SCCOOS Pier Sampling Program Protocol
Location: Cal Poly Center for Coastal Marine Sciences (CCMS) Pier in Avila Beach

FIELD SAMPLING AT CAL POLY PIER

Bottle Preparation and Labeling

1. Label a French square bottle with "Whole water", "CPYYMMDD", "10mL 37% formalin"
2. Label a 20mL glass vial with "Net Tow", "CPYYMMDD", "3mL 37% formalin"
3. Label 2 cryovials with "CPYYMMDD" and "DA#1" or "DA#2"
4. Label 2 cryovials with "CPYYMMDD" and "CL#1" or "CL#2"
5. Label a 20mL plastic scintillation vial with "CPYYMMDD" and "Nutrient"

Collecting Environmental Conditions and Contextual Data

1. Obtain sky, wind and water conditions from the live data online and record in the HAB database. Cpool1.marine.calpoly.edu/cpool
    a. Make sure to go into "CCMS Metstation" then "cpp met current.js" in order to get data that corresponds to the exact time the sample was collected.
    b. If conditions were not recorded immediately before or after sampling, the data is stored in the "MET DataLog" csv files. These files can be accessed at any time, but it is important to note that the time zone used is UTC (Coordinated Universal Time).
    c. To convert UTC to PST (Pacific Standard Time, Nov-Mar) subtract the UTC time by 8 hours (i.e., UTC files will be 8 hours ahead of PST). To convert UTC to PDT (Pacific Daylight Time, Mar-Nov) subtract the UTC time by 7 hours (i.e., UTC files will be 7 hours ahead of PDT).
2. Note the visibility of the ocean water.
3. Make note of any net discoloration, film, or smells (anything abnormal and just general observations) under "Net Tow Observations"

Sample Collection

1. Collect **surface water sample** (for quantitative phytoplankton counts, domoic acid concentration, and chlorophyll a concentration) in a bucket by lowering the bucket into the water, rinsing once before pulling the bucket up full. Record the local time and location sample was taken.
    a. Take the temperature of the bucket sample immediately using a Traceable Digital Dial Thermometer (CAT# 4344) and record in the notebook.
2. Collect depth-integrated water sample (for qualitative phytoplankton counts) from 6m using net tow with cod end attached (Figure 1). Lower the net down 6m (see tape mark on line) then raise net up out of the water and allow the water to filter through. Repeat 1 more time (2X total). Collect sample in net cod end.
3. Begin processing samples in the classroom at CCMS
    a. Fill two 1L Nalgene bottles with the bucket sample water. (Submerge the Nalgene bottles carefully in the sample water in order to fill them. This will keep cells from exploding).

Figure 1. Net and cod-end for qualitative sample

Chlorophyll Sampling

1. Chlorophyll sampling should be completed before other sampling procedures to minimize exposure to light.
2. Slowly invert one of the 1L Nalgene sample bottles several times to ensure a homogenous sample.
    a. Avoid long exposure of filtrate and filters to light or room temperature throughout processing
3. Place a 25mm GF/F Whatman filter (0.7 pore size) (CAT# 1825-025) over funnel base on top of an Erlenmeyer flask connected to a vacuum (Figure 3).
4. Use a graduated cylinder to measure out 100ml of unfiltered surface water sample.
5. Transfer the 100ml into the funnel. Ensure no leakage.
6. Turn on vacuum pump to no more than 5 mm of Hg. Allow sample to filter through.
    a. If there is too much biomass in the water sample, the filter will clog. Therefore, only filter 50 mL and record this deviation from the protocol.
7. Stop vacuum as soon as the water has passed through the filter to avoid damaging cells.
8. Take off the top of the funnel.
9. Fold filters in half onto themselves using forceps and place in a cryovial (CAT# 1050025). Immediately place in the -20C freezer.
10. Repeat this process once more to make a replicate filter.
11. Store samples in -80C freezer when on campus at Cal Poly until ready to process for fluorometry.

Domoic Acid (DA) Sampling

1. Slowly invert one of the 1L Nalgene sample bottles several times to ensure a homogenous sample.
    a. Avoid long exposure of filtrate and filters to light or room temperature throughout processing
2. Place a 25mm GF/F Whatman filter (0.7 µm pore size; CAT# 1825-025) over the funnel base on top of an Erlenmeyer flask connected to a vacuum (Figure 2).
3. Use a graduated cylinder to measure out 200ml of unfiltered surface water sample.
4. Transfer the 200ml into the funnel. Ensure no leakage.
5. Turn on vacuum pump to no more than 5 mm of Hg
6. If there is too much biomass in the water sample, the filter will clog. Therefore, only filter 100 mL and record this deviation from the protocol.
7. Stop vacuum as soon as the water has passed through the filter to avoid damaging cells.
8. Fold filters in half onto themselves using forceps and place in a cryovial. Immediately place in the -20C freezer.
9. Repeat this process once more to make a replicate filter.

