SCCWRP Harmful Algal Bloom Response Standard Operating Procedures

Sampling for chlorophyll and algal toxins using hand pump and filtration unit

Samples to obtain:

- Domoic acid filters (250 mL water filtered) for each filter x 2
- Chlorophyll-*a* filters (100 mL water filtered for each filter) x 2

Tutorial video:

https://www.youtube.com/watch?v=94uFFSWNcmE

Materials:

- Plastic bucket with line
- 1 L HDPE amber bottle
    - Or any bottle for collecting water sample
- Cooler and ice packs
- Graduated cylinders
    - 100 mL
    - 250 mL
- Hand pump with tubing
    - Thermo Scientific 6132-0010 (Nalgene™ Repairable Hand-Operated PVC Vacuum Pumps with Gauge 15 cc/stroke | Buy Online)
- Filtration unit with 47 mm filter holder
    - Thermo Scientific 300-4050 (Nalgene™ Reusable Filter Units 250 mL | Buy Online | Thermo Scientific™)
- 47 mm GF/F filters
    - Sterlitech F4700 (Grade F Borosilicate Glass Microfiber, 47mm, 100/Pk)
    - Cytiva 1825047 (Cytiva Whatman Binder-Free Glass Microfiber Filters GF/F Circles 47 mm | Buy Online | Cytiva | Fisher Scientific)
- Filter forceps
    - MilliporeSigma XX6200006P (MilliporeSigma Filter Forceps Beveled, Unserrated | Buy Online | MilliporeSigma™ | Fisher Scientific)
- 5 mL plastic tube for storing filters
- Sharpie & Labels
- Aluminum foil
- Clean freshwater for cleaning between stations

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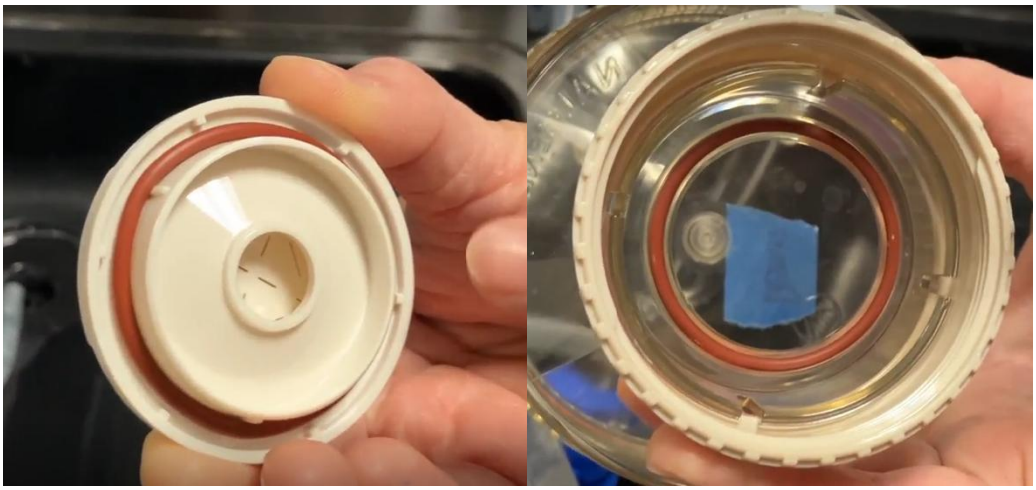
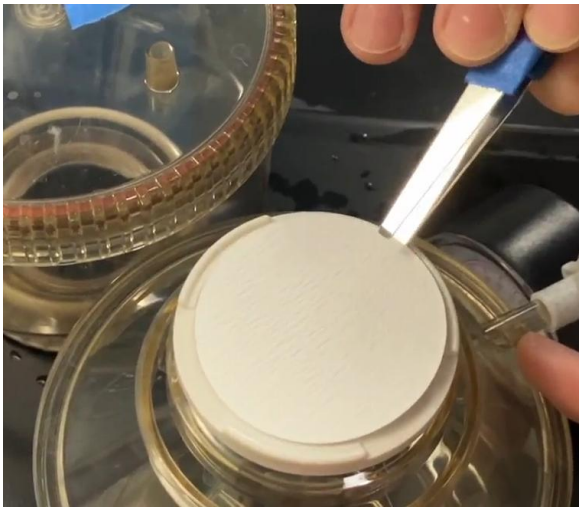
Procedures:

1. Collect sample from water surface using a bucket and line.
    a. Toss bucket into water pull up and swirl water around in bucket to rinse the bucket with sample water. Toss water back.
        i. Repeat two more times to rinse bucket a total of three times.
    b. Now that bucket is rinsed, toss again and collect a full bucket of water.
    c. Rinse out the sample collection bottle with collected sample water three times.
        i. Add a small amount of sample water (at least 20 mL) into the bottle.
        ii. Place the bottle lid back on and shake the sample water in the bottle vigorously for 10 seconds.
        iii. Repeat two more times.
    d. Fill bottle to the brim with sample water.
        i. Filling the bottle completely minimizes air bubbles that avoids sloshing and damage of cells during transport.
    e. Keep collected sample away from direct sunlight and cool (~4 °C) if transport is necessary.
    f. Record sampling info in field datasheet (included at the end of this SOP).
2. Set the filtration unit up
    a. Make sure one of the hose barb sidearms on the filtrate receiver (bottom part of the filtration unit) is sealed with a stopper.
    b. Connect the hand pump to the other hose barb sidearm with tubing adapter (or tubing directly if the adapter is not available).
    c. Remove the sample funnel (top part of the filtration unit) off by unscrewing if from the filtrate receiver.
    d. Make sure that there is an o-ring at the bottom part of the filter support and the bottom of the sample funnel.



    e. Place one GF/F filter onto the filter support. Align it so it lies flat against the support base/ridges of the filter support.

i. Using the filter forceps, grasp only the edge of the filter. Avoid touching the center of the filter where the sample water will pass through.



f. Place the sample funnel back on the filtrate receiver, and screw it back on tightly to ensure water will not leak through.

