Chapter 12

Fieldwork

Site inspection and sampling

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CONTENTS

INTRODUCTION

This chapter is primarily intended for readers that are not running a routine monitoring programme but need to organise fieldwork and sampling from scratch. Local, national and regional guidelines may also exist and should be followed, when appropriate. This is especially important if the results of sampling are intended to inform any type of public advisory postings. If guidelines

are not available for a specific region, it may be nonetheless helpful to consult the existing guidelines from neighbouring regions or regions with similar conditions. Under local conditions, however, it may be difficult to fully comply with the existing guidelines for various reasons, for example, lack of material or deviating seasonal patterns of phytoplankton dynamics. It is then preferable to organise fieldwork with the locally available means rather than to suspend fieldwork completely due to the lack of specific material requested in guidelines. This could be, for example, sample containers such as wide-mouthed amber glass bottles that are not available or unaffordable and have to be replaced by ubiquitous plastic bottles. This chapter is therefore more of a blueprint to develop locally adopted guidelines than a guideline itself.

The following is largely focused on the sampling of plankton and the measurement of hydrophysical parameters in the pelagic of waterbodies. The sampling of sediment and benthic cyanobacteria is briefly discussed.

A number of practical issues need to be considered when sampling for cyanobacteria and cyanotoxins and good preparation greatly facilitates on-site work. Sampling campaigns can be considerably impeded by weather conditions that make manoeuvers that appear very simple from behind a desk more challenging in the field. For this reason, sampling campaigns should be prepared in a way that reduces on-site handling steps to a minimum.

In addition, samplers should be prepared to address questions and concerns from the general public when sampling recreational sites (see Chapter 15).

12.1 PREPARATIVE STEPS

Before fieldwork is conducted, the monitoring programme should be consulted and for each task verified that it can be conducted as planned (see Chapter 11). Staff responsible for collecting samples needs to be trained on the entire process, including completing sampling protocols, handling of sampling devices and storage of samples during transport. Further, basic knowledge of cyanobacterial biology is favourable to decide on deviations from the sampling scheme or to collect additional samples when the actual conditions indicate this to be appropriate, for example, scum formation at an unexpected shore site due to unusual wind directions.

Preparative steps include:

- preparation of checklists for materials required for the on-site sampling (sampling devices, storage containers, vehicles, etc.);
- preparation of easy-to-fill-in protocol forms that can be completed under adverse conditions in the field (may include field data collection sheets, sample submission forms and chain of custody forms);
- consistent use of unique sampling location names for all sampling sites. This will greatly simplify data management and avoid confusion;
- establishing a sample labelling scheme that allows the unambiguous back-tracking of samples. This is especially important when samples

are diverted later, that is, to be sent to different laboratories/analysts. When multiple institutions and laboratories are involved, the consistent use of a labelling scheme must be asserted;
- verification that the sampling can be practically fulfilled as intended, with sufficient time buffer to compensate possibly occurring delays;
- planning and organisation of transport to and from sampling locations, including access permissions for restricted areas;
- planning of transport of samples to other laboratories when not analysed in-house, including measures to preserve samples appropriately;
- contact information for laboratories conducting analysis (in case questions arise during sampling, or sample transport will be delayed), sampling site owners or managers, and emergency contacts.

Good logistical preparation prior to fieldwork requires that equipment is checked to ensure that it is functioning properly, for example, regular testing and calibration of electrodes; testing and changing of batteries; and keeping operation, maintenance and calibration records, respectively. It is essential to prepare a sampling checklist that includes maps of sampling site locations, a list of required equipment, a detailed explanation of the methods for sample collection, lists of the types and numbers of samples to be taken at each site, as well as of the required volumes of samples. The labelling of sample containers (with water-proof markers) should follow a consistent system to make every sample traceable at any later time. As a minimum, sample container labels should include a unique and consistent sample site code (e.g., a code for the waterbody and a code for the sampling point) and the type of sample or the intended analysis, respectively. In combination with the collection date and time on the sample protocol, a unique sample identifier is created. It is imperative that unique and specific site names be established and consistently used by all sample collectors. Developing an electronic master site list linked to geographic information and other pertinent metadata (laboratory methods used, reporting limits, sample collector name, etc.) is encouraged. This will greatly simplify data storage, retrieval and future data analysis. Whenever possible, extra sample containers and labelling tools should be included in the material taken to collection sites. The extra containers can be used if additional samples are deemed necessary while on site (detection of scums or shifting bloom location) or if containers become broken or contaminated during transit or while on site.

12.2 DETERMINATION OF KEY HYDROPHYSICAL CONDITIONS

Among the hydrophysical conditions affecting cyanobacterial occurrence, the most important ones are turbidity, temperature profiles (stratification), pH, oxygen concentration and – for rivers or streams – flow rate (Chapter 3).

12.2.1 Turbidity

Turbidity is easily assessed with a Secchi disc. It is slowly submerged into the water at a line to the point where it is just still visible (or no longer visible) and this depth is termed "Secchi depth" (Figure 12.1). The depth down to which photosynthesis is possible in aquatic ecosystems, the euphotic depth, is 1.5–2.5 fold the Secchi depth (Preisendorfer, 1986), and in freshwater studies, the factor 2.3 is widely used (Chapter 4). More precise determinations of the euphotic depth are possible by photon flux measurements requiring a submersible quantum sensor (for photosynthetically active radiation; PAR). However, for the assessment of conditions favouring cyanobacterial proliferation, the much cheaper and simpler determination of Secchi depths is usually sufficient and allows reproducible measurements also by untrained persons after a brief introduction to the method (for an example, see Box 11.1).

