'Calibrating %DO' on the display. The instrument will automatically calibrate the sensor to the current barometric pressure. If DO Local% is enabled, the sensor will calibrate to 100%. This may take up to 2 minutes depending on the age of the sensor and membrane. You can press the Cal key at this time to cancel the calibration.
- 'Calibration Successful' will display for a few seconds to indicate a successful calibration and then the instrument will return to the Run screen.
- If the calibration is unsuccessful, an error message will display on the screen. Press the Cal key to exit the error message and return to the Run screen. See the Troubleshooting guide for possible solutions.

SENSOR MAINTENANCE: DO

Polarographic Sensors – Model # 605203

The cap membrane and KCl (potassium chloride) electrolyte solution should be changed every 2-4 weeks during regular use. In addition, the electrolyte solution and membrane should be changed if (a) bubbles are visible under the membrane; (b) significant deposits of dried electrolyte are visible on the membrane; and (c) if the sensor shows unstable readings or other sensor-related symptoms.

During membrane changes, examine the gold cathode at the tip of the sensor and the silver anode along the shaft of the sensor (figure 12). If either the silver anode is black in color or the gold cathode is dull, the sensor may need resurfaced using the 400 grit wet/dry sanding discs included in the membrane kit. Do not sand the electrode every membrane change as this is not routine maintenance. In fact, visually, the anode may appear tarnished and operate properly. YSI recommends using the sanding disc if the sensor has difficulty stabilizing or calibrating after a regular membrane change.

Gold Cathode

For correct sensor operation, the gold cathode (figure 12) must be textured properly. It can become tarnished or plated with silver after extended use. Never use chemicals or abrasives that have not been recommended or supplied by YSI.

First dry the sensor tip completely with lens cleaning tissue. Wet a sanding disc and place it face up in the palm of your hand. Next, with your free hand, hold the sensor in a vertical position, tip down. Place the sensor tip directly down on the sanding disc and twist it in a circular motion to sand the gold cathode. The goal is to sand off any build-up and to lightly scratch the cathode to provide a larger surface area for the electrolyte solution under the membrane. Usually, 3 to 4 twists of the sensor are sufficient to remove deposits and for the gold to appear to have a matte finish. Rinse the sensor thoroughly with distilled or deionized water and wipe the gold cathode with a wet paper towel before putting on a new cap membrane. If the cathode remains tarnished, contact

YSI Technical Support or the YSI authorized dealer where you purchased the instrument. Figure 12, DO sensor with membrane removed.

Silver Anode

After extended use, a thick layer of Silver Chloride (AgCl) builds up on the silver anode reducing the sensitivity of the sensor. The anode must be cleaned to remove this layer and restore proper performance. The cleaning can be chemical and/or mechanical: Mechanical cleaning: In order to sand the silver anode along the shaft of the sensor, simply hold the sensor in a vertical position. Wet the sanding disc and gently wrap it around the sensor and twist it a few times to lightly sand the anode (the goal is to simply sand off any build-up without scratching or removing layers of the anode itself). Usually, 3 to 4 twists of the sanding disc are sufficient to remove deposits. After completing the sanding procedure, repeatedly rinse the electrode with distilled or deionized water and wipe with lens cleaning tissue to remove any grit left by the sanding disc. Thoroughly rinse the entire tip of the sensor with distilled or deionized water and install a new membrane.

IMPORTANT: Be sure to: (1) Use only the fine sanding discs provided and (2) Sand as mentioned in the above procedures. Not adhering to either of these instructions can damage the electrodes. i 34 35 Chemical cleaning:

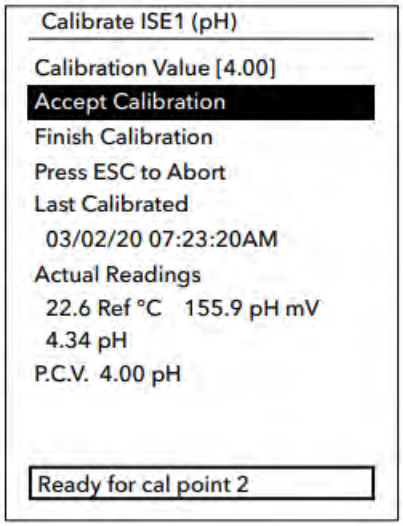
Remove the cap membrane and rinse the sensor with deionized or distilled water. Soak the sensing section of the sensor in a 14% ammonium hydroxide solution for 2 to 3 minutes or in a 3% ammonia solution overnight for 8-12 hours (most household ammonia cleaners are typically around 3%). Rinse heavily in cool tap water followed by a thorough rinsing with distilled or deionized water. The anode should then be thoroughly wiped with a wet paper towel to remove the residual layer from the anode. Trapping residual ammonia under the new membrane cap can quickly tarnish the electrode and/ or give false readings and should therefore be avoided. After performing a chemical cleaning on the polarographic sensor, lightly sand the cathode and anode following the mechanical cleaning procedures described previously. If this procedure is unsuccessful, as indicated by improper sensor performance, contact YSI Technical Support or the YSI authorized dealer where you purchased the instrument.

pH Calibration Protocol

YSI ProQuatro

The YSI ProQuatro requires a two-point calibration for pH using buffer solutions of 7.0 pH and 10.0 pH.

- Begin by filling the calibration cup with 7.0 pH buffer and the other with 10.0 pH buffer. Use fresh pH solution for each week of calibration. Calibration cups should be clean and dry before use.
- Remove the guard from the probe and thread the YSI onto the calibration cup.
- The ProQuatro will automatically generate a calibration value that compensates for temperature and corrects the buffer value. This value should not be changed. Record the calibration values for both YSIs on the datasheet.



- Keep an eye on the “ph mV” readings. In the 7 buffer, the pH mV values should be between –50 and +50. In the 10 buffer, the reading should be 165 to 185 mV lower than the reading in the 7 buffer. Once the pH reading is stable, select “accept calibration”.
- Remove the YSI from the solutions and rinse with DI. Avoid directly spraying the pH sensor bulb with DI. Instead, use a little bit of tap water to rinse the pH sensor bulbs. Shake the probes to rid of excess water droplets and thread them back into the opposite calibration cups.
- Repeat the process, ensuring that the pH readings are stable and that the ph mV readings are within the thresholds.
- The bottom of the screen should say “add second cal point”. Once the readings are stable, select “accept calibration”.
- The meters display a post-cal value at the bottom of the screen with the abbreviation “P.C.V.”. However, this value is not live. Select “finish calibration” and return to the main readings screen to record the post-cal value for that 2nd calibration point buffer. To get a post-cal reading for the 1st calibration point buffer, you will need to rinse the sensors, return them to the original solution, and record the pH value from the main readings screen.

Calibration for FH950 Flow Meter

Calibrate the FH950 weekly as a part of routine equipment calibration.

1. Suspend the sensor in a plastic 5-gallon bucket with water at least 6 in. deep. Leave at least 3 in. between the sensor and the sides and bottom of the bucket. Sensor ca be suspended with a flat object spanning the top of the bucket.
2. Wait until water in the bucket is *completely still* before continuing with the calibration process and proceeding to Step 3.
3. When the water is still, power on the FH950. From the Main Menu screen on the FH950, select *Setup*, then select *Velocity Calibration*.
4. Allow the meter to complete one velocity cycle (the default is 10 seconds and cannot be changed) If the meter reads 0.00 feet per second (ft/s), select *OK*. If the water is still and the meter does not read 0.00 ft/s, wait for the reading to stabilize and select *Zero Velocity*, then select *OK*.
5. The zero stability of this instrument is +/- 0.05 ft/s (Hach 2018). If a velocity of greater than +/- 0.05 ft/s is read, repeat the zero adjust process starting with Step 2. Consider cleaning the sensor electrodes or ensuring the water inside the bucket is completely stable before repeating the process.

   Contact HACH directly if issues persist.

Calibration Sheet: Date: _____ **Time:** _____ **Initials:** _____

Weekly Calibration Tests:

Flow Meters (FH950): Use a bucket of tap water to calibrate flow meters. Check that units are in ft/s with 30 second fixed-period averaging (FPA). Check handheld unit battery level and charge if needed.

FLOW-MBV-	
Pre-Cal	
Post-Cal	

FLOW-MBV-	
Pre-Cal	
Post-Cal	

FLOW-MBV-	
Pre-Cal	
Post-Cal	

Conductivity (YSI Pro2030, YSI ProQuatro): Remove sensor guard and rinse meter with last week's solution before calibrating. Pour fresh solution into calibration cup. For creeks, use 1413 us/cm, for Dawn Patrol use 50,000 us/cm. Rinse well with DI after.

DOE-MBV-	
Standard	
Pre-Cal	
Post-Cal	
Temp (°C)	

DOE-MBV-	
Standard	
Pre-Cal	
Post-Cal	
Temp (°C)	

DOE-MBV-	
Standard	
Pre-Cal	
Post-Cal	
Temp (°C)	

DOE-MBV-	
Standard	
Pre-Cal	
Post-Cal	
Temp (°C)	

***Dissolved Oxygen (YSI Pro2030, YSI ProQuatro):** Check there is a clean, damp sponge in each end cap. Calibrate 100% Air Saturation calibration in sleeve. Then put all meters into beaker with spinner. Turn dial up to around 6-7 (no air bubbles present). Record values when stable.

DOE-MBV-	
DO (%)	
DO (mg/L)	
Temp (°C)	

DOE-MBV-	
DO (%)	
DO (mg/L)	
Temp (°C)	

DOE-MBV-	
DO (%)	
DO (mg/L)	
Temp (°C)	

DOE-MBV-	
DO (%)	
DO (mg/L)	
Temp (°C)	

pH (YSI ProQuatro): Remove sensor guard and rinse meter with **tap water** before calibrating. Use pH 7 rinse for both sensors, then pour fresh pH 7 solution into calibration cup. Calibrate sensors using the pH 7 solution first. From the calibration screen, record the Actual Reading and the Pre-Cal PCV. When the screen displays "Ready for cal point 2," rinse the sensors thoroughly with tap water then repeat the process with pH 10 solution.

DOE-MBV-		
	pH 7	pH 10
Pre-Cal (Actual Reading)		
Pre-Cal PCV		
Post-Cal		

DOE-MBV-		
	pH 7	pH 10
Pre-Cal (Actual Reading)		
Pre-Cal PCV		
Post-Cal		

Turbidity (HACH 2100 Q): Gently invert all sealed standards except <0.10 vial 5-6 times, or until fully mixed. Use a KimWipe to clean standards before calibration.

TUN-MBV-				
Standard	20	100	800	10: Verification
Pre-cal				—
Post-cal				

Additional notes (use reverse if needed):

Dissolved oxygen **must be calibrated weekly. If short on time, other analytes can be skipped.*

Bi-Weekly Calibration Test: Run DI water blank using boxed lab-grade water.

Phosphate Blank	COL-MBV-
Blank (DI)	

Monthly Calibration Tests: Run phosphate blank and 1mg/L; change pipette filters

COL-MBV-	
Standard	Reading
Blank (DI)	
1 mg/L	

☐ Change pipette filer

Routine Checklist:

Weekly Tasks:

- ☐ Stock and clean out WQ and DP kits (datasheets, WhirlPaks, pencils/Sharpies)
- ☐ Fill all DI squirt bottles
- ☐ Toss WhirlPaks that have exceeded hold time (48-hr)
- ☐ Empty non-toxic waste
- ☐ **File datasheet in archive, bring up any issues**

Bi-Weekly (or as needed):

- ☐ Download all photos from WQ cameras
- ☐ Charge FH950 and WQ cameras
- ☐ Empty phosphate waste
- ☐ Rinse pipette tips
- ☐ Check First Aid kits / safety items
- ☐ Clean out debris from kits
- ☐ Straighten up back room
- ☐ **Enter data into Excel calibration logs**

Monthly (or as needed):

- ☐ Supply inventory (check calibration fluids for re-stock, check expiration dates)
- ☐ Vacuum equipment room
- ☐ Take out trash and recycling
- ☐ Wipe out coolers, tables, frequently touched surfaces
- ☐ Clean lab table, remove cobwebs

Bacteria Laboratory Equipment

SHEL LAB SMI2 Incubators and Thermometers

This instrument is to provide a controlled dry heat environment from 8ºC to 70ºC ± 0.35 ºC for culture tubes or Petri dish incubations.

Each time a volunteer or staff member places samples in the incubator or removes samples, the temperature on the certified thermometer inside the incubator is recorded on a log sheet. The temperature on the certified thermometer should be at 35ºC ± 0.5ºC for IDEXX Colilert-18 incubation and 41ºC ± 0.5ºC for IDEXX Enterolert incubation. If the incubator is not at the desired temperature, the temperature control will be adjusted until the temperature is correct.

If the incubator thermometer is not within ± 0.5ºC of the certified thermometer, the temperature difference will be recorded and the incubator temperature will be adjusted accordingly.

Morro Bay Volunteer Monitoring Program
Bacteria Laboratory Equipment Calibration Log

Date	Incubator ID	Certified Thermometer Reading (ºC)	Incubator Thermometer Reading (ºC)	Temperature Difference (ºC)	Incubator Setting Adjust

APPENDIX O. MORRO BAY NATIONAL ESTUARY PROGRAM DATA MANAGEMENT PROTOCOLS

Morro Bay Volunteer Monitoring Program
Data Management Protocols

The Morro Bay National Estuary Program saves all data to cloud storage via SharePoint with routine backups on an external hard drive.

