Sampling protocol for qualitative (net) and quantitative phytoplankton

1. Qualitative Phytoplankton Samples

Qualitative samples are taken to give a quick overview of the phytoplankton community throughout the water column. These samples are easily obtained with a net and can be used for finer taxonomic identification than quantitative methods. The analysis of these samples is quick (less than an hour) but offers only limited information in the form of relative abundance between species. These samples are especially suited for harmful algae monitoring.

Materials

Phytoplankton net: 20µm mesh net that can be obtained at local vendors such as Dynamic Aqua Supply.

Sample jars or bottles: 200-500ml capacity amber glass jars. Caps can be lined or not. Common in catalogs of local and international vendors.

Lugol's iodine solution: this fixative is a water-based saturated solution of iodine and iodide that is usually pH neutralized with ascetic acid. Can be found in catalogs of local and international vendors, but a diluted version is common. Recommend preparing from scratch using the recipe at the end of the document.

Procedure for qualitative phytoplankton sample collection:

1. Before starting the sampling make sure your bottles are correctly labelled.

2. Attach a calibrated line to the 20µm net. Check that both the calibrated line and the collection cup are firmly attached to the net.

3. Lower the net from the sampling platform (pier, boat, etc.) into the water using the calibrated line. Be careful not to lower it all the way to the bottom, stirring up the sediments (refer to tide tables to anticipate the depth of the water and stop at least 0.5m before the bottom).

4. **Slowly** pull up the net. If it is pulled up faster than 1 m/s, turbulent flow may washe out some of the collected material.

5. When the excess water has drained from the net it may be necessary to use the squirt bottle to wash material down the net into the collection cup.

6. If the sample is too dilute, reduce its volume by filtering with one side of the net near the collection cup, and wash the material back into the cup with the least amount of water possible.

7. Pour the contents of the collection cup into an appropriately labelled amber glass bottle.

8. Because this is a qualitative sample you can repeat the steps outlined above as many times as necessary to obtain desired amount of material.

9. Fix the sample using approximately 20 drops of concentrated Lugol's iodine solution for every 250ml of sample, producing a tea color in the sample. This sample must be stored at room temperature (avoid freezing) protected from the light (hence the amber glass).

10. Place the wet net in a plastic bag and once back in the lab, rinse it with fresh water and let it hang to air dry. **Never** let seawater dry on a net as the salt will destroy the nylon fibers.

Notes:

- **Do not freeze, boil or otherwise physically or chemically change the samples.**
- **Seawater samples preserved in this way should be viable for analysis for at least one year. If longer storage is needed, replenish the Lugol once a year, making a note for the volume increase.**
- **Most particles smaller than 20µm will be lost due to the use of a net, limiting the scope of these samples. Smaller mesh sizes are not practical due to reduced flow rate and increase in turbulent flow.**

2. Quantitative Phytoplankton Samples

Whole seawater samples are collected for quantitative analysis (i.e. counting). These samples are used to determine the number of phytoplankton species and individuals present at discreet depths. The analysis of this kind of samples is slow (3 hours + prep) and requires a qualified expert for adequate taxonomical determinations, but the information is detailed and quantitative.

Materials

Sample jars or bottles: 200-500ml capacity amber glass jars. Caps can be lined or not. Common in catalogs of local and international vendors.

Lugol's iodine solution: this fixative is a water-based saturated solution of iodine and iodide that is usually pH neutralized with ascetic acid. Can be found in catalogs of local and international vendors, but a diluted version is common. Recommend preparing from scratch using the recipe at the end of the document.

Procedure for quantitative phytoplankton sample collection:

1. Attach the calibrated line to a water sampler and check that it is firmly attached.

2. Arm the water sampler and lower it to the depth required. Wait for 1 minute to ensure water has flown through the sampler before you close it.

3. Place the messenger on the line, secure it, and release it. Once the water sampler is closed, pull it up.

4. Once the sampler is on the pier, use the outflow to pour water into an appropriately labelled amber glass bottle.

5. Fix the sample as specified above, using approximately 20 drops of Lugol's iodine solution for every 250 of sample.

6. Repeat the steps outlined above for any other depths required. **Never** add water, mix samples or let samples dry.

Notes:

- **Do not freeze, boil or otherwise physically or chemically alter the samples.**
- **Seawater samples preserved in this way should be viable for analysis for at least one year. If longer storage is needed, replenish the Lugol once a year, making a note for the volume increase.**
- **Most particles smaller than 20µm will be lost due to the use of a net, limiting the scope of these samples. Smaller mesh sizes are not practical due to reduced flow rate and increase in turbulent flow.**

Lugol's iodine solution recipe

Dissolve 100 g potassium iodide (KI) in 1 L of distilled water. Dissolve 50 g iodine (crystalline) in 100 ml glacial acetic acid. Mix these two solutions. Remove any precipitates (can be filtered through a coffee filter). Store in the dark in amber glass bottles. Both iodine compounds dissolve easily in water and the acid addition doesnt produce much heat, so the procedure is quite safe besides the handling of the concentrated acetic acid. The recipe can be reduced for any volume you may want to prepare with half liters being easier to handle.

Lugol's solution is the preferred fixative used for coastal marine phytoplankton as it is nontoxic (unless ingested in quantity) and non-volatile. It is easy to handle and any contact with the skin will only produce a temporary stain. This solution does not produce a significant change in the volume of the samples, which can be an important factor in quantitative samples.

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