

A Phytoplankton Methods Manual for Australian Freshwaters

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Glossary

Accuracy	The closeness of a measured or computed value to its true value.	Cyanoprokaryote toxins	Substances formed by cyanoprokaryotes that act as liver and nervous toxins in animals and humans.
Advected	Moved downstream with the current.	Diatom	An alga of the class Bacillariophyceae, characterised by a silicified cell wall (frustule) and yellow-brown chloroplasts containing fucoxanthin.
Alga	A non-stem-forming plant, may vary in size from microscopic single cell organisms to gigantic seaweeds.	Diatom frustule	The silicified cell wall of a diatom cell, consisting of two halves, the epivalve and the hypovalve.
Algal unit	The natural formation a species occurs in; for example, single cell, filament or colony.	Diurnal	On a daily basis.
Anthropogenic	Made or caused by human influence.	Detritus	Non-living organic matter.
Aqueous solution	Solution based on water.	Dominant	The species which is most abundant in the waterbody.
Autochthonous	(Energy or matter) derived from within the waterbody.	Enumeration	The scientific counting and simultaneous identification of algal cells.
Benthos	Organisms that live on and in the sediment of waterbodies, often only for part of their life cycle.	Epilimnion	The mixed surface layer in a stratified waterbody.
Biomass	The amount of biological material present.	Ergonomic	A physical workplace arrangement designed to prevent operator injury from long-term overstraining because of wrong posture.
Benthic algae	Algae that live on or in the sediment of a waterbody; often diatoms, but also mats of cyanoprokaryotes.	Eutrophication	The progressive enrichment of surface waters with nutrients. This process can be anthropogenic or occur naturally.
Chrysophyte	An alga of the class Chrysophyceae, typically having two flagella, a red eyespot, yellow-brown chloroplasts and often silica scales on the outside (eg. <i>Synura</i>).	Euphotic zone	The water column between the surface and the depth at which the light intensity is 1% of that at the surface (Schwoerbel, 1970).
Colony	A group of algal cells of the same species that normally live together but can function separately.	Filament	A type of algal colony in which cells are adjoined to each other to form a chain-like structure.
Cyanobacteria	See 'cyanoprokaryotes'.	Flagellates	Unicellular organisms; for example, algae that have a flagellum and are therefore motile.
Cyanoprokaryotes	A group of very primitive, alga-like organisms (previously called cyanobacteria) that do not contain any cell structures. Contain blue-coloured accessory pigments (phycocyanin and phycoerythrin) which mask the green chlorophyll. Some species form blooms which may be toxic.	Flagellum	A whip-like structure occurring in motile algae which propels the cell.

Gas vesicles	Gas-filled intracellular membrane structures in cyanoprokaryotes which regulate buoyancy.	Photomicrograph	A photographic picture taken of an object through a microscope.
Habitat	The specific environment an organism lives in. Examples of habitat for algae include any waterbody, moist soil, on stones or other plants and hot springs.	Phycologist	A biologist specialising in the study of algae.
Halocline	Layer of largest density change in a waterbody due to saline concentration.	Phytoplankton	Algae that live suspended in the water.
Homogeneous	Uniform.	Picoplankton	Phytoplankton species with a cell size from 0.2 μm to 2 μm .
In situ	Directly in the waterbody, not in the laboratory.	Plankton	Community of plants (phytoplankton) and animals (zooplankton) of microscopic size which are adapted to suspension in water and which are liable to passive movement by wind and current.
Inverted light microscope	Used to count algae; objectives are situated underneath the stage.	Plankter	A member of the plankton.
Lentic	Standing waterbody.	Poisson distribution	Similar to a normal distribution but truncated on the left-hand side close to the mean. Describes the probability of random occurrences in space or time; for example, the distribution of algal cells in a water sample.
Lotic	Flowing water.	Potamoplankton	True river plankton, which sustains itself and grows under river conditions.
Macrophytes	Rooted aquatic plant with stems and leaves; can be submerged or emerged.	Precision	The closeness of repeated measurements of the same quality.
Meroplanktonic	Organisms that live partly in the plankton and partly on the sediment.	Primary producer	Organism which builds organic substances from inorganic material using photosynthesis to capture light energy. All plants are primary producers.
Metalimnion	Steep density gradient between the epilimnion and the hypolimnion in standing waterbodies.	Retentive zones	Parcels of water under the banks or in backwaters which are incompletely mixed with the bulk of the flow and might act as incubators for phytoplankton.
Mixed zone	The well-mixed, warmer surface layer of a stratified waterbody.	Stratification	Formation of layers of different density (temperature, conductivity) within a waterbody which can prevent the exchange of small particles and gases between the layers.
Motile	The ability of an alga to propel itself, mostly by means of a flagellum or through mucus secretion (in diatoms).	Thermocline	The plane at which greatest temperature change occurs with depth in a stratified waterbody.
Multiple counter	A mechanical apparatus to register counts for several categories simultaneously. Commonly used for bacteriological and algal counts.		
Nanoplankton	Phytoplankton species with a cell size from 2 μm to 20 μm .		
Nomenclature	The rules on which taxonomic categories are based and which form an ordering system for organisms based on their evolutionary similarity.		

Taxon	Any category used in the classification and identification of living organisms; for example, class.
Transect	A strip across the bottom of a counting chamber.
Vacuole	An intracellular membrane structure containing an aqueous solution; often takes up a considerable volume of the cell.
Whipple graticule	A graticule, inserted in one of the microscope eyepieces, with a square of 1 mm x 1 mm etched onto it. The square is divided into 100 smaller squares.
Zooplankton	Organisms feeding on particles and living in the plankton; for example, the water flea <i>Daphnia</i> .

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List of abbreviations

ANZECC	Australian and New Zealand Environment and Conservation Council
APHA	American Public Health Association
ARMCANZ	Agriculture and Resource Management Council of Australia and New Zealand
ASU	Area Standard Units
EPA	Environment Protection Authority
GPS	Global Positioning System
ISO	International Organization for Standardization
LWRRDC	Land and Water Resources Research and Development Corporation
NATA	National Association of Testing Authorities
PAR	photosynthetically active radiation
PET	polyethylene terephthalate
TASS	Taylor sphere sampler
TISA	Taylor integrated sampler
UNESCO	United Nations Educational Scientific and Cultural Organization

Executive summary

The Phytoplankton Methods Manual for Australian Freshwaters describes suitable methods for the sampling, fixation, preservation, identification and enumeration of phytoplankton in Australian surface waters, and recommends procedures for quality control, data storage and occupational health and safety. While covering all aspects of phytoplankton monitoring in Australia and providing benchmark methods, the manual leaves many choices and flexibility to the user. Guidelines for all aspects of phytoplankton monitoring in Australian freshwaters are provided without attempting to impose a strict national standard. The manual has been developed after extensive consultation with and input from algal workers and water managers within the Australian water resource industry and gains part of its significance from this process.

Potential users of the manual are algal workers (people who do the counting) and water quality and water resource managers (people who design and run the programs and analyse the data). The document addresses their needs for undertaking routine monitoring as well as research-orientated programs.

Although the manual deals with all phytoplankton, it contains many specific recommendations on the sampling and counting of cyanoprokaryotes (blue-green algae), for example, dealing with potentially toxic scum or buoyancy. The Agriculture and Resource Management Council of Australia and New Zealand's 'National protocol for the monitoring of cyanobacteria and their toxins in surface waters' (Jones, 1997b) deals specifically with the management of cyanoprokaryote blooms by government bodies and is complementary to *The Phytoplankton Methods Manual for Australian Freshwaters*. Where appropriate, cross-references have been made and consistency in methods has been agreed with the Agriculture and Resource Management Council of Australia and New Zealand National Algal Manager.

The imperative for monitoring phytoplankton populations is to understand and manage the ecological functioning of our standing and running waters. The term 'phytoplankton' encompasses all suspended microalgae in a waterbody belonging to all taxonomic algal groups and includes the cyanoprokaryotes or blue-green algae. Phytoplankton, together with other aquatic plant life, are the primary producers in aquatic

ecosystems and form the basis of the food web. Freshwaters are complex systems in which a component can only be managed if its links to the others are well understood. Phytoplankton is particularly sensitive to inorganic and organic nutrient levels and heavy metals, and responds to changes in nutrient levels faster and at an earlier stage of pollution than other groups of organisms. Due to their short life cycle, planktonic algae respond quickly to environmental changes and are thus a valuable indicator of water quality.

Objectives of phytoplankton monitoring in Australia and elsewhere are numerous and vary from case to case (see section 2.2, 'Objectives of phytoplankton sampling'). While this manual provides guidelines for the design and conduct of phytoplankton monitoring programs, it is recommended that each program be designed by an experienced algal ecologist with appropriate statistical advice. A monitoring program will always be specifically designed for the waterbody in question and cannot be transferred to other waters. The manual contains, apart from the pure technical information, a wealth of limnological background information relevant to the design and running of phytoplankton monitoring programs.

For most monitoring programs, data on the development of the phytoplankton population (ie. species composition and abundance) are collected. Often, chlorophyll-*a* concentration is also determined as a measure of phytoplankton biomass. The objectives of a monitoring program will determine the associated costs. The more specific the identification of the algae is required to be, the higher the costs of analysing a sample. For example, if taxonomic identification to species level is required (as is the case for most blue-green algae programs) adequately trained staff must be employed and the time required for counting the sample and cost of the program necessarily increase.

Information on sampling site selection in running and standing waters and details on how to take representative samples are given in chapter three. Considerations for sampling weir pools and reservoirs are included. The preservation, transport and storage of algal samples is discussed in detail.

Field and laboratory procedures for the analysis of algal samples are detailed in chapter four, giving a choice of three different counting chambers, all

currently used in Australia. Statistical background information pertaining to algal counting is given, addressing issues of accuracy and precision of counting results. Chapter five provides recommendations regarding quality control and quality assurance matters, while occupational health and safety issues and training for field and laboratory work are discussed in chapter six. It is pointed out that regular and meaningful training of field staff does increase sample quality and accuracy of data and therefore justifies the additional costs involved. In the event of a laboratory wanting to change to a new method, chapter seven details how to proceed with the changeover.

Much useful and practical information is contained in the appendices. In addition to an extensive list of taxonomic algae literature, including relevant Australian material, field and laboratory standard data sheets and listings of required equipment are presented.

I. Introduction

In 1995, under the Monitoring River Health Initiative, a project was initiated to develop a phytoplankton bioassessment protocol for Australian rivers. There were two reasons for the project:

1. To include phytoplankton as a representative of primary producers in the spectrum of organisms to be used to assess 'river health'.
2. To meet the need for a generally agreed manual for phytoplankton assessment in Australian rivers for use by the water resources industry. The manual was published in 1998 as LWRRDC Occasional Paper 18/98 and is superseded by the current manual which, due to industry demand, includes, among other additions, a section on standing waters and estuaries. *While covering all aspects of phytoplankton monitoring in Australia and providing benchmark methods, the current manual leaves many choices and flexibility to the user.* The Agriculture and Resource Management Council of Australia and New Zealand's 'National protocol for the monitoring of cyanobacteria and their toxins in surface waters' (Jones, 1997b) is a document dealing specifically with the management of cyanoprokaryote blooms by government bodies and is complementary to *The Phytoplankton Methods Manual for Australian Freshwaters*. Where appropriate, cross-references have been made and consistency in methods has been agreed with the Agriculture and Resource Management Council of Australia and New Zealand National Algal Manager.

This manual has been developed at a time when lakes and rivers have become the focus of many water resource issues in Australia, in particular the need to ensure ecosystem sustainability. On the one hand, the public has become aware of the occurrence of toxic cyanoprokaryote blooms in surface water systems and is demanding action; on the other, there is less than complete scientific understanding of what determines the development of phytoplankton communities, of which the cyanoprokaryotes are only one group, in these systems.

The manual covers both lakes and rivers. Historically, it was believed there was no true potamoplankton (riverine plankton) because the

residence time in rivers was too short for algae to reproduce and the light and hydrodynamic conditions were too unfavourable for them to survive. The algae found in rivers were believed to come from sources other than the rivers themselves – either from upstream lentic waterbodies and tributaries, or from the benthos (Reynolds, 1988). *We now have confirmation that planktonic algal species do reproduce within rivers and that many species, although originating from upstream lentic waterbodies, develop substantial populations in situ* (Reynolds, 1988) and contribute significantly to the productivity of individual systems. The occurrence of true potamoplankton, as confirmed in recent literature, may be partially an outcome of the drastic hydrological changes imposed on many rivers by human intervention, resulting in increased residence times (Tubbing et al., 1994), as well as an effect of anthropogenic eutrophication. This could well be true in Australia, for instance, for the Murray, Lower Goulburn and Murrumbidgee rivers.

The concept of the hydrological dynamics of river flow has developed from a simple model of an open pipe, well-mixed in its entire cross-section, to one of a much more differentiated waterbody with a highly variable lateral profile of velocities and residence times. The observation that parcels of water are entrained in pockets of river bed or bank has led to *the concept of 'retentive zones'*, describing water that is incompletely mixed with the bulk of the flow and that might travel at a different velocity to that of the main current (Reynolds, 1988). It is important to keep these concepts in mind in order to sample algae in rivers in a representative fashion, as the physical environment largely determines the distribution of phytoplankton within the water.

Standing waterbodies (lagoons, lakes and reservoirs) are equally important in our consideration of surface waters. In many regions of Australia, they are the major sources of drinking water, apart from providing irrigation supplies and areas for recreational use. In particular, many of our reservoirs have been plagued by toxic cyanoprokaryote blooms in recent decades, jeopardising the use of the water for human purposes.

The ecology of phytoplankton in lakes has been studied for over a century and is relatively well-known.

However, *reservoirs or storages do not always function in the same way as lakes*, since their water level variations are often unseasonal and water levels may be drawn down so low that large areas of lake sediment are exposed. Such events may drastically alter the ecology of a reservoir for years to come.

In general, phytoplankton development in standing waters is determined by the same physical and chemical parameters (eg. temperature, light availability, nutrients) as in rivers, although these parameters create a different type of environment. Most standing waterbodies undergo diurnal or seasonal thermal stratification. A consequence of stratification might be the permanent loss of algal cells from the euphotic zone due to sinking below the thermocline. Also, during stratification, nutrient concentrations in the epilimnion may be reduced to growth-limiting concentrations by algal growth. Wind action may determine vertical and horizontal distribution of phytoplankton. In standing waters at the scale of the whole waterbody, two nutrient pools may influence phytoplankton growth:

1. those nutrients present in the water column; and
2. those trapped in the sediment if they are released into the hypolimnion under anoxic conditions and distributed throughout the waterbody in the next mixing event.

At times, other biota such as zooplankton and parasites may alter phytoplankton cell density considerably.

In the past, the monitoring of algae in Australian surface waters has not always had clearly defined objectives, and comparison of results obtained by different laboratories has been difficult because of the use of different methodologies. It is hoped that these difficulties will be largely overcome with the implementation of this manual and that information gathering by individual organisations will become compatible across Australia. *The manual has been developed after extensive consultation with and input from algal workers and water managers in the water resource industry* and gains part of its significance from this process. Monitoring and assessing the development of phytoplankton will remain an integral part of biological monitoring in Australian surface waters. It is recommended that the manual be re-evaluated from time to time, initially in the year 2002.

2. Objectives of phytoplankton monitoring

The objectives of the manual are described in this section, together with the reasons for and objectives of phytoplankton monitoring programs. The design of such programs is addressed with the suggestion that a pilot study be conducted for unknown waterbodies before a conceptual model is formed. Water quality parameters to be measured in conjunction with algal samples are listed.

2.1 Objectives of the phytoplankton methods manual

The objective of this manual is to describe suitable methods for the sampling, fixation, preservation, identification and enumeration of phytoplankton in Australian surface waters and to recommend procedures for quality control and data storage. While providing guidelines for all aspects of phytoplankton monitoring in Australian freshwaters, the manual does not attempt to impose a nationwide standard. While implementation of the manual will lead to greater uniformity in the methods applied in both field sampling and laboratory work around the country, details of individual programs will depend on program objectives, client needs and available resources and methods may need to be modified accordingly. *For some procedures a benchmark method has been highlighted* against which other methods, if used, should be compared for equality of results. Specific methods for managing potentially toxic cyanoprokaryote blooms are addressed in the Agriculture and Resource Management Council of Australia and New Zealand cyanobacterial protocol.

