

Measuring Chlorophyll-*a* in plankton.

1. Collect phytoplankton sample using a Van-Dorn, Niskin, or similar bottle. Note depth, time of day, date and location of sampling. Process without delay. Onboard the ship, each Niskin bottle is numbered and corresponds to a discrete sampling depth that is written on a white board and posted at the rosette deck on the *Lake Guardian*. Rinse a container with the sampled water then place at least 2 L of water sample into the container. If for some reason the sample cannot be readily processed then keep the bottle in the dark and cool to prevent the sample from changing appreciably prior to the filtration (place in walk-in cooler or refrigerator and process within 1 hour).
2. Conduct a parallel filtration by filtering known volumes onto different pore-size polycarbonate membrane filters. For oligotrophic and mesotrophic waters, filter 200-400 mL onto a 20- μm pore size filter, 200 mL onto a 2- μm pore size filter and 50 mL onto a 0.2- μm pore-size filter (note: any volume can be filtered but the volume of lakewater filtered must be determined). Use a graduated cylinder to measure lakewater. Conduct a duplicate filtration for each pore-size. Make sure to re-suspend seston by gently inverting the bottle before measuring the volume of lakewater to be filtered. Rinse the sides of the filtration rig with filter-sterilized ($<0.2 \mu\text{m}$) lakewater to ensure filtration of all particles in the sample; this is particularly important if the sample is particle rich and filtration is slow (in which case a “scum” rim will form around the filtration tower). In eutrophic situations (noticeable water color in a 1 L volume use 50-100 ml for the 20- μm filter, 25-50 ml for the 0.2- μm filter, and 10-20 mL for the 0.2- μm filter. Use an appropriate size graduated cylinder (or pipette) for each measurement of lakewater.
3. Extract in 10 mL of 90% acetone by immersing the filter fully into the acetone solution contained in a 15 ml capped and numbered polypropylene centrifuge tube. [Prepare 90% acetone by adding 100 mL of de-ionized water to 900 mL of acetone.] Use the number to track the sample identity. It is easiest to fill the tube with 10 mL of 90% acetone prior to adding the filter since spillage will occur more frequently otherwise. Place in dark (fluorescent lights will degrade chl-*a*) and cold (4°C; a fridge).
4. After an extraction period of 8-24 h, measure chl-*a* content in the extract using fluorometry (Welschemeyer technique; Welschemeyer 1994). The fluorometer used is a Turner Designs, TD-700 (model 7000-000 with far-red photomultiplier tube is required). Calibrate the instrument with the solid standard prior to each series of measurements. Make sure the acetone warms to room temperature before adding to a disposable glass tube in order to prevent condensation that will interfere with measuring the fluorescence. Samples from very turbid waters may require settling or centrifugation to reduce the interfering particle suspensions in the acetone extract.
5. Calculate the chl-*a* concentration in the water sample (Equation 1).
6. Express chl-*a* content in the respective size fractions, as determined by the method of difference (equations 2-4).

Calculation of chl-*a* content:

[chlFL] = concentration of chl-*a* ($\mu\text{g/L}$) measured in acetone extract by the fluorometer.

V_{extract} = volume of acetone extract (L)

V_{sample} = volume of water sampled filtered (L)

[chl-*a*] = concentration of chl-*a* in water sample; $\mu\text{g/L}$

$$[\text{chl-}a] = [\text{chlFL}] \times V_{\text{extract}} / V_{\text{sample}} \quad (1)$$

Express chl-*a* concentration as the average of the duplicate filtrations. Calculate the size fractionated chl-*a* concentrations as follows:”

Size fractionated chl-*a* concentrations:

$$\text{Microplankton (<20 } \mu\text{m)} = [\text{chl-}a]_{20 \mu\text{m}} \quad (2)$$

$$\text{Nanoplankton (2-20 } \mu\text{m)} = [\text{chl-}a]_{2 \mu\text{m}} - [\text{chl-}a]_{20 \mu\text{m}} \quad (3)$$

$$\text{Picoplankton (0.2-2 } \mu\text{m)} = [\text{chl-}a]_{0.2 \mu\text{m}} - [\text{chl-}a]_{2 \mu\text{m}} \quad (4)$$

Note: A size fraction can be measured by using filters of different pore-size. The parallel filtration technique described here provides the same accuracy as a serial filtration but precision is greater with the latter method. Parallel filtration gives precision of about 5%.

Reference

Welschemeyer, N.A. 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and phaeopigments. *Limnol. Oceanogr.* 39: 1985-1992.

Some notes on filtration.

1. Filtration is used to separate particles from water and to separate a size class of particles from another size of particles. A filtration is conducted to recover particles, recover filtrate, or both.
2. Convention has the “dissolved” phase as being $<0.45 \mu\text{m}$ or $<0.2 \mu\text{m}$. However, colloids $<0.2 \mu\text{m}$ will be present in solution that passes such a cut-off and colloids are not truly dissolved solutes. No organisms are $<0.2 \mu\text{m}$.
3. Filtration will alter the chemistry of a water sample. Some solutes are in chemical equilibrium with the solid phase. As a sample is filtered the concentration of particles on the filter surface increases and thus water passing over the filter “cake” will tend to be enriched relative to the water that is not yet filtered. This phenomenon can be avoided by filtering smaller volumes of water.
4. Rules of thumb for filtering:
 - Filter only as much sample as necessary to avoid overloading a filter;
 - Use as low a vacuum pressure as possible to avoid rupturing cells that will release their contents to the “dissolved phase”. Use 10-15 “inches of mercury” as a low vacuum.
 - Use as large a filter surface area as possible to increase filter efficiency.
 - If a filter plugs then change the filter rather than continuing with a filtration that may lead to an artefact.

- Take care not to tear or contaminate a membrane filter when placing it on the filtration apparatus (check the filtrate for the presence of particles; particles $>20\ \mu\text{m}$ will diffract light and be detectable).
 - Do not let a filter run dry for a long time (membrane filters will bond to the filter holder and tear upon retrieval).
 - Use only the membrane for filtering; discard the blue parchment paper used to separate the membranes.
5. Check to ensure that the vacuum bottle does not overflow when filtering.
 6. Run the vacuum pump for 5 minutes with no load following a series of filtrations to allow the mechanism to cool down.
 7. A hand-held vacuum pump (with a gauge) is an adequate substitute for an electric pump.
 8. If a filtration is not working to check for: a) vacuum, b) vacuum leaks, c) plugged filter (due to too great a particle load).
 9. Remember to take notes of the volume filtered, sample origin, and tracking number of filter in extraction tube.
 10. Remember to periodically empty the vacuum trap (containing filtrate) or else the pump will fail.

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