Figure 2. Complete vacuum set up with funnel, Erlenmeyer flask, graduate cylinder and filters (left) and box of Whatman GF/F filters (right).

Nutrient Sampling

1. Rinse a 140ml In-line Syringe three times with sample water. Fill the syringe completely and remove the air bubbles
2. Place a 47mm Swinnex filter holder with a 47 mm Nucleopore PCTE filter (CAT# 930069) on the end of the syringe and push about 20ml from the unfiltered surface water sample through the syringe to wash the filter and discard this water (Figure 3).
3. Rinse a pre-labeled 20ml plastic scintillation vial 3X with filtered sample water from the syringe. Then fill the scintillation vial about **2/3 full** with the filtered sample (not above the neck). Place samples in cooler to bring back to Cal Poly. Store samples upright in -20C freezer. Freezing samples sideways can compromise the sample.

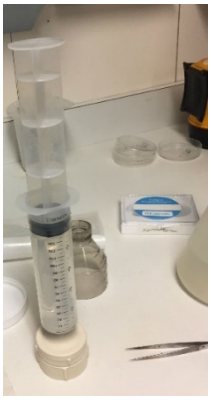

Figure 3. Syringe with filter attachment for nutrient sampling.

Fixing and Processing Whole Water Sample

1. Pipette 10mL of 37% formaldehyde (stabilized with 10-15% methanol) into a 125mL French square bottle
    a. Use gloves and goggles to pipette formaldehyde.
2. Slowly invert one of the 1L Nalgene sample bottles several times to ensure a homogenous sample.
3. Using a graduated cylinder, add 90ml of the unfiltered sample water into the French square bottle containing fixative (Figure 4).

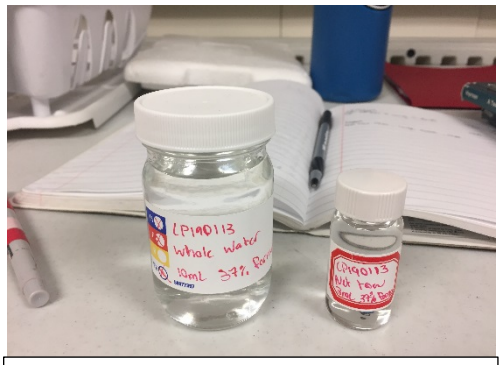

Figure 4. Fixed whole water sample (left) and net tow sample (right).

Fixing and Processing Depth-integrated Net Tow Sample

1. Pipette 3mL of 37% formaldehyde (stabilized with 10-15% methanol) into a 20mL glass vial.
    a. Use gloves and goggles to pipette formaldehyde.
2. Put away formaldehyde.
3. Slowly invert net tow sample cod end bottle several times to ensure homogenous sample. Pour small sample into small beaker to make pouring easier
4. Fill the glass vial containing fixative to the neck with unfiltered net tow sample water (~17mL) (Figure 4).

Qualitative Phytoplankton Analysis

1. Note that this is done at the pier within 30 minutes of collection.
2. Slowly invert net tow sample cod end bottle to ensure a homogenous mixture.
3. Using a Palmer slide with a cover slip, pipette 1ml of the unfiltered net tow water onto the slide.
4. Examine the slide at 100X under a light microscope (Figure 5). Scan the entire slide and record relative abundance of species present using the index below.

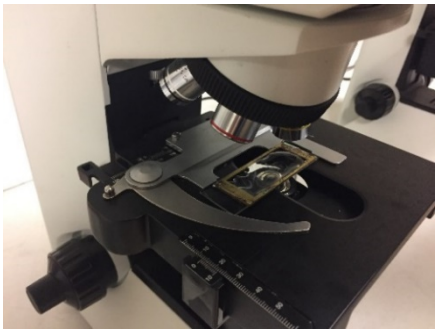

Figure 5. Light microscope with Palmer slide.

Relative Abundance Scale:

R (Rare): <1%
P (Present): 1-9%
C (Common): 10-24%
A (Abundant): 25-49%
D (Dominant): >50%

LABORATORY ANALYSIS AT CAL POLY

Quantitative Phytoplankton Analysis

Settling the Sample in Utermöhl Chambers:

Figure 6. Settling tray with labeled samples and 25 mL graduated cylinder.

1. Settle the sample as soon as possible upon return to the lab.
2. Obtain plastic slides and apply silicon grease around rim of sample area (Figure 6).
3. Attach slide to settling tube and screw into settling tray, making sure the seal is tight to prevent any leakage.
4. Wearing gloves, invert whole water sample several times, and measure out 25mL in graduated cylinder.
5. Add the 25mL to settling tube and let sit for 24 hours.
6. After 24 hours, wearing gloves, unscrew the settling tube, clean up any spillage, and add a coverslip to plastic slide. Make sure and label slide with the correct sample ID. Make sure to discard all formalin waste into labeled waste container.