All of the data that is collected for the volunteer monitoring program is managed in either an Access database or in Excel spreadsheets. Data managed in the Morro Bay Monitoring Program (MBMP) Access database, which includes water quality, bacteria and bay water quality data, are submitted to CEDEN. Bioassessment data is managed in a separate Access system designed by SWAMP. The remaining projects have separate datasheets that were created by the VMP specifically for each project. Following is a list of the data that is collected and the file where it is entered.

Project	Data Entry File
Bacteria	Database - Documents/MBMP.accdb
Water Quality and Flow	Database - Documents/MBMP.accdb
Water Quality and Bacteria QA	Database - Documents /MBMP.accdb
Dawn Patrol	Database - Documents/MBMP.accdb
Seeps	Database - Documents/MBMP.accdb
Bioassessment	DATA - Documents/Monitoring/Data Management/Macro Database
Continuous Water Temperature	DATA - Documents/Monitoring/vmp/VMP Data/Temperature (TIDBIT)
Continous Water Quality (EXOs)	DATA - Documents/Monitoring/vmp/VMP Data/Sonde Data
Continuous Water Depth	DATA - Documents/Monitoring/vmp/VMP Data/Flow
Eelgrass Monitoring	DATA - Documents/Monitoring/vmp/VMP Data/Eelgrass
Eelgrass Restoration	DATA - Documents/Projects/Restoration/Eelgrass
Bay Macroalgae	DATA - Documents/Monitoring/vmp/VMP Data/Algae - Bay
Bird Surveys	DATA - Documents/Monitoring/vmp/VMP Data/Bird Surveys
Stream Profiles	DATA - Documents/Monitoring/vmp/VMP Data/Stream Profiles
Stormwater Monitoring	DATA - Documents/Monitoring/vmp/VMP Data/Stormwater
Volunteer Hours	DATA - Documents/Monitoring/vmp/vmp admin/Recognition/Volunteer Hours
New Volunteers	Google Forms
Calibration	DATA - Documents/Monitoring/vmp/vmp admin/Equipment & Supplies/Calibration

CEDEN

The California Environmental Data Exchange Network (CEDEN) is a cooperative effort by many partners, including the Surface Water Ambient Monitoring Program and the Water Boards to exchange environmental data and make it accessible to the public. In order to load data into CEDEN, it must be entered into a series of upload templates.

The VMP created an Access-based data management system to manage data, import lab data, and export data into the CEDEN template formats. These templates are then submitted to the appropriate Regional Data Center for checking and loading into CEDEN. We call this database the Morro Bay Monitoring Program (MBMP) Database.

The following types of data are managed in the MBMP Access system:

- Creek water quality – conventional analytes and nutrients
- Creek instantaneous flow data
- Bacteria data for creeks and bay
- Dawn Patrol – bay water quality data
- Seeps water quality data
- QA data for water quality and bacteria monitoring
- Monitoring sites
- Monitoring equipment

Bioassessment data for macroinvertebrates, physical habitat and water quality are managed in the SWAMP format templates. Templates are fed into CEDEN via an online server managed by the California Water Board.

Bacteria / Bacteria QA

1. Use the printed IDEXX table, or the IDEXX MPN Generator (in the Start Menu), to verify that the volunteer correctly identified the MPN.
2. If the sample was diluted, verify that the MPN was multiplied by the dilution factor.
3. For *E. coli*, if multiple dilutions were run, enter data from the results that are closest to 80% positive wells (77 of 97 wells). This may be from separate dilutions for the separate types of bacteria. Indicate on the datasheet and in the Notes column of the database what dilution, for each type of bacteria, was used on the data entered.
4. For total coliform, always enter the result for the dilution that is less than 80% positive wells. This may not be the result that is closest to 80% positive wells.
5. If the results for a site are above this range then increase the dilution factor the following month, and likewise, if the results for a site are below this range, decrease the dilution the following month.
6. The data is entered in the Bacteria Monitoring form in the MBMP.accdb
7. For entering the QA data:
    i. For precision data: In the detail record, choose Hydrologic Unit = QA. For QA Purpose, choose 'Volunteer Precision'. Fill in the QA Result, QA Qualifier and QA Dilution. If the results are within the R_{log} range, then the precision test is a 'pass'.
    ii. For accuracy data: For QA Purpose, choose 'CRM'. Fill in the QA Result, QA

Qualifier and QA dilution. If the volunteer result is within the target range provided by the manufacturer, then the accuracy test is a 'pass'.

iii. For sterility data: For QA Purpose, choose 'Sterility'. Enter the QA Result, QA Qualifier and QA dilution. If the QA Result is less than the MDL, then the result is a 'pass'.

iv. When finished entering data, initial and date the datasheet in the bottom left hand corner then file in the folder 'Data Entered – Needs QA'. The program manager will then double check the data, initial and date it, then either file it into the 'Need to File' folder or the appropriate project folder.

Dawn Patrol

1. The data is entered in the MBMP database in the Dawn Patrol form.
2. When finished entering data, initial the datasheet in the upper right hand corner then file in the folder 'Data Entered – Needs QA'. The program manager will then double check the data, initial it, then either file it into the 'Need to File' folder or the appropriate project folder.

Water Quality

1. The data is entered in the Water Quality section of the MBMP database.
2. In the Field Observations section, enter observations about the site regarding algae, flow conditions, etc. If no field observations were collected, check the 'No Field Observations Made' box. Also enter any notes about equipment problems, site conditions, etc.
3. If data was discarded due to failure of QA criteria, flag the data as 'Exclude' so that it will not be included in analysis.
4. If duplicate readings are taken for any of the analytes (e.g. dissolved oxygen), enter the second reading in the Volunteer Precision field. The % RPD will be calculated. For RPD values < 25%, choose 'Pass'. For RPD values > 25%, choose 'Fail'.
5. For the nutrients, if there were not any levels detected then the Result entered would be the PQL of the equipment and the Result Qualifier would be '<'.
6. For QA accuracy data entry:
   a. This lab-generated data is imported in an automated process. From the main menu, choose Lab Import and then Water Quality Lab Import.
   b. Hit 'Choose File' to select the file to import. Then choose the 'Import' button.
7. For QA precision data entry:
   a. Complete the Result and Result Qualifier information for the precision result. The %RPD will automatically calculate. If the RPD is > 25%, choose 'Fail'. If the RPD is < 25%, then choose 'Pass'.
8. For Sterility data entry:
   a. Enter the Result and Result Qualifier information for the blank. If the Result is less than the PQL, then enter 'Pass'.
9. Flow data is entered in the flow detail record. The Q value is automatically calculated by the system.
10. When finished entering data, initial the datasheet in the upper right hand corner then file in the folder 'Data Entered – Needs QA'. The program manager will then double check the data, initial it, then either file it into the 'Need to File' folder or the appropriate project folder.

Bioassessment

1. Bioassessment data is entered into CEDEN, per the protocol provided by SWAMP.
2. Data entry protocols are saved on SharePoint under: DATA - Documents\Monitoring\Data Management\Macro Database\Database Protocol.
3. All bioassment Access database files should be saved under DATA - Documents\Monitoring\Data Management\Macro Database\YYYY.

Eelgrass

Permanent Transects

4. Field data from permanent transect monitoring is located on SharePoint under
5. DATA - Documents\Monitoring\vmp\VMP Data\Eelgrass\Permanent Transects\Database - Data Analysis. There is an Excel database with all data from each site, called "Eelgrass Transect Database.xlsx."
6. Enter all data as shown on the datasheet, calculate average coverage for each site.
7. When finished entering data, initial the datasheet in the upper right hand corner.
8. The datasheet should be QA'd by a secondary staff member before filing electronically.
9. After the data has been entered and QA'd, file a scanned datasheet under DATA - Documents\Monitoring\vmp\VMP Data\Eelgrass\Permanent Transects\Datasheet Scans.

Bed Condition Monitoring

1. Field data from bed condition monitoring is located on SharePoint under DATA - Documents/Projects/Restoration/Eelgrass/Montioring/ Bed Condition Monitoring/Database.
2. There is an Excel database with all data from each site where all Bed Condition Monitoring data should be entered.
3. Enter all data as shown on the datasheet. When finished entering data, initial the datasheet in the upper right hand corner.
4. The datasheet should be QA'd by a secondary staff member before filing electronically.
5. After the data has been entered and QA'd, file a scanned datasheet under DATA - Documents/Projects/Restoration/Eelgrass/Montioring/ Bed Condition Monitoring/Scanned Datasheets.
6. Photos should be uploaded to …Restoration/Eelgrass/Monitoirng/Bed Condition Monitoring/Survey Photos. All photos should be named using the following nomenclature: YYYYMMDD_SITE_Q#_ORIENTATION.jpg.

Stream Profiles

1. The stream profile data is entered in a separate excel database for each site. These files are located in the folder DATA - Documents\Monitoring\vmp\VMP Data\Stream Profiles\Data - Summaries\Profile Data

2. The file All Sites – Channel Data.xls is where all of the descriptive channel data is entered. This includes bankfull measurements and calculations of slope and entrenchment for all of the sites.
3. For each of the profile sites, open the file corresponding to that site's name. Under the 'Stadia Reading' heading, create a column that corresponds to the current year and enter the stadia reading data.
4. Under the 'Elevation' heading, also create a column that corresponds to the current year then enter the formula to compute the elevation data from the stadia data. Drag the formula down through all of the cells.
5. Open the profile plot worksheet. Update the source data by adding a new series in order to display the new data. Then remember to update the heading on the plot to reflect the correct time frame.
6. There is also a file, DATA - Documents\Monitoring\vmp\VMP Data\Stream Profiles\Data – Summaries\Dates Sites Surveyed.xls, which shows a table that indicates which of the sites were measured in a given year. Update this file as well as sites are revisited.
7. When finished entering data, initial the datasheet in the upper right hand corner then file in the folder 'Data Entered – Needs QA'. The program manager will then double check the data, initial it, then either file it into the 'Need to File' folder or the appropriate project folder.
8. FYI – in the past, veg and substrate data was collected in conjunction with the stream profiling data. This data is saved in CCAMP ## in the TransData2 worksheet.

Pressure Transducers

See Appendix P for description of data protocols.

EXO3 Sonde

See Appendix C for description of data protocols.

Volunteer Hours

1. There is a separate worksheet for each year, which has listed only the volunteers that participated during that year, and shows both monthly and annual participation hours.
2. The 'All Volunteer Hours' worksheet lists the names of all the volunteers that have ever participated, their annual participation hours and overall total for all years. This worksheet should be manually updated each December.
3. The volunteer list is sorted by last name so search for the appropriate location to add the volunteer.
4. Insert a row then in the first cell, type in the volunteer's last name. In the second cell, type in the volunteer's first name.
5. Now monthly hours can be added for that volunteer.
6. The previous worksheets have been protected to prevent accidental data loss from those years.
7. Should these sheets need to be edited, the protection can be removed by

clicking on the pull down menu 'Tools', selecting 'Protection', and 'Unprotect Sheet'.

8. Similarly, to protect a sheet once data has been entered click on the pull down menu 'Tools', select 'Protection', and 'Protect Sheet'.
9. You will be prompted for a password which, in this case, should be left blank.

New Volunteers

1. All participants in program activities need to fill out a contact information/liability release form.
2. If the volunteer is interested in receiving monthly emails with program updates, enter their contact information into the Access database. Open the database and choose Data Input/Lookup.
3. Enter contact info into appropriate cells.
4. Save the release form so we can add their email address to the active mailing list. File in the volunteer info binder.
5. If someone only participates in a one-time event and is not interested in getting email updates, do not enter their contact info into the database. Simply file their sheet in the brown Pendaflex folder.
6. Enter completed volunteer training in the Volunteer Training Log Sheet located at DATA - Documents\Monitoring\VMP\vmp admin\Recognition\Volunteer Hours – Fiscal Year.xls. Enter the volunteer name, hours of training, type of training, location of training, and staff member conducting training.

APPENDIX P. MORRO BAY NATIONAL ESTUARY PROGRAM PRESSURE TRANSDUCER PROTOCOL

Morro Bay National Estuary Program Pressure Transducer Protocol

The MBNEP is responsible for the maintenance and monthly data download of three pressure transducers located throughout the Chorro Creek Watershed. Data is downloaded approximately monthly, during which staff collect stage height data using a staff plate, and record streamflow velocity/discharge using a flow meter.