12.2.1.1 Equipment

Secchi discs can be self-made, but convenient ones are available from companies that provide field-sampling equipment. They should be 25 cm in diameter, made of sufficiently heavy material to be readily submersible, may include holes to ensure easy horizontal sinking and be attached to a chain or cord of sufficient length with depth marks (Figure 12.1).

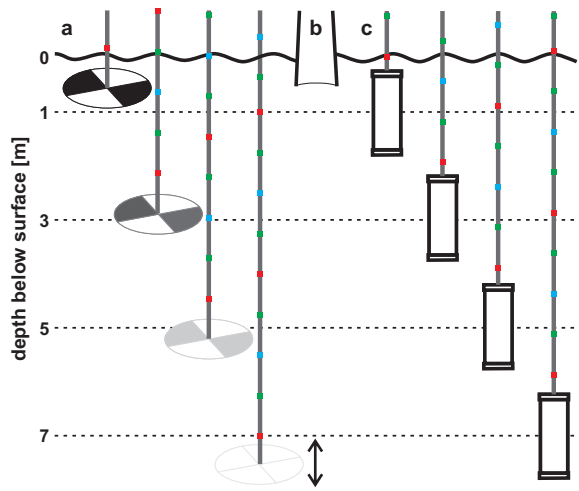

Figure 12.1 (a) Secchi depth measurement: the Secchi disc is lowered at the graduated rope to the depth where it is no longer visible. At this point, the disc is repeatedly lifted and lowered to determine accurately the depth at which the disc becomes visible, and this depth is read from the markings on the rope; the reading can be improved by using an underwater viewer to avoid reflection from the water surface (bathyscope, b). (c) Discontinuous depth-integrated sampling: with a water sampler, samples are taken at predefined (exemplary) depths and then combined.

12.2.1.2 Procedure

- Lower the disc into the water in the shade of a boat (or a pier) as reflections from the surface may distort the reading.
- Lower it to the depth at which it is just still visible; move it up and down several times to confirm that depth.
- If the water surface is very turbulent (e.g., through strong wind), it may help to create a quiet surface with a box without a bottom.
- Blooms may be very patchy, and immersing the disc will move them away from that spot. In such cases, wait a few seconds until they have redistributed.
- Do not wear sunglasses during the procedure as that may distort (i.e., reduce) the reading.
- Comparing readings between fieldworkers is an easy, but important exercise to reduce uncertainty, and it generally leads to remarkably similar results once the procedure has been discussed, understood and agreed.

For measuring transparency in shallow depth such as bathing sites, a Secchi disc with a smaller diameter can be mounted on a graduated rod instead of a rope. This allows rapid and precise measurements while wading in the water up to depth of about one meter.

For greater depths or under poor light conditions, the reading can be improved by using an underwater viewer or bathyscope (Figure 12.1) made of a wide box or tube with a transparent bottom on one side.

12.2.2 Temperature, oxygen and pH profiles

Whether a lake or reservoir is thermally stratified or totally or partially mixed can be determined from temperature, oxygen and pH depth profiles, usually measured at a central location. Modern fieldwork equipment includes multiprobes on long cables that can be lowered stepwise, taking readings at defined depths. A simplified approach is the measurement of temperatures in water samples taken at the defined depth, either directly after hauling the water sampler to the surface or with a thermometer mounted on the water sampler. For the latter approach, sufficient time needs to be allowed for an accurate reading and the haul to the surface has to be rapid enough to avoid errors through changes in the water's temperature when moved from deep layers to the surface.

More precise and continuous data are obtained by installing thermistor chains permanently in the water column. This may be of interest when raw water offtake sites are located at a depth close to the thermocline (see Chapter 8).

From such depth profiles, thermal and chemical stratification can be determined as described in Chapter 4 and Box 4.3.

12.2.3 Additional parameters measured on site

The availability of field-portable sensors enables quick data collection for a suite of informative water quality parameters. This includes multiparameter datasondes that are capable of simultaneously measuring chlorophyll-*a*, phycocyanin and turbidity along with the other parameters mentioned above. More sensitive multispectral sondes may also be able to discern between different types of phytoplankton and estimate their relative abundance through fluorescence measurements (see section 13.6). These tools can be used to help verify the presence of cyanobacteria while on site (through detecting phycocyanin/phycoerythrin) and can help direct sampling to locations of cyanobacteria maxima. For example, a datasonde profile can be collected throughout the water column or along a horizontal gradient, and samples can be collected at discrete depth or locations with elevated phycocyanin or chlorophyll-*a* concentrations.

12.2.4 Flow rate and discharge

In running waters such as rivers and streams, the determination of flow velocity and discharge is of interest for aspects such as estimates of nutrient input to a lake or reservoir, or turbulent mixing (see Chapter 7). Flow velocity is measured with a current meter. Current meters commonly can be mechanical with a propeller or based on Doppler acoustics. Since most running water show turbulent flow and pronounced gradients within the transversal section, a measurement of average flow velocity can be only achieved by measurements at multiple points in the profile. For some purposes, the temporal and seasonal variation at defined measurement points is more important than an exact determination of average flow velocity or discharge, and a measurement of flow velocity at a single, well-defined point in the middle of the stream or river may be sufficient for cyanobacterial monitoring and management purposes because in longer time series (frequent) data on relative changes in flow velocity are more meaningful than (a few) accurate measurements of absolute discharge. Correlating measured flow velocities with precipitation in the catchment may be helpful.