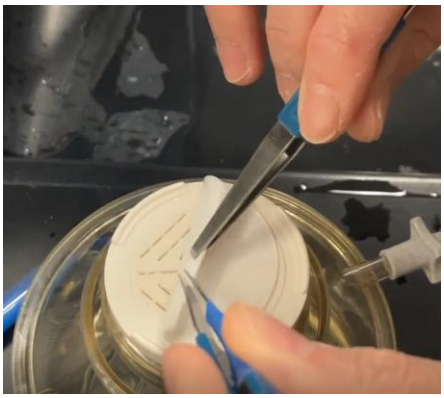
3. Prepare labelled tubes for storing filters in advance.
4. Place water sample into the sample funnel.
   a. Mix the water in the sample collection bottle gently by doing a gentle figure-8 rotation (again, to avoid damaging cells prior to filtration).

   b. Pour a small amount (10 – 20 mL) of sample water into the graduated cylinder to rinse it. Dump the rinse water.
   c. Measure out the required amount of sample water using the graduated cylinder.
      i. Ensure the bottom of the meniscus aligns with the desired volume mark at eye level.
   d. Pour the measured water gently into the filtration tower.
      i. Unscrew the covering lid on the sample funnel if necessary.
   e. Check that no water is leaking out the connection part between the sample funnel and the filtrate receiver.
5. Apply vacuum using the hand pump.
   a. As you pump, you'll notice that the gauge needle will rise. Avoid letting the pressure go beyond 15 in. Hg.

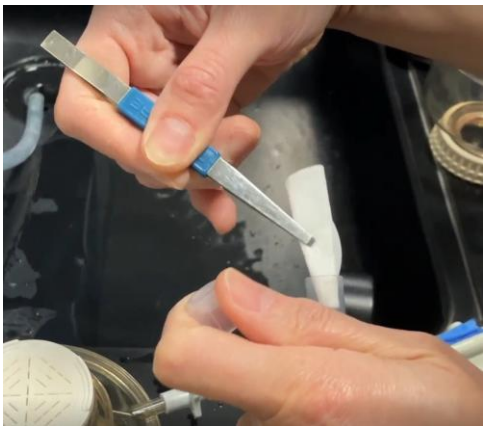
b. It may take a few pumps before pressure builds up enough to pull water through.
c. There's no need to pump fast. A slow and steady pump is sufficient.

6. When all water has passed through the filter, release the pressure built up in the system by pressing the lever at the front of the hand pump. A hissing sound indicates pressure is being released.

7. Retrieve the filter from the filtration system.
   a. Unscrew and remove the sample funnel.
   b. Using the filter forceps, grab the side of the filter and gently roll it up.



   c. Avoid touching the middle of the filter where the plankton has been collected via filtration, but it is ok to touch the backside of the filter.
   d. Place the rolled-up filter into the prelabelled filter storage tube.



e. Protect sample from light by wrapping the tube with aluminum foil and store sample in freezer (-20 °C) as soon as possible.

8. Clean equipment between stations and at the end of the day.
    a. Rinse graduated cylinders with clean freshwater.
    b. Rinse forceps with clean freshwater.
    c. Disassemble filtration unit and rinse each component with clean freshwater.
        i. Beware that the mesh of the filter holder can fall out.

        ii. The filtrate receiver only needs to be rinsed at the end of the day.
9. Ensure that sample collection field sheet with sampling information are filled in at the end of the day (Next page).

Field Sheet for HABs sampling for Southern California Coastal Water Research Project

Abbreviated sampling instructions

1. Using a bucket and line, toss bucket into water pull up and swirl water around in bucket to rinse with sample water. Toss water back.
2. Now that bucket is rinsed, toss again and collect full bucket of water
3. Pour small amount (less then ¼-ish full) of water into selected bottle, put cap on and shake vigorously to rinse bottle with sample water, do this 3 times
4. After rinsing sample bottle, fill to top with water from bucket
5. Record latitude and longitude, date, and time of water collection on labels and on field sheet
6. Stow bottle back into cooler and keep cool and dark until filtration
    a. If you have been provided a filtration kit, filter samples according to the collection protocol
    b. Quick "cheat sheet" on sample types and volumes requested.
        i. duplicate domoic acid filters (250 mL volume for each replicate)
        ii. duplicate chlorophyll-a filters (100 mL volume for each replicate)
7. After all samples are collected, store filters in freezer and coordinate a pick up of samples with Alle (allel@sccwrp.org)

Date	Time	Bottle/Station ID	Latitude	Longitude

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