Site Code	Creek Name	Site Description	Latitude	Longitude
310CCC	Chorro Creek	Chorro Creek upstream from Chorro Flats at Chorro Creek Road. PTD located on right bank about 10m downstream of road	35.35 762164	-120.8123446
310CHO	Chorro Creek	Chorro Creek on Camp SLO property, PTD located on the left bank of bedrock pool	35.32196696	-120.7222985
310CPN	Pennington Creek	Pennington Creek below bridge, PTD located on the left bank about 10m downstream of the bridge	35.345201	-120.728915
310DAM	Dairy Creek	Dairy Creek at dog park, above the bedrock pools, PTD located on the left bank	35.33558057	-120.7279324
310SBC*	San Bernardo Creek	San Bernardo Creek upstream of water quality monitoring site at bridge	35.382038	-120.781535
310BSL	San Luisito Creek	Upper San Luisito Creek at fish barrier (Turri), PTD located directly above barrier on right bank	35.362351	-120.756821
310CLV*	Upper Los Osos Creek	Los Osos Creek at Clark Valley Road (Forest Nurseries), PTD located downstream	35.289187	-120.803851

**The PTD at 310SBC and 310CLV are different from the established WQ site. The coordinates listed in this table reflect the actual location of the PTD.*

Flow

Flow is measured at each site using the MBNEP protocol. Generally, the measurements should be taken at the same location each month, however, as flow regimes change, taking measurements at the same location may not be feasible. The location can be moved to another spot in the general location, just as long as there are no tributaries, seeps, springs etc. contributing to the flow of the new monitoring location.

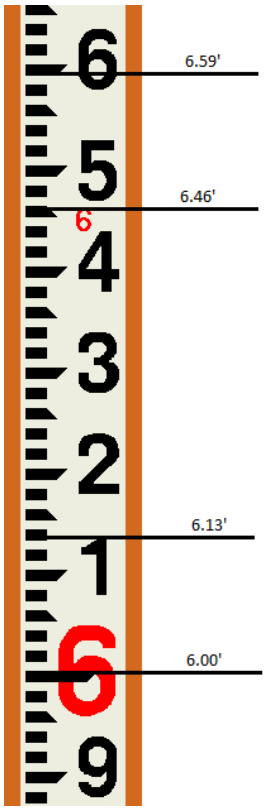
During low flow conditions it is especially difficult to take flow measurements. It is important to move any rocks, organic material, or anything that may cause an eddying effect in the water column. If any "grooming" of the channel is conducted be sure to do it before and not during measurements. Please review the MBNEP flow monitoring protocol prior to heading out into the field.

It is important to have at least 20 measurements in the transect, with no more than 5% of the flow in any one section. Thus, if the majority of the flow is concentrated in one section of the channel, it is appropriate to have smaller segments in that portion and larger segments in the slower moving portions.

Stage Height

Depending on the monitoring location, stage height is measured using a semi permanently installed staff plate or stadia rod. At most sites,a staff plate is installed directly adjacent to the pressure transducers. The stage height will be read directly off these plates.

Record stage height **even if there is no flow**.
All stage measurements are in tenths of feet.



Site Maintenance

In addition to the flow measurement and stage readings, it is important to perform a few maintenance measures to insure that the equipment is functioning properly.

Clearing of Debris

It is important, especially after a storm event, to clear any debris or sediment that has accumulated around the pressure transducer. DO NOT clear small debris (such as leaves and small branches/twigs) on the downstream control. Do clear large debris such as big logs and/or unembedded large cobbles that have obviously been deposited recently and will likely be mobilized in future storm events. The idea is to create and maintain a non-erodible cross-section on the downstream control. Also, check to see if the pressure transducer is secure to the creek bank and channel bottom. If the PTD is loose, take notes and photos. Then, re-secure with zipties.

Downstream Control

While it is important to clear debris that is in the immediate vicinity of, or in contact with, the pressure transducer, DO NOT clear small debris (such as leaves) that have accumulated on the downstream control. It is okay to clear large branches or logs that have clearly been deposited as a result of a storm event. The leaves that you clear will likely build up again in a day or two after you leave the site anyway.

The downstream control is a feature, such as bedrock, embedded cobbles/boulders, or simply a tail of a pool, that controls the water level of the reach behind it (upstream of it). Think of the control something that, if moved, would cause the water level around the PTD to drop.

With any debris that has accumulated around the downstream control, take notes about how much, what type, and if it appears to be backing up water around the PTD. When making rating curves, a separate curve will likely be created for “fall” when falling leaves consistently build up on the downstream control.

Coiling the Cable

The PTD cable should be spiraled in the PVC housing and should not ever be kinked when stowed away. Sharp turns in the cable could allow moisture to accumulate in the cable and interfere with pressure readings.

Desiccant

Each cable end is equipped with a chamber filled with desiccant. Desiccant protects the electronics from condensation, which can cause irreparable damage and loss of data. The indicating desiccant changes from blue to pink as it becomes saturated with moisture. Check the condition of the desiccant each time you visit the site.



Desiccant stages from left to right: new (left); nearly expired, should replace now (center); expired (right).

If the desiccant appears to be a dull color of purple or pink, replace the desiccant. Unscrew the chamber cap and pour the old desiccant into the jar labeled "OLD." Refill the chamber with the new desiccant which should be stocked in the PTD backpack. You can use your hands as a funnel, or a small piece of paper to do this. If there is no desiccant stocked, leave the existing desiccant in the chamber, and return to the Cuesta lab to re-bake the desiccant.

To refresh the desiccant, bake at 225 °C for approximately 30 minutes or until the desiccant has turned blue. Desiccant should be baked at the Cuesta College sediment lab per the instructions below.

- Place the Maximum Registering Thermometer (MRT) inside the oven to ensure the oven goes up to temperature. Adjust the red dial to the lowest possible temperature on the MRT.
- Flip the middle switch to 'Power' to turn on the oven.
- Flip the lower switch upwards to 'Heat'.
- Set the dial thermostat to 225° C or about 440° F.
- The red (Power) and orange (Heating) indicator lights on the front of the oven should come on.
- When the orange indicator light has gone off, open the oven door and record the temperature from the MRT. In the logbook, record the date, time, and temperature read out.
- When the internal temperature is at approximately 225° C, place desiccant on tray and bake for 30 minutes or until the desiccant has turned dark blue/purple.

Data Download

The pressure transducer data is downloaded approximately month using a Bluetooth connection (Wireless TROLL Com) or a laptop installed with WinSitu5 application.

Connecting the Pressure Transducer to a Bluetooth device

1. To access the end of the pressure transducer cable, remove the cap from the PVC pipe that is located on the stream bank. To remove the desiccant housing, hold the textured section of the cable connecter in one hand and the desiccant in the other. Twist in opposite direction to unlock the desiccant housing from the cable.

2. Position the Wireless TROLL Com and the cable connector so they will connect properly. Insert the Wireless Com connector firmly into the cable connector.



Troll Com Cable Connector

3. Hold the textured section of the sleeve in one hand and the TROLL Com in the other. Push and twist until you hear a click. The click ensures the cable and TROLL Com are securely attached.

4. Press the power button on the Wireless Com to active Bluetooth connection, and launch the VuSitu app on your mobile device (smart phone or tablet), or launch the WinSitu5 desktop application. If this is a new device, choose “Add new device” and VuSitu or WinSitu5 application will display the connected instrument screen when pairing is complete.

5. To download the data in VuSitu:
    a. Select the log you wish to download by tapping on it
    b. Export either **All Data** or **New Data** and then tap to download. This can take between one and fifteen minutes depending on the size of the log.
    c. Confirm that the file has downloaded using the tab option on the upper left side of the screen. Select **Data Files** to view downloaded data. You should be able to see the file name, date of download, and site name.
    d. Click the checkbox and then use the export function to email yourself the file. (This step can be saved for when you are connected to WiFi, if necessary.)

6. To download the data in WinSitu5:
    a. Connect to the Insitu sensor using the twist lock cable. Make sure the cable is fully snapped into place.
    b. Open Winsitu5. If prompted to “Connect to Device Now?” select ‘**Yes**.’ Otherwise tap **File >> Connect**. It make take a moment, and if any error messages pop up tap **Okay** or **Yes** . Once the device is found it should open a screen with real-time readings of the probe in the first tab.
    c. Navigate to the second tab, where there should be logs with the name of the site and the water year.
    d. To download the data:
    e. **Select the log you wish to download** by tapping on it. **Tap the curved arrow** at the bottom of the screen to open the download dialog window

    a. In that window select either **All Data** or **New Data** (usually we select **New Data** unless we need the entire log for some reason) and then tap the arrow to download
    b. This can take between one and fifteen minutes depending on the size of the log
f. Once the data has finished downloading a window listing the time elapsed will pop up—**tap OK**. A window will pop up asking if you would like to view the data—**tap No**
g. Ensure log is still running (the icon next to it should be a running figure) and then tape **File >> Disconnect**. You can now remove the cable, reattach the desiccant, and return it all to the housing
h. If you cannot connect to the sensor:
    a. In the **Preferences >> Comm Settings** window ensure the port displayed in the **Port Number** dropdown is correct. If there are other options in the dropdown menu try selecting those to see if a connection can be made
    b. You can also select **Reset All Devices** which will reset the sensor's setting and should make it possible to connect
    c. **Make sure you software is updated**—the automatic checks for software updates often don't work, so it is good to manually check your software version vs the newest available version on a regular basis.

Data Management

PTD files should be exported from VuSitu via email. When you receive the email, unzip the HTML file and select the option to "Export a CSV." Save the raw CSV file under its respective creek mainstem folder under DATA-Documents/Monitoring/vmp/VMP Data/Flow. Place raw data files in the correct site folder in a subfolder called "Data Files."

Performing QA/QC on the Data:

1. Open the raw data file in the Excel app. Visually assess the data using the scatter line plots. Normal PTD data should be a relatively smooth line, with drastic jumps in stage only coinciding with storm events. Prior experience with stage hydrographs will help in determining when the data has outliers.
2. It is not uncommon to have one to a few data points that obviously do not follow the trend of the surrounding data. PTDs are not perfect and can sometimes spike for no reason. If this is a consistent occurrence, consult a supervisor.
3. Common and acceptable sources of variations or spikes in the data include:
    a. **Storm events.** Storms can produce drastic and sudden changes in water level, sometimes as much as a few feet over an hour. A standard stage hydrograph during a storm event will usually exhibit a sharp increase in stage, followed by a slightly less intense drop in stage that indicates surface runoff that is draining quickly. The data might then hit a point where it lowers at an even slower rate, indicating water draining out of the hyporheic zone. Eventually the data should level out or drop at a very slow rate, indicating baseflow conditions.
    b. **Daily fluctuations of a few hundredths to one tenth of a foot in stage caused by evapotranspiration.** Riparian vegetation can sometimes have significant effects on water level, drawing the level down during the late afternoon/early evening after a long day of taking in water through their root systems. The water level should bounce back overnight and be evident in the data. These minor fluctuations might appear unnoticeable when the y-axis is elongated due to elevated stage during storm events.

c. **Unusual data during periods of drying and rewetting.** PTDs do not perform normally in dry conditions, and the transition period between dry and wet can produce weird variations. Only clip data after the date/time that the site has been visually confirmed dry.
d. **Sudden drops in stage and rebounding associated with pumping.** Certain sites are subject to influences from surrounding landowners suddenly turning their wells on or off. This usually looks like a sudden drop in water level for a short period of time (usually several hours) and then a sudden rebound back to its original water level. Keep an eye out for this at CCC or CAN (county site), which is located close to municipal wells.
e. **Slow leveling out or increase in stage, or sudden small drops in stage, associated with "Fall" conditions.** Given the small, low flow nature of Morro Bay's tributaries, Fall conditions can have a strong influence on PTD stage. When leaves and small branches start falling in late Summer and throughout Fall, they can accumulate on the downstream controls of the PTDs and artificially bolster the stage. This is noticeable by a leveling out (from a previously decreasing trend) or a slight increase in stage. Additionally, these artificial leaf/twig dams can suddenly give out and cause a drop in water level, sometimes by as much as a few tenths of a foot. It is important to remember to not artificially remove these small debris pileups because they will just reform a few days later.

Flagging Data for Removal

If erroneous data points exist, and do not follow under the above acceptable variations, then it may be necessary to flag these data points for removal in the master spreadsheet. Do so carefully, and only after consultation with a supervisor. Be sure to interpolate as best as possible between the ends of the clipped data to ensure data resolution is not lost.

Storing Data in Master Excel Databases:

When all data has been reviewed and cleaned, proceed to edit the data and combine it into the master Excel sheets for each site, located in each site folder and labelled with "_Master" at the end of the site name.

1. Double check that the CSV file you are working with matches the _Master file.
2. Ignoring the top metadata-style information (usually the top 80 rows or so), copy and paste all headers and data into the _Master sheet. Place the information in the appropriate sheet, labelled by water year.
    a. Water year = October 1st of the previous year to September 30th of the year for which it is labelled. Example: WY2020 = 10/1/19 – 9/30/20
3. Be sure that the date and time lines up exactly with existing data already in the sheet. Data points are collected every 15 minutes.
4. Plot Depth (ft) over time using a scatter line plot using the Excel app.
5. Navigate to the "Master Chart" sheet and edit the data series so that it includes the newly added data. Each water year should have its own series, so add a new series for a new water year when appropriate.

Compiling Flow and Stage Data into Rating Curves and Tables:

Sometimes, it is necessary to provide flow data and stage data to a project partner or consultant, or simply to create a rating curve. To do so, simply query the flow data for the desired sites out of the Access database. Add the data to the already existing flow data/rating curve documents that exist in each project folder. Be sure to include notes taken during each flow measurement, as these are critical to interpreting the data when creating a rating curve.

