Sampling freshwater and marine microalgae and harmful algal blooms (HABs)

1 Purpose and scope

This document outlines standard procedures to collect and preserve freshwater and marine microalgae from aquatic environments. General methods are also described for safely collecting and handling water samples of known or suspected harmful blue-green algae (cyanobacteria) that may contain toxins.

This document does not provide information on the collection of algae samples for toxin analysis, nor the collection of tissue samples of livestock, wildlife, fish or shellfish that are suspected to have been in contact with, or have ingested harmful algal toxins. These methods are provided in *Queensland Harmful Algal Blooms Operational Procedures* (DNRM 2014). The *Queensland Harmful Algal Blooms Operational Procedures* also describes how to report suspected blue-green algal blooms to the appropriate authorities.

2 Associated documents

Biological assessment: Background information on freshwater and marine microalgae and harmful algal blooms (HABs)

Sampling design and preparation:

- *Permits and approvals*
- *Record keeping including taking field photographs and videos*

3 Health and safety

Some algal species can cause health issues. Before following the methods contained in this document, a detailed risk management process (identification, assessment, control and review of the hazards and risks) must be undertaken. All work carried out must comply with the Queensland Work Health and Safety legislative obligations.

4 Permits and approvals

Permits and approvals may be required to conduct activities involving animals, plants and/or in protected areas (for example National Park/Regional Park, State Forest or State Marine Park). See *Permits and approvals* document for more information on requirements.

5 Skills, training and experience

Staff using this method should have previous training or experience in:

- recognising algal blooms and associated surface scums
- how to collect, transport and deliver samples to a laboratory for analysis.

6 Equipment

See Appendix 1 for equipment check list and Appendix 2 for preparation of Lugol's solution.

7 Procedure

7.1 Preparation for sampling

Note: Samples should be collected in 500-1000mL plastic or glass sampling bottles.

7.1.1 Ecosystem surveys

When undertaking ecosystem surveys:

- Be prepared to collect samples that represent a range of habitats
- Prepare to collect water samples at a particular site at approximately the same time of day each time, preferably between 8.30am and 12.00pm. This is because the algae move up in the water towards the surface in the morning, and tend to sink to lower regions in the afternoon. By sampling at roughly the same time on each occasion the survey results can be directly compared over time.

7.1.2 Algal blooms

Algal blooms (including cyanobacterial blooms) can be extremely patchy in distribution, both spatially and temporally. Buoyant species tend to accumulate near the surface or along the shoreline at the downwind or downstream end of reservoirs or river reaches. In view of this, "depth integrated" open water sampling is the preferred option, because it provides a better representation of the "true" or average algal population in a water body.

When monitoring algal blooms, the selection of sampling sites will depend on a number of factors, including:

- the time of day
- prevailing winds
- proximity to tributary inflows i.e. where a stream flows into a larger body of water
- the proximity to potential nutrient input sites.

Collect samples at any time if an algal bloom has been reported.

Note: Some species of blue-green algae can cause skin irritation. If sampling from an area that has a high level of phytoplankton, wear gloves and appropriate protective clothing to minimise contact with the water. Where skin contact does occur, the skin should be washed immediately.

7.2 Collection of algae samples from a stream or small river

7.2.1 Collecting samples directly from a stream or river

1. Choose a site that is representative of the bulk water being assessed.
2. If sampling an algal bloom, take notes that describe the surface scum (colour, odour, presence of dead organisms etc.).
3. Label sample bottles with a water proof pen.
4. Enter the water, move towards midstream and face upstream to collect the sample (Figure 1).
5. If the bottle needs rinsing (i.e. if reusing bottles from previous samplings) wash the sample bottle with ambient water three times, discarding the rinse water downstream.
6. To fill the sample bottle the following method should be used to avoid sampling surface scum.
7. Grip the bottle in one hand around the base and remove the lid with the other hand.
8. Invert the sample container fully and submerge to a depth of 0.2m below the surface.
9. Turn the mouth of the bottle upwards and towards the current.