Discharge, the volume of water that flows through a transect per unit of time, usually in m^3/sec, is estimated from measurements at multiple points in the profile. This may require additional expertise or training. Discharge data may be available from regional water authorities.

12.3 ON-SITE INSPECTION AND DATA COLLECTION

A protocol for on-site inspection and data collection should be established, allowing a rapid and easy entry of data that are not logged electronically. Data to be registered include date and time, air and water temperature,

wind and general weather conditions, observations such as surface blooms, smell, dead fish, reports from the local population or the like. For recurrent questions, multiple choice-type questionnaires are recommended as these allow a rapid entry, even under adverse conditions, and have the benefit of a consistent and comparable recording of key data, in particular when multiple institutions exchange data.

Sites used for drinking-water abstraction or recreation should be subject to inspection by trained staff, and preferably in conjunction with sampling expeditions. Careful inspection and reporting can assist in the interpretation of results from laboratory analysis. Moreover, the development of personal expertise in relation to specific waterbodies can provide the best form of early warning system, and hence, staff continuity has a high value.

When scums appear on the water surface, cyanobacteria may be present in densities hazardous to human health, and thus, appropriate responses should be initiated quickly (see Chapters 5 and 6), and samples for further analysis should be taken considering safety aspects (see section 12.10). Sampling of scums outside designated or habitual bathing sites is also of great value for determining and predicting hazards, for example, in case of a change in wind direction.

A well-prepared sampling protocol greatly facilitates on-site work. It should be easy to fill in under field conditions, that is, by using checkboxes or multiple-choice options. Information to be collected on site is as follows:

- *General information*: date, time, waterbody, sampling site, staff;
- *Weather conditions*: air temperature, precipitation, wind direction and speed;
- *Water conditions*: water temperature, water transparency, water colour, pH, conductivity, oxygen concentration;
- *Samples*: volume of specific samples, split samples;
- *General observations*: visibility of cyanobacterial (surface) blooms, odour, reports from local stakeholders;
- *Delivery of samples*: handover protocol to cooperating laboratories.

12.4 TAKING WATER SAMPLES

A variety of commercially available water sampling devices have been developed for specific purposes (Figure 12.2). Before purchasing a water sampler (or building one in-house), a limnologist should be consulted to select an adequate type. For practical reasons, the dimensions of a sampler should also be considered as the manual lifting of a filled sampler can be challenging, in particular when working from a small boat.

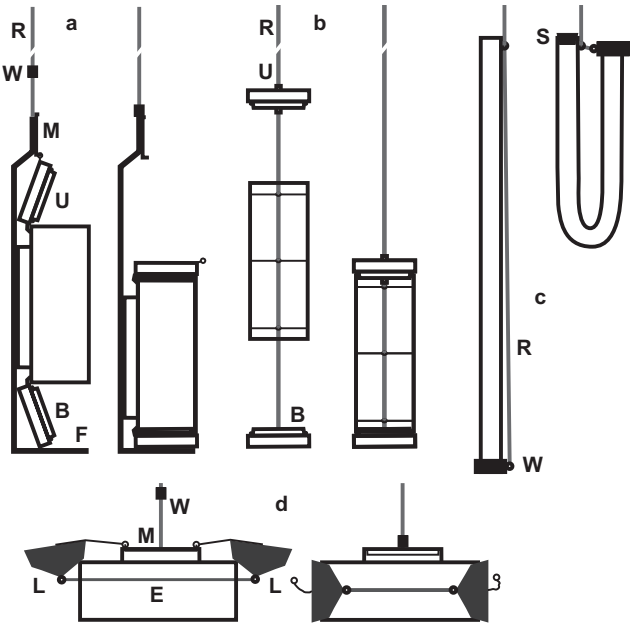

Figure 12.2 Water sampling devices. (a) Limnos-type sampler: the sampler is mounted on a frame (F); for sampling, the upper (U) and bottom (B) lids are held by a release mechanism (M). When the sampler is lowered to the desired depth, a weight (W) is let loose and slides down the rope (R) and hits the release mechanism, thereby unlocking the lids and closing the sampler. After bringing it to the surface, it is emptied. (b) (simplified) Ruttner- or Kemmerer-type water sampler: the bottom lid (B) is lowered to the desired depth, and the tube and the upper lid (U) are released and slide down along the rope to close the sampler. (c) Hosepipe sampler: the weighted end of the hosepipe (W) is lowered to the desired depth at a rope (R); the upper end is closed with a stopper (S) before the lower end is brought to the surface and the hosepipe is emptied. (d) Van Dorn-type sampler: the lids (L) are held open by a release mechanism (M) against the tension of an elastic strap (E); at the desired depth, the lids are released by the weight (W) and close the sampler.

The total volume of the water sample to be taken is determined by the sum of volumes of all subsamples for individual analyses (see below). The calculated total volume needed for all individual analyses is best exceeded about twice to ensure a sufficient sample volume even in case of accidental loss during sample processing.