The manual has been written to address the needs of algal workers (people who do the counting) and water quality and water resources managers (people who design and run the programs and analyse the data) to undertake routine monitoring as well as research-orientated programs.

2.2 Objectives of phytoplankton sampling

Phytoplankton, together with benthic algae and macrophytes, constitute the autochthonous primary producers in aquatic ecosystems and, as such, form part of the basis of the food web in terms of energy and material input. To understand the biological functioning of individual rivers, lakes and reservoirs, and detect changes in them, it is essential to investigate the development of their phytoplankton populations. Phytoplankton are particularly sensitive to changes in nutrients, responding rapidly when levels increase. *Due to their short life cycle, planktonic algae respond quickly to environmental changes and are thus a valuable indicator of water quality.* Most of our standing waters contain viable phytoplankton populations which, in many instances, are monitored regularly to ensure the availability of safe water for human consumption. It is well-known that many of our rivers contain substantial phytoplankton populations which might, in their lowland sections, play a major role in the carbon cycle and thus need to be assessed as part of effective river management. If the processes that drive phytoplankton development in Australian rivers were better understood, managers might be in a position to manipulate environmental conditions to achieve particular outcomes.

The term 'phytoplankton' encompasses all suspended microalgae in a waterbody belonging to all taxonomic algal groups and includes the cyanoprokaryotes, or blue-green algae, which might be present in insignificant numbers or constitute the dominant group in a waterbody at a particular time. The importance of cyanoprokaryotes for water managers arises from their possible toxicity to animals and humans and the ecological and aesthetic consequences of their blooms to individual aquatic systems.

To obtain baseline data for water management, the entire phytoplankton (eg. 'total cell count' or 'phytoplankton biomass') needs to be assessed. This is because the system is a complex one in which a component can be managed only if its links to the other components are well-understood. To understand and

manage nutrient, carbon and energy cycles in a running or standing water, the entire phytoplankton community needs to be evaluated.

The objectives of particular phytoplankton sampling programs will vary and may include some of the following:

- monitoring the concentration and composition of phytoplankton as indicators of water quality, in particular for nutrients and heavy metals;
- assessing ecosystem health;
- monitoring the abundance and composition of major phytoplankton groups to assess the consequences of, for example, land use and eutrophication on water quality within a river basin or reservoir catchment;
- monitoring the effects of remedial management measures aimed at improving water quality or restoring system health;
- providing data to determine long-term trends in phytoplankton composition and abundance within a particular waterbody, including 'problem species', in order to assess, for example, the effect of sewage discharge and agricultural run-off containing fertiliser and harmful chemicals;
- monitoring the effects of management measures such as river regulation, river-to-river transfer, reservoir regulation and water abstractions within a particular system;
- detecting the presence of and examining short-term trends in the growth of 'problem species' (eg. algae or cyanoprokaryotes producing taste and odour problems or toxins, and algae responsible for filter clogging in drinking water supplies) to determine the suitability of a particular water for drinking, recreational use, spray irrigation or stock watering;
- providing ecological data on particular phytoplankton groups;
- monitoring the raw water intake for public health risks in drinking water supplies; and
- increasing the knowledge of phytoplankton ecology, limnology and the state of the environment.

2.3 Design of phytoplankton monitoring programs

The details of a program will vary from case to case and from waterbody to waterbody. The design of an individual phytoplankton program requires careful consideration of the precise aims of the program and of the inherent and potential variability of the system being studied. *It is recommended that each individual phytoplankton program be designed by an experienced algal ecologist with appropriate statistical advice.*

For an unknown waterbody, a 'pilot study' should be conducted to determine the hydrodynamics of the system, horizontal and vertical distribution of phytoplankton, species composition, range of cell densities and frequency of change in species composition and abundance. Details of the sampling program such as sampling frequency (daily, weekly, fortnightly or variable), spatial distribution of sampling sites and location of sample (surface or depth integrated) will then be based on the background information gathered in the pilot study or on data from previous investigations. Depending on the project objectives, the sampling regime may vary over time. For example, if the effects of floods are studied, samples would be taken around flood events rather than at regular intervals.

The major considerations in designing a sampling program are the program objectives and associated information needs and how the program will meet them, taking into account the heterogeneity and variability of the physical environment, and the distribution and likely behaviour of the phytoplankton within it. The morphological, hydrological and geographical characteristics of a waterbody largely determine the spatial and temporal development of the phytoplankton within it. Consequently, a monitoring program will always be specifically designed for the waterbody in question and cannot be transferred to other waters. A useful guide in designing a program is to ask what level of information will be obtained with a given sampling pattern. For example, if phytoplankton abundance and composition in a stratifying weir pool were to be studied, a subsurface sample would be insufficient to obtain the required information. In contrast, a subsurface sample would be sufficient to obtain the same information from a well-mixed river. The spatial spread of sampling sites will relate, among other factors, to water travel times down

the river or to lake morphology. The temporal spread of sampling will most importantly consider cell doubling times of algae which vary from less than a day to more than a week. Depending on weather and conditions in the waterbody, the population concentration and taxonomic composition can change drastically within a few days. Therefore, a weekly sampling frequency is generally accepted as adequate for phytoplankton programs (see 3.3, 'Sampling in estuaries'). In addition to investigating species composition and abundance, a sample to determine algal biomass such as chlorophyll-*a* may be taken.

Detailed advice on designing sampling programs is given in *Standard methods for the examination of water and waste water* (APHA, 1995), and in ISO 5667-1, *Water quality – Sampling – Part 1: Guidance on the design of sampling programs* (ISO, 1980). Additional advice on the statistical and comparative aspects of biomonitoring are provided in the Australian and New Zealand Environment and Conservation Council 'National Water Quality Management Strategy Guidelines for Fresh and Marine Water Quality', a draft of which is currently in use. The design of programs to monitor potentially toxic cyanoprokaryotes and associated health risks in drinking water supplies and recreational waters is described in detail in Jones (1997b) for Australia and in Chorus and Bartram (1999) for other parts of the world. Jones (1997b) gives cell concentrations for the alert levels framework system which the Australian states agreed on in 1998.

Physical and chemical data sampled simultaneously

Generally, the following water quality data are collected concurrently with algal samples to help with the understanding of the phytoplankton data: discharge rate and velocity (for rivers), temperature, pH, electrical conductivity, dissolved oxygen concentration, turbidity, photosynthetically active radiation (PAR), determination of light extinction coefficient, Secchi disk transparency, and, if required, concentrations of the nutrients phosphorus, nitrogen and silica. Silica is an essential nutrient for diatoms, which often dominate riverine plankton. In standing waters, water temperature is usually measured at discrete depths over the whole water column to determine the presence of stratification. Often the ratio of euphotic zone to mixed zone is calculated as an indication of light availability to the phytoplankton (Harris, 1978). Total phosphorus concentrations give an indication of the carrying

capacity (maximum possible algal population size) of the water and thus, in conjunction with other parameters, the potential for algal blooms (Chorus and Bartram, 1999).

Conceptual model

Once an understanding has been gained of the spatial and temporal distribution of the algae and their responses to environmental factors in the waterbody in question, a conceptual model can be formed and specific sampling programs tailored. For instance, if previous studies have shown little change over winter in the phytoplankton community under investigation, a monthly sampling frequency for this season may be adequate. *Again, it is recommended that an experienced algal ecologist be involved in this type of interpretation and design work.* Monitoring programs should be reviewed regularly for their effectiveness in providing the required data and for the appropriateness of objectives under current circumstances.

3. Taking samples

This section provides information on sampling site selection in running and standing waters under different flow and mixing conditions. Special consideration is given to the sampling of weir pools and reservoirs. Details are given on how to take a representative sample in relation to program objectives and on the preservation, transport and storage of algal samples. This section also contains limnological background information relating to phytoplankton populations in both running and standing waters.

3.1 Sampling in rivers

3.1.1 Site selection

Site selection will depend on the objectives of the individual phytoplankton program. Two aspects are to be considered:

1. choosing *the site* along the stream or within the river basin; and
2. choosing the *exact location* of sampling at the sampling site.

Sites are often chosen:

- upstream and downstream of a *point source* such as a sewage treatment plant, weir pool or tributary;
- upstream and downstream of a source of major *ecological impact* such as a reservoir or weir pool; or
- at certain intervals along the river stretch under investigation, in order to explore longitudinal distribution of phytoplankton.

If samples are collected upstream and downstream of a point source or tributary, care needs to be taken to choose the downstream site at a point where complete mixing has occurred, or to take samples at two or more locations across the width of the river if lateral mixing is incomplete. Vertical mixing of incoming waters might also be incomplete in a slow-moving river as a result of thermal or other density stratifications. In some Australian rivers, a vertical conductivity gradient develops under low-flow conditions because of the inflow of saline groundwater. If the influence of an in-

stream reservoir or weir pool on the phytoplankton population is to be traced further downstream, water travel times should be taken into account when selecting sampling sites.

Since the habitat of the phytoplankton is the running water itself, and the algae are continuously carried downstream (advected) with the water, phytoplankton is not site-specific; rather the phytoplankton community sampled at a particular site is the result of conditions experienced further upstream. Therefore, when taking phytoplankton samples in rivers, the actual site is often less important than the upstream stretch of river. This should also be kept in mind when taking samples for physical and chemical water quality variables. A river is often conceptualised as a continuum which is intercepted at certain sites for sampling to provide a 'snapshot' of present conditions.

3.1.2 Location within the river

Considerations of where in the stream to sample and how many samples to take are guided by the principle of taking a *representative sample of the community*. Within the river it is preferable to sample from the main current by boat, from a bridge or using a sampling device which locates itself in the current (eg. Taylor integrated sampler, Appendix H). *Rivers are not homogeneous waterbodies and they may stratify thermally and chemically, either horizontally or vertically.* Depending on circumstances, it may be appropriate to take several samples across the river to account for horizontal heterogeneity caused by, for example, variations in flow velocity. If the river is not well-mixed vertically (eg. under low-flow conditions or stratification in weir pools), depth-integrated or discrete depths samples should be taken.

The counting of phytoplankton is generally time consuming and the degree of accuracy chosen should be related to the representativeness of the sample. A better statistical result may be achieved by collecting several samples from slightly different spots at one site and counting them to a lower level of precision (Vollenweider, 1969).

3.1.3 Sampling methodology

The sampling method chosen should:

- a. guarantee a representative sample; and
- b. be easy to handle.

The method will vary according to in-stream conditions. *The preferred method is a depth-integrated sample taken from the main current.* Water to be analysed for all variables (eg. algae and chemical and physical data) should be collected in a single grab then subsampled.

If the river appears *well-mixed* vertically and horizontally (presence of turbulence, lack of temperature variability), a sample taken at mid-stream 0.5 m below the surface (APHA, 1995) will suffice to characterise the phytoplankton present. For specific purposes, for example sampling at a water supply offtake for drinking water, samples from a particular depth may be needed. Avoid sampling backwaters or backcurrents when sampling for phytoplankton. For procedures to monitor cyanoprokaryote blooms in rivers, refer to Jones (1997b). If a boat is not available, obtain a sample from the shore with either a dip stick sampler (more than 3 m long), carrying a one litre glass sample bottle at the end, or a Taylor sphere sampler (see Appendix G). Buckets are not recommended for sampling from the shore, or if the water surface is contaminated with non-planktonic material. In the former instance, the water contained in the bucket when pulling it out of the water is not the same as when it first filled up out in the stream because of flow dynamics developing in the bucket. Further investigations will be needed to ascertain whether the phytoplankton is evenly distributed, vertically and horizontally, in a well-mixed river (see O'Farrel and Lombardo, 1998).

Under *conditions of incomplete mixing or slow flow* the collection of a depth-integrated sample from the main current is recommended. Sampling may be by hosepipe sampler (Appendix F), requiring two operators and a boat, or using a Taylor integrated sampler (Appendix H), an apparatus designed to take depth-integrated samples from the middle of the stream without the need of a boat. Difficulties may be encountered when using the hosepipe sampler in a strongly flowing stream as the hosepipe will be dragged with the current, making vertical sampling impossible. If there is uncertainty about even distribution of the phytoplankton across the river, take a sample from each bank and from the middle, mix together even volumes in a container, and then subsample the usual sample volume.

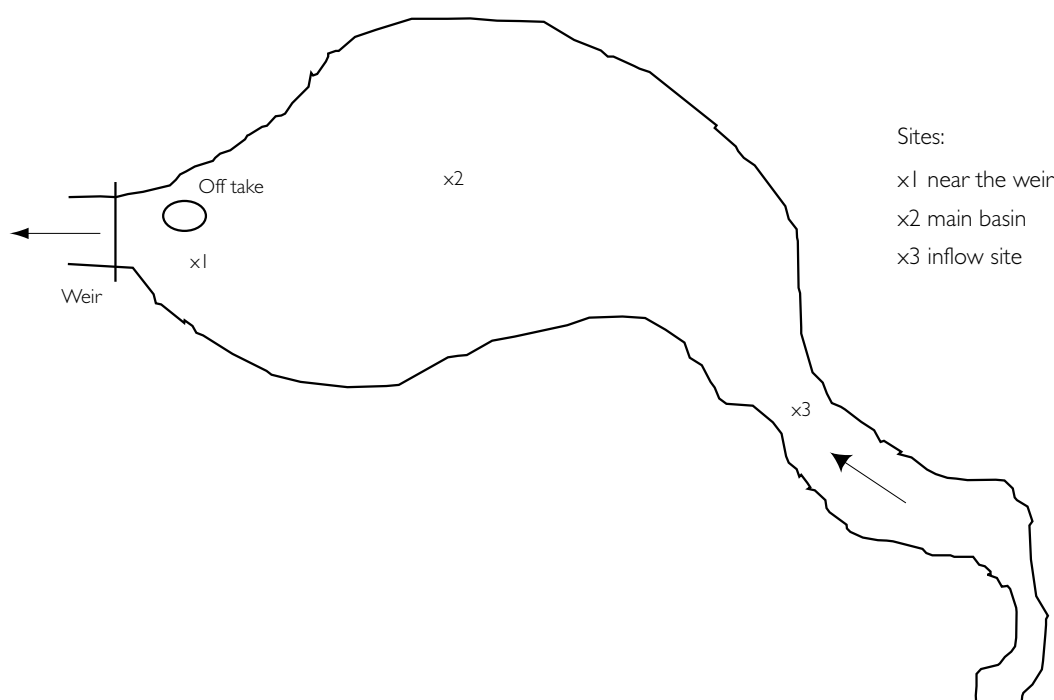
The sampling regime in weir pools depends on the prevailing flow conditions. Under high flow or conditions of incomplete mixing, sample as for a river. Under low flow conditions, where the water is likely to stratify diurnally (on a daily basis) or persistently (day and night), depth-integrated samples are taken. For detailed information, see section 3.2, 'Sampling in standing waters'. A temperature profile is taken to verify the presence of stratification. It is also recommended that an oxygen profile is taken to check for anoxic conditions in bottom waters. If stratification is present in a weir pool, certain algae might layer at certain depths. If project objectives are not achieved by taking an integrated sample under such circumstances, then samples from *specific depths* are taken with a standard discrete-depth water sampler, such as a Ruttner or Van Dorn sampler or a Niskin bottle (see APHA, 1995). For some monitoring programs where the whole weir pool is to be monitored, it may be appropriate to take one depth-integrated sample close to the weir wall for full counts to be entered onto a database and, for operational use, several subsurface samples around the weir pool for immediate estimation of cell numbers.

The methods of sampling recommended for different locations within a river are summarised in Table 3.1 together with codes suggested for use in data recording. Each three-letter code stands for a combination of sampling method and location.

Table 3.1: Methods for taking phytoplankton samples in rivers

Equipment	Location within the river		
	Shore/Jetty	Bridge	Boat
Dip stick (DS)	SDS		
Sphere sampler (SS)	SSS	BSS	
Discrete depth sampler (DD)		BDD	KDD
Integrated sampler (IS)	SIS	BIS	
Hosepipe (integrated sampler) (HP)		BHP	KHP

Notes: Codes indicate where to use each method. The three-letter code indicates where and how the sample was taken. The first letter indicates the location: S, shore; B, bridge; K, boat. The remaining two letters indicate the method used. The code is for use on the 'Phytoplankton Field Sampling Sheet' (Appendix E) and in the sample management database. For a technical description of samplers see Appendices F, G and H.