10. When the bottle is full remove it from the water rapidly and replace the lid. Take care to keep fingers clear of the lid liner and neck of the bottle.
11. If sampling an algal bloom, surface scums can be targeted by moving the sample bottle through the surface.
12. If preservation with Lugol's solution is required to stain and preserve the sample, add a couple of drops of Lugol's solution into the sample to preserve it. The sample should turn the colour of weak tea.
13. Check that details on the sample container are correct.
14. Place the sample bottle(s) in a cool box (with ice or ice bricks) or portable refrigerator that is suitable for transport to the analysing laboratory. Record the site, sample name, date and time, sampler name and any general observations about the site.

Note: Accumulations of surface scums are useful for identification purposes, but once diluted they should not be used for counting purposes (quantitative analysis).

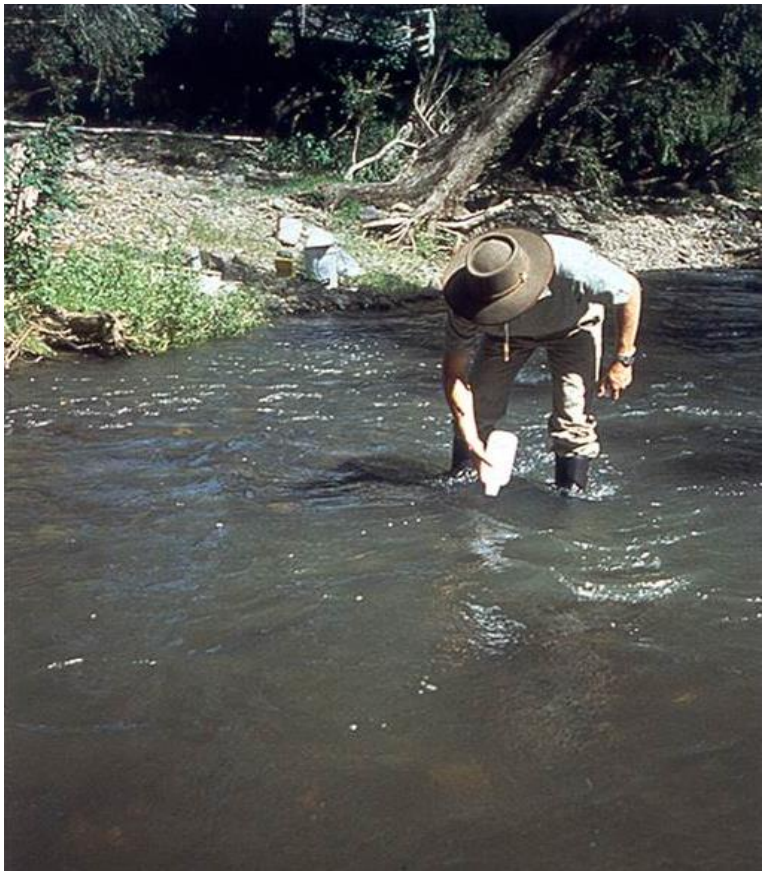

Figure 1: Collecting a grab sample

7.2.2 Collection of samples using a pole-type sampler

1. Follow steps 1–3 in Section 7.2.1.
2. Extend the sampling pole to the required length and check that all surfaces have been cleaned.
3. Remove the lid of the sample container.
4. Place the sample container into the bracket of the sampling pole.
5. Invert the sample container and submerge to a depth of 0.3m below the water surface.
6. Rotate the sample container into the direction of flow and fill the sample bottle.
7. Turn the sample bottle upright, remove from the water and replace the bottle lid. Take care to keep fingers clear of the lid liner and neck of the bottle.
8. Follow steps 11-14 in Section 7.2.1.

Figure 2: Using an extendable pole type sampler

7.3 Collection of algae samples from large rivers, lakes or the ocean

Samples in large rivers, estuaries, oceans and lakes can be collected using a hose-pipe sampler (Figure 3) or using a depth sampler, e.g. Van Dorn (Figure 4).

Note:

- When sampling from a boat, rinse the bottle on the side of the boat opposite from where samples are collected in order to avoid disturbance of the surface algal community.
- When a fuel-powered boat is used, collect samples from the bow to avoid contamination from the motor.