Two principle types of samples are distinguished, grab samples and integrated samples. Grab samples, either from the surface or from a defined depth, provide information restricted to a specific spot in the waterbody, for example, for a site used for recreation or for drinking-water offtake, whereas for assessing conditions in the whole waterbody, integrated samples are more appropriate.

12.4.1 Grab samples

For surface samples, the easiest way is to submerse the sample container or another vessel. In the presence of surface blooms or scums, preferably multiple samples are taken to account for spatial heterogeneity that can be substantial within distances of a few metres. It is often helpful to collect grab samples at discrete depths, using either Van Dorn- or Kemmerer-type samplers. This is especially useful for determining source water conditions for drinking-water systems, which often draw water from deeper intake locations. For offtake systems with the flexibility to draw water from multiple intake depths, sampling each discrete intake depth can help inform water system operators of the region with the best water quality.

It is important to note exactly where and how the samples have been taken. When surface blooms or scums are present, highest concentrations are expectedly found in the uppermost centimetres, but once disturbed, for example, by wading in the water, the scum may be redispersed in the water column, thus lowering concentrations. It has therefore to be specified what individual samples should represent: maximum concentration or averaged for a water volume resembling the situation of a frequented bathing site.

12.4.2 Integrated samples

Thermal stratification usually results in inhomogeneous distributions of oxygen, nutrients and populations of algae, cyanobacteria and other organisms. For this reason, depth-integrated samples are generally more adequate than (surface) grab samples for the assessment of the size of a cyanobacterial population and nutrient concentrations. However, even when temperature profile is uniform throughout depth, stratification of organisms may develop on calm days. Depth gradients of oxygen concentration and pH are good indicators of this.

Depth-integrated samples are taken by either continuously sampling the entire water column from the surface to a defined depth or by taking several individual samples from defined depths and combining them into a larger volume. A vessel of sufficient volume needs to be available, such as a polyethylene barrel or canister.

Continuous depth-integrated samples are often adequate for shallow and waterbodies of moderate depths. A simple depth-integrating pipe or tube sampler for shallow water columns (up to 5 m depth) or for the surface layers of deeper waterbodies is shown in Figure 12.2. This sampler is made of a piece of flexible tubing of several centimetres in diameter and sufficient length, one end of which bears a weight and is open at both ends. Preferably, the tube is transparent to allow easy recognition of any contamination that may attach to the inner wall. The weighted end is lowered slowly into the water on an attached cord. When the tube has been lowered to the desired depth, it contains an integrated volume of the water column. Before hauling in the lower

end with the attached line, the upper end is closed to avoid the loss of water once the lower end emerges from the surface. Hoses need to be thoroughly cleaned after use and stored preferably dry between sampling trips to avoid cross-contamination, for example, by microbial growth. In case multiple sites or waterbodies are sampled during a sampling trip, the hosepipe needs to be conditioned at each sampling site by repeatedly lowering it on one side of the sampling vessel or dock before the sample eventually is taken at the opposite side to avoid cross-contamination. Alternatively, continuous depth-integrated samples can be obtained using a submersible water pump attached to a hose that is operated at a steady pumping rate while the water inlet is drawn upwards between the desired depths at a uniform speed.

In deeper lakes or reservoirs with thermal stratification, depth-integrated samples can be obtained by taking multiple grab samples at defined depths, for example, at 1, 3, 5 and 7 m below the surface and combined to an integrated sample. If background information on the typical stratification characteristics of a given lake is available (e.g., from long-term monitoring), sample numbers can be reduced by selecting adequate depths to represent specific strata. If depth intervals are unequal and samples are to be integrated, the volume of each subsample must be chosen to represent the actual fraction of the vertical stratum it represents.

In the case of surface bloom-forming cyanobacteria, wind-driven inhomogeneity can be considerable with a variation in concentrations of cells and toxins by orders of magnitude across the lake's surface. Before a single sampling location is chosen as representative for a given waterbody – generally a central location is chosen – this should be confirmed by sampling at different locations and by visual inspection. When available, remote sensing data of the waterbody can give indications on heterogeneous horizontal distribution of phytoplankton or chlorophyll-*a*, respectively (see Chapter 11).

12.4.3 Sampling bulk material

For a number of purposes, the sampling of bulk cyanobacterial material is of interest, for example, in-depth chemical analysis of toxins and other metabolites, isolation of cyanobacterial strains or toxicological studies.

Sampling scums is carried out most easily with a wide-necked plastic or glass container submerged only to a depth corresponding to the thickness of the scum.

Cyanobacteria distributed in the water column can be concentrated with a plankton net. The plankton net is lowered to the desired depth and slowly hauled to the surface. The depth at which the plankton net is deployed depends on the taxa of algae and/or cyanobacteria present. Floating cells (e.g., *Microcystis*, *Dolichospermum, Aphanizomenon*) are harvested within the upper metres of the water column, while the sampling of well-mixed or stratified waterbodies with distinct depth distributions of cyanobacteria (e.g.,

Planktothrix) may include deeper water layers. The mesh size of the net needs to be appropriate for the taxa present, and for most cyanobacteria of interest, 20 μm will suffice. A plankton net sample is not fully representative for the sampled waterbody, especially not in quantitative terms because the efficiency with which the net can retain organisms depends on their size: it will be less effective for filaments with a small diameter (e.g., *Limnothrix* sp.) or picoplanktonic organisms (e.g., *Synechococcus* sp.), and this reduced efficiency cannot be quantified. Further, mucilaginous species (e.g., *Microcystis* sp.) may rapidly clog the mesh, thus reducing further passage of water.