Figure 3.1: Suggested sampling sites in a weir pool

Depending on the life cycle of the algae present, it is advisable to *sample both water column and sediment surface*. It has been reported from tropical rivers in Central Queensland (Larelle Fabbro, pers. comm.) that cyanoprokaryote species (eg. *Limnothrix*, *Planktolyngbya*, *Nostoc*) form metalimnetic and merolimnetic populations under certain flow conditions and later appear in the plankton. In other rivers, for example the Lower Murray, large numbers of akinetes have been found on the river sediment. These will form a cyanoprokaryote bloom under the right conditions. In such cases, it is advisable to take sediment surface as well as water column samples at the same site.

Whole *water samples* (unfiltered) should be collected for quantitative evaluation of cell density. Sample volume should be between 100 mL and 1,000 mL (up to several litres in upland streams), depending on the number of cells present as judged from the results of the pilot study. In general, cell density is expected to be lowest in fast-flowing, clear upland streams and highest in large lowland rivers. A *live sample* might be taken, especially when flagellates or other delicate cells are likely to be present, and filtered or centrifuged for same-day species identification. These organisms may not be readily identifiable from preserved material as cell shape and colour can be distorted and cells may lose their flagella.

For chlorophyll-*a* analysis, a separate sample of 0.5 L to 1 L is taken.

It is advisable to use a *standardised phytoplankton field sampling sheet* (see Appendix E) for each program to ensure all samples and measurements taken in the field are properly recorded in the field. The field sampling sheet will also facilitate sample registration in the laboratory and later data reporting. The sheet presented in Appendix E is an example only and should be modified for individual programs.

In addition to taking water samples for phytoplankton and water quality parameters, it is useful to record observations such as water colour, smell and scum formation as well as wind direction and strength.

3.2 Sampling in standing waters

Standing waters with regard to this manual include lakes, reservoirs, billabongs, lagoons and weir pools as well as rivers under unusual low-flow conditions.

3.2.1 Site selection

Sampling sites may be chosen following a statistical approach or according to a set of criteria related to the aim of the program. The aim of the statistical approach is to entirely select sites by random, for example, by using a grid network or transects and choosing sites thereon at random and changing them on each occasion. In practice, it may be convenient to change sites only annually or every two years (Jones, 1997b). The statistical approach requires a large number of sites

to be sampled and is particularly useful for a pilot study. For most monitoring programs, however, due to resource limitations, only a minimum number of permanent sites are established. Most often, these sites are chosen with the objectives of the sampling program in mind to best represent the true phytoplankton population in the waterbody, considering the information from the pilot project and the criteria listed below:

- to represent all lake basins or different arms of a reservoir of importance (morphological and water quality heterogeneity);
- to sample inflowing and outflowing waters;
- (in a reservoir) to investigate and monitor water quality of the outlet point (about 100 m from the dam wall) and water supply off-take point;
- to monitor water quality and health hazards of recreational waters;
- to monitor accumulation of algal matter at the downwind end of lake or shore; and
- to sample the deepest point.

The number of sampling sites and samples taken is a function of the aims of the study, the morphology of the waterbody and the financial resources available. In the following paragraphs, many detailed recommendations are made on how to select sampling sites. For an individual program design, only some of these may be applicable.

For a round basin, choose sites along two perpendicular transects which extend from one shore to the other. For a long and narrow basin, choose at least three sites covering the length of the basin, including one site near the inlet and one near the outlet. A change in species composition between sites close to the inflow (fluvial species) and those distant from the inflow (lacustrine species) has been reported for Australian reservoirs (May, 1988). For a weir pool (see Figure 3.1) select at least one site near the weir; if the water backs up for a considerable distance, select sites as for a long and narrow basin.

In the presence of different morphological basins or several arms, it is recommended that several sites be established (see Figure 3.2). In a reservoir with two arms, for example, a site would be chosen in each of them and another near the dam wall to monitor the water quality of the downstream releases. Bays and inlets are often poorly mixed with little exchange with the main basin and therefore might develop a water quality which is

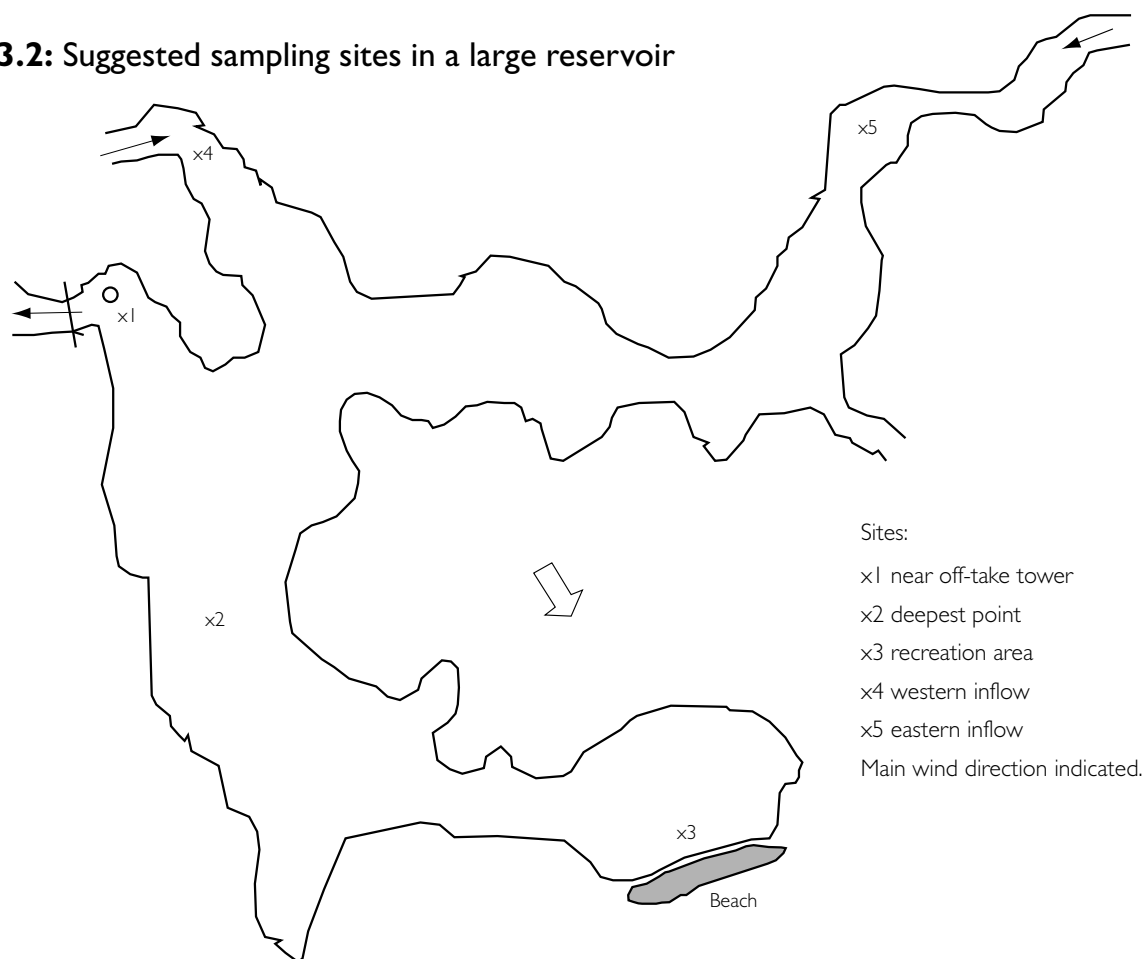
different from that of the main basin. A site may be established here, if the bay is important ecologically or for human use. If there is a prevailing wind direction, one site should be situated at the downwind end of the waterbody if the area is used for recreation. Commonly, a site is established at the deepest point (if different from the dam wall site) to take depth profiles and, if applicable, at the drinking water off-take.

Sampling sites should be spaced far enough apart to ensure spatial independence. Thus, the distance between sites depends on the size of the waterbody, sampling objectives and representativeness of the phytoplankton population. For many reservoirs, a minimum distance of 100 m between sites is recommended. Samples should be taken in the open water more than 50 m from the shore to avoid contamination of the sample with benthic species and wind-accumulated scums. For sampling of near-shore areas to monitor for health risks of recreational areas due to cyanoprokaryote blooms, refer to the criteria for sample collection in Jones (1997b).

When sampling phytoplankton in lakes and reservoirs, one must consider the patchiness of the phytoplankton distribution across the area of the water body (Wetzel, 1975). Patchiness refers to the uneven (for no apparent reason) horizontal distribution of phytoplankton across a waterbody. Thus, to adequately represent the phytoplankton assemblage, samples must be taken at several sites and depths (Moss and Hunter, 1992).

If the waterbody is heterogeneous, it is recommended that several sites are sampled. The samples are then treated as individual samples and each counted separately. If resources are very limited, a representative sample is obtained by sampling several sites and pooling these samples at equal volumes into a composite sample rather than sampling only one site. Subsamples of the composite sample are then treated as usual for algal identification and enumeration or for chlorophyll-*a* analysis. The counting of individual samples from several sites provides greater statistical power to the results than counting only one 'pooled' sample for the whole water body. The latter reduces algal counting costs significantly.

Standing waters, under certain weather conditions, may thermally stratify. Many weir pools in Australia also stratify under certain weather and flow conditions. During stratification, exchange between the warmer top water layer (epilimnion) and the deeper waters (hypolimnion) becomes much reduced due to the

Figure 3.2: Suggested sampling sites in a large reservoir

temperature-driven vertical density gradient. The layer of transition between the epilimnion and hypolimnion is called the metalimnion or thermocline. The epilimnion and hypolimnion often develop different physico-chemical characteristics during stratification. While deeper water bodies often stratify persistently (over many days or weeks) shallow (less than 2 m) water bodies may stratify diurnally or continuously for only a few days (Sherman and Webster, 1994). Usually, in shallow waters such as billabongs and lagoons, convective cooling overturns the water column each night.

Stratification is considered a dynamic phenomenon. Its stability and duration depend not only on the size of the temperature difference between the top and bottom layers, but also wind force, intensity of solar radiation and, in some water bodies, the mixing effect of seiches (internal waves). Australian research has shown that a temperature difference as small as 0.05°C , equivalent to a minimum temperature gradient of $0.25^{\circ}\text{C m}^{-1}$, was sufficient to separate the surface mixed layer from the bottom layer in Chaffey Dam (Sherman et al., 1999). In many circumstances, a temperature difference of 0.2°C is appropriate to define the bottom of the surface mixed layer (B. Sherman, pers. comm.). Heating of the top

water layers by solar radiation is intensified in coloured or highly turbid waters (such waters are typical in many parts of Australia) due to increased energy absorption. Thus in shallow and turbid billabongs or lagoons, heating of the top layer and the onset of stratification occur faster than in comparable waterbodies with clear water.

Phytoplankton is not evenly spread through the water column. In most waterbodies with stable stratification, a large part of the phytoplankton biomass is found in the euphotic zone because its downward transport is prevented. Many members of the plankton (eg. motile algae) actively vary their position in the water column and often avoid the surface layer. Others (eg. buoyant cyanoprokaryotes) may accumulate at the water surface under calm conditions or concentrate at a particular depth, for example, *Anabaena* (see Figure 3.3).

3.2.2 Sampling methodology

A vertical temperature profile should be taken at 1 m intervals to verify the presence of stratification (Bartram and Ballance, 1996). The presence of stratification is best recognised if the rate of temperature change with depth at the thermocline is significant

($0.2^{\circ}\text{C m}^{-1}$, B. Sherman, pers. comm.). In shallow waters, a temperature difference between the top and bottom layers of 1°C or greater under low wind conditions may be taken as an indication of stratification.

The specific sampling method for phytoplankton depends on the depth of the water body. In shallow waters (lagoons, billabongs) with a depth of less than 2 m, a subsurface grab sample at 0.5 m is taken if the water body is well mixed. However, if the water body is stratified or motile algae are present, a depth-integrated sample or discrete depth samples are taken. Surface samples such as subsurface grab samples are not adequate in water bodies deeper than 2 m because such samples are unrepresentative of the vertical spread of the phytoplankton population. *In deeper waters, it is recommended that a depth-integrated sample is taken from a boat over either the epilimnion (the mixed surface layer during stratification) or, if there is no stratification, the euphotic zone.* The euphotic zone is commonly determined as 2.5 times the Secchi depth and is the water column between the surface and the depth at which the light intensity is 1% of that at the surface (Schwoerbel, 1970).

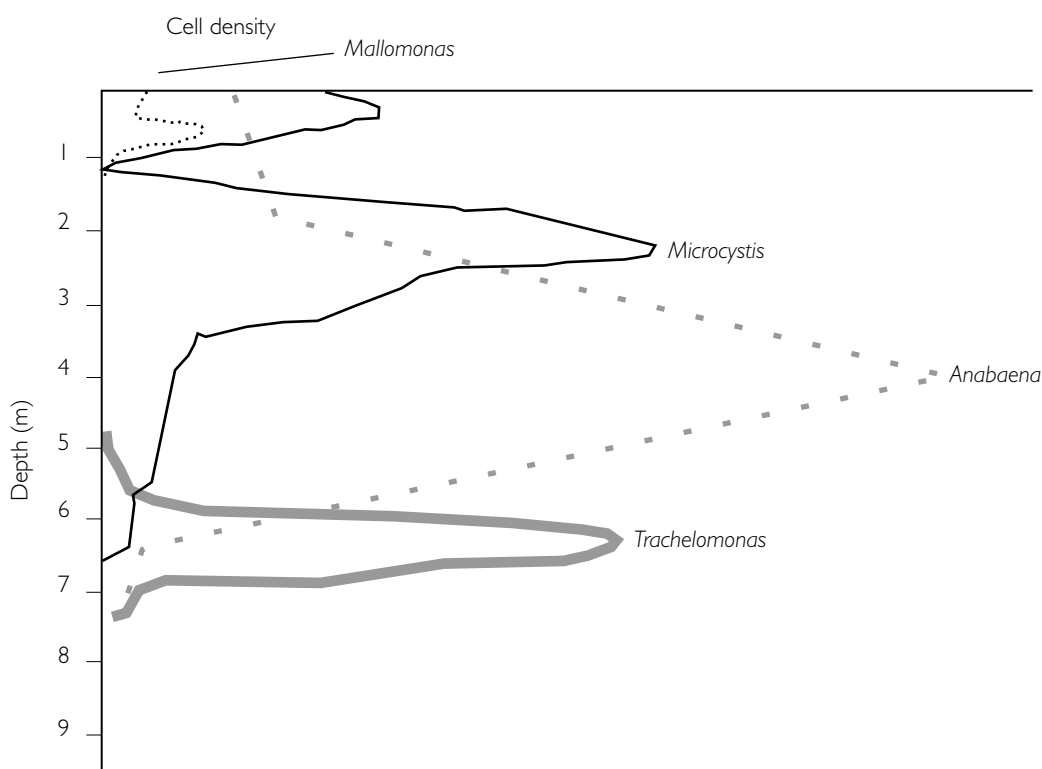
At each site, three samples are taken within an ecologically relevant sized area, mixed at equal volumes

in a clean container and the required volume subsampled. For work on large reservoirs, where usually only a limited number of sites are sampled, at each site take five integrated samples within an area of 100 m by 100 m and mix even volumes into a composite sample. In this way, the sampling regime takes into account the horizontal patchiness of the phytoplankton without increasing the number of samples.

For deep lakes and reservoirs, in addition to the integrated sample over the epilimnion or euphotic zone, deeper grab samples at 5 m intervals are recommended for at least one site. In some instances, discrete grab samples above 10 m are mandatory if layering of algae has been identified as a problem (eg. *Anabaena* in a weir pool or reservoir) (see Figure 3.3). Similarly, a halocline might exist with some algae layered above it.

In reservoirs, in addition to a depth-integrated sample, discrete samples are often taken at the depth from which the water is to be withdrawn or from the depths at which alternative water off-takes exist. The discrete depth samples give an indication of algal cell density in the water to be released into the downstream river stretch or used as raw water for drinking water treatment, while the integrated sample detects the possible presence of motile algae or distinct algal layers due to seiches over the whole depth of the water column.