7.3.1 Hose-pipe sampler (for depth integrated samples)

A hose pipe sampler is a weighted hose-pipe that can be used to obtain a water column sample. It is usually used for collecting depth integrated samples when a representative sample of the water column is desired. A hose-pipe sampler can be constructed using a garden hose. The length of the hose-pipe should be chosen to reflect the appropriate depth to which the cells are likely to be mixed. A temperature probe can be used to determine the mixing status of the waterbody and depth of any thermocline present. Water samples can then be collected from the surface to the thermocline.

Note: It is possible to use a rigid pipe fitted with a one-way valve, instead of an integrated hose-pipe sampler. It can simplify the operation of withdrawing the pipe and sample from the water.

- If mixing status is unknown, a 5m long hose-pipe is recommended.
- At shallow sites (less than 3m water depth) a 2m long hose-pipe should be used.
- At deeper sites longer hose-pipes (up to 10m) may be useful.

The method for collecting samples using a hose-pipe sampler is shown in Figure 3. The procedure for collecting a sample using an integrated hose-pipe sampler is:

1. Determine the depth of any thermocline present using a temperature probe and collect samples from the surface to the thermocline.
2. If you do not have a temperature probe use a 5m long hose-pipe as a default.
3. Attach a cord to one end of the hose and the boat to prevent accidental loss of the hose-pipe.
4. Holding the hose-pipe at the top end, rapidly drop the weighted end of the hose-pipe into the water to a depth of approximately 5m.
5. Pull the bottom of the hose-pipe into the boat using the cord without inserting the rubber cork.
6. Rinse the hose-pipe.
7. Repeat steps 3-4, but this time insert the cork into the top end of the hose-pipe before pulling the hose-pipe into the boat.

8. Pull the bottom end of the hose-pipe to the surface using the cord, so that the tube is in a U-shape (Figure 3).
9. Lower the weighted end of the hose into a bucket and remove the cork. Ensure that the entire contents of the hose are emptied into the bucket.
10. Mix the contents of the bucket and then transfer part of the contents into a sample bottle. Discard the rest of the contents of the bucket.
11. Record the site, sample name, date and time, sampler name, depth and any general observations about the site.
12. Clean the hose-pipe and sampling bucket (rinse several times thoroughly with clean water).

When not in use, the hose-pipe sampler and bucket should be kept clean and stored in a dark shed or cupboard.