12.5 SAMPLING IN THE DRINKING-WATER TREATMENT TRAIN

If cyanotoxins are detected at the raw water intake at concentrations of concern, treatment train samples are relevant for validating the efficiency of cyanotoxin removal at each treatment step (see also section 5.1, Chapters 10 and 11). Of critical importance for treatment optimisation is whether cyanotoxins are predominantly extracellular or intracellular and therefore at the raw water should be analysed for both intracellular and extracellular cyanotoxins. As cells may lyse and release toxins during treatment, analysing both fractions in every treatment train sample may be relevant.

Preferably, sampling is timed with the flow through the plant, so the effect of processes on the same parcel of water can be determined. This is most important for systems that experience large fluctuations in intake water quality. Most water plant operators understand flow rates and hydraulic residence time through their plant, and sampling times can be adjusted accordingly. If the entire treatment train cannot be sampled, at least the major processing steps that are anticipated to provide the bulk cyanotoxin removal should be sampled, for example, prior and after flocculation and filtration, and after oxidation, prior to distribution (see also Chapter 10).

Any sample collected after oxidant addition should be immediately quenched during sample collection. The quenching agent used will depend in part on the method selected to analyse the sample and must be chosen in contact with the laboratory. For example, sodium thiosulphate is a commonly used quenching agent when analysing a sample using an ELISA-based method, but ascorbic acid is more typically used when analysing a sample via an LC-based method (see Chapter 14).

Treatment train sampling may require some specialised sampling equipment. Swing samplers on telescoping poles are especially useful for sampling the top of deep sedimentation basins or filter beds if a dedicated sampling line is not available. A simple bucket attached to a rope can also work in many situations. Whichever sampling equipment is used, it should be cleaned and conditioned between sampling sites (at minimum, triple rinse).

Sampling programmes may include further water quality parameters, including those that serve as potential surrogates for, estimating cyanobacterial cell and cyanotoxin removal throughout the treatment plant: Operators have used portable multiparameter datasondes to collect real-time phycocyanin and chlorophyll-*a* measurements throughout the treatment train. Such real-time data can be useful for quickly estimating the presence of cyanobacterial cells and their removal throughout the plant – although not for dissolved toxins. If a datasonde is not available, grab samples can also be collected from the intake and throughout the treatment train, and analysed in a laboratory with a spectrophotometer. Turbidity reduction is associated with particle removal, including cyanobacterial cells, and is generally a valuable operational monitoring parameter for the efficacy of filtration methods. Critical turbidity limits are therefore frequently used in treatment plants.

12.6 SAMPLE CONTAINERS

It needs to be decided in advance whether it is more practical to subdivide a water sample into subsamples for each subsequent analysis (plankton, toxins, nutrients, etc.) prior to transportation, or whether a single larger sample is to be divided upon receipt in the laboratory (Figure 12.3). In both cases, for subdividing a larger sample, it needs to be ensured that the sample is well mixed. Especially, buoyant cyanobacteria (*Microcystis*, *Dolichospermum*, etc.) can float up within minutes and hence bias subsampling.

Bottles – or containers in general – used for the storage and transport of samples are ideally chosen by the laboratory that will conduct the analyses to avoid later problems due to inappropriate materials or insufficient volumes, respectively (see Chapter 14.1). Accordingly, the cleaning and preparation of the containers is most efficiently defined by the analysing laboratory because the staff can best estimate the risks of carryover effects due to inappropriately cleaned sample containers. This is particularly important for highly sensitive analytical procedures that can detect trace amounts (e.g., for soluble reactive phosphate).

Preferably, containers are prelabelled and well arranged in a suitable rack or box to allow rapid and easy handling under field conditions. To avoid cross-contamination, it is advisable to always use the same bottle for an individual site and individual parameter. For most samples, glass bottles are most appropriate due to the chemical inertness of glass. However, for safety reasons, plastic containers may be more adequate and can be used for most sampling purposes, for example, wide-mouthed polyethylene or polycarbonate bottles. Sample containers have to be checked for their appropriateness, including their volume, ease of cleaning and testing for possible adsorption of analytes (toxins, nutrients, etc.) to the material.