Figure 3.3: Possible vertical distribution of different algae in a stratified lake



Do not sample for phytoplankton in an area with algal scums unless the program objectives include the targeting of algal blooms and scum material is needed. The following observations with regard to wind conditions are important when sampling specifically for buoyant algae. If buoyant algae are present and a constant wind has blown, their largest concentration will be found at the downwind end of the lake or reservoir. These algae will be dispersed through the whole water column in that area, not just concentrated near the surface. If wind blows consistently from one direction, the depth of the surface layer containing buoyant algae will be larger at the downwind end than at the upwind end.

Permanent sites should be marked with a buoy or by the Global Positioning System (GPS), greatly simplifying work from a boat in windy conditions. If sampling from a boat, approach the sampling site at low speed, and take samples away from the motor and bow waves to avoid the disturbances they cause in the water.

To take a depth-integrated sample, use a hosepipe sampler (see Appendix F) of appropriate length. Alternatively, several grab samples at discrete depths could be taken with an appropriate sampler (for example, Van Dorn, Ruttner, Niskin bottle), pooled in a container (composite sample), thoroughly mixed and a subsample taken. To take a sample with a discrete depth sampler, open the bottle and 'load', lower to the desired depth, send a 'messenger' or weight to close the bottle and then lift it into the boat. For details of such samplers see APHA (1995) or Bartram and Ballance (1996). Fill the sample bottle from the sampler outlet or empty sample into a bucket and take a subsample.

In oligotrophic (nutrient-poor) waters take a sample volume of up to 6 L; in eutrophic (nutrient-rich) waters, 0.5 L to 1 L is usually sufficient. A phytoplankton sample is always taken as a whole water sample (unfiltered and unstrained). An additional sample of 0.5 L to 1 L is taken for chlorophyll-*a* analysis.

Test for inflowing waters

If inflowing waters are colder than the main waterbody they will sink to the layer of equivalent density. If they pose a problem (eg. high nutrient load) and their distribution in the waterbody needs to be tracked, the sampling program needs to be designed accordingly.

Visual inspection

In addition to taking water samples, a visual inspection of the water and the shoreline should take place, recording colouration and smell of the water, algal

colonies visible to the naked eye, the accumulation of algal matter and its colour, consistency (use gloves) and smell. If the inspection is undertaken as part of cyanoprokaryote monitoring, follow the details outlined in Jones (1997b).

3.3 Sampling in estuaries

An 'estuary' can be defined as a semi-enclosed body of water having an open or intermittently open connection with the ocean. The term 'estuary' further applies to the tidally influenced lower reaches of a creek, river or lake where freshwater meets saltwater. An estuarine system will therefore vary in water composition due to the seasonal and periodic influences of catchment areas and tidal movements. The phytoplankton community will therefore be a diverse mix of fresh and marine species.

Within an estuary, the water quality and associated phytoplankton distribution will be determined by the interactions of a number of processes, including imports and exports of materials, physical transportation and mixing, and the various chemical and biological processes.

Therefore when deciding where and when to sample phytoplankton in an estuary, consideration needs to be given to the physico-chemical condition of the system. Salinity stratification needs to be assessed in addition to temperature stratification and integrated samples taken from layers of interest. When samples are integrated across salinity layers, the total biomass sampled will include both fresh and marine species. The vertical and horizontal salinity gradients move upstream or downstream within the estuary according to changes in seasonal flow and weather conditions.

3.3.1 Site selection

It is recommended that an experienced ecologist and/or statistician be consulted before commencing a phytoplankton monitoring program in an estuary.

3.3.2 Sample collection

When sampling phytoplankton in an estuary, consideration should be given to both salinity stratification and temperature stratification. It is therefore necessary to measure the temperature and electrical conductivity profile at the sampling site and determine which depths of the water column are to be sampled in accordance with project objectives. It may be that only the freshwater or saltwater layer is of interest,

or it may be that both are. Samples are then taken accordingly. To obtain an integrated sample, use a hosepipe sampler of appropriate length. To obtain samples from different depths employ a discrete depth sampler. The discrete depth samples may then be pooled into a composite sample if required.

3.4 Frequency and timing of sampling

The sampling frequency is determined by the growth rate of the organisms under study. The generally recommended *frequency* of sampling for phytoplankton is weekly for both lotic and lentic waters. This will detect the often rapid changes in phytoplankton species composition and abundance; *generation times of algae vary from less than a day to several days* (Reynolds, 1984). Consequently, phytoplankton cell densities in natural environments can double under favourable conditions in two to three days. In standing waters, fortnightly sampling may be adequate in times of low cell growth. If the sampling specifically targets cyanoprokaryote blooms, a frequency of two to three days, or even daily, is recommended, depending on river levels, weather conditions, anticipated releases from upstream and current cell densities. Details are given in Jones (1997b). The same recommendation applies for standing waters under stratified conditions. Many programs reduce sampling frequency in winter due to the belief that algae grow less quickly then. However, increased cell numbers have been observed in winter in rivers as a consequence of low-flow conditions and lower turbidity (Hötzel and Croome, 1994; Hötzel and Croome, 1996), while increased cyanoprokaryote concentrations have occurred in a Victorian storage in midwinter and winter diatom blooms have been observed in rivers in the Sydney region (D. Cannon, pers. comm.).

In a standing waterbody, the time of day at which sampling occurs may be critical to the result. Buoyant alga may rise to the surface in the latter half of the day under calm, sunny conditions and be dispersed to deeper layers again by evening winds or through cooling at night (G. Jones, pers. comm.). In contrast, under calm, stratified conditions at night, surface scums may be found in the morning which are mixed back into the water column later by increased wind activity (Jones, 1997a). Hence it is recommended that routine sampling be conducted within the same predetermined time period in the day for each sampling event. The pilot

study should provide the necessary information about temporal changes in vertical phytoplankton distribution.

3.5 Phytoplankton nets

Phytoplankton nets catch only the algae that are larger than their mesh size. They are *unsuitable for taking a quantitative or even a presence/absence sample* because they do not collect the smaller organisms. Algae in the size ranges of picoplankton (less than 2 μm) and nanoplankton (2 μm to 20 μm) will pass through a standard phytoplankton net. The picoplankton and nanoplankton fractions of the phytoplankton, which include most flagellates, small green algae and the small diatoms, often constitute the major proportion of a river phytoplankton population. Nevertheless, a live sample taken with a plankton net (mesh size of 25 μm to 35 μm), in addition to a whole water sample, may aid the identification of the larger species.

3.6 Sample containers

Dark brown glass or PET (polyethylene terephthalate) bottles are suitable *sample containers* for phytoplankton. Other plastic bottles such as polypropylene bottles are unsuitable since iodine fumes (corrosive) diffuse through them. Samples for chlorophyll-*a* analysis are preferably taken in brown glass bottles (PET, polyethylene or clear glass are acceptable, but see section 3.7 'Preservation, transport and storage' for transport details). The sample bottle should be rinsed thoroughly (at least three times), away from the sampling site (or the side of the boat) to avoid disturbing the water to be sampled. Correct and immediate labelling of all sample containers with a durable label is essential.

3.7 Preservation, transport and storage

Samples for later counting should be preserved immediately at the sampling site by adding Lugol's solution at a ratio of 1:100 (Vollenweider, 1969). This gives the sample a weak tea colour. If a higher ratio is needed to preserve the sample, this should be noted on the field data sheet and taken into account during enumeration when calculating cell concentrations. Samples so preserved will keep for several years if stored correctly and topped up annually with the preservative (see below). Lugol's solution is made by mixing 20 g of

potassium iodide (KI) with 200 mL distilled water, then dissolving 10 g of pure iodine in this solution. Glacial acetic acid (20 g) is added a few days before use (Schwoerbel, 1970). The stock solution must be stored in a dark and well-ventilated space (Vollenweider, 1969) in a glass bottle and remains effective for at least a year.

An advantage of Lugol's solution is that flagellates preserved with it retain their flagella. A disadvantage is that the frustules of delicate diatoms and the scales of some Mallomonads may dissolve over a few months due to the acidity of the solution. The type and degree of alterations caused by fixation with Lugol's solution depend on the composition of the algal population and the water sampled. Cryptomonads, large diatoms, Peridineae, coccoid green algae, Desmidiaceae and single cells and trichomes of cyanoprokaryotes are least affected by this preservation and may keep intact for more than two years. Identification might be difficult if cells are overstained, although this might be overcome by adding sodium thiosulphate (Thronsdén, 1978). The iodine in Lugol's solution not only preserves the algal cells, but also increases their specific weight, so facilitating sedimentation. Samples fixed with iodine should be stored in the dark in amber glass bottles with an insert in the cap made of teflon to prevent iodine fumes from escaping (P. Baker, pers. comm.). For long-term storage, add one to three drops of Lugol's solution saturated with iodine to the already preserved samples and check annually for iodine content. The potential health hazards of storing such samples are covered in section 6.1, 'Occupational health and safety issues'.

Samples earmarked for identification only can also be preserved with formaldehyde acidified with acetic acid. To make a 20% aqueous solution of formaldehyde (HCHO), mix equal parts of formalin (40% HCHO) and concentrated acetic acid. For fixation, add 100 mL of the water sample to 2 mL of the acidified formaldehyde (the final concentration of HCHO should be 0.4%) (Thronsdén, 1978). The advantage of this agent is that samples can be stored for several years. The disadvantages are that the cell shape of naked flagellates can be distorted, flagella will be thrown off and the cell content will bleach out. Samples can also be preserved with neutralised glutaraldehyde (see section 6.1, 'Occupational health and safety') to a final concentration of 1% to 2% (APHA, 1995) and then stored for several years. Formaldehyde and glutaraldehyde are both toxic to humans (see section 6.1, 'Occupational health and safety').

Preserved samples should be kept in the dark (for iodine preservation) during transport with the usual care taken when handling chemicals. Live samples should be kept cooler than 10°C and in the dark. They need to be processed within 12 hours of taking the sample (Klee, 1993). Water quality in an unpreserved sample can change within hours, and zooplankton grazing can reduce algal numbers. Unpreserved samples for chlorophyll-*a* analysis should be filtered in the field and the filter frozen immediately in liquid nitrogen or a car fridge and kept in the dark. The frozen filters may be stored for up to three weeks before processing. Alternatively, unpreserved samples are kept cool (but not frozen) and in the dark and filtered in the laboratory within 12 hours of taking the sample in the field.

4. Analysis of samples

This section details procedures for the analysis of algal samples, beginning with the subsampling and concentration procedures and then discussing identification, enumeration and calculation of final results. Recommended benchmark methods are clearly identified, incorporating a choice between the three different counting chambers currently used in Australia. Statistical information pertaining to algal counting is given, addressing issues of accuracy and precision of counting results. The section closes with suggestions for an algal coding system.

4.1 Enumeration

Accurate identification and enumeration of algal cells under a microscope requires an experienced taxonomic phycologist, and a good quality instrument. The level of taxonomic identification will depend on the aim of the study, but it should be noted that habitat requirements in algae are species specific rather than genus or family specific. For some groups (eg. the diatoms and chrysophytes), special preparation is needed before identification to species level can occur.

The time required to count each sample will vary greatly depending on cell density, ease with which the cells can be identified, the desired level of precision and the amount of detritus/turbidity in the sample.

Counting is recommended for Lugol's preserved samples only. The counting of live samples is considered inaccurate since flagellates and diatoms move around in the counting chamber. The phytoplankton cell density and species composition in a live sample may also change in the time between sampling and counting as a result of zooplankton grazing and disintegration of cells. The basic procedure for counting algae is to fill a counting chamber with a subsample of the preserved sample and, after a period of settling, simultaneously identify and count the algal cells under a light microscope. *Because of the statistics used to calculate the precision levels of algal counts (see section 4.1.10, 'Statistics of counting'), algal units must be enumerated.* 'Algal units' may be single cells, filaments or colonies depending on the species' usual life form.

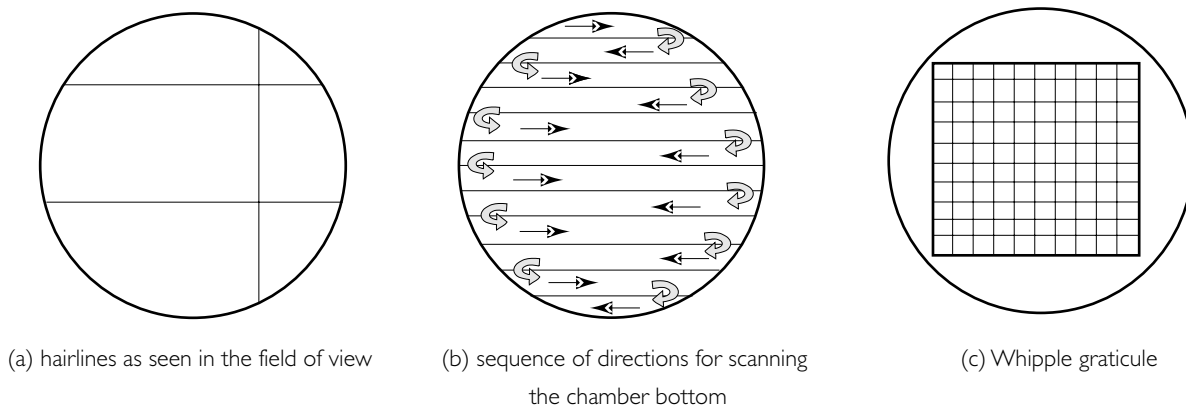
4.1.1 Microscope requirements

An upright or inverted light microscope with white light achromatic objectives of 10x, 20x, 40x and 100x magnification is required. If an inverted microscope is used, the 20x and 40x objectives must be of long working distance and a suitable condenser is required. To use a Sedgwick-Rafter chamber on an upright microscope, the 40x objective needs to be of long working distance, but the 20x objective may not. For the 20x and 40x magnifications, the use of phase-contrast objectives would be an advantage, especially for the identification of live cells. For use with the Utermöhl chamber (see below), three hairlines or threads are needed in the optical path (Lund et al., 1958). Two hairlines are arranged parallel and the distance between them may be adjustable, while the third one runs at a right angle. For most modern inverted microscopes a special part with adjustable lines can be obtained. Otherwise fixed hairlines can be inserted into one of the eyepieces. Make a thin cardboard or plywood ring with an outer diameter so that it will lie on the flange inside the eyepiece. Glue three fine glass threads onto the ring with epoxy adhesive as shown in Figure 4.1(a). The ring is then inserted into one of the eyepieces. If hairlines are unavailable, a Whipple graticule (Figure 4.1(c)) placed in one of the eyepieces can be used instead.

Most often the sample is counted at more than one magnification depending on the size of the phytoplankton present. Large cells or colonies are counted at 100x while the majority of cells is counted at 200x. If picoplankton is present in considerable numbers, a count at 400x may be performed. Viewing at 1,000x is used for identification only.

4.1.2 Subsampling preserved samples

To avoid subsampling errors, gently shake and invert the preserved sample thoroughly for at least 30 seconds so that it is well mixed before subsampling. For the original Utermöhl chambers (made by Zeiss) a separate filling chamber (Füllkammer) is available which ensures even distribution of algal units in the sedimentation chamber. The break-up of filaments and colonies during shaking is often unavoidable. The subsample is immediately transferred into either a counting chamber or into a sedimentation vessel. The subsampling technique should

Figure 4.1: Microscopic details for Utermöhl method

be checked regularly by taking several successive subsamples from the same storage bottle and comparing the counts of some species (Hasle, 1969). Ten subsamples should be taken and the five dominant species counted.

If the cell density is less than 10^5 cells per litre, the sample needs to be concentrated before counting (McAlicie, 1971), preferably by sedimentation (for filtration see section 4.2, 'Semi-quantitative enumeration'). If cell density is too high, dilute the sample with distilled water. If cell density in the counting chamber is too high, smaller cells will be overlooked and counting will take too long. Additionally, the count will be incorrect due to easy tiring and the observer's inability to accurately count so many units in the field of view. Dilution or concentration factors need to be taken into account in calculating the final results.

Usually, only 'live' cells (cells with cell contents) are counted, but if the age status of the population is of interest, 'live' and 'dead' cells should be counted separately.