Starting New Logs

New data logs should be started at the top of each water year (October 1). If all data has been downloaded, older logs can be deleted to free up memory on the PTD.

1. Delete any old logs on the device that are no longer needed. **Make sure you have downloaded any necessary data.** Tap on the log you wish to delete and then tap the Stop option. Select the stopped log again and choose Delete.

2. To start a new log, tap the icon at the bottom showing a list with an asterisk. The site name should already be correct unless you are moving the sensor or creating a new site, in which case you can add a new site. The log name should be the correct water year (e.g. WY2020 or WY2021).
    a. Set the measurements should be set to Linear Logging, Surface Water, and the units of C, PSI, and Feet
    b. Set the Logging Interval to **fifteen minutes and** ensure that there is no stop time. Use the “overwrite memory when full” option.
    c. Set the start time to the next fifteen-minute interval in the future (i.e. on the hour, 15, 30, or 45 minutes after). This is an important step so that the logs do not have funky increments like 00:03:17.
    d. Start the log, then disconnect when done.

APPENDIX Q. MORRO BAY NATIONAL ESTUARY PROGRAM RECREATIONAL USE MONITORING PROTOCOL

MBNEP Recreational Use Monitoring Protocol

The objective of Recreational Use monitoring is to keep track of the visitation at eight sites across the estuary and establish a time series of estuary use. Each site is a parking lot primarily used for bay recreation and does not include parking lots along the Embarcadero that have mixed uses or parking lots in residential areas. The methods outlined in the protocol are based on a case study from Cape Cod (Mulvaney et al. 2020).

Sample Sites

Site Name	Description
Coleman Beach (COL)	All parking north of the last building along the Embarcadero to the south end of the basketball courts.
Tidelands Park (TID)	All parking on both sides of the Embarcadero south of the last building and north of the park.
Tidelands Boat Launch (TBL)	All parking in the lot directly south of Tidelands Park. Trucks with boat trailers count as one vehicle. If boats are left unattended on a trailer with no truck, it is not counted.
Windy Cove (WIN)	All parking along the low-lying stretch of sand on the west side of Main St from the north to south end of Windy Cove. Also includes the small dirt lot facing the golf course on the south side of the cove.
State Park Marina (SPM)	All parking in the Marina lot, both on the east and west side of the Bayside Café. This site is home to the Bayside Cafe. People counts will be important for distinguishing between recreators and restaurant patrons.
Pasadena Point (PAS)	All parking along Pasadena Dr between Santa Ysabel Ave and Baywood Way.
Baywood Pier (BAY)	All parking in the dirt lot where 1st St dead ends. Also includes the parking lot for Nautical Bean and Back Bay Inn, as well as parking along the west side of El Moro Ave until 3rd St. People counts will be important for distinguishing between recreators and hotel/cafe patrons.
Pecho Road (PCH)	All parking in the dirt lot on the corner of Pecho Rd and Binscarth Rd.



Establishing Stock Curves for Each Site

Background:
Visitation at coastal recreation sites varies throughout the day, and the presence of people at a site at a specific time is a function of both the arrivals and departures throughout the day. By taking into account both of these factors, a stock curve can be established that represents proportional visitation throughout an average day. Once a stock curve is established, instantaneous counts can be conducted at any time of day during future monitoring and can be corrected to daily visitation rates. Estimates of estuary use will utilize the sum of visitation across all 8 sites, but observing trends in site-specific use is also possible. New stock curves for each site will likely need to be re-established every few years, but an exact time frame has not yet been

established. Due to the difficulties of scheduling all-day surveys, we are opting to utilize the four-hour survey method as outlined in the EPA guidelines for this project (Mazzotta et al 2021).

Preparation:

1. Estuary Program staff will coordinate with students and/or volunteers to develop a sampling schedule. Continuous counts are time-consuming, so working with partners outside of the Estuary Program will be necessary.
2. To fully develop a stock curve, each site must be visited multiple times for continuous counts within a calendar year.
3. An effective sampling schedule must consider the availability of all participants, the tide height during the survey, and the day of week it takes place on. At a given site, surveys should be conducted at varying tidal heights and on different days of the week to represent the alterations in visitation introduced by each of these factors.
4. While survey dates should be somewhat random, the idea is to sample "ambient conditions" (borrowing some terminology from water quality monitoring), meaning we want to capture average visitation, ideally without large gatherings taking place at the site or during major storm events. However, sometimes this may be unavoidable, and it should be documented. To plan ahead, visit this link for upcoming events in Morro Bay and this link for events in Los Osos/Baywood Park.
5. When working with new students or volunteers, take them on a tour of all the sites and show them the specific site boundaries. Crossing into these boundaries indicates an arrival. For maps of site boundaries, see the Detailed Site Maps section below.

Sampling:

1. Surveyors should bring the following gear with them:
    a. Clipboard and pencil
    b. Continuous Count datasheets
    c. EXO3 sonde and handheld
    d. Hip waders
    e. Water and snacks
    f. Foldable chair or beach towel
2. Meet at the site by 11:45am or earlier. This will ensure there is enough time to count the initial number of cars and people. All survey sites require at least two surveyors to be present, even at smaller sites, so they can take a break if needed.
3. Have one surveyor collect the first set of water quality data with the EXO3 sonde. The surveyor will put on hip waders, wade out into knee-high water, and let the sonde stay submerged until the readings stabilize.
4. Once it is 12pm, begin tallying all arrivals. One surveyor will be in charge of tallying cars, and the other will be in charge of tallying people and their respective activities.
5. After each 15-minute interval, start tallying in the next box. For car counts, count all the cars present in the lot at the end of the hour and record in the shaded box. For people counts, record the total number of people present at the end of each hour at the bottom of the respective datasheet.
6. During periods of downtime, one of the surveyors will fill out the shore, water, and weather conditions.
7. Make note of any unusual recreation activities or community events taking place. *Staff should attempt to schedule sampling for days when large community events are not taking place.*
8. At 2pm, collect the next set of EXO3 readings as was done in step 3.

6. Continue the sampling until 4pm, making note of how many cars and people are in the lot upon finishing the sampling. Take the last set of EXO3 readings and record on the datasheet.
7. Return the EXO3 sonde and completed datasheets to the Estuary Program.

Conducting the Periodic Counts

Background:
Even before the stock curves have been established (or updated), periodic car counts can be conducted and converted into visitation upon completion of the stock curve development. Twice per month, volunteers will survey each site and take an instantaneous count of cars. Each site's count will be multiplied by their respective extrapolation factors (specific to site and time) and then combined from all sites to get an overall estimate of estuary use for that day. While this will underestimate total usage, the idea is that by using a composite value of recreational use from several representative locations, relative use can be accurately compared over time.

Sampling:
1. Each month, Estuary Program staff will generate a new schedule for volunteer recreational use monitoring.
2. Pick up Periodic Count datasheets from the office or print them out at home before conducting the monitoring.
3. At the top of the datasheet, record your name, the date, and the current weather.
4. Visit each of the monitoring sites between 12pm and 4pm. A more specific sampling time may be required if the periodic count is designed to capture particular tide conditions.
5. To conduct a periodic count, simply count all of the cars within the boundaries of the sample site. If cars leave/arrive during your count, make note of this on the datasheet.
6. At each site, record the car count, the time, and circle "Yes" or "No" to designate whether the lot was full or not. The Tide Height column can be left empty, staff will fill this in later using the NOAA tide data from station 9412110.
7. Make note of some of the recreational activities you observe at each site, as well as any unusually large gatherings or weather events in the Additional Comments section.
8. After visiting all of the sites, drop off the datasheet at the office or scan and email them to the Monitoring Coordinator.

APPENDIX R. MORRO BAY NATIONAL ESTUARY PROGRAM CONTINUOUS TEMPERATURE LOGGER PROTOCOL

MBNEP Temperature Logger Standard Operating Procedure

Overview: The HOBO MX2203 TidbiT is a continuous temperature monitor that can log up to 96,000 measurements and is accurate to ±0.2°C (or ±0.36°F). These are sealed units that require a Bluetooth-enabled device and an Android or iOS device with the HOBOmobile app to interface with the TidbiT software. These devices are left in streams to monitor the daily fluctuations and seasonal trends of water temperature in Morro Bay watershed creeks. In addition to detecting small changes in temperature when submerged in water, the Tidbits can also be used to detect when the monitored section of a stream runs dry.

Before Deploying: New loggers must be configured and loggers that have been deployed previously may need to be re-configured if they are being used at a new site.

1) Connect to TidbiT using HOBOmobile application. Press down on the magnetic button to activate the device's Bluetooth signal.
2) Configure the TidbiT settings. If this is a new logger, create a new page in the equipment log book following the naming scheme used in previous loggers. The HOBOmobile configuration options are listed below:
    a. Name: Our convention for these models is "SO-MBV-# - SiteCode" e.g. *SO-MBV-6 – SLU*. Beside the site code, these names should not change, so that they are consistent with database entry. TidbiTs may be moved from one creek to another, so in the above example, the logger could be renamed to *SO-MBV-6 – DCH* if the logger was redeployed at Dairy Creek. Serial numbers always remain constant, so these are the most reliable way to check records for errors in naming.
    b. Group: Not used as of 2020
    c. Location: Not to be relied upon for deployment history – the logger only takes the mobile user's current location.
    d. Deployment Number: Not edited by users
    e. Logging Interval: Loggers should be set to 30 minute intervals.
    f. Start Logging: Start the log on an interval of 30 minutes e.g. 1:00, 1:30, or 2:00 PM on the day you plan to deploy.
    g. Stop Logging: Never (wrap option). Data should be downloaded with enough frequency that the memory does not fill. Complete a final download and delete on-board data at the end of each water year.
    h. Alarm: Not used
    i. Bluetooth/Power Saving Mode: Always Off. This will save battery. Bluetooth connection is only needed for a few moments to download. Note that the Bluetooth signal does not penetrate water.
    j. Logging Mode: Normal, fixed interval. Statistics may be enabled if desired, but each of them can be obtained easily in Excel.
    k. Show LED: On. This serves as a visual check in case the mobile device cannot connect.
3) Start the TidbiT. After completing the configuration settings above, select "Start" so that the configuration file is sent to the TidbiT. The main page should then show you that the logger is "Awaiting Delayed Start". At this point, the logger is ready to be placed in the creek.

Note: HOBOmobile also allows users to adjust the app's settings. Select SI units for downloads. US units are acceptable, but require conversion in Excel.

Deploying: These devices need to be placed in an appropriate location within the creek and properly secured.

1) Use four (4) zip ties to secure the TidbiT to one looped end of a steel cable. Normally, once these devices are attached to a cable, they should not be removed unless their attachment seems faulty or they need to be sent for servicing.
2) Using additional zip ties, attach the other looped end of the cable to a sturdy root or branch that would be unlikely to come loose in a large flow event. Attach them to live branches and roots, not dead ones.
3) Site selection is important – pools and deep runs are ideal. If possible, the TidbiT should be placed out of direct sunlight. Its depth should be below the level of downstream control. The cables are flexible and may move around, so imagine the TidbiT's possible locations in fast and turbulent flow and whether it could be "stranded" somewhere dry after a passing high flow.
4) Take photos of the spot where the cable is anchored.
5) Take a temperature reading with a handheld device such as a YSI meter to confirm the downloaded temperature later.

Note: Certain TidbiTs require a MBNEP secured password in order to access data. All passwords are 3834.

After Deploying: After leaving the device, it is important to make permanent notes of the deployment details. For a new year of deployment, create a folder under DATA-Documents\Monitoring\vmp\VMP Data\Temperature (TIDBIT)\Deployment Notes. For 2020, there is a folder called "2020 Deployment" and a file called "2020 TIDBIT Deployment Log.docx," which gives a detailed record of the deployment site, including a written description and photos of the site. For each new year, create a folder and a corresponding deployment log. Upload any photos you took of the TidbiT deployment site, as well as a detailed written description. Other deployment details, such as YSI temperature comparisons, last time checked, and previous deployment sites should be put under DATA-Documents\Monitoring\vmp\VMP Data\Temperature (TIDBIT)\Deployment Notes in a file called "Deployment Record Database.xlsx."

Downloading and Data Management: TidbiT data should be downloaded during regular site visits for water quality or other regular monitoring events. Additional download intervals are only necessary when retrieving a device before a storm or if the data is needed sooner for a report or research.

1) Take a temperature reading with a YSI 2030 or similar handheld device. Write down the temperature.
2) Pull the device completely out of the water and press the magnetic button to activate the Bluetooth signal while the HOBOmobile app is open. The app should automatically detect the signal and display the active TidbiT.
3) Note the amount of used memory and battery level.
4) Select "Readout" under the Actions menu. The file will begin to download and can be found in the Data Files tab afterward. Open the data file and review the data for

anomalous behavior like spikes or erratic recordings.