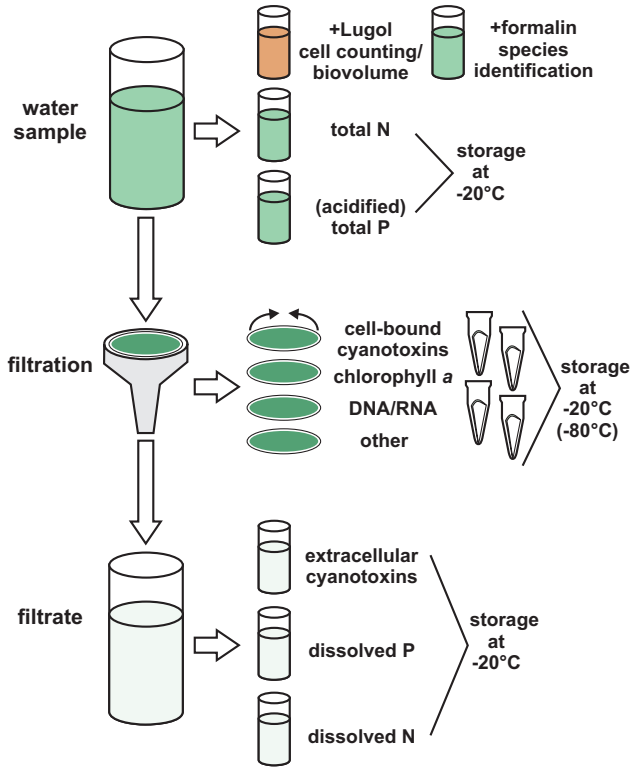

Figure 12.3 Scheme for the splitting of a water sample into multiple subsamples for particular analyses. The list of subsamples is not exhaustive; further parameters could be pigments, iron, dissolved organic carbon, etc. Filter types to collect cells must be chosen to be compatible with the particular downstream analyses. Subsamples can be kept at 4 °C; for later analyses, storage at –20 °C is adequate for most chemical analyses, while for molecular analyses (in particular, RNA), storage at –80 °C may be required.

All samples taken in the field should be stored cool and dark until returning to the laboratory. Sunlight and heat inevitably leads to changes of the samples and eventually to biased data. Insulating boxes such as camping boxes are widely used.

The following containers are recommended for the transport of samples taken for particular analyses. Before filling the individual containers with the samples for analysis, they need to be rinsed with the sample at least twice to minimise cross-contamination from previous samples.

Total phosphorus analysis (for various fractions, see Chapter 13): 100-mL glass bottles prewashed with and stored until usage containing a residual of sulphuric acid (4.5 M) or hydrochloric acid. Since the determination of dissolved phosphorus is done at low µg/L concentrations, care must be

taken to avoid cross-contamination of samples. Contamination may arise from phosphate-containing detergents or from previous storage of samples with very high phosphorus concentrations. Phosphates are easily adsorbed to glass surfaces, and the residual sulphuric or hydrochloric acid serves to minimise this effect.

Total nitrogen analysis: 100-mL glass or polyethylene bottles. Transformations between nitrate and ammonium may occur if samples are not properly stored (cooled).

Samples used to quantify dissolved nutrients have to be filtered as soon as possible. On-site filtration can be achieved with cellulose acetate syringe filter (ca. 0.2 µm pore size) or (manually operated) vacuum pumps and adequate filtration devices. Ammonium (NH_4^+), nitrate and nitrite concentrations have to be analysed rapidly (within 24 h of sampling) using a spectrophotometric method. Whether or not on-site filtration is required depends on the time delay between sampling, temperature control and the arrival in the laboratory; it is often necessary to find a compromise between the amount of sampling to be achieved on a given field trip and possible sample degradation on the way to the laboratory. Preferably, possible effects of delayed filtration are evaluated by parallel processing of a few samples.

While it may be possible to use filtrates for several different analyses (e.g., of dissolved nutrients and toxins; Figure 12.3), it may, however, be necessary to use specific types of filters (e.g., different pore size or filter material) for specific analyses.

Cyanobacteria (phytoplankton) identification by microscopy: 100-mL wide-mouthed polyethylene bottles for fresh grab or net sample (see below). Samples can be stored with ethanol at a final concentration of 30% v/v or neutral-buffered formalin at a final concentration of 4% v/v.

Cyanobacteria (phytoplankton) quantification by microscopy: 100-mL clear glass bottles prefilled with 1 mL of Lugol's iodine solution (see below) or neutral-buffered formaldehyde solution (final concentration 4% v/v), respectively (in this case, of course, rinsing with samples is not done). Alternatively, the preservative is added immediately after filling the bottles with the sample. Bottles have to be stored in the dark to avoid photodegradation of iodine. Brown glass bottles are more protective but render later control of stored samples for sufficient iodine residue more difficult (see Chapter 13; also Catherine et al., 2017).

- *Preparation of Lugol's iodine solution*: Dissolve 20 g of potassium iodide (KI) in 200 mL of distilled water; add 10 g of sublimated iodine and 20 mL of glacial acetic acid. Test the solution by diluting 1 mL with 100 mL water. The diluted solution must be clear and have the colour of whisky. Samples fixed with Lugol's iodine and stored need to be checked regularly for decoloration (see Chapter 13).

Cyanotoxins: 1.0-L (minimum for some chemical analysis; depending on methods used in the laboratory) wide-mouthed glass or polyethylene bottles are preferred. For the detection of cyanotoxins by ELISA, smaller sample volumes are sufficient (100 mL). Cyanotoxins may bind to other types of plastic containers, which could reduce the measured concentrations. If only plastic containers are available, before filling the container, rinse it at least twice with the sample. This procedure will encourage binding during the rinsing steps and minimise potential for under-reporting cyanotoxin concentrations in the sample. Containers must be cleaned thoroughly with nonphosphate detergent and rinsed with distilled/deionised water to prevent contamination, especially from dried cells attached to internal surfaces, between sampling events. Generally, the denser a phytoplankton sample, the less volume is needed for a chemical analysis of cell-bound cyanotoxins. If dissolved cyanotoxin analysis was envisaged, a subsample can be filtered in the laboratory, possibly requiring a larger sample volume. To represent source water conditions, filtration should occur as soon as possible, preferably within 24 h of sample collection and prior to freezing the sample which could lyse cells and release cell-bound cyanotoxins. Filtration can also be done on site, but since this is a time-intensive step, it may not be feasible.