4.1.3 Concentration of phytoplankton by gravity

While other methods are available, *the concentration of algae by sedimentation is the preferred method.*

After mixing, a subsample of between 5 mL and 1,000 mL (depending on cell density) is taken and sedimented in a measuring cylinder of suitable size, allowing two hours for each 1 cm of water column at 20°C. If small diatoms are present, a settling time of six hours for every 1 cm of water column is recommended (Furet and Benson-Evans, 1982). For example, if a

100 mL subsample is sedimented in a standard 100 mL measuring cylinder (18.5 cm in height), sedimentation will take 2.3 days for average plankton and 4.6 days for small centric diatoms. *For most algal populations a sedimentation time of 48 hours for a standard 100 mL measuring cylinder is recommended.* Such a sedimentation time will also settle 'difficult' species such as *Cylindropermopsis* and *Planktolyngbya*. There are shorter 100 mL measuring cylinders available which will reduce average sedimentation time to 24 hours. For occupational health and safety reasons, place sedimentation cylinders under a fume hood or use stoppered cylinders. If these options are not available, at least cover the top of the cylinders with a protective film or foil.

After sedimentation, the top 90% of volume is carefully siphoned off without disturbing the sedimented algae, the remainder is shaken gently and a subsample of appropriate volume (see sections 4.1.7 to 4.1.9) is transferred to the counting chamber and allowed to settle before counting. From time to time, examine the supernatant for cells that have not sedimented. If the supernatant is siphoned off from a point well below the meniscus, cells floating on the surface will be pulled down into the concentrate and will mix with the other cells when the sample is shaken before taking the subsample for counting.

The choice of sedimentation vessels should be guided by the following considerations. In tall settling chambers an error might be introduced due to cells adhering to the walls (Utermöhl, 1958). Investigations have shown that this might happen with chain-forming and setae-bearing marine diatoms (Paasche, 1960) while

most other forms of phytoplankton are unaffected (Margalef, 1969; Hasle, 1978). The settling containers (usually measuring cylinders) should have a height:diameter ratio not exceeding 5:1. Convection currents will occur if the sedimentation chamber is higher than five times its diameter (Nauwerk, 1963) resulting in a considerable amount of plankton failing to settle, regardless of length of sedimentation time.

To satisfy client needs of short turnaround time, concentration by continuous-flow centrifuge, which would cut down the required time to a few minutes per sample, is currently under investigation. The method has been used for marine work but is not documented for freshwater samples.

4.1.4 Removal of buoyancy

Species containing gas vesicles (that is, cyanoprokaryotes) are unlikely to fully settle despite the iodine fixation. The gas vesicles need to be collapsed before the sample is set up for sedimentation. Keep some untreated sample for identification purposes. In order to collapse the gas vesicles, expose the sample to brief (approximately 10 seconds) ultrasonication (Furet and Benson-Evans, 1982). Alternatively, apply pressure to the sample as follows: after placing the sample in a strong rigid bottle which is closed with a tightly fitting rubber stopper, bang the stopper several times with a mallet. After such treatment the sample is sedimented as usual. It is advisable to check the water surface in the sedimentation vessel for the presence of buoyant algal cells before siphoning off the supernatant. Note that experience indicates that the distinction between certain cyanoprokaryote taxa is no longer possible after gas vesicles have been collapsed. In this case, perform identification on the untreated sample and estimate the proportions of the different cyanoprokaryotes present, so that cell counts can be apportioned accordingly. Examine the surface within the settling chamber to ascertain whether or not other algae may be floating rather than sedimenting; for example, the green alga *Botryococcus* which floats because it contains a light hydrocarbon oil.

4.1.5 Turbidity

Water samples from rivers frequently contain high levels of fine inorganic particles which obscure the algal cells. This is particularly the case during floods. These particles are in the same size range as many algal cells, making it impossible to separate the two by a single procedure such as filtration. In this case, the subsample

can be diluted so that cells are visible enough to count and identify. This involves a trade-off between diluting the subsample in order to see the cells and reducing cell concentration below statistically acceptable levels. To overcome this problem, some authors suggest that several subsamples should be counted and the results summed.

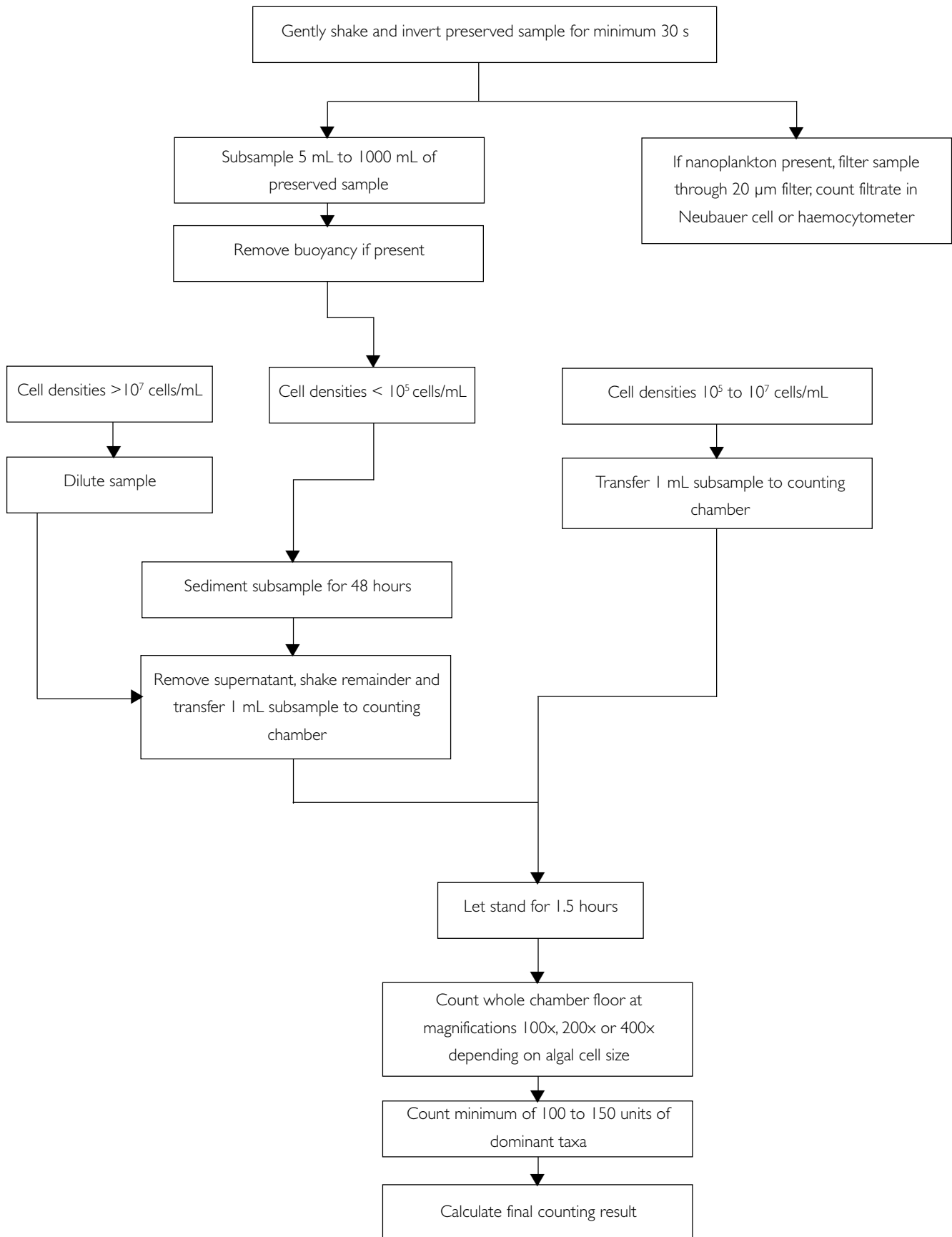
4.1.6 Choice of chamber

It is recommended that an Utermöhl chamber be used with an inverted microscope, or a Sedgwick-Rafter chamber or a Lund cell with an upright microscope. The choice of chamber and microscope will depend on the experience of the algal worker and the financial resources available for the project. *The use of any of the chambers is acceptable, even though the results obtained using different chambers are not strictly comparable,* as has been reported by many algal workers. Their results are comparable in terms of general trends of phytoplankton development and common species, but, because of technical differences, for the same sample, the overall number of species detected and the cell densities of each species may vary considerably between chambers. Therefore, an acute statistical analysis of results obtained using different chambers is not possible.

For studies in which small nanoplankton and picoplankton are prevalent, filtration through a mesh of pore size 20 µm is recommended, followed by counting of the cells in the filtrate in a Neubauer cell or haemocytometer (APHA, 1995). Small phytoplankton (less than 10 µm) found in Australian rivers in high cell densities include the genus *Synechococcus*. As the algal cells will not be randomly distributed across the floor of any of the above counting chambers, the whole floor usually needs to be scanned to avoid serious errors (Lund et al., 1958; UNESCO, 1974). For organisms occurring in large numbers, in the absence of an obvious non-random distribution, transects or a suitable number of fields may be counted (Lund et al., 1958). The filling of cells (eg. Sedgwick-Rafter chamber or Lund cell) by pipetting does not ensure a random distribution of the algal cells on the chamber floor (UNESCO, 1974).

An *Utermöhl chamber* (Figures 4.2 and 4.3) is a combined chamber (Röhrenkammer) consisting of a bottom counting chamber plus a chamber cylinder which is temporarily attached for sedimentation. A round hole in the bottom plate (up to 2.5 cm diameter) closed with a coverslip on one side forms the chamber. The algae are sedimented directly onto the coverslip and can thus be viewed with an inverted microscope without

Figure 4.2: Quantitative algal enumeration with Utermöhl chamber



optical impairment by the water column in the chamber. Tubes of varying heights and volumes are placed onto the bottom slide, filled with the subsample and removed after sedimentation. Various Utermöhl-type chambers are available from manufacturers of inverted microscopes (see Appendix D) but simple chambers can be made readily at low cost (see Appendix D for details). The commercially available chambers with a hole of 2.5 cm diameter allow direct sedimentation of volumes between 5 mL and 100 mL, in contrast to the chamber detailed in Appendix D which may require an extra sedimentation step because its tube holds only 4 mL. The commercially available chambers are also faster to set up and clean because the coverslip that constitutes the 'bottom' of the chamber is fixed in the bottom slide with a metal ring that can be easily unscrewed to allow replacement of a broken coverslip.

A *Sedgwick-Rafter chamber* consists of a 50 mm by 20 mm microscope slide with a grid floor (1,000 fields) and a raised-rim well holding 1 mL (Figure 4.4). The Sedgwick-Rafter chamber is placed directly under an upright microscope. The chamber can be used with a 10x, 20x and 40x objectives but the 20x and 40x should be of long-working distance since for total magnifications greater than 100x, the working distance of most objectives greater than 10x is less than the depth of the chamber. If only 10x and 20x objectives are available, the examination of nanoplankton (less than 20 μm) is excluded. Glass Sedgwick-Rafter chambers, although considerably more expensive, are often preferred to plastic ones because the latter are easily scratched.

A third type of chamber is the *Lund cell*. It consists of an ordinary microscope slide with two long, thin pieces of brass shim or glass glued to the longer edges.

Figure 4.3: Diagram of Utermöhl chamber

(a) bottom counting chamber; (b) bottom counting chamber and chamber cylinder set up for sedimentation. See text and Appendix D for further explanation.

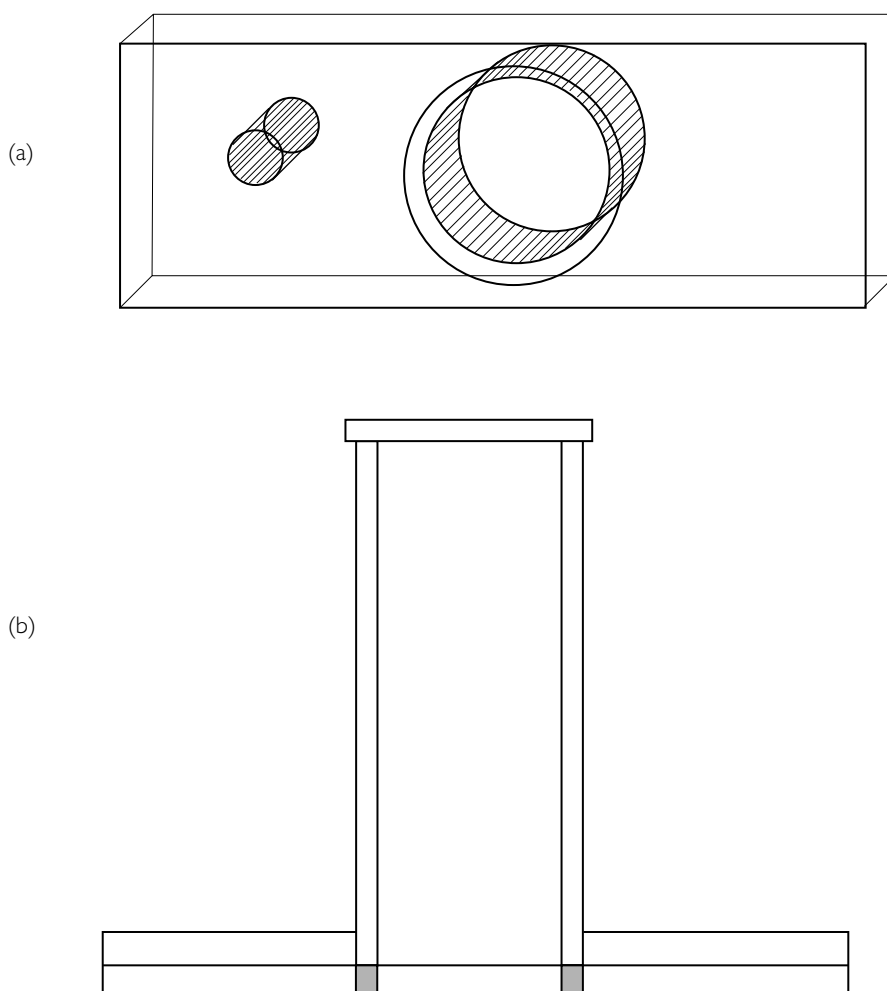


Figure 4.4: Diagram of Sedgwick-Rafter chamber with coverslip in 'fill position'

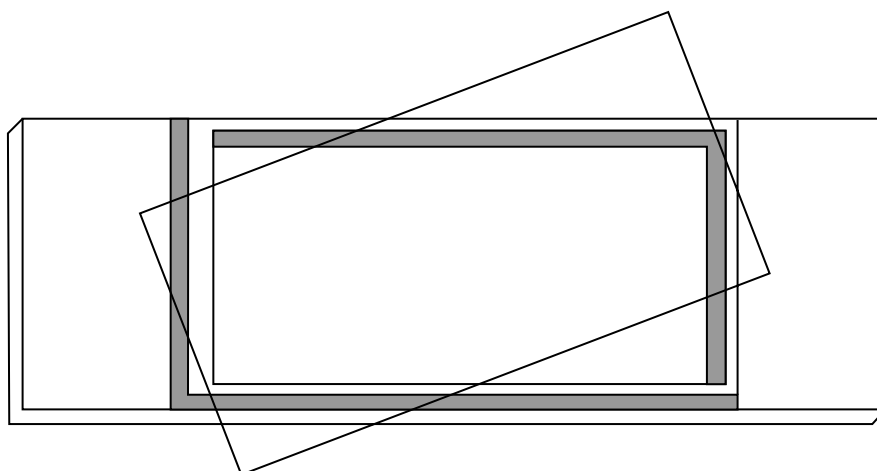
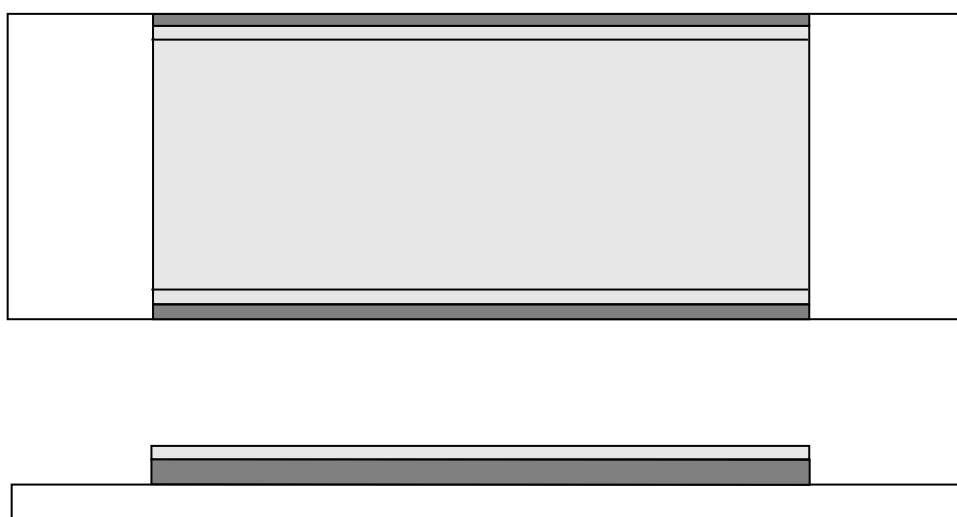


Figure 4.5: Diagram of Lund cell with coverslip in place



A rectangular coverslip is layed on top to form the chamber (Figure 4.5). A subsample of the original concentrated or diluted sample is placed in the chamber and, after short sedimentation, is counted under an upright microscope.