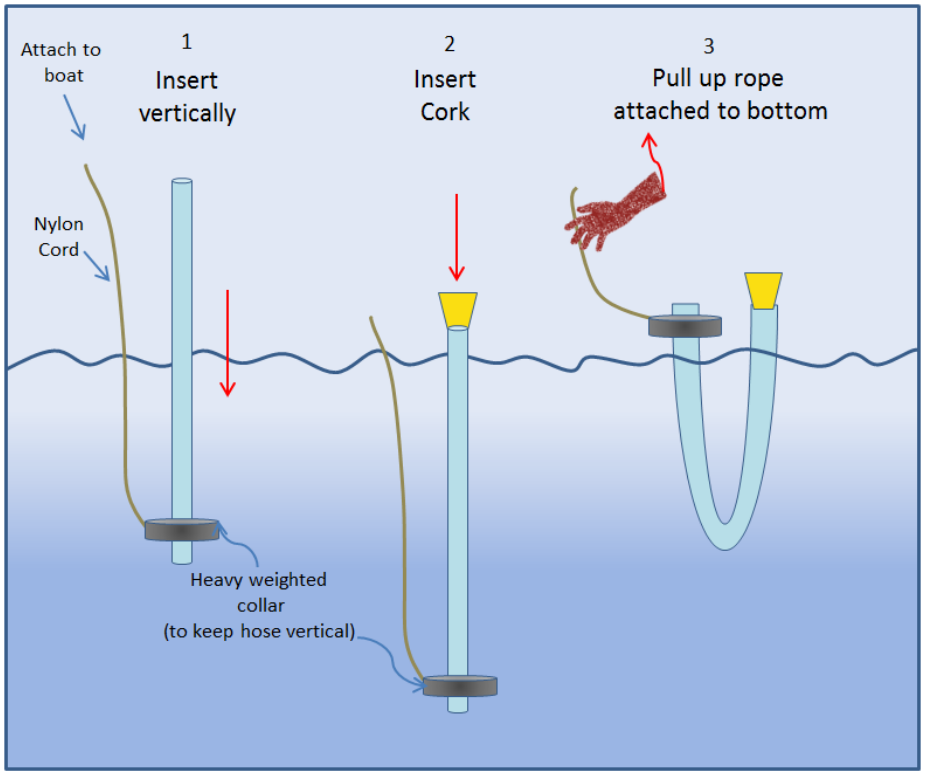

Figure 3: Procedure for use of the integrated hose-pipe sampler

7.3.2 Collecting samples using a depth sampler

Samples can be collected at discrete depths using a sampler such as a Van Dorn sampler (Figure 4) or Niskin bottles. Some Niskin bottles are fitted with a thermometer to record the temperature of the water at the sampling location.

The general procedure for collecting samples using a depth sampler is:

1. Cock the sampling bottle as per the manufacturer's instruction.
2. Lower the bottle into the water to the required depth.
3. Trigger depth sampler as per the manufacturer's instruction.
4. Remove the device from the water.
5. Gently transfer the volume required to labelled sample bottles.
6. Record the site, sample name, date and time, sampler name depth, and any general observations about the site.
7. Fix the samples using Lugol's solution (if required).
8. Rinse the sampling bottle thoroughly between each site.

Figure 4: Van Dorn sample bottle

7.3.3 Preservation and transport of microalgae samples

Samples should be examined as soon as possible after collection while the algae are still alive, as some identifying features are more clearly seen in live algae. Consult with the analytical laboratory as to their preferred method for maintaining, preserving and transporting live samples. For example, unpreserved samples received by the laboratory greater than 48 hours after collection may not be analysed. Algal samples should be kept cool or cold on ice in a cool box or in a portable refrigerator and stored in the dark until samples can be examined. Samples should not be allowed to freeze.

Samples may be preserved using Lugol's solution if required. Lugol's solution can be added to the water samples drop by drop, using a disposable pipette until the sample is a weak tea colour (i.e. approx. 0.5mL Lugol's solution to 100mL of sample). If there is a high concentration of algal cells (such as in a bloom event), it may be necessary to add more Lugol's solution to the water sample. Lugol's solution is commercially available or can be prepared easily as described in Appendix 2.

Note: Algal samples may be considered 'dangerous goods'. Commercial carriers have shipping regulations—ensure the sample packaging and labelling meet the requirements. Contact the courier company for details prior to sampling.

7.4 Freshwater and marine benthic microalgae

Filamentous and other benthic algae can usually be found attached to substrates such as rocks, sand and gravel beds, woody debris, buoys and mooring fixtures where they form mats. These attached algae can be collected for taxonomic and qualitative analysis as mats or strands of filaments.

Note: Samples should be collected in a high density polyethylene screw top jar and filled with surrounding water and fixed with Lugol's solution (if required).

7.4.1 Collecting samples from shallow water

Small samples from shallow near shore sites can be collected by hand or scrapers/spatulas (sample size around the size of a ten cent coin). Larger samples can be collected using nets or rakes, ensuring that the full varieties of habitats present at a site are sampled. Duplicate samples across the area of interest should be collected.

If there is little variation in the type of material across the area of interest, a composite sample may be taken incorporating multiple subsamples.

7.4.2 Collecting samples from deep water

Benthic algae samples in deep water sites using a benthic sampler such as a Van Veen grab sampler (Figure 5) or an Eckman grab sampler.

Figure 5: Van Veen Grab Sampler collecting benthic mud in sample container

7.4.3 Preservation and transport of benthic algae samples

Samples from different habitats and substrates should be separately bottled or bagged.

1. Refrigerate or chill immediately after collection (to 4˚C) but do not allow the sample to freeze. Samples will remain fresh for approximately two days.
2. When ready to send for analysis, place the sample jar into a zip lock bag and put it into a small plastic cool box with one or two freezer bricks.
3. Pad the spaces with absorbent material in case the sample leaks and tape up the package securely. Send the samples as soon as possible.