Chlorophyll-a analysis: 1.0-L (minimum) wide-mouthed bottles are preferred. Samples must be stored dark and cool to minimise chlorophyll degradation during transport.

Bulk cell material for toxin content, structural analysis of toxins or toxicity assays: Wide-mouthed bottles with volume according to the desired amount of sample material. For smaller volumes, containers for urine samples are particularly cheap and suitable. If samples are to be freeze-dried later on, the sample is preferably frozen in layers not thicker than 2 cm to reduce drying time. To produce frozen plaques, robust household plastic bags can be used when stored in watertight cooling boxes and immediately transferred to a freezer in the laboratory.

12.7 SEDIMENT SAMPLING

Sediment sampling may be helpful if there is a likelihood of high internal nutrient loads to the waterbody of interest (see Chapters 4 and 8). Waterbody management strategies that aim to limit internal nutrient loading often require baseline sediment nutrient data if they are to be successful. A limnologist should be involved in the selection of appropriate sample sites. In general, one to three sediment samples should be collected in small waterbodies, and more locations may be needed to collect representative data for larger waterbodies. Within larger waterbodies, both deep and shallow sites should be selected, representing inlets and some shallower bays. Ideally, sediment cores

(at least 30 cm depth) should be collected and each 2 cm segment analysed for phosphorus (P) fractions, total aluminium, total iron and percent solids to help determine potential for sediment nutrient flux.

Sediment cores can also be analysed for cyanotoxins and may be able to provide a record of historic cyanotoxin occurrence within the waterbody (Waters, 2016; Zastepa et al., 2017).

Sediment corers, usually simple sampling devices, can be made in-house or purchased in a variety of materials. They are preferred compared to samples collected using a dredge sampler, because corers can maintain a representative vertical profile of the sediment stratigraphy, create less disturbance by shock waves and can collect more highly consolidated deposits. Sediment corers are slowly lowered to the substrate (gravity corers are released at the water surface and allowed to fall freely); they then penetrate the sediment under the sampler's own weight or are pushed or vibrated into the sediments. Commercial corers often contain core catcher inserts and one-way valves that allow the sample to enter the tube, but not exit and to hold it in place. Inserts should not be reused between sample locations unless properly cleaned. Inserts made of plastic should not be used when collecting samples for organic analysis. Upon retrieval, the corer can be disassembled (e.g., split spoons, some core tips unscrew) and the sample laid in a container or a prepared surface for further processing. Cores from simple tubes and most other corers often drop out or can be pushed out with a clean rod. Plastic or thin-walled metal corers (or core liners) can be cut, the ends capped and secured with tape, and the entire segment sent to the laboratory. This process and the split spoon sampler reduce contamination from one segment to another in vertically stratified samples (OEPA, 2018).

12.8 SAMPLING OF BENTHIC CYANOBACTERIA

Benthic cyanobacteria can be a source of cyanotoxins (as well as taste and odour compounds) and are typically more difficult to monitor than planktonic cyanobacteria. In shallow waters, such as wadable streams, a visual inspection to identify patches of possible benthic cyanobacterial growth is advised prior to the actual sampling. Wood et al. (2009) outline the estimation of cyanobacterial coverage of streambeds with the aid of an underwater viewer.

Limited established guidance is available on benthic sampling, but a variety of techniques have been used to varying degrees of success. Distribution of benthic cyanobacteria can be very heterogeneous, typically occurring in spatially limited patches with high density next to bare areas. Therefore, collecting samples from numerous sites and compositing may be appropriate for determining average conditions or assessing whether benthic cyanobacteria may be a concern. One method of collecting epilithic

cyanobacteria (i.e., those growing attached to hard substrate) from streams or littoral zones is scraping a predefined area of representative rocks or substrate. Since variability in epilithic cyanobacteria can be high, multiple rock scrapes from a sampling site can be composited into a single representative sample (Bouma-Gregson et al., 2018). Samples can be collected by hand in wadable areas and by SCUBA divers in greater depths. Epiphytic cyanobacteria are collected together with the macrophytes to which they are attached (see Chapter 4).

Benthic cyanobacteria can also be sampled using a dredge sampler (for larger areas) or sediment corer (for fine-grained sediments). Discrete depth samplers can also be lowered to the bottom of a waterbody to collect samples near the benthic zone. Unfortunately, the dredge, corer and discrete depth sampling methods can displace benthic cyanobacteria during the sampling process and may result in underestimating benthic cyanobacteria occurrence. Since distribution of benthic populations is generally highly variable, these methods may also miss significant benthic mats that are not visible from the surface.

Analytical results of benthic cyanotoxins generally relate them to sediment area, for example, μg/cm², to (cyanobacterial) biomass, for example, μg/g fresh or dry weight. A transformation to volumetric units, this is, true concentrations, can only be tentative.