The advantages of the Lund cell are:

- it is easy to construct, does not have to be precision-made and is therefore inexpensive;
- it is easy to set up and clean; and
- it is used with an upright microscope with normal-distance 10x and 40x objectives to reach final magnifications of 100x and 400x.

With the Lund cell it is optically difficult to identify and count nanoplankton. Users of the Lund cell have reported that the algal units do not always distribute randomly within it.

4.1.7 Counting with the Utermöhl chamber

A suitable volume of either the original sample or the concentrated or diluted subsample is placed in the assembled chamber and put aside for sedimentation. If the perspex chamber described in Appendix D is being used, 1 mL is sedimented for 1.5 hours before enumeration. Usually, the whole floor is examined since the distribution of the algal units on it is not always

random. For very abundant taxa it might be sufficient to count every second or third strip or several central transects in different directions across the floor. By moving the mechanical stage, the chamber bottom is traversed backwards and forwards along adjacent strips (see Figure 4.1(b)) so that eventually the whole bottom has been covered. All algal cells lying between the two parallel hairs (Figure 4.1(a)), or within the edges of the Whipple graticule if no hairs are present (Figure 4.1(c)), are counted, as they seem to pass the vertical line. Cells or units lying across the top hair are counted, but those across the bottom hair are not. Care needs to be taken not to double count long filaments that lie across both hairs. Different magnifications may be used to count different-sized organisms within the same sample; for example, 10x objective for taxa greater than 30 μm , such as *Ceratium*, 40x objective for cells less than 5 μm , and 20x objective for most other taxa. This approach is considered preferable to the suggestion of some authors that several subsamples of different concentrations be counted, an approach which introduces another level of error.

The final result, expressed as number of cells per millilitre, is calculated employing factors relevant to the volume sedimented, including *concentration* or *dilution factors*. The cell concentration C for each taxon is calculated according to:

- (a) for a concentrated sample, C [cells mL^{-1}] = cells counted/concentration factor; or
- (b) for a diluted sample, C [cells mL^{-1}] = cells counted \times concentration factor.

For example, after sedimentation, 100 mL of original sample is reduced to 10 mL (concentration factor of 10), and a 1 mL subsample is taken for enumeration. In this, 580 cells of species A are counted. The concentration of species A in the original water sample is calculated according to:

$$C = 580/10 = 58 \text{ cells mL}^{-1}.$$

If central transects are used, a factor is derived from the ratio of the chamber floor covered by the transect to the whole chamber floor. This factor is multiplied by the number of cells counted to obtain the concentration in cells per millilitre for each particular taxon. To obtain total cell density per millilitre, sum all counting results of individual taxa expressed as cells per millilitre. Where colonial taxa are counted as units, multiply the count of units by the average number of cells per unit before calculating the cell concentration in 1 mL (see section 4.1.11, 'Remarks on counting filaments and

colonies' for details). For the dominant (most abundant) taxa, a minimum of 100 to 150 units should be counted per sample.

Willen (1976) suggested a simplified version of the Utermöhl method in which a limited number of species (six to eight) is counted so that approximately 90% of total phytoplankton volume is included in the count. This reduces the counting time by as much as half. Additionally, the number of units counted for each species may be limited, for example, to 60, resulting in a maximum estimated error of $\pm 26\%$ according to equation 8 (below). This recommendation was based on several years of algal counts in Swedish lakes of varying nutrient status. This approach appears preferable to imposing a time limit per count set *a priori*, or to counting only abundant taxa, but its applicability would need testing for individual programs.

If the genuine Zeiss chambers are employed (diameter of bottom plate = 2.5 cm), only part of the bottom is counted; that is, in several traverses of a width between 50 mm and 200 mm. The number of traverses depends on the cell density on the chamber floor. After each traverse the chamber is turned a predetermined angle, relating to the number of traverses counted (eg. for four traverses counted, angle is $360/4 = 90$). A factor is calculated expressing the ratio between the area counted (the transects) and the whole bottom area. This factor is included in the calculation of final count results.

4.1.8 Counting with the Sedgwick-Rafter chamber

Each chamber needs to be calibrated before use. The volume is determined by weighing, 10 times, the cell filled with deionised water and calculating the average. For calibration the chamber is filled in the usual manner. Calibration of each Sedgwick-Rafter chamber also requires determination of its area (nominally $50 \times 20 \text{ mm} = 1,000 \text{ mm}^2$) with a planimeter and its depth (nominally 1 mm) with a micrometer. A record should be kept of the calibration measurements for each chamber. If the chamber volume differs from 1 mL by 5% or more a factor should be calculated to correct to 1 mL. This factor should be marked permanently on the chamber and used when calculating final counting results. Recalibrate each Sedgwick-Rafter chamber annually.

A Pasteur pipette is used to subsample 1 mL of either the original sample or the concentrated or

diluted subsample (Gilbert, 1942). The subsample is then run into the chamber at one corner with the coverslip lying at an angle across the chamber (see APHA, 1995). Once the chamber is filled, the coverslip is moved to cover the whole chamber and the sample is left to settle for 30 minutes. To prevent formation of air bubbles due to evaporation of sample during counting, distilled water may be added to the edge of the cover slip from time to time. The cells are counted on the bottom of the chamber. *The counting of live samples is not recommended since flagellates and buoyant cyanoprokaryotes may accumulate under the coverslip and constitute a source of error.* All cells or units within randomly selected fields or traverses are counted. Depending on the density of the algae, a smaller or larger number of traverses or fields is counted until the minimum number of units set for each species has been counted. Since the units do not always distribute randomly in the cell, space the traverses or fields along the whole chamber to overcome this bias. A convention needs to be followed for cells or units lying on a boundary line of a field; for example, all cells or units overlapping the right-hand and top boundary would be counted, but those overlapping the bottom and left-hand boundary would not. *It is recommended that 30 fields be counted within the chamber so as to include 90 to 95% of the species present.* Counting 25 fields will include 80 to 90% of species present (McAlice, 1971).

For a field count, the cell concentration C , expressed as the number of units per millilitre for each taxon, is calculated according to:

$$C [\text{cells mL}^{-1}] = \frac{N \times 1,000 \text{ mm}^3}{A \times D \times F} \quad (1)$$

where:

N = number of cells or units counted

A = area of field (mm^2)

D = depth of a field (Sedgwick-Rafter chamber depth) (mm)

F = number of fields counted.

For colonial taxa, multiply the count of units by the average number of cells per unit (see section 4.1.11, 'Remarks on counting filaments and colonies' for details) and use the resulting value as N in equation 1. To adjust for sample concentration or dilution, the result is divided or multiplied by the appropriate factor. To obtain total cell density per millilitre, sum all counting results of individual taxa expressed as cells per millilitre.

If cell density is low (less than 10 units per field), counting of long traverses to cover a larger proportion of the chamber floor is more appropriate. Several traverses with a width of a chamber field are counted. The number of traverses depends on the required precision and the phytoplankton density.

For a traverse count, the cell concentration, C , expressed as number of units per millimetre for each taxon, is calculated according to:

$$C [\text{cells mL}^{-1}] = \frac{N \times 1,000 \text{ mm}^3}{L \times D \times W \times S} \quad (2)$$

where:

N = number of cells/units counted

L = length of each traverse (mm)

W = width of traverse (mm)

D = depth of a field (Sedgwick-Rafter chamber depth) (mm)

S = number of traverses counted.

Treat counts of units as described above. To adjust for sample concentration or dilution the result is divided or multiplied by the appropriate factor.

4.1.9 Counting with the Lund cell

Lund cells must be calibrated before use. Determine the volume of each chamber by weighing it before and after filling with deionised water. Repeat this 10 times and calculate the mean. The chamber should be filled in the same manner as for counting, and completely dried between measurements. Calculate the area of the chamber by multiplying the length of the coverslip by the distance between the two side pieces. These lengths are measured either with the vernier scales on the microscope table or with a separate vernier gauge. Repeat measurements at intervals along the length several times. After initial calibration always use the same coverslip with each cell and recalibrate annually. If the coverslip is broken, repeat the calibration with a new coverslip.

Measure the area of the Whipple graticule at each magnification under the microscope using a stage micrometer. The Whipple graticule, located in one of the eyepieces, defines the field of view, or width of the traverse counted. Since only part of the chamber (either several fields of view or traverses) is counted, a conversion factor needs to be derived to relate the area counted to the total area of the chamber. By knowing the volume of the Lund cell and the area being

examined it is possible to relate the algal count recorded as a number of units per area to cells per millilitre in the original sample.

The total number of Whipple graticule fields within the total chamber area is calculated as:

$$\text{Total number of fields} = \frac{\text{Total area of chamber [mm}^2\text{]}}{\text{Area of Whipple graticule [mm}^2\text{]}} \quad (3)$$

The conversion factor Ff for counting fields of view is calculated as:

$$Ff = \frac{1}{\text{Lund cell volume [mL]}} \times \text{total number of fields} \quad (4)$$

The conversion factor Ft for counting short traverses is calculated as

$$Ft = \frac{Ff}{(\text{Cell/Whipple graticule length})} \quad (5)$$

To *perform a count*, place the coverslip onto the cell (Figure 4.5) and run in a volume as determined by the calibration of either the original sample or a concentrated or diluted subsample into the chamber by placing the tip of the pipette close to the open edge of the coverslip. The liquid will be sucked under the coverslip by capillary action. Avoid the formation of air bubbles in the chamber. Let the filled chamber stand for 10 minutes for the algae to settle onto the surface of the slide. If buoyant cyanoprokaryotes are expected, scan the optical plane directly under the coverslip as well as the depth of the chamber for their presence.

Only part of the chamber floor is counted. A number of randomly selected fields of view or short or long traverses are counted until a minimum of 100 units for each of the dominant species is counted. The number of fields of view or traverses counted depends on the cell density in the chamber. Cells greater than 12 μm are counted at 100x magnification, while smaller cells are counted at 200x magnification. If high numbers of picoplankton are present these cells are counted at 400x magnification.

Counting fields of view: The size of a field of view is determined by the outline of the Whipple graticule.

Counting traverses: The traverses are as wide as the Whipple graticule. The traverses are placed evenly across the length (short traverses) or the width (long traverses) of the cell, avoiding the area close to the open edges.

Calculate the cell concentration C , expressed in numbers of units per millilitre, for each taxon according to:

$$C [\text{cells mL}^{-1}] = \frac{N}{F} \times Ff \quad (6)$$

where:

N = number of cells or units counted

F = number of fields counted

Ff = field conversion factor.

To adjust for sample concentration or dilution, the result is divided or multiplied by the appropriate factor. To express final cell count results of colonial species in cells per millilitre, follow the procedure in section 4.1.11, 'Remarks on counting filaments and colonies'.

4.1.10 Statistics of counting

The purpose of phytoplankton counting is to gain an estimate of the population in the sampled waterbody that is as close as possible to the true size of the population. Due to errors inherent in the methodology, we will never know the *true value*, but we can use statistics to evaluate the probability of the *measured value* lying within a certain range or confidence limits around the true value. The true value of a phytoplankton count is a function of both the accuracy and precision of the count. This section gives the steps and knowledge needed for the determination of accuracy and precision of counts and explains sources of errors in algal counts, while in-depth treatments of the subject can be found in Lund et al. (1958), Lund (1959), Baker (1986) and Laslett et al. (1997).

At the beginning of a program it is essential to evaluate the precision of the algal count method used and to decide on the desired level of precision. To obtain a precise and reproducible result, amenable to statistical comparison, representative samples need to be collected in the field (see section 3, 'Taking samples') and representative subsamples need to be taken from the preserved material in the laboratory (see section 4.1.2, 'Subsampling preserved samples').

The agreed subsampling procedure should be tested at the beginning of the project since it is not practical to do this for each sample counted. It is advisable to keep the number of subsampling steps to a minimum because each one introduces a new source of error. There are generally two subsampling steps in algal enumeration: (1) taking a subsample from the storage bottle, and (2) counting only part of the chamber floor (strips or fields), thus introducing two sources of error – filling error for (1) and clumping error for (2). Determine for

each subsampling step the type of distribution of algal units among repeated subsamples in order to estimate the error introduced. Depending on the chamber used and the mode of counting, compare either results from several whole chamber floors filled from the same sample (Utermöhl chamber) or results from several fields or strips in one subsample (Lund cell, Sedgwick-Rafter chamber). *The statistics describe the distribution of entities within the samples with regard to calculating error levels.* These entities, referred to as *algal units* in this text, may be single cells, filaments or colonies, depending on the species.

As regards the filling error, the distribution of counting units of individual species among subsamples from the same storage container can generally be described with a Poisson distribution, but it is advisable to test this using the statistic D after Fisher et al. (1922).

$$D = \frac{\sum_{i=1}^k (x_i - \bar{x})^2}{\bar{x}} = \frac{(n-1)s^2}{\bar{x}} \quad (7)$$

This variance test has the chi-square (χ^2) distribution with $k - 1$ df, where k is the maximum number of individuals a sampling unit could contain and df is degrees of freedom. The outstanding feature of the Poisson distribution is that the variance is equal to the mean ($\sigma^2 = \mu$). The assumption of $H_0: \sigma^2 = \mu$ (the null hypothesis) is tested against $H_1: \sigma^2 \neq \mu$ (the alternative hypothesis) where the rejection criteria are $P(\chi^2) > 0.975$ and $P(\chi^2) < 0.025$ using the statistic D, which is the index of precision (the ratio of standard error to the mean). Acceptance of the null hypothesis indicates a non-random distribution, that is, a Poisson distribution.

If only part of the chamber floor is counted (field or strip count) and the counting chamber is filled with a subsample of the original sample, random distribution of algal units within the counting chamber needs to be verified. If algal units are not randomly distributed the data need to be normalised before errors and confidence limits can be estimated.

With regard to the clumping error, the distribution of counting units among subsamples within the one sample (fields or strips) does not usually follow a Poisson distribution. This should be tested by performing a one-tailed test for $H_0: \sigma^2 = \mu$ against $H_1: \sigma^2 > \mu$ with $P(\chi^2) < 0.05$. The null hypothesis can also be tested graphically by plotting sample mean squares against the sample means. Most points should lie on or close to the 45° line $\sigma^2 = x$. The null hypothesis is rejected when points fall outside the upper [$P(\chi^2) < 0.025$] or lower

[$P(\chi^2) > 0.975$] limit for the sample size used (McAlice, 1971). To obtain confidence limits for cell counts of colonies or filaments, multiply the confidence limits for the count of units by the mean number of cells per unit.

The level of precision relates to individual taxa, not to the overall sample, since it depends on the number of algal units counted. A predetermined level of precision should be set for the most abundant taxa by the program designer, which in many cases will be the client. The level of precision should relate to the aim of the program, the ability to make correct management decisions based on such algal counts and the desired cost of the algal counts. The program designer needs to decide whether real change in cell abundance (for example, cell doubling) can be detected in successive samples and what the risks are of having a false positive (false indication of change) or a false negative (false indication of no change) at the chosen level of precision. For recommended precision levels in cyanoprokaryote counts, see the paragraph on counting filaments below. For other phytoplankton, such as species with individual cells or small colonies, it is recommended to set the level of precision to $\pm 20\%$ error for the most abundant taxa following Lund et al. (1958). This level of precision is reached by counting a minimum of 100 algal units of the taxon in question per sample. The counting precision for less common taxa will always be lower than that for common taxa since the required minimum of 100 algal units will not be found in the sample.