5) Compare the last reading with the YSI result. Temperature values should be within 0.5°C of each other if they are taken at the same time.
6) Put the logger back into the water or cut it from the anchor if it needs to be removed. Note any significant changes in the site since the last visit.
    a. If you are ending a deployment, select “Stop Logging” to end the current program. Data can still be downloaded after it is ended.
7) Back at the office, use the HOBOmobile app to export the downloaded graph to an .XLSX file. The file should be added to the appropriate site folder under DATA-Documents\Monitoring\vmp\VMP Data\Temperature (TIDBIT). Different mobile devices will have different methods to upload the data file – one reliable method is to send the Excel file in an email to your MBNEP account.
    a. Update the “Deployment Record Database.xlsx” file with YSI and TIDBIT temperature readings and note if you have completed a deployment, specifying the TidbiT’s new location in the VMP room or wherever it is stored.
8) Review the file again in Excel using a graph. Flag data points that may have resulted from exposing the logger to the air during a download or before/after deployment.

Morro Bay National Estuary Program Stormwater Monitoring Protocol

Goal

The purpose of the monitoring effort is to identify the presence of typical pollutants in the stormwater runoff from the State Park Marina parking lot before and after construction of a project to reduce runoff to Morro Bay.

Sampling Preparation

1. Note all coolers and sample bottles labeled with Site and Sample ID codes
2. Note site locations and site codes.
3. Visit sites during daylight to become familiar with monitoring locations and site needs.

Storm Event Sampling

1. Assemble all equipment:

Coolers and ice packs	Rain jackets and boots
Gloves	Sampling Pole
Bucket and Cup	Datasheets and Pencils

2. At the site, rinse the bucket or sampling cup 2-3 times in the outflow before filling it.

3. Use the bucket to fill the sample bottles. DO NOT RINSE SAMPLE BOTTLES as they have been sterilized and contain preservatives. DO NOT SUBMERGE PRESERVATIVE BOTTLE TO FILL as the preservative could mix with and contaminate the sample.

4. Record time of sample collection.

5. Photograph each outfall at the time of sampling.

6. Complete datasheet.

Note for Post-project sampling:

All samples will be collected from the outfall pipe the drains each bioswale. If the outlet pipe is submerged by the high tide, then it cannot be sampled.

Stormwater Sampling Bottles

Oil and Grease

- Pace Analytical Analysis
- 1-quart glass bottle, sulfuric acid preservative
- Fill as far as possible, a little head space is all right
- 28-day hold time

Dissolved Metals: Copper, Zinc, and Lead

- Pace Analytical Analysis
- 1-Liter plastic bottle, no preservative
- Leave some head space in the bottle
- 181-day hold time

Total Suspended Solids

- Pace Analytical Analysis
- 1-Liter plastic bottle, no preservative
- Leave some head space in the bottle

- 7-day hold time

Total Petroleum Hydrocarbons (TPH)-Gasoline

- Pace Analytical Analysis
- VOA, Glass Vial 40 mL, 200 uL HCl preservative
- Try to leave NO head space when filling
- 14-day hold time

Total Petroleum Hydrocarbons (TPH)-Diesel

- Pace Analytical Analysis
- 1 Liter glass amber bottle, no preservative
- Leave some head space in the bottle
- 14-day hold time

Bacteria (*E. coli*, Total Coliform)

- San Luis Obispo County Public Health Agency
- 1 sealed sterile IDEXX bottle with sodium thiosulfate
- Leave some head space in the bottle
- 24-hour hold time

Upon Completion of Sampling:

1. Return to office.
2. Confirm that all sample bottles are labeled correctly.
3. Store samples in refrigerator.
4. Review datasheet for completeness.
5. Complete sample chain of custody forms for SLO County Lab and analytical lab.
6. Contact lab to notify that samples have been collected.
7. Notify lab courier or bring samples to laboratory directly.
8. Conduct bacteria analysis for E. coli. Analyze with a 1:10 dilution and a 1:100 dilution. If the SLO County lab is available, they should conduct the analysis using the IDEXX method.

Pace
ANALYTICAL SERVICES 4100 Atlas Ct. – Bakersfield, CA 93308 – 661.327.4911 – Fax: 661.327.1918 – www.pacelabs.com

Chain of Custody Form

Page 1 of 1

Report To
Client: Morro Bay National Estuary Program
Attn: Ann Kitajima, Makenzie O'Connor
Street Address: 601 Embarcadero, Suite 11
City, State, Zip: Morro Bay, CA 93442
Phone: 805-772-3834 Fax:
Email: annk@mbnep.org; moconnor@mbnep.org
Work Order #:

Project #:
Project Name:
BID#
Sampler(s) Name Printed:

Analysis Requested

Sample #	Description	Date Sampled	Time Sampled	TPH-Gas	TPH-Diesel	Oil & Grease	Total Suspended Solids	Dissolved Lead	Dissolved Copper	Dissolved Zinc			Soil	Sludge	Drinking Water	Ground Water	Waste Water	Other	Notes
																		Water	

Comments:

Sample Matrix

Result Request **Surcharge
STD (10 Day) ☐ 5 Day** ☐ 4 Day**
☐ 3 Day** ☐ 2 Day** ☐ 1 Day**
Rush requests must be approved

Notes

Billing
☒ Same as above
Client:
Address:
City: State Zip
Attn:
P.O. #:

System # (Needed for CLIP)
GIS/Key ☐ Well Star ☐

EDF Required Geotracker ☐ Yes ☒ No Global ID

	Relinquished By	Date	Time	Received By	Date	Time
1.						
2.						
3.						

Pace Analytical Bakersfield does not accept samples containing radioactive material above background levels. Samples containing radioactive material must be disclosed prior to receipt. Any samples suspected of containing radioactive material above background levels will not be accepted and will be returned to client.

MBNEP Stormwater Sample Collection

Sample ID	Time Collected	Collected by	Bottle Type

Site Conditions: (please circle one)

Precipitation:	Mist	Drizzle	Rain	Downpour	
Water Clarity:	Clear	Cloudy	Murky	Muddy	Dark/Black
Wind:	Calm	Light	Medium	Strong	
Flow:	5 gal/min	10 gal/min	25 gal/min	50 gal/min	

Observations:

Oily Sheen?	Yes	No
Odor?	Yes	No
Floating Trash?	Yes	No
Bubbles/Foam Plume at outlet?	Yes	No
Dark Plume at outlet?	Yes	No

Other Observations and Notes:

APPENDIX T. CAL POLY HYDROCAT STANDARD OPERATING PROCEDURE

Cal Poly HydroCAT Standard Operating Procedure

All equipment will be factory calibrated by the manufacturer prior to deployment. Instruments will be powered by a lithium battery enclosed in a waterproof unit. These batteries will be powered by a solar panel and charge controller setup.

All batteries and the solar panel are inspected on an approximately monthly basis. The Hydrocat and battery will be connected to an Fondriest X2 data logger, which will provide power to the instrument and also log data. This unit also contains a cellular modem that will transmit the data in near real-time to Fondriest's online server (WQDatalive). These data are then pulled and backed up at Cal Poly and sent to CeNCOOS for their near real-time data portal. The instruments at both sites (BM1 and BS1) are mounted on a mooring setup and attached using clamps designed for the instruments.

All sensors are deployed with the correct pump orientation to alleviate bubbles in the conductivity cell (arrows facing up on the unit). At BM1 the instrument pole and mooring mount are pulled up manually using a pulley system. At BS1, the instruments can also be deployed using a pulley system. The instruments are cleaned and inspected on an approximately monthly basis at both sites, and each system's pumped volume is purged on these visits to remove any sediment build-up. At BM1, instruments are pulled up and cleaned using the pole mount and pulley system. Upon return to the water, the pumped volume is again purged to remove air bubbles. At BS1, this process is similar; however, at certain high tides, scuba divers are required for instrument access and cleaning (all divers are American Academy of Underwater Sciences certified). All of the instruments are stable and are not expected to drift during the approximately annual deployment period. After approximately a year, the instruments are pulled and sent into the manufacturer (Sea-Bird) for inspection and calibration.

Datastreams are available on the California Ocean Observing Systems Data Portal (https://data.caloos.org/?&sensor_version=v2#metadata/45/sensor_source) in near real-time and available for download on ERDDAP. The Data Portal uses an automated quality assurance and quality control based on the Quality Assurance for Real Time Oceanographic Data (QARTOD) standards. For each site, a series of tests are applied for the parameters measured including a gross range test, a flat line test, a rate of change test, and a spike test.

Additional details can be found at the Data Portal site for BM1 and BS1, as well as the Integrated Ocean Observing System's Quality Control Github page: https://github.com/ioos/ioos_qc

APPENDIX U. CAL POLY SEABIRD SEAFET PH SENSOR DEPLOYMENT AND CALIBRATION PROTOCOLS

Cal Poly SeaBird SeaFET pH Sensor Deployment and Calibration Protocols

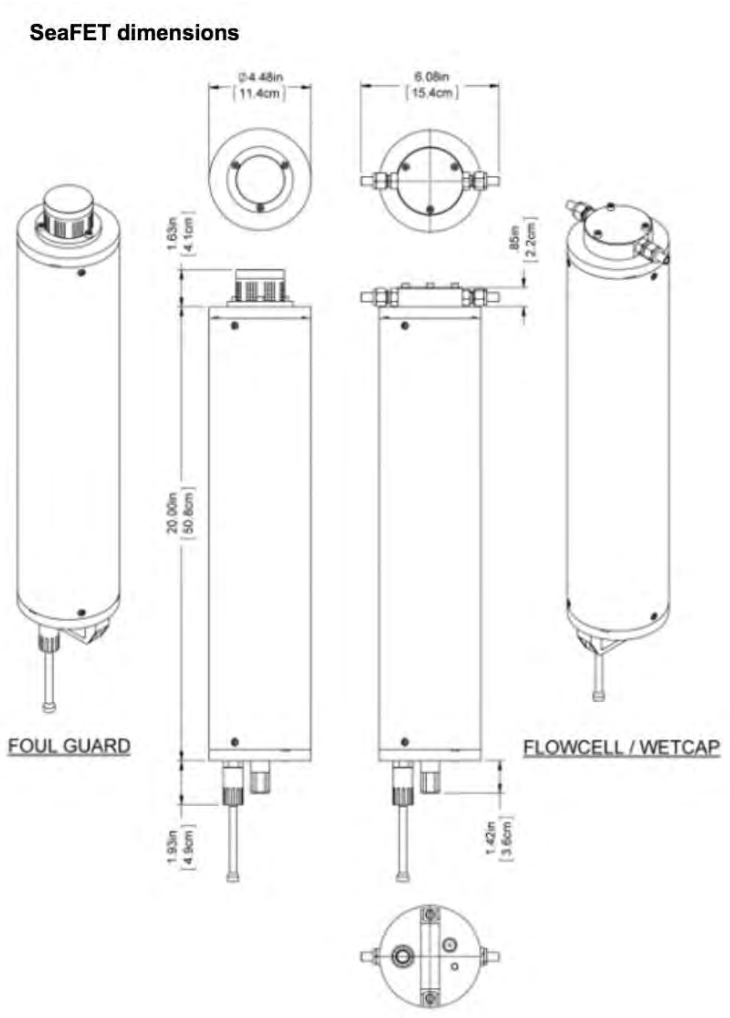
Maintenance, calibration, deployment, and recovery can only be performed in direct consultation with Emily Bockmon.

*Much of the information included in this SOP is collected and synthesized from the SeaFET User Manual and Best Practices Guide.

Instrument Specifications

The SeaFET V2 is a commercial oceanographic pH sensor available from Sea-Bird Scientific. It uses an ion sensitive field effect transistor to sense pH in marine environments. Two reference electrodes incorporated within the SeaFET allow for extra verification of the data validity. The internal reference is an electrode in a saturated KCl solution which is incorporated within the DuraFET® sensor. The external reference is an Ag/AgCl reference electrode that responds to the seawater pH and salinity directly. In addition to pH the DuraFET sensor also measures and records temperature.

Diagram of the instrument (per User Manual):



According to the manufacturer (Sea-Bird User Manual) the SeaFET has the following specifications:

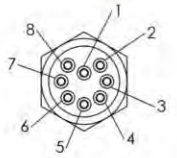
- Rated depth: 50m
- Temperature range: 0-50 ˚C
- pH measurement range: 6.5 – 9.0
- pH accuracy +/- 0.05 pH units
- pH precision: <0.001 pH units
- pH Resolution: 0.0001 pH units
- pH Stability: 0.005 pH units per month

Communication and Power

The SeaFET communicates using a bulkhead connection with pinouts as listed in Table 1.1.1 of the User Manual. For independent deployment, the SeaFET is is connected to a computer using RS232 protocol through a USB convertor for communication and data transfer. For cabled deployment, real-time communication is done through a SeaBird StorX cable connected to the bulkhead.

- Examine bulkhead connectors before every deployment.
- If needed, clean with isopropyl alcohol and then blow dry with air
- Apply a small quantity of 3M Spray Silicone Lubricant to the pin end of the connector. Let it dry.

1.1.1 Bulkhead connector

Contact	Function	MCBH-8-MP
1	Voltage in	
2	Power supply return-signal ground	
3	USB V in	
4	CTD/pump V in (12 V, 650 mA, optional)	
5	TXD/D+	
6	RXD/D-	
7	CTD TXD (optional)	
8	CTD RXD (optional)	

The SeaFET operates powered by 12 D batteries. It can also be deployed with an external power source (e.g., through a StorX cable for communication relay), but the internal batteries must be installed so that the sensing element has uninterrupted power.