Microscopic Analysis

1. Use settled slide for quantitative observations.
2. Count 10 fields of view at 100X total magnification (10X eyepiece and 10X optical) on inverted microscope (Figure 7) and record the species and number of phytoplankton cells observed (Note - alternative magnifications can be used for ID, but all counts should be done at 10X).
3. To get a better view of smaller cells, the pull tab on the right-hand side can be extended to increase magnification by 1.5. DO NOT conduct the quantitative counts with this tab extended. Push the tab back in before continuing counts.
    a. For dense samples, 5 fields of view may be counted. Record this in the notebook.
    b. For particularly abundant species, 5 fields may be counted and then the total multiplied by 2 at the end of the count. In order to get an accurate count of other, less abundant species, 10 fields of view may still be used to count them.
    c. Sizes of cells can be estimated for identification purposes using the ocular measurements below:

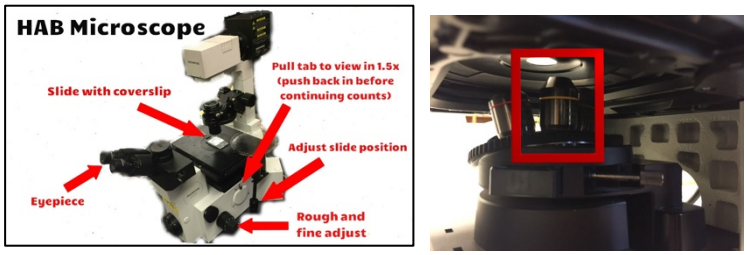

Figure 7. HABs microscope (left) and 10X Objective (right).

Ocular micrometer measurements:
@40x 1mm = 40 ticks, so 1 tick = 25 um

@100x 1mm = 100 ticks, so 1 tick = 10 um
@200x 1mm = 200 ticks, so 1 tick = 5 um
@400x 1mm = 400 ticks, so 1 tick = 2.5 um
@600x 1mm = 600 ticks, so 1 tick = 1.6 um

Chlorophyll Extraction and Fluorometric Analysis:

1. Put on goggles and gloves
2. Extract GF/F filter in 7 mL of 90% acetone in glass tubes and leave in fridge for 24 hours prior to analysis. Cover tubes with parafilm.
    a. It is important these samples are kept dark – therefore cover the rack with aluminum foil during the extraction processes.
3. All samples are analyzed on a 10-AU fluorometer (Figure 8). Turn on fluorometer and remove chlorophyll samples from fridge ½ hour before analysis.

Figure 8. 10-AU Flourometer.

4. Put on goggles and gloves
5. Run blank (7 mL of pure acetone)
    a. Wipe off tube with a Kimwipe prior to placing in fluorometer.
    b. Use the following sequence of buttons to complete the blank: ENT, 2:Calibration, 1:Blanking, 1:Run Blank.
6. Record blank value.
7. Measure the solid red standards from box (Figure 9) and record values on the calibration data sheet.
    a. Wait for reading to reach 8 secs.
    b. Use the following sequence of buttons to take the readings: ENT, 3:Diagnostic, ENT to next screen
    c. Record fluorescence readout and FS% for high and low standards.
    d. (NOTE - Reading should not drift more than 5% from calibration values)

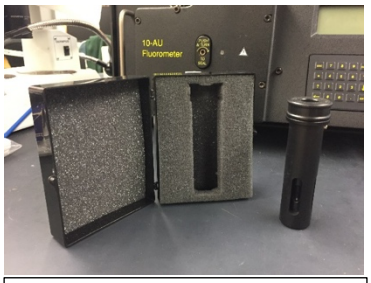

Figure 9. Rhodamine Standard (Cat # 10-AU-904)

8. For each sample do the following:
    a. Remove the filter from the tube and invert the extracted sample falcon tube to mix.
    b. Wipe off tube with kimwipe and place in the fluorometer.
    c. Wait for value to stabilize to 8 secs., press *, and then record the first value (this is the before acid value).
        i. If the value exceeds the detection limits of the fluorometer, perform a 1:5 dilution (1 mL of chlorophyll sample + 4 mL 90% acetone) and record.
    d. Add 150 µL of 0.1N HCl (no bubbles, cover with parafilm, invert 3 times, and place the tube back in the fluorometer. Wait 90 seconds and then take the second reading (this is the after acid value).
    e. Record stabilized value.
9. Dispose of samples in acetone waste.

Supply Information Table

Item	Vendor	Catalog Number
Thermometer	Traceable.com	666431
Glass fiber filter	Sigmaaldrich.com	987464
Nucleopore filters	Sigmaaldrich.com	930069
Cryo Vials	Fishersci.com	1050025
Falcon tubes	Corning.com	352096
5mL pipette tips	Eppendorf.com	224920890
Filter forceps	Emdmillipore.com	CP9AA4587
Glass bottles	Wheaton.com	W216924
Gloves	Fishersci.com	19-130-1597B
Counting cell	Sciencefirst.com	3-1801-G20