The *counting error* (precision) can be estimated with a 95% confidence limit by:

$$\frac{2}{\sqrt{N}} \times 100 = \text{counting error } (\pm \%) \quad (8)$$

where N is the number of units counted, if the algal units do follow a Poisson distribution for both subsampling steps. For example, if 100 units are counted, the error is $\pm 20\%$; if 400 units are counted the error is reduced to $\pm 10\%$. Thus a fourfold increase in counted units is required to halve the error. Generally, 100 to 150 units of the common taxa are counted, giving an approximate error of $\pm 20\%$. Of course, within a sample the error for individual species will vary widely according to their individual densities.

One should be aware of the causes and magnitude of all errors in one's methods, firstly in order to reduce them, and secondly in order to decide on the level of precision wanted and the time involved to reach it. *Two types of errors are generally recognised when estimating*

phytoplankton densities: systematic errors and random errors. Systematic errors, which affect the accuracy of the results, are inherent in the method used and introduce a bias for all counts. They can be eliminated by appropriate change of methods. Sources of random error are the naturally occurring non-random distribution of algae in the water (patchiness), subsampling, observer error, and errors in estimating the number of cells per colony or filament. Random error affects both the accuracy and precision of counts. The size of the errors also depends on the counting methods used and the size of samples.

The accuracy of algal counts will never be very high due to patchiness of the phytoplankton in the water. Most ecological studies that involve sampling natural populations are concerned with generations or an abundance change of 100%, where a precision of $\pm 50\%$ would be quite adequate. Assuming the algal units are distributed randomly on the chamber floor, it can be calculated how many algal units need to be counted to achieve a desired degree of precision for a given confidence level. *The precision of a count varies inversely with the square root of the number counted (Lund et al., 1958); for example, a count of 100 cells has a precision of $\pm 20\%$ compared to a count of 400 cells which has a precision of $\pm 10\%$ at the same level of confidence (95%).* Consequently, to increase the precision level from $\pm 20\%$ to $\pm 10\%$ at the same level of confidence, the counting time per sample increases fourfold. For most algal enumeration of field samples a precision of $\pm 20\%$ is generally accepted, in which case a count of 100 units/mL for a taxon based on 100 randomly distributed units in the chamber would mean that the estimated value lies somewhere between 80 and 120.

As a preferred method for reporting results, for each algal count the precision for the five most abundant taxa should be recorded and presented together with the counting results in the data report. It is vital to interpret algal counting results with their precision in mind. For example, if a count of 1,000 cells/mL with a precision of $\pm 20\%$ is reported, the true cell concentration in the sample may be between 800 and 1,200 cells/mL. If the counts from two samples were to differ by one order of magnitude, this might be a true difference in population density or may have resulted from counting imprecision (McAlice, 1971).

4.1.11 Remarks on counting filaments and colonies

To obtain an accurate count, the number of cells per filament or colony needs to be enumerated in each sample, since the size of these algal units can vary greatly. For example, *Anabaena* filaments may contain between five and several hundred cells, and the size of a *Microcystis* colony can range from fewer than 10 cells to several thousand cells.

For filaments in which the individual cells are easy to recognise and are of regular length (eg. *Aulacoseira*), the number of cells per filament for the first 30 filaments observed is counted and the mean number of cells per filament for the sample is calculated. In your samples, establish whether the number of cells per filament is normally distributed. If a skewed distribution is apparent, calculate the median rather than the mean number of cells per filament for the species concerned.

For filaments of cyanoprokaryotes, it is recommended to follow the procedures in Laslett et al. (1997) for calculating the precision according to the Sichel distribution. The equation used:

$$\sqrt{\frac{2}{N}} \times 100 = \text{counting error } (\pm \%) \quad (9)$$

where N is the number of units counted, calculates the overall counting error in terms of variance and standard error, thus taking into account the clumping error and the error related to the variability of cells per algal unit. To achieve a $\pm 20\%$ precision, 50 trichomes need to be counted; to achieve a $\pm 30\%$ precision, 23 trichomes need to be counted. In either case, the number of cells for each trichome counted must also be determined. For specific recommendations for the precision levels of cyanoprokaryote counts please refer to the final Agriculture and Resource Management Council of Australia and New Zealand national protocol for the monitoring of cyanobacteria (Jones, 1997b).

For filaments in which cells are not distinguishable at 100x or 200x magnification (eg. *Limnothrix*) in each sample determine the average length of 30 cells at 400x or 1,000x magnification and measure the length of filaments during counting. Then calculate cell concentrations of the species in question from these two measures. If the 1,000x objective cannot be used with your counting chamber, after counting transfer some sample to a normal microscope slide and view at 1,000x.

In some genera of filamentous forms (eg. *Oedogonium* and *Lyngbya*) individual species can be identified only when mature thalli or reproductive stages

are present. Nevertheless, size classes of varying filament width can be readily distinguished and cell counts for these genera should be recorded in separate size classes. The width of such filaments is measured between the cell membranes; for example, the sheath of cyanoprokaryotes is not included.

To determine the average number of cells per algal unit for colonies shaped differently from filaments, record the number of cells in the first 30 colonies encountered and calculate the mean if normally distributed. If these measurements are skewed, calculate the median. The number of units per millilitre counted in the sample is then multiplied by the mean or median to obtain a final result of cells per millilitre.

Some species under bloom conditions form colonies so large that only an estimate of cells per colony can be made unless the colonies are dispersed and individual cells are counted. Some cyanoprokaryotes (*Coelosphaerium*, *Gomphosphaeria*, *Microcystis*) form large, three-dimensional, mucilage-bound colonies within which counts of individual cells are all but impossible. Reynolds and Jaworski (1978) recommend rapid (less than 1 minute) ultrasonic disintegration of colonies as a quick, routine procedure in this situation, followed by a count of the individual cells at 400x magnification. Alternatively, it may be appropriate to estimate the number of cells in each colony examined, rather than estimate the average number of cells per colony for a given sample size. For particularly large colonies, this can be achieved by counting the number of cells in a small proportion (area) of the colony (using an eyepiece grid) and then estimating the total number of similar areas in the total colony. If, however, estimates rather than accurate counts are made, this needs to be recorded in the laboratory work sheet and indicated in the final report to the client.

4.1.12 Taxonomic identification

The level of taxonomic identification depends on the objectives of the program, the training of staff and the financial resources available. *Dominant species and problem species should be identified to species level in any case.* For a general study, identification to genus level might suffice, while for ecological studies all the species important with respect to abundance and biomass should be identified to species level. Although identification to species level often takes considerably longer than working to lower taxonomic levels (phylum or class), it will also supply the information essential for studies of phytoplankton community structure and

succession (McAlice, 1971). Ecological studies require identification to species level to draw on the information on autecology of the algae. *The ecological requirements of individual algae can vary considerably within one genus* and, although tedious, *correct taxonomic identification to species level still forms the basis of any in-depth ecological investigation.* In water quality monitoring, identification to species level is critical for highlighting the presence of potentially toxic species; for example, *Anabaena circinalis* appears to be the only species of *Anabaena* to produce toxins in Australia.

For identification, fresh live samples are recommended, especially if flagellates are thought to be present.

Some taxa, in particular the diatoms, cannot be identified to species or even genus level at the magnification used for counting. It is convenient to group such taxa into size classes and count the cells accordingly. For instance, single centric diatoms might be grouped into size groups of less than 5 µm, 5 µm to 10 µm, and greater than 10 µm. After additional preparation of sample material, diatom species can be identified and the size classes related to species names. If quantitative results for the diatoms are required, 300 valves on a permanent slide are counted and the relative proportion of each species in the sample calculated.

Diatoms are often counted in collective categories with defined size ranges such as 'centric diatoms 5 µm to 10 µm', because detailed identification is impossible in the counting chamber. Special preparations of the frustules and viewing at 1,000x on a microscope slide are required for their identification. Diatoms are probably the best-studied algal group with respect to autecology and there is now a large body of literature describing ranges of environmental conditions such as temperature, pH and ion concentration for many species.

For taxa occurring rarely within a particular algal program, it may be convenient to simply count them in groups; for example, 'cryptomonads'. When counting preserved samples, the observer needs to distinguish between cells that were alive at the time of fixation and those that were not. *Only the cells that were alive are counted.* Complete and healthy looking chloroplasts and cell membranes are often used to characterise healthy cells. Such distinction is especially important when counting diatoms since their frustule can exist for many years after the cell has died.

For ongoing monitoring programs it is strongly recommended that a collection of photomicrographs

and permanent wet mounts or dry mounts (for diatoms) of the taxa observed be established for future reference and training of new staff. Such collections can also be handy in identifying possible changes in shape and size in particular species (eg. *Ceratium*) over the seasons. Keeping preserved material and photomicrographs will also facilitate information exchange between workers at different agencies. In addition to the photomicrograph and permanent slide collection, *written records need to be kept of unidentified taxa* in a standard format containing information such as cell dimensions, colour, number and shape of chloroplasts, presence or absence of cell organelles, unusual features and a drawing. Identification by an algal taxonomist can then be sought at a later stage.

4.1.13 Recording results

A *standard algal counting worksheet* (hardcopy or electronic form) should be used for all samples. It should give essential information about the sample and have space to record the counting results; for example, see Appendix E. The sheet should contain information on laboratory reference number, date and site of collection, number of the counting chamber used, magnification used, the volume of subsample for sedimentation or filtration, area counted and factors used to calculate the final cell count. The sheet should also contain the name of the person identifying and counting the sample and of the person entering the counting results onto the database. *A record should always be made of whether it was cells or colonies or filaments that were counted.* The worksheet should also provide a space for general comments. To save time it is useful to employ a multiple counter or, even better, one of the software programs designed for this purpose (see Appendix D).

The taxonomic level at which the counts are recorded depends on the aim of the study. It may vary from recording only counts summed by algal class with the exception of full identification of the dominant taxa (eg. green algae, diatoms, Euglenophyceae, cyanoprokaryotes, *Aulacoseira granulata*, *Microcystis aeruginosa*, *Actinastrum falcatus*) to identification to species level wherever possible.

Despite differing levels of accuracy, it is preferable to enter the enumeration results of all taxa, including the rare ones, onto the database. Time will be saved during data entry if species names are ordered by taxonomic classes. For routine monitoring at established sites, the 15 or so most common taxa should be prelisted on the worksheet.

Phytoplankton density is a concentration measure and is usually expressed in cells per millilitre. For certain studies, the *phytoplankton load* will be of interest. This can be calculated as the product of the cell concentration and the discharge. *For statistical reasons, phytoplankton is usually counted in units.* Units may be either single cells, colonies or filaments depending on the usual form of the species. If units are not single cells, the average number of cells per unit can be estimated by counting the cells per unit for the first 30 units observed and calculating an average. The number of units counted in the sample is then multiplied by the average number of cells per unit. This procedure needs to be repeated for each sample since the number of cells per unit can vary considerably in space and time.

4.1.14 Algal coding system

To facilitate electronic data storage, reporting and analysis, it is essential to use a coding system for the algal taxa when recording the counts. Several coding systems for algae have been suggested (for example, Lhotsky et al., 1974; Whitton et al., 1978; Williams et al., 1988). The latest and most comprehensive list is 'A Coded List of Freshwater Algae of the British Isles', a digital version of which is also available (Whitton et al., 1999). Although this list cannot be adapted directly for Australia because of differences in species, it is an excellent example of such an endeavour. *Any such system assigns a unique code to each individual taxon.* The code can comprise a series of numbers or letters or both. According to Whitton (1991), all of the suggested algal coding systems have shortfalls when screened against four criteria. One criterion demands the flexibility to accommodate future nomenclatural changes. Such changes can be anticipated, since algal taxonomy is still very much subject to change and opinions about the validity of various taxonomic systems vary. A second criterion requests 'dumping ground' categories on the master list for organisms that cannot be fully identified.

Setting up a comprehensive algal coding system will always meet taxonomic difficulties since many organisms have yet to be clearly identified. *Algal taxonomy, in contrast to that for higher plants, for example, is still at a stage of continuous revision.* In Australia, phycologists routinely resort to overseas texts for identifications because an Australian key has yet to be published. The lack of local taxonomic texts adds uncertainty to taxonomic identifications. Therefore, the proposed algal coding system contains 'dumping

ground' categories in which cells that cannot be identified to genus or species level are recorded. Of course, such cells and the category they were counted in need to be documented (see section 4.1.13, 'Recording results') to avoid recording the same cell in more than one category.

The development of a national master list of freshwater algae, the centrepiece of such a coding system, is beyond the brief of this current project, but the establishment of such a master list *for use by all water resource agencies and other algal workers would be highly desirable*. Use of such a system would greatly enhance information exchange between agencies and improve the usefulness of algal and related environmental databases in describing freshwater systems across basins or States.

The structure for the coding system suggested here incorporates elements of Whitton et al. (1978) and the code includes information on the phylum, genus and species of the algal cells. In the absence of an Australian flora, the phylum structure in *Algae, an introduction to phycology* (van den Hoek et al., 1995) is followed. This phylum structure has been adopted for the 'Algae in Australia' series of taxonomic texts (see Appendix C). Additionally, the following considerations were taken into account for the suggested format of the code:

- an eight-digit code can be used on all computer systems; and
- by allocating the first two digits for the protista and algal phyla, the code meshes well with the eight-digit code developed for all Australian organisms by the Victorian Environment Protection Authority (J. Dean, pers. comm.).

The adoption of the DIATCODE format for genus and species caters for the needs of diatom taxonomy. DIATCODE, a coding system for diatoms developed internationally, is already being used in Australia by diatom taxonomists. DIATCODE can be obtained by contacting Dr Helen Bennion, Environmental Change Research Centre, University College of London, 26 Bedford Way, London WC1H0AP, UK or on <http://www.geog.ucl.ac.uk/ecrc/>.

By choosing to include both a low taxonomic level (phylum) and a high taxonomic level (species and varieties), the coding system is useful for both general monitoring work and specific, in-depth taxonomic and ecological research such as nutrient transfer functions for diatoms where species identification is required. The phylum level is included to allow a grouping of detected algal taxa into relatively general categories

which are relevant for managers as well as scientists. Possible groupings here could be, for example, 'cyanoprokaryotes' versus 'other groups' for operational purposes or 'green algae', 'diatoms', 'chrysophyta', 'cryptophyta', 'cyanoprokaryotes' and so on, to look at different pigment composition or selective use of nutrients (eg. SiO₂ by diatoms). The level of genus is a relatively certain taxonomic unit. The identification to genus level is quite specific but does not require as many resources (time and taxonomic expertise) as identification to species level. Identification to species level is relevant for a range of different purposes such as the identification of potentially toxic cyanoprokaryote blooms, identification of odour-producing or filter-clogging 'nuisance algae', and detailed ecological work, including the use of benthic diatoms as water quality indicators.

4.1.15 Details of code

The suggested code consists of eight digits, the first four being alpha characters and the remainder usually being numeric characters. The first two digits code the highest taxonomic category (phylum), characters three and four represent the genus, characters five, six and seven represent the species and character eight is reserved for variety or form. Three characters for the species are needed to record some genera of diatoms and desmids which contain more than 99 species. Varieties and forms are most common among the diatoms. The codes for the phyla (the first two digits) are given in Table 4.1. The genera within a phylum and the species within a genus are ordered alphabetically.

Example for code:

CCAU003A	<i>Aulacoseira granulata</i>
CC	Phylum Protista, Bacillariophyta
AU	Genus <i>Aulacoseira</i>
003	Species <i>granulata</i>
A	type species

Example for varieties:

CCAU003B	<i>Aulacoseira granulata</i> var. <i>angustissima</i>
CCAU003D	<i>Aulacoseira granulata</i> var. <i>granulata</i>

Example for different species:

CCAU030A	<i>Aulacoseira crenulata</i>
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The designation of the last alpha character (digit eight) is arbitrary and can be assigned to either the type species or a variety. Normally, the type species gets a priority designation 'A', except where it has a nominated

variety. This is often the case, as in the following example.