- Change the 12 D cell batteries prior to each deployment.
  - Ensure the wet cap is attached to the sensors
  - Remove the vent plug
    - Put the sensor on its side on a flat surface.
    - Put the 8/32" thumb screw into the center thread of the vent plug.
    - Pull on the thumb screw until it does not come out any further.
  - Use a 5/32" hex wrench to remove the three 10-31 x 3/8" screws from the battery end flange.
  - Insert a flat head screwdriver into the slot between the pressure housing and the end flange.
  - Loosen the end flange with the screwdriver until the end flange can be pulled from the pressure housing with one hand.
    A safety line attaches the end flange to the battery plate to keep the battery connector wires attached.
  - Press the locking tab on the white end flange connector to disconnect and gently pull it out.
  - Remove the desiccant packs and put them in a sealed bag.
  - Remove the two 1⁄4" nuts that hold the battery plate.
    - Loosen evenly so that battery plate does not get stuck
  - Remove and replace batteries
    - Do not mix used and new batteries
    - Use only industrial 1.5V alkaline D-cell batteries
  - Examine, clean, and if necessary, lubricate the O-rings on the end flange.
  - Examine and clean the surface on the inside of the battery compartment where the O-rings sit.

- Replace the desiccant packs.
- Put the battery plate into position and tighten the two nuts equally until the battery plate is flush with the white connector.
- Connect the battery connector. Make sure that it "locks" in the receptacle.
- Install the end flange. Make sure that the wiring is not pinched.
- Use a hex wrench to install the three 10-31 x 3/8" screws.
- Fully insert the vent plug.
- Connect the sensor to the software and turn on the internal batteries.
    - The voltages in the sensor *Dashboard* are 12V and 6V. If they are not, open the sensor and correct the problem.

- Remove batteries if storing the sensor for an extended time

Storage between deployments

Both the SeaFET sensor and the external reference electrode should be kept wet at all times (dry for a maximum of ten minutes). Allowing the sensors to dry can cause leaking of KCl gel, rapid sensor drift, and other damage.

- Clean the sensors using high-purity isopropyl alcohol (90% or higher) and lint- free wipes and swabs.
    - First rinse thoroughly to remove any grit that may scratch the ISFET
    - Polish the external reference electrode using the supplied polishing film.
- Use a dilute hydrochloric acid solution to remove mineral deposits.
- Fill the flow cell with filtered natural seawater and seal with plastic plugs. Do not use artificial seawater.

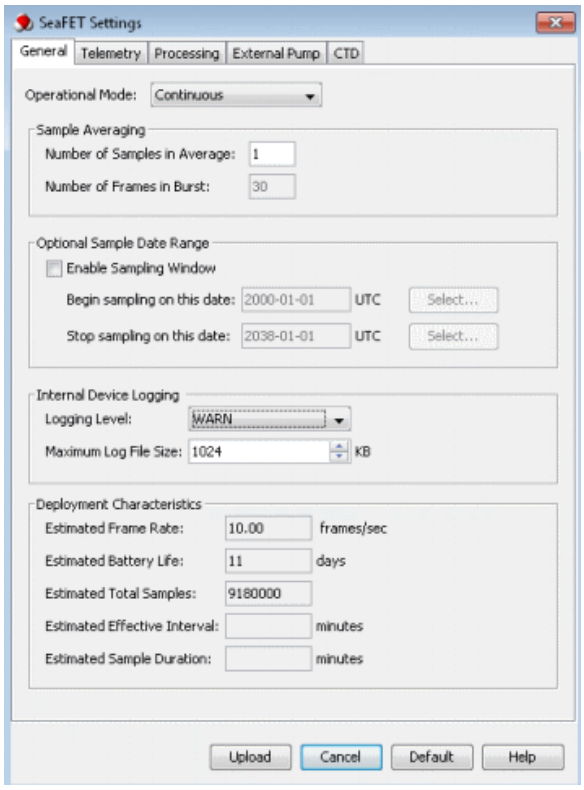
Deployment and Recovery

Preparation
To prepare the sensor for deployment, the following steps must occur:

1. Make sure that the batteries are installed and turned on.
2. Make sure that the sensor is connected to the software and PC and has power.
3. Set the sensor internal clock.
    a. Start the software.
    b. Select Sensor, *Set Clock* from the main menu.
    c. Push Synch Time to align the sensor clock with the clock of the PC.
    d. Note that the sensor clock is always set to Coordinated Universal Time (UTC). To change this, go to the *Preferences* menu, then *Display*, and remove the check in the box next to "Use UTC Time."
4. Select the Sensor menu, then *Settings* and change the settings in the General tab.
    a. Set periodic mode. The sensor will collect data at a user-specified sample interval
        i. "Sample Interval": Enter the interval between data collection events: 15 minutes
        ii. Sample Averaging
            1. "Number of Samples in Average:" Enter the number of pH measurements that are averaged into a data frame: 10
            2. "Number of Frames in Burst:" Enter the number of data frames in a "burst" of data frames.
            3. "Enable Sampling Window"

a. Put a check in the box to specify when data collection starts and stops.
b. Set Sample Date Range

5. Internal Device Logging
    a. Choose the INFO level of recording, keeping a log (file) of all high-level operations about its performance.
    b. These files are saved as MESSAGES.LOG until they are at the maximum size specified in the "Maximum Log File Size" box. The first file is backed up as MSG_OLD.LOG and MESSAGES.LOG is overwritten.
6. Deployment Characteristics
    a. "Estimated Frame Rate:" The frame rate, the data that is output from the sensor per second. Frame rate is inversely proportional to the "number of samples in average."
    b. "Estimated Battery Life:" Related to the nominal battery capacity, the number of data collection events and the duration of each event.
    c. "Estimated Total Samples:" Related to the frame rate, the number of data collection events, and the duration of each event.
    d. "Estimated Effective Interval:" Periodic mode only. Related to the "Sample Interval" setting except when the "Estimated Sample Duration" is longer than the specified "Sample Interval."
    e. "Estimated Sample Duration:" Periodic mode only. Related to the "Number of Samples in Average" and the "Number of Frames in Burst" settings.



4. Make a summary report recording status of the sensor
    1. Select Sensor at the main menu, then *Summary Report*.
    2. Enter the "Operator Name" and any "Summary Report Comments."
    3. Put a check in the "View Report" checkbox to see the report in the default .pdf

software on the PC.
    4. To change the directory in which the summary is saved, push Browse.
6. Deploy the instrument and disconnect the communication cable.
7. Remove the wet cap that the sensor is stored with.
    1. Unplug the cable from the sensor.
    2. Remove the two stainless steel plugs from the wet cap. Keep them for future use.
    3. Put the sensor over a sink or bucket and empty the solution of artificial seawater from the wet cap.
    4. Put the sensor upright again.
    5. Use a 5/32" hex key to remove the three 10-32 x 5/8" socket head cap screws from the wet cap. Keep the screws to use with the anti-fouling guard.
    6. Remove the wet cap.
    7. Use a clean dry cloth to clean the sensor and the wet cap of any artificial seawater.
    8. Make sure to keep the wet cap, O-rings and stainless-steel plugs for future use.
8. Install the anti-fouling guard and connect the dummy plug.
    1. Put the sensor on a flat surface with the external reference electrode end flange face up.
    2. Put the anti-fouling guard over the ISFET and external reference electrode.
    3. Use a 5/32" hex key to install the three 10-32 x 5/8" socket head cap screws that secured the wet cap.
        1. Make sure to tighten completely.
    4. Disconnect the test cable from the sensor and attach the dummy plug and lock collar onto the connector.

Placement of SeaFET

Exact placement and positioning of the sensor is dependent on location.
Planned deployments include:

Morro Bay T Pier	35°22'10.1"N	120°51'22.2"W
Bay South Station	35°19'58.2"N	120°50'56.0"W

Alternate location:

State Park Marina	35°20'45.4"N	120°50'35.9"W
Grassy Bar Oyster Co	35°20'26.6"N	120°50'56.7"W

- When submerging the SeaFET, deploy vertically so that all bubbles escape.
- Then, invert the sensor to deploy facing downwards.
    - The ISFET is sensitive to light – limit exposure when possible.
- Secure SeaFET to piling using large hose clamps

Mid-deployment checks and cleaning

Sensor will be returned to quarterly for:

- Collection of discrete samples for calibration
- Biofouling check and cleaning
- Instrument status verification

- Apply a magnet to the indicator and evaluate per the table:

No Flash: **SeaFET V2 batteries/memory are not ready for deployment**	**Red Flash:** **SeaFET V2 has not received a sampling command**	**Green Flash:** **SeaFET V2 is sampling or waiting to begin sampling**
The LED will not flash if any one of the following conditions are true: • The RTC battery is not sufficient (below 2.5 V) • The isolated battery is not sufficient (below 4.0 V) • The main battery or external power supply is not sufficient (below 7.0 V) • The memory is full	The SeaFET V2 batteries and memory are ready to deploy, but the instrument has not received a sampling command. Connect the SeaFET to a computer and click on "Start" in UCI, or send "**startnow**" or "**startlater**" before deploying.	The SeaFET V2 batteries and memory are ready to deploy, and the SeaFET V2 has received the "**startnow**" or "**startlater**" command. It is either sampling or waiting to begin sampling at the time predetermined in UCI or with the "**startdatetime=mmddyyyyhhmmss**" command.

Recovery

Upon completion of a deployment prepare for instrument recovery:

- Divers will collect final discrete check sample next to the sensor
- Loosen hose clamps to free SeaFET from piling or post
- Return to surface
- Remove anti-biofouling cap and replace with wet cap filled with seawater
- Return sensor to lab for cleaning and data download.

Instrument Calibration

SeaFETs are exceptionally stable instruments, but need calibration to produce accurate data. This is accomplished through collection of discrete samples alongside the sensor throughout deployment. These data will allow for calibration as well as an estimate of error associated with the data.

Three separate calibrations will be performed.

1. Sensor will be returned to manufacturer for calibration (and maintenance) annually
    a. Gel cap will be replaced
    b. External reference electrode is replaced (as needed)
2. Verify calibration in laboratory prior to deployment
    a. Fill cap with sample
    b. Record data until stable
    c. Compare to sample analyzed on spectrophotometric pH instrument
    d. To save new coefficients to the sensor, go to Menu, Sensor, Advanced, Upload Coefficients.
3. In situ discrete sample calibration

Upon deployment the sensor can be slow to equilibrate. Thus, in situ calibration during deployment, using a discrete seawater sample analyzed in the laboratory is essential.

- Discrete sampling will be completed monthly at a minimum, or whenever the sensor

is cleaned.
- An additional sample will be taken directly before instrument recovery.

Discrete sample collection

Equipment – this kit is stored in Dr. Bockmon's lab at Cal Poly. Building 52-D20.

- Niskin
- Yellow Rope
- Bottom Weight
- Trigger Weight
- Notebook & Pen
- Vinyl Tubing
- Headspace syringe
- Grease
- Kim-Wipes
- Black Bands
- White Clips
- Mercury solution
- Mercury pipet & tips
- Safety Goggles
- Gloves
- Sharpie

Procedure

Niskin Deployment from Dock or Boat:

1. Attach Bottom weight to rope and make sure rope is secured to Niskin
2. Cock open both ends of Niskin
3. Pull out nipple to its closed position & tighten top valve
4. Lower Niskin into ocean (1-2 meters) and wait 1 minute for the water to flush
5. Gently deploy trigger weight to close Niskin
6. Record exact time of sample.
7. Pull back up to dock or boat to transfer to bottle.

Alternative Niskin Deployment by Diving:

1. Remove rope and bottom weight from Niskin
2. Cock open both ends of Niskin
3. Pull out nipple to its closed position & tighten top valve
4. Driver submerges, holding Niskin as close to sensor as possible, wait 1 minute for the water to flush
5. Deploys trigger by hand to close Niskin.
6. Record exact time of sample.
7. Surface for transfer to bottle.

Niskin to Sample Bottle:

1. Label the sample bottle with sharpie: YYMMDD Location SampleID
2. Attach Vinyl Tubing to nipple and put the other end into the sample bottle
3. Push in nipple; no water should flow.
4. Slowly open (rotate) the top valve on Niskin to allow water flow

5. As the water flows through the tubing, squeeze & move the tubing to remove all bubbles
6. Rinse: Allow a small amount of water sample into bottle, tighten valve to stop flow, rinse bottle, and pour water out.
7. Start flow into sample bottle again taking care to avoid bubbles.
8. Allow water to gently fill the bottle, and then overflow at least half the sample volume.

Preparing Bottle for Storage/Transport:

1. Put Sample Bottle into secure holder
2. Use syringe to remove water to create headspace
3. Use Kimwipe to dry the inside of the bottle neck
4. Add 120 micro liters of 100% saturated Mercury II Chloride to sample via pipet.
    a. Or, add 240 micro liters of 50% saturated Mercury II Chloride to sample via pipet.
5. Grease glass stopper, push it into bottle, then rotate until tight to securely seal bottle
6. Pull down black band and connect white clip around bottle neck
7. Invert bottle several times to fix thoroughly

Bottle may be stored in the laboratory for up to 6 months before analysis.