12.9 SAMPLES FOR MOLECULAR ANALYSES

DNA and RNA sample collection may require specific on-site sample preparation and handling protocols due to the potentially rapid degradation of DNA and, especially, RNA. Ideally, samples collected for molecular analyses should be filtered on-site and the filters placed on ice (or as cold as possible). In some cases, DNA sampling protocols may call for in-laboratory filtration, as long as samples are received and filtered by the laboratory within a narrow time frame (preferably within 24 h). RNA sample collection is typically more rigorous, requiring immediate filtration after sample collection, placement of samples onto dry ice to quickly flash freeze filtered material, and holding on dry ice until sample can be transferred to a laboratory or low-temperature freezer prior to extraction and analysis. Due to the extremely high sensitivity of molecular methods, care must also be taken to ensure all sampling equipment is thoroughly cleaned and sterilised. Preferably, sterile, disposable sampling supplies (syringes, cartridge filters, etc.) are used to avoid cross-contamination. In all cases, the validity of sampling protocols should be verified by the laboratory conducting the analyses (see Chapter 14).

12.10 SAFETY CONSIDERATIONS

Caution and attention are appropriate while working with cyanobacteria, particularly when they are highly concentrated in scums. It is wise to treat all blooms as potentially toxic. Contact with water should be minimised during sampling, and gloves and rubber boots should be worn because cyanobacteria (and organisms associated with them) might also have a high allergenic potential.

However, during sampling, cyanobacteria actually are often not the most important hazard and general safety considerations for water sampling need to be implemented. In some areas of the world, other water-based hazards (e.g., organisms causing schistosomiasis or bilharziosis) may also be present. In such circumstances, water contact should be minimised, and following contact, the skin should be immediately rinsed with clear water and dried.

Although glass is generally the most inert material, avoiding glassware for fieldwork enhances safety. For most samples, polyethylene bottles are appropriate.

Inhaling spray or getting spray in eyes from boats, wind or irrigation water from areas with cyanobacteria blooms has to be avoided. Under conditions that promote spray formation, eye protection and a mask are recommended, especially while sampling cyanobacteria scums.

Last but not the least, sampling preferably is always conducted in a team – a basic rule for fieldwork. This has practical reasons when handling water samples, sample bottles, conserving agents, field log sheets, etc. Furthermore, sampling a waterbody involves the risk of serious injury and drowning, even in shallow but turbid waters where dangerous objects may not be visible from the surface and especially when working from a boat. Wearing a life jacket on a boat is strongly recommended and may be mandatory by local safety regulations.

12.11 QUALITY ASSURANCE AND CONTROL

Quality control policies are required for many operators of water supplies, laboratories and public authorities conducting surveillance, and they are important. A subset of samples can be collected for quality control purposes. Duplicate samples can be used to determine laboratory method precision. Replicate samples can be used to determine representativeness of sampling. Field samples may also be split for interlaboratory comparisons. Field blanks consisting of distilled deionised water and preservative, where appropriate, should be submitted along with regular samples to establish practicable detection limits and to monitor for levels of contaminants to which field samples may be exposed. In addition, if sample bottles are being reused, after cleaning, a subset of reused sample bottles should be periodically filled with distilled or deionised water and analysed for the parameters of interest

to verify the adequacy of the cleaning procedure. All field instruments used in the measurement of physical, chemical or biological parameters must be properly calibrated and maintained, with records kept of observations for each instrument. Laboratories should consider a regular participation in proficiency testing studies conducted by accredited providers or, more informally, in cooperation with other laboratories in the same region. Quality assurance sampling is especially important if the sample results will be used for regulatory purposes, to document human health impacts or where decisions based on the data could be disputed in court. The following sections describe different types of quality control samples and their intended purposes (OEPA, 2018).

Field duplicate samples (also known as field splits) are used to assess the variance of the total method of sampling and analytical procedures. Duplicate samples demonstrate the precision of the sampling system, from initial sample collection through analysis. A field duplicate is done by thoroughly mixing one sample, dividing it into two separate sets of containers and analysing as (blinded) independent samples.

Field replicate samples are used to measure sampling repeatability and natural variability within the sampled water. A field replicate is done by collecting two or more separate samples from the same site and time using the same sampling method (replicates A, B, …) and analysed as independent blinded samples. The variability of replicates should be compared to duplicate variability. Replicate sampling is often used to estimate heterogeneity, for example, in sediments. Field duplicate and field replicate sampling may be combined to allow a full assessment of the validity of the entire sampling procedure.

Blank samples are used to evaluate the potential for contamination of a sample by contaminants from a source not associated with the water being tested. Blanks may be used to demonstrate that no contamination occurs from equipment, reagent water, preservatives, sample containers, ambient air, etc. Field blanks are used to evaluate the potential for contamination of a sample by site contaminants from a source not associated with the sample collected (i.e., air-borne dust, etc.). Equipment blanks are collected to verify that cleaning techniques are sufficient and that cross-contamination does not occur between sites, for example, by using the same water sampler. At least one equipment blank per equipment type per field season should be collected. One equipment blank container should be prepared for each type of preservative used. Container blanks are normally tested by the analysing laboratory (see Chapter 14).

12.12 PERMISSIONS AND DECLARATIONS

Sampling a waterbody may require permission, either because private property has to be accessed or because national regulations generally restrict the removal of organisms from the environment. The Nagoya Protocol (UN, 2011),

an international agreement on the protection of economic interests possibly arising from natural biodiversity, has been implemented in the legislation of many countries.

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