Note: Algal samples may be considered 'dangerous goods'. Commercial carriers have shipping regulations—ensure the sample packaging and labelling meet the requirements. Contact the courier company for details prior to sampling.

8 Analysis of samples

Once the microalgae samples have been collected and/or fixed with Lugol's solution the samples can be transported to the analytical laboratory and provided to the phytoplankton expert for identification and counting purposes.

9 References and additional reading

Hötzel, G, Croome, R 1999 *A Phytoplankton Methods Manual for Australian Freshwaters*, LWRRDC Occasional Paper 18/98.

Hallegraeff, GM, Anderson, DM, Cembella, AD (eds) 2003, *Manual on Harmful Marine Microalgae*, UNESCO Publishing, Paris.

WQRA 2010, *Management strategies for cyanobacteria (blue-green algae) and their toxins: a guide for water utilities*, Water Quality Research Australia Limited, Research report 74.

DNRM 2014, *Queensland Harmful Algal Bloom Operational Procedures*, Department of Natural Resources and Mines, Brisbane. Available from: https://publications.qld.gov.au/storage/f/2014-10-01T05%3A41%3A36.624Z/hab-operational-procedures.pdf

Appendix 1 Equipment checklists

Table 1: Equipment checklist for phytoplankton water samples

Equipment and consumables	✓
Note book or equivalent	
Fresh water for washing skin and hand washing equipment	
Gloves	
Integrated hose-pipe sampler – 5m length of 2.5cm diameter plastic piping with a weighted collar at one end	
Rigid pipe fitted with a one-way valve (if preferred to a flexible hose-pipe)	
Cord (attached to the hose and boat)	
Rubber cork that fits one end of the hose	
5L bucket	
Pole type sampler; sampling pole with attachment to hold sample bottle/container	
200mL and 500mL sample bottles and lids	
Lugol's solution and disposable pipette	
Cool box and ice	

Table 2: Equipment checklist for benthic algae samples

Equipment and consumables	✓
Note book or equivalent	
Fresh water for washing skin and hand washing equipment	
Gloves	
Benthic sampler (e.g. Eckman grab) or a rigid plastic corer (e.g. PVC or polycarbonate pipe)	
Nets or rakes	
Container with a fitted lid or a zip lock bag	
Lugol's solution and disposable pipette	
Cool box and ice	

Table 3: Equipment checklist for marine benthic algae identification

Equipment and consumables	✓
Gloves	
Rake or garden fork	
150mL high density polyethylene screw top jar	
Zip lock bag with absorbent material	
Portable refrigerator	
Cool box and ice	

Appendix 2 Preparation and use of Lugol's solution

To make up Lugol's solution

Chemicals

- Potassium iodide (KI)
- Pure iodine crystals
- Glacial Acetic acid
- Distilled water

Note: Take care when handling, avoid contact with skin because the glacial acetic acid is highly corrosive and Lugol's solution will readily stain skin (brown/orange colour).

Method

1. Mix 20g of potassium iodide (KI) with 180mL distilled water.
2. Dissolve 10g of pure iodine crystals in this solution.
3. Add 20mL glacial acetic acid.
4. Store the solution in a dark coloured glass bottle inside a dark chemical storage cupboard. The bottle should be labelled with:
    - the date of preparation
    - name of the analyst who prepared the solution
    - a use by date.
5. This stock solution should not be used 6 months after the date of preparation.
6. The bottle should be sealed around the cap with Parafilm®.
7. If using polyethylene bottles for water samples, note that these bottles will absorb the iodine in Lugol's solution very quickly, and should not be used for long term storage. Staining also occurs when numerous water bottles (containing Lugol's fixed samples) are stored in a plastic container (i.e. an esky). If Lugol's fixed water samples are to be stored prior to concentration by settling or centrifugation in the laboratory, they should be stored in amber glass bottles in the dark and processed as soon as possible.

Note: The iodine in Lugol's solution not only preserves and stains the phytoplankton but it also increases their density making them heavier so that they sink more readily to the bottom of a settling cylinder.