Discrete sample analysis

Equipment

- Custom Dissolved Inorganic Carbon instrument using a Licor 7000 NDIR analyzer
- Custom Total Alkalinity titrator built according to Dickson et al. 2003
- Custom automated spectrophotometric pH instrument using *m*-cresol purple indicator dye.

Procedure

Sample analysis for each bottle starts on pH:

1. Lamps must be on for 45 minutes before they are stable
2. Warm-up instrument and confirm reproducibility (precision).
    a. Minimum 3-4 replicate junk samples
    b. Do not start sample analysis until DIC instrument is warm and ready
3. Run a Standard Reference Material (SRM) to check instrument accuracy and provide value for instrument calibration.
    a. Visually inspect these data before continuing.
    b. SRMs should be run every ~10 samples, or when concerned about the instrument.
4. Check seal on bottle for salt and cracks in grease before opening
5. Analyze samples in duplicate at a minimum
    a. Run a third if values are not reproducible or if you suspect instrument problems.
6. Mark date & time opened on bottle.
7. Re-insert stopper.

Pass bottle to DIC for immediate analysis:

DIC provides a backup value and a second parameter to characterize the seawater carbonate system.

1. Warm-up instrument and confirm reproducibility (precision).
    a. Minimum 3-4 replicate junk samples
2. Run a Standard Reference Material (SRM) to check instrument accuracy and provide value for instrument calibration.
    a. Visually inspect these data before continuing.

b. SRMs should be run every ~10 samples, or when concerned about the instrument.

3. Analyze samples in duplicate at a minimum
    a. Run a third if values are not reproducible or if you suspect instrument problems.
4. Insert stopper and band & clip.

Perform Alkalinity analysis within 48 hours:

Alkalinity is a conservative parameter not impacted by gas exchange.

1. Warm-up instrument and confirm reproducibility (precision).
    a. Minimum 3-4 replicate junk samples
2. Run a SRM to check for instrument accuracy.
    a. Visually inspect these data before continuing.
    b. SRMs should be run every ~10 samples, or when concerned about the instrument.
3. Begin sample analysis.
    a. If system is running well (both the VSD and fit are good) samples can be run a single time.
    b. Otherwise run duplicates for value confirmation.
4. When finished with a sample, pouring remaining sample into Junk Seawater Carboy.

Discrete data processing and calibration

- Instrument data is processed according to best practices and calibrated using reference materials. The pH data is also corrected for dye purity and dye perturbation. These finalized pH values are used for calibration.
- After recovery of the instrument and downloading the data, the data will be adjusted multiplicatively or by offset according to a single calibration point from a discrete bottle pH. See Kapsenburg et al. 2017.Additionally, data can be further temperature and salinity corrected if data is available.

Quality Control and Data Management

Discrete pH samples are also used to assess error; establishing an error envelope for the sensor time-series calculated as a function of the discrete pH error. This error will be reported with the dataset.

Error analysis should be performed, per Bresnahan et al 2015, by creation and analysis of the following plots:

a. Time-series anomaly plots to identify periods of sensor conditioning, drift, fouling, and failure.
b. Property-property plots to examine agreement between the sensor pH and independent reference pH values from bottles or other calculated means (e.g. O2).

For each deployment, once satisfied that the data is robust and well-calibrated, it will be finalized and a report created. The report will include:

- Deployment details including location, dates, and depth.
- Calibration procedures applied to the dataset
- Estimates of accuracy and precision
- Plots of the pH (total pH scale) and temperature vs date & time

The data will be archived as a csv file (easily accessible both by Excel and MATLAB) and stored along with the report in the Cal Poly One Drive. Additionally the raw data, plots, and report will be sent to the NEP and eventually published on the NEP website.

References

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APPENDIX V. CAL POLY SHORELINE NUTRIENT MONITORING PROTOCOL

Cal Poly Shoreline Nutrient Monitoring Protocol

Water Quality Measurements

The sensor is a SeaBird 19plus from SeaBird Scientific. It measure conductivity, water temperature, and water depth (using pressure) and is thus known as a CTD sensor.

Prior to use, the CTD is sent out for servicing and calibration with the manufacturer to ensure that it is ready for deployment.

To collect data, the CTD is lowered and raised in the water using a davit pole and capstan setup. The sensor is lowered and raised through the water column at a rate of ~1 m/sec. The CTD will equilibrate at the surface for at least 1 minute prior to lowering, per recommended practices.

Upon completion of data collection, the CTD will be properly rinsed and flushed prior to storage, per manufacturer recommendations.

Sample Collection

Equipment – this kit is stored in Dr. Bockmon's lab at Cal Poly. Building 52-D20.

- Niskin sampling bottle with trigger weight
- Rope and bottom Weight
- Notebook & Pen
- Sharpie
- 50 mL Falcon tube, plastic

Procedure

Niskin Deployment:

1. Cock open both ends of Niskin.
2. Pull out nipple to its closed position & tighten top valve.
3. Lower Niskin to desired depth and wait 1 minute for the water to flush.
4. Deploy trigger weight to close Niskin.
5. Record exact time of sample.
6. Pull back up to dock or boat to transfer to bottle.

Transfer to Sample Bottle:

1. Label the Falcon tube and cap with sharpie: YYMMDD Location SampleID. (Note: Do not use tape as labels as they tend to fall off when shipped with dry ice.)
2. Push in nipple and open (rotate) the top valve on Niskin to allow water flow.
3. Rinse the Falcon tube and empty.
4. Fill Falcon tube. Leave 10 ml headspace to allow for expansion upon freezing.
5. Store in cooler on ice.

Sample Storage:

1. Upon return to the lab, filter samples using 0.4 um polycarbonate filters.
2. Freeze sample as soon as possible.
3. Bottle may be stored in the laboratory freezer for up to 6 months before analysis.

APPENDIX W. MORRO BAY NATIONAL ESTUARY PROGRAM FRESHWATER SEEPS MONITORING

Morro Bay National Estuary Program
Freshwater Seeps Monitoring for Nitrates

Overview:
To assess the impact of the Los Osos Water Reclamation Facility project, monitoring of freshwater seeps along the bay shoreline was begun in the spring of 2014. Sites are monitored on a bi-monthly basis for nitrates as N, with analysis conducted by Fruit Growers laboratory.

Sites:
All sites are located along the shoreline in Los Osos. Access is from Third Street at the ramp south of the intersection with El Morro Avenue.

- NPS: North of Pump Station
- SOD: South of Dock
- PSS: Third Street/Pismo Seep
- NPW: North of Pismo Wetland
- SSO: Sweet Springs Outlet

Scheduling:
Monitoring should be scheduled for a low tide to allow access to the shoreline sites. The tide height should be less than 2'.

Fruit Growers can accept nutrient samples at their office in San Luis Obispo Monday through Thursday. Sampling cannot take place on a Friday because the lab cannot process samples on a Saturday. The samples have a 48-hour hold time.

Equipment:

- Knee boots
- Cooler and ice packs
- 8 oz. plastic lab bottles
- Sharpie
- YSI 2030 or YSI ProQuatro meter (calibrated for marine environments)
- DI water

Protocol:

- At each site, measure specific conductance and salinity.
- Label the bottle.
- Collect a sample, ensuring that it is free of sediment.
- Store in cooler.

At the office, complete a chain of custody form for the lab. Fill out the date and time of sample collection. Sample is picked up by a courier for delivery to the lab.

APPENDIX X. MORRO BAY NATIONAL ESTUARY PROGRAM TOXICITY MONITORING PROTOCOL

Morro Bay National Estuary Program
Toxicity Monitoring Protocol

Toxicity monitoring occurs at four locations in the Morro Bay watershed. Three sites are located in the Chorro Creek subwatershed (310CAN, 310SLU, 310ACR), and one site is located in the Los Osos Creek subwatershed (310TUR). Water toxicity is assessed twice per year, including one "wet" sampling event and one "dry" sampling event. Sediment toxicity is assessed once per year during the dry season.

Sampling Coordination and Hand Off

Sample collection must be coordinated with the UC Davis Granite Canyon (UCD-GC) laboratory **approximately one month in advance** of the prospective sampling date (minimum of two weeks prior for sediment and three weeks prior for water).

- Bushra Kahn, bhkhan@ucdavis.edu
- Randi Barton, rlbarton@ucdavis.edu
- Bryn Phillips, bmphillips@ucdavis.edu

When possible, samples should be physically handed off to UCD-GC at a central location to avoid shipping costs and uncertainty. If overnight shipping samples, ensure shipping logistics comply with the 48-hour hold time. A courier can be requested from UCD-GC approximately one month in advance. Historically, we've met up around 11:15am at the Starbucks in Paso Robles (1205 24th Street) since it's immediately off the freeway.

For dry season sampling (sediment/water), **two trained sampling teams are recommended** in order to meet the courier in Paso Robles by 11:15am. It will take each team approximately three hours for two sites (one team samples TUR/CAN, and the other at ACR/SLU). For wet season sampling (water only), two teams are still recommended but can also be completed by one team if needed.

Water Toxicity (wet/dry season)

Equipment:

- Two 4L amber glass bottle per site (provided by UCD-GC)
- One clean 1L glass/HDPE bottle per site (optional, must be requested from UCD-GC)
- One pair of clean gloves per site
- SPoT datasheet (unique datasheet for each site)
- Pencil and Sharpie
- YSI ProQuatro or EXO3
- Extra-large cooler (provided by UCD-GC)
- Ice packs for cooler

Procedure:

1. Survey the sampling before stepping into the stream. Find a spot in the centroid of the stream that is deep enough to submerge the toxicity bottle.

2. Label the 4L bottles with the site code (eg. “310SLU”). On the datasheet, record the sample time, personnel names, YSI/EXO equipment ID, field observations, and coordinates (list as upstream, and NR for downstream).
3. Use the standard subsurface grab sample collection method for water samples (sampling depth = 0.1m). Fill the two 4L amber glass bottles per site for a total of 8L.
    a. If there is no sampling point deep enough to submerge the 4L toxicity bottle, a clean 1L glass or HDPE bottle can be used. Triple-rinse the bottle with creek water before collecting the subsamples.
    b. If the stream is not deep enough to submerge any bottle, then comments should be made on the field data sheets that surface water was collected (sampling depth = 0m).
4. All samples should be put on ice and cooled to 4°C.
5. Fill out the datasheet using YSI measurements and observations.

Sediment Toxicity (dry season only)

Before sampling, sediment grain size should be checked in the field. The goal is to collect **recently deposited fine sediment only**. Avoid hard clay, bank deposits, gravel, disturbed and/or filled areas. Ideally, only fine sediments of less than 64 um in diameter should be collected.

The target is for fine-grained sediments to make up more than 50% of the sample (>50% fines). Sediment that feels smooth when rubbed between gloved fingers is preferred, and sediment that feels gritty should be rejected unless finer sediment is unavailable in depositional areas. Avoid periphyton (mixture of algae, microbes, and detritus) that may accumulate on the surface of sediment deposits. This will feel more slimy than smooth when rubbed between gloved fingers.

If no suitable depositional areas can be found, sampling personnel should not collect the sediment sample and should discuss alternative sampling locations and/or approaches. Sites that are routinely difficult to collect should be considered for elimination or relocation.

Equipment:

- One 4L glass compositing/homogenizing container per site
- Plastic bags (large enough for 4L container)
- One pair of clean gloves per site
- SPoT datasheet (unique datasheet for each site)
- Pencil and Sharpie
- YSI ProQuatro or EXO3
- Extra large cooler (provided by UCD-GC)
- Ice packs for cooler

Optional Equipment:

- One 4oz glass jars per site
- One pre-cleaned polyethylene scoop per site (in plastic bag)
- One pre-cleaned core per site (in plastic bag)

Procedure:

1. Survey the sampling site before stepping into the stream. Choose a sampling site with low hydrologic energy, such as the inner depositional side of bends or eddies where the water movement may be slower. Woody debris and vegetation often create low hydrologic energy areas with ample fine sediment, especially when found along banks.
2. Label the 4L jar with the site code (eg. "310SLU"). On the datasheet, record the sample time, personnel names, YSI/EXO equipment ID, field observations, and downstream coordinates.
3. Carefully enter the stream and start sampling at the closest appropriate reach, then continue sampling UPSTREAM. As you approach the sample location, avoid disturbing any possible sediment collection sub-sites nearby. Do not advance downstream, as this could lead to sampling disturbed sediment.
4. Using a gloved hand, retrieve a small amount of recently deposited fine sediment, aiming only for **the top 1-2 cm**. Inspect the sediment making sure it is a consistent grain size throughout the sample. If it is, add it to a 4L glass compositing/homogenizing container. Typically, at least five or more fine-sediment sub-sites are sampled into the composite jar within a 100m reach.
    a. If using a clean polyethylene scoop, scoop the top 1-2 cm of sediment from the sampling location, then add it to a homogenizing jar. Stir (do not shake) collected sediment with a polyethylene scoop for at least 5 minutes, making sure sediment is completely homogenized. Quickly scoop sediment out of the homogenizing jar into desired sampling jars making sure to stir the sediment in the homogenizing jar in between each addition.

    b. If using a core, push the tube vertically into the sediment to beyond the desired sample depth. Cap the bottom using a gloved hand or HDPE core cap to create suction. Pull the core out of the sediment and slowly decant any overlying water. Push the sediment out of the core, discarding all but the top 2cm. Place the remaining sediment into the collection jar.

5. As sampling is underway, draw a picture of the sample reach on the backside of the datasheet, showing the downstream/upstream ends and any significant features such as debris jams, riffles, pools, side channels, pipes, large woody debris, etc. On the drawing, label each sampling sub-site with a number. This number should correlate with the area # from the datasheet.
    a. On the datasheet, mark each sub-sample area # with the quality of overlying water (eg. underwater, damp, dry), presence of sample debris, depth of collection (1-2cm), and type of equipment used.
6. Continue sub-sampling at up to 10 locations over a 100m reach. At the end of the reach, collect downstream coordinates and record them on the datasheet.

7. Overfill the sampling jar to ensure that enough sediment has been collected (**at least 75% after sediment has settled**). Wipe off the jar and make sure all containers are capped tightly.
8. Single bag sediment sample containers to prevent cross contamination.
9. Stored in a cooler on ice in the dark at 4°C until delivery to the laboratory.

10. Fill out the datasheet using YSI measurements and observations. Make sure to record any field notes that are not listed on the provided data sheets. This information can be reported as comments with the sediment analytical results.

University of California, Davis - Marine Pollution Studies Laboratory
Chain of Custody

34500 Highway One
Monterey, CA 93940

Final Destination: MPSL
34500 Highway One
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Contact: Bushra Khan
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Project: Morro Bay 2026 (CitMon_MorroB_2026)
Morro Bay National Estuary Program
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Morro Bay, CA 93442

Contact: Morro Bay NEP
805-776-3778
annk@mbnep.org
moconnor@mbnep.org

Sample Name	Date Sample	Time Sampled	Analysis	Quantity
310CAN			Water Toxicity	8 L
310SLU			Water Toxicity	8 L
310TUR			Water Toxicity	8 L
310ACR			Water Toxicity	8 L

Signature and Print Name	Date/Time	Location

APPENDIX Y. MORRO BAY NATIONAL ESTUARY PROGRAM MASTER SITE LIST

Site Code	Site Description	Latitude	Longitude	Monthly Water Quality	Bacteria	Pressure Transducer	Dawn Patrol	Bi-monthly Nutrients	Agricultural Monitoring	Continuous Temp	Instantaneous Flow	Bioassessment	Stormwater	Seeps	Sediment Elevation	Stream Profiling	Toxicity	Continuous Bay Water Quality	Bay Macroalgae	Bay Nutrients	Recreational Use
310CCC	Chorro Creek upstream from Chorro Flats	35.35762	-120.812	X	X	X				X	X										
310CPN	Pennington Creek @ bridge	35.3453	-120.729	X	X	X				X	X										
310PEN	Pennington Creek @ Rancho El Chorro	35.33999	-120.736	X	X						X										
310DAM	Dairy Creek near dog park	35.33558	-120.728	X	X	X		X			X	X									
310SLU	San Luisito Creek @ Adobe Rd	35.35595	-120.786	X	X			X	X	X	X						X				
310TWB	Chorro Creek at South Bay Boulevard	35.35433	-120.828	X	X			X			X	X									
310UCR	Chorro Creek @ upper Chorro Creek Ecological Reserve	35.33862	-120.768	X	X			X		X	X										
310BAY	Morro Bay Shoreline at Baywood Pier	35.32678	-120.841		X																
310CIN	Morro Bay Shoreline at Cuesta Inlet	35.31986	-120.849		X																
310COL	Morro Bay Shoreline at Coleman Beach	35.3722	-120.86		X																
310PAS	Morro Bay Shoreline at Pasadena Point	35.32999	-120.844		X																
310SIN	Morro Bay Shoreline at Sharks Inlet	35.31805	-120.857		X																
310SPM	Morro Bay Shoreline at State Park Marina	35.34627	-120.843		X																
310TID	Morro Bay Shoreline at Tidelands Park	35.35982	-120.852		X																
310WIN	Morro Bay Shoreline at Windy Cove	35.34934	-120.844		X																
310GS1	Turri Rd. 0.35 mi from South Bay Blvd	35.33382	-120.819		X																
310APN	Pennington Creek upstream from horse corral	35.35371	-120.722	X							X										
310ATP	Morro Bay offshore at Tidelands Park	35.35738	-120.851				X														
310CAN	Chorro Creek at Canet Road	35.35315	-120.789						X								X				
310CHD	Chorro Creek below Chorro Creek Dam	35.3343	-120.689							X		X									
310CHI	Morro Bay offshore at North of Cuesta Inlet mouth	35.32375	-120.855				X														
310CLK	Los Osos Creek Clark Valley Road	35.28187	-120.79									X									
310CSI	Morro Bay offshore at Near Cuesta Inlet	35.32042	-120.85				X														

Site Code	Site Description	Latitude	Longitude	Monthly Water Quality	Bacteria	Pressure Transducer	Dawn Patrol	Bi-monthly Nutrients	Agricultural Monitoring	Continuous Temp	Instantaneous Flow	Bioassessment	Stormwater	Seeps	Sediment Elevation	Stream Profiling	Toxicity	Continuous Bay Water Quality	Bay Macroalgae	Bay Nutrients	Recreational Use
310DAL	Dairy Creek at bottom of El Chorro Regional Park	35.33039	-120.73																		
310DAU	Dairy Creek above Cattle Enclosure	35.34752	-120.715							X		X									
310LO2	Morro Bay offshore at Los Osos Creek outlet	35.336	-120.841				X														
310LSL	San Luisito Creek Lower	35.35961	-120.775									X									
310MNO	San Bernardo Creek Middle	35.37584	-120.791									X									
310MSL	San Luisito Creek Middle	35.36241	-120.757																		
310OSL	San Luisito Creek above upper site	35.37027	-120.739																		
310PSP	Morro Bay offshore at Pasadena Point	35.3337	-120.848				X														
310SHI	Morro Bay offshore at Sharks Inlet	35.3144	-120.866				X														
310SPO	Morro Bay offshore at State Park Marina	35.3468	-120.845				X														
310SYB	Los Osos Creek @ Turri Rd	35.33065	-120.817																		
310USL	San Luisito Creek Upper	35.36592	-120.751							X		X									
310UWR	Warden Creek 1.5 mi upstream from Turri Rd	35.29384	-120.753																		
310WLB	Walters Creek @ bridge	35.3466	-120.755																		
310WLF	Walters Creek @ flume	35.34811	-120.75																		
310WLM	Walters Creek downstream 0.2 mi from bridge	35.34578	-120.757																		
310WRP	Warden Creek @ Wetlands Restoration Preserve	35.3206	-120.806																		
310PFL	Pennington at the former Fish Ladder	35.34464	-120.73			X				X	X										
310SBE	San Bernardo Creek @ Adobe Rd	35.36174	-120.805																		
310UPN	Pennington Creek @ corrals	35.34798	-120.726					X		X	X	X									
310CER	Chorro Creek in the Ecological Reserve	35.3474	-120.773	X							X	X									
310COO	Coon Creek at Pecho Valley Road	35.25423	-120.885	X							X										
310LVR	Los Osos Creek at Los Osos Valley Rd.	35.30589	-120.812	X							X	X									
310CHO	Chorro Creek at Highway 1 bridge on Camp SLO	35.32197	-120.722	X				X		X	X										
310CLV	Los Osos Creek 1.0 mi downstream from 310CLK	35.28809	-120.802	X		X		X			X										
310TUR	Warden Creek @ Turi Road	35.30231	-120.776	X	X			X	X		X						X				

Site Code	Site Description	Latitude	Longitude	Monthly Water Quality	Bacteria	Pressure Transducer	Dawn Patrol	Bi-monthly Nutrients	Agricultural Monitoring	Continuous Temp	Instantaneous Flow	Bioassessment	Stormwater	Seeps	Sediment Elevation	Stream Profiling	Toxicity	Continuous Bay Water Quality	Bay Macroalgae	Bay Nutrients	Recreational Use
310ACR	Chorro downstream of CMC WWTP on CDFW property	35.32822	-120.758									X					X				
310UCD	Chorro Creek Above Reservoir	35.34552	-120.6828									X									
310UMP	Upper Middle Pennington	35.363384	-120.7119									X									
310UNP	Upper Northern Pennington	35.366615	-120.7133									X									
310SSO	Sweet Springs Outlet freshwater seep	35.32288	-120.841											X							
310NPW	North Pismo Wetland freshwater seep	35.32374	-120.841											X							
310PSS	Pismo freshwater seep	35.32418	-120.841											X							
310SOD	South of Dock freshwater seep	35.32494	-120.84											X							
310NPS	North Pump Station freshwater seep	35.3257	-120.84											X							
B High	Marsh	35.34717	-120.833												X						
C High	Marsh	35.34787	-120.829												X						
A High	Marsh	35.34585	-120.833												X						
B Low	Marsh	35.34115	-120.837												X						
A Low	Marsh	35.34183	-120.842												X						
C Low	Marsh	35.33852	-120.83												X						
CHN 01	Chorro Creek stream profiling site	35.35334	-120.829													X					
CN2	Chorro Creek stream profiling site	35.35223	-120.83													X					
CS2	Chorro Creek stream profiling site	35.35207	-120.829													X					
CU1	Chorro Creek stream profiling site	35.33379	-120.69													X					
CU2	Chorro Creek stream profiling site	35.33297	-120.691													X					
CU3	Chorro Creek stream profiling site	35.33149	-120.692													X					
CU4	Chorro Creek stream profiling site	35.33052	-120.694													X					
CU5	Chorro Creek stream profiling site	35.32909	-120.696													X					
DAM0	Dairy Creek stream profiling site	35.34027	-120.721													X					
DAM1	Dairy Creek stream profiling site	35.34026	-120.721													X					
DAM2	Dairy Creek stream profiling site	35.33792	-120.724													X					

Site Code	Site Description	Latitude	Longitude	Monthly Water Quality	Bacteria	Pressure Transducer	Dawn Patrol	Bi-monthly Nutrients	Agricultural Monitoring	Continuous Temp	Instantaneous Flow	Bioassessment	Stormwater	Seeps	Sediment Elevation	Stream Profiling	Toxicity	Continuous Bay Water Quality	Bay Macroalgae	Bay Nutrients	Recreational Use
PEN2	Pennington Creek stream profiling site	35.33727	-120.74													X					
PEN3	Pennington Creek stream profiling site	35.33637	-120.74													X					
PEN4	Pennington Creek stream profiling site	35.33615	-120.741													X					
PEN5	Pennington Creek stream profiling site	35.33483	-120.743													X					
XS1	Chorro Flats stream profiling site	35.36041	-120.818													X					
XS3	Chorro Flats stream profiling site	35.35899	-120.815													X					
XS4	Chorro Flats stream profiling site	35.35836	-120.814													X					
XS5	Chorro Flats stream profiling site	35.3581	-120.815													X					
XS6	Chorro Flats stream profiling site	35.36026	-120.821													X					
CS3	Chorro Flats stream profiling site	35.35196	-120.829													X					
NSS	North Sandspit, across channel	35.367285	-120.8569																X		
REF	Reference, across channel from Tidelands Park	35.358333	-120.8538																X		
WIN	Windy Cove	35.348648	-120.8442																X		
MAR	State Park Marina	35.345937	-120.8463																X		
OSB	Oyster Bed	35.340892	-120.8479																X		
PAS	Pasadena, across channel from Pasadena Point	35.333084	-120.8495																X		
S1	North T-Pier	35.370833	-120.85866																	X	
S2	South Embarcadero	35.359638	-120.85193																	X	
S3	Kayak Dock	35.346124	-120.84335																	X	
S4	Pasadena Point	35.329841	-120.84441																	X	
S5	Yellow Cottage	35.32144	-120.85080																	X	
S6	End of Butte Road	35.317983	-120.85708																	X	
BM1	Front bay CeNCOOS sensor	35.3708	-120.8589															X			
BS1	Back bay CeNCOOS sensor	35.3338	-120.8473															X			
COL	Recreational use monitoring at Coleman Beach	35.3725	-120.860																		X
TID	Recreational use monitoring at Tidelands Park	35.3600	-120.852																		X

Site Code	Site Description	Latitude	Longitude	Monthly Water Quality	Bacteria	Pressure Transducer	Dawn Patrol	Bi-monthly Nutrients	Agricultural Monitoring	Continuous Temp	Instantaneous Flow	Bioassessment	Stormwater	Seeps	Sediment Elevation	Stream Profiling	Toxicity	Continuous Bay Water Quality	Bay Macroalgae	Bay Nutrients	Recreational Use
TBL	Recreational use monitoring at Tidelands Boat Launch	35.35806	-120.851																		X
WIN	Recreational use monitoring at Windy Cove	35.34917	-120.843																		X
SPM	Recreational use monitoring at State Park Marina	35.34611	-120.842																		X
PAS	Recreational use monitoring at Pasadena Point	35.32972	-120.844																		X
BAY	Recreational use monitoring at Baywood Pier	35.32667	-120.841																		X
PCH	Recreational use monitoring at the end of Pecho Road	35.31889	-120.852																		X