Example for variety being the type species:

CCA005A *Aulacoseira distans* var.
distans

To describe an unknown variety of a known species, the last digit would be a '1', if it was the first unknown variety found. Using the numbers '1', '2' and so on instead of a '9' will make it easier in the context of a database to rename records once the variety has been identified or described.

The code for the remaining six digits is found in the master list that is to be developed. For diatoms the existing DIATCODE may be used.

Where a taxon is recorded in several size classes use the last digit to indicate the size class. An alpha character other than the ones employed for variations is chosen.

4.1.16 'Dumping ground' categories

An unidentified taxon is represented by a sequence of '9's. Where an alga can be recognised only at phylum level it would be recorded as

for example
CG999999 Unidentified
Euglenophyte

or at genus level as
CGEU9999 Unidentified genus Euglena.

Where an alga can be recognised only to genus level, three possibilities exist for it to be recorded:

- (a) If it is recognised as an alga belonging to a certain genus, it will be recorded under the genus

for example
CCA09999 *Aulacoseira* sp.

- b) If it can be clearly distinguished as a separate species, it will be coded as unknown species 1, 2 etc.

for example
CCA09919 *Aulacoseira* sp. 1

- c) Similarly, if it can be distinguished into a particular size category but not as a species, it will be coded as an unknown species using the last digit to indicate the size class according to the rules above.

for example
CCA0999R *Aulacoseira* size class
'small'

In some genera (eg. *Lyngbya*, *Oedogonium*), although the cells show a large morphological range, species cannot readily be recognised in the absence of reproductive stages or mature thalli. In these instances,

it is suggested that the genus be divided into size classes which are coded in the same manner as an unknown species with different size classes. Size classes for such genera are established in accordance with those occurring in the geographical area sampled.

The codes for the first two characters representing the phyla are as follows in Table 4.1.

Table 4.1: Higher taxa codes for algae

Code	Regnum	Phylum
A	Archaeobacteria	
AA		Archaeobacteria
B	Eubacteria	
BA		Other phyla of Eubacteria
BB		Cyanoprokaryotes
BC		Prochlorophyta
C	Eukaryota	
CA		Glaucophyta
CB		Rhodophyta
CC		Heterokontophyta (incl. Chrysophyceae, Xanthophyceae and Bacillariophyceae)
CD		Haptophyta
CE		Cryptophyta
CF		Dinophyta
CG		Euglenophyta
CH		Chloroarachniophyta
CI		Chlorophyta

4.1.17 Software for counting

Software is now available for recording cell counts directly into a computer data file during counting. Use of such programs saves time and eliminates data entry as a separate source of error. Most programs have minimal hardware requirements (eg. IBM-compatible 386 machine). Depending on the program, it will calculate final counts and give statistical error margins for the

results obtained. Counting results can be directly imported into analytical software. For details of programs and suppliers, see Appendix D.

4.1.18 Area Standard Units

Area Standard Units (ASU) are calculated as the sum of the maximal cross-sectional areas of all the algal cells found in a sample.

The use of ASU in Australia is limited, the only known example being in licence agreements for water treatment plants in the Sydney region. The sole purpose of ASU is to provide to the plant managers an indication of the filter-blocking capacity of the phytoplankton present in the raw water. *ASU are not a biomass measurement. The parameter ASU has no significance with regard to the ecological state of a waterbody and is of little relevance in water quality studies.*

Method

For each species occurring in the sample, the two longest cell dimensions (without spines or appendices) of a minimum of 30 individual cells are measured and the mean cross-sectional area calculated. Cell areas are estimated for each algal species from formulas for solid geometric shapes that most closely match the cell shape. Multiply the value of the cross-sectional area by the number of cells per millilitre for each species found in the sample. To obtain ASU for the sample, sum all products from all species found in the sample. It is self-evident that cell size measurements need to be repeated at different seasons and on algae originating from different waterbodies. For ongoing monitoring programs it is possible to set up a database of ASU for species occurring by measuring at least 100 cells over several seasons.

4.2 Semi-quantitative enumeration

If time restrictions do not allow a full quantitative analysis of the preserved material, a semi-quantitative estimate of cell numbers can be made from live samples not older than 24 hours. Semi-quantitative enumeration is not recommended as a benchmark method. Cells are concentrated by filtration, then counted in a Sedgwick-Rafter chamber or Lund cell as described above. Always

indicate in the data report that results are only semi-quantitative.

4.2.1 Concentration by filtration

A defined volume of sample is filtered using a membrane-type filter (for example, cellulose) of pore size 0.45 μm at low suction pressure to a point where the filter is still just wet. The algae are then brushed off and resuspended in 5 mL of distilled water. A 1 mL subsample is used to fill a Sedgwick-Rafter chamber or the appropriate volume to fill a Lund cell. Observations then proceed as usual. If motile forms are present, narcotisation is recommended (McAlice, 1971) with drops of isotonic magnesium chloride solution or one drop of saturated uranylacetate (UNESCO, 1974). Alternatively, adding methyl cellulose will increase the viscosity of the sample (UNESCO, 1974).

The advantage of counting live samples is the short turnaround time. The disadvantages are that naked algae or thin-walled diatoms may be damaged during filtration (UNESCO, 1974) and that the count is only semi-quantitative. This procedure might be used for operational purposes such as monitoring the development of an algal bloom at short intervals, but weekly or fortnightly counts from preserved material for verification should be maintained in parallel.

4.3 Biomass measurements

While cell counts give information on community structure and abundance of individual species they are not satisfactory as a measure of algal biomass. The two most common approaches to determine algal biomass are volumetric biomass determination and chlorophyll-*a* measurements.

4.3.1 Volumetric biomass determination

An inherent difficulty in relating algal densities to environmental parameters such as nutrients is the enormous size range of algal cells, from 1 μm to 10 μm (van den Hoek et al., 1995). *A cell count value of one can refer to a vast range of cell volumes.* There is no easy way to transform cell counts into volumes of living biomass. The most common method used by phycologists is to measure the dimensions of a representative number of cells (minimum of 30, but most researchers measure 100 to 200 cells) for each species and to calculate an average volume for each species using formulae for geometrical shapes closest to the cell's shape. A more sophisticated

approach includes the subtraction of the volume of the cell vacuole from the overall cell volume to gain a more realistic value. The average volume for each species is then multiplied by the cell count for the species in question and all the products summed to gain a biovolume per sample in mm^3 per millilitre. The total wet algal volume is calculated according to (APHA, 1995):

$$V_t = \sum_{i=1}^n (N_i \times V_i) \quad (10)$$

where:

V_t = total plankton cell volume (mm^3/L)

N_i = number of organisms of the i th species/mL

V_i = average volume of cells of i th species (μm^3).

The described procedure is time-consuming and tedious, but gives reliable results. *The average size of cells of a species may change by a factor of up to 10 during a growing season or its life cycle.* Lists of the mean biovolumes of species are available in the literature (Reynolds, 1984) and from some of the Australian algal counting laboratories. In using such values, keep in mind how much the average size of cells of a given species may vary within during a growing season and that the size of the same species may vary from one waterbody to another.

Although such a detailed study may be reserved for special investigations, because of the required resources, *data from monitoring programs that investigate the ecological context of algal populations would be much more useful if they included some indication of algal biomass.* It is therefore suggested that data on volumetric biomass be determined at a lower frequency within the regular sampling program (eg. monthly for a weekly program) and that emphasis be placed on the dominant species and very large species. Useful information will thus be gained for relating the development of phytoplankton populations to in-stream nutrient concentrations or to the biomass of higher levels in the food web (eg. zooplankton), since in most instances the dominant species make up 70 to 80% of the biomass.

4.3.2 Chlorophyll-*a*

In exploiting the fact that algae, like all plants, contain the pigment chlorophyll-*a*, one can measure its concentration in a water sample, then calculate algal biomass using an average factor for the chlorophyll-*a* concentration per cell: approximately 1 to 2% of dry weight in planktonic algae (APHA, 1995). This method

is faster than algal counts and can be performed with less training. It has two disadvantages: the loss of information on the species composition of the phytoplankton, and the large natural variation of chlorophyll-*a* concentration per cell which depends on such factors as species and physiological light adaptation. Cyanoprocaryotes contain less chlorophyll-*a* per cell weight than eukaryotic algal groups.

*Samples taken for chlorophyll-*a* analysis cannot be preserved.* To determine the chlorophyll-*a* concentration, a straight water sample of between 0.5 L and 5 L, depending on cell density, is taken and preferably filtered in the field. The sample is thoroughly mixed by shaking and a measured volume of it passed through a glass fibre filter (free of organic binder) with a pore size of 0.45 μm , using a suitable holder and hand pump (inexpensive hand pumps are commercially available). The filter is immediately placed in an opaque bag and deep-frozen in liquid nitrogen or in a car freezer. *Chlorophyll-*a* breaks down if exposed to light.* The filters are kept frozen until analysed in the laboratory. Processing of filters ideally occurs on the same day or, if this is not possible, they may be stored deep-frozen for up to three weeks (APHA, 1995) before analysis. Whichever procedure is adopted for the program, its validity should be tested beforehand. The high turbidity often present in Australian inland waters may interfere with the filtration and thus reduce the volume filtered.

There are various methods for measuring the chlorophyll-*a* concentration in water samples, including spectrophotometry, fluorimetry and high-performance liquid chromatography. In recent years, successful *in situ* fluorescence methods have been developed. The recommended spectrophotometric method is that described in ISO 10260:1992 (E), which uses hot ethanol as the solvent. It is most commonly used in routine and research work both in Australia (Clark and Lidston, 1993) and overseas (Tubbing et al., 1994). Many workers prefer hot ethanol because it is non-toxic and yields a better extraction than, for example, cold acetone. Further information on chlorophyll-*a* methods can be found in Nusch (1980), Marker et al. (1980) and Nusch (1984).

5. Quality assurance

Recommendations are provided on how to apply quality control and quality assurance principles to algal sampling and enumeration and how to correctly report data to clients. Correct database creation and maintenance are emphasised.

5.1 Quality assurance

Quality assurance consists of a set of guidelines that, if followed strictly for both field and laboratory procedures, will produce analytical results of known accuracy and precision. Quality assurance has two aspects: internal quality control and external quality assessment (Figure 5.1).

Quality control extends to areas of:

- employing suitably trained staff;
- implementing a quality system including a laboratory quality manual;
- providing reasonable facilities and suitable equipment;
- documenting analytical methods;
- documenting procedures for handling and analysing samples; and
- documenting procedures for data reporting and record keeping.

Basic principles of quality assurance can be found in the literature (NATA, 1992; NATA, 1993; ISO, 1994; APHA, 1995), so only the main issues in algal enumeration are addressed here.

Internal quality control in algal enumeration involves documenting the procedures employed for all steps involved and making them readily accessible to all staff concerned. The documentation will include acceptable error sizes and confidence limits for every step of enumeration. Steps include, for example, subsampling from the original sampling bottle and subsampling within a counting chamber if only part of the floor is counted. Quality of counting results will be enhanced by regularly (eg. annually) revising the procedures with staff and documenting such changes. This process will create consistency amongst operators in terms of procedures and taxonomic identification, and will ensure that improvements are incorporated.

Another element is operator competence. *This includes training staff in correct application of sample collection and analysis procedures.*

To check the *validity* of algal counting results and estimate their accuracy, the following procedures are implemented. To test *individual operator precision* (operator error) a count on three subsamples of one sample is conducted every three months and related errors and confidence limits calculated. To test for *precision within the laboratory*, every six months the same three subsamples of a sample are counted by each operator and the error and associated confidence limits calculated. Single species samples (cultures) are suitable for these two tests. To test consistency of species identification among operators and ensure that all operators reach the predetermined level of precision, the latter test should be repeated every six months with mixed species samples. A different operator should recount 5% of the samples in the same laboratory to test for precision of cell counts and species identification.

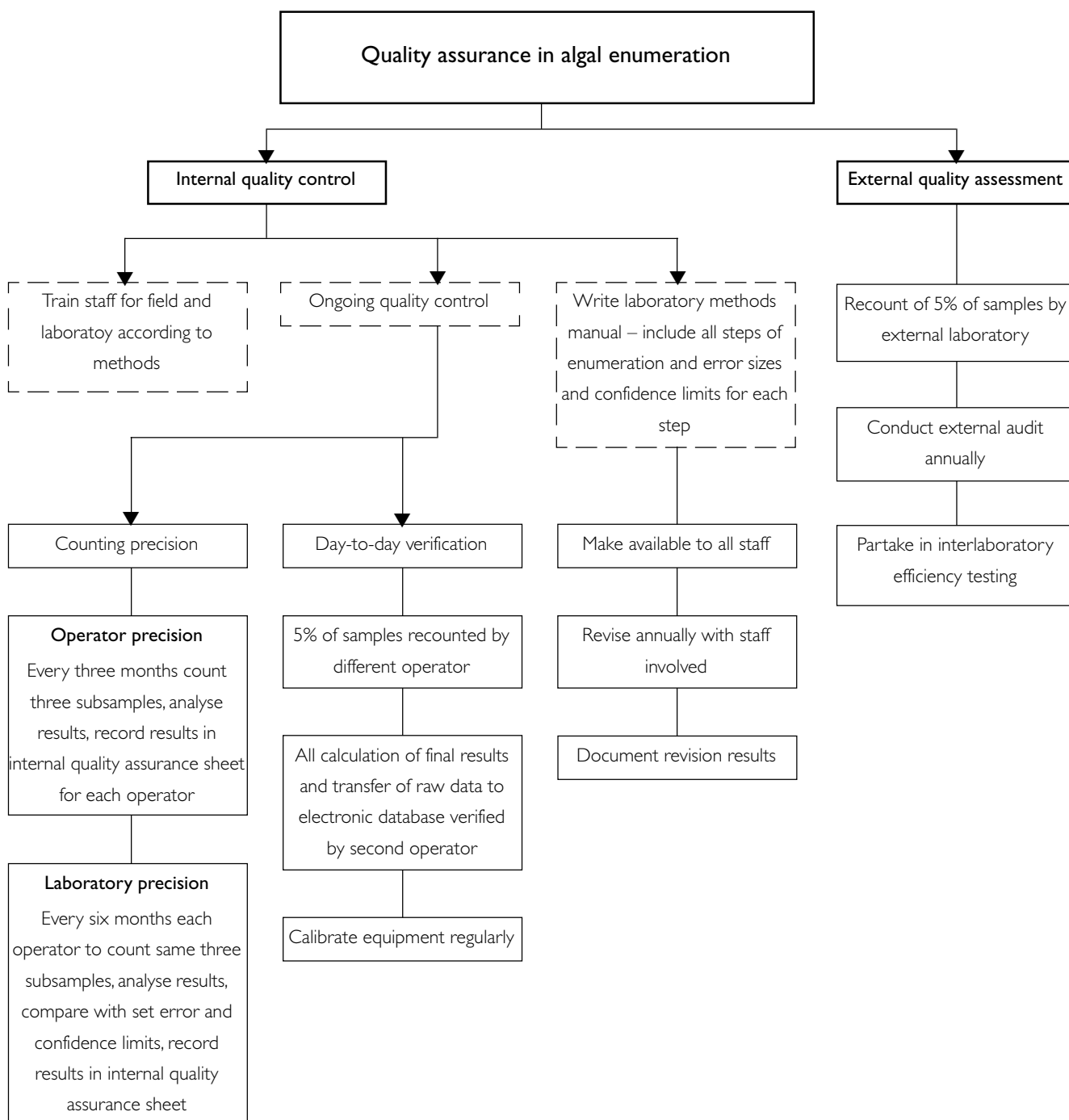
Equipment such as micropipettes, counting chambers and microscopes need to be regularly calibrated and maintained.

There is no detection limit for algal counts inherent in the method, rather the number of taxa found in a sample depends to some degree on counting time and subsample volume, and on the taxonomic proficiency of the operator. *The precision and accuracy of counting results always need to be stated* (see previous statistical section) *in the final report.*

Each step in the algal enumeration should be verified by a person other than the operator who performed that step. For instance, calculation of final count results should be verified by a second person. Correct data entry could be checked by comparing a print-out of the entered data with the original work sheet. At the point of data entry some automatic checking procedures, such as acceptable ranges, reporting of outliers and so on, should be built into the database program.

The second part of quality assurance, *quality assessment*, is the implementation of external checks to ensure adherence to the documented procedures. This includes interlaboratory tests and external audits at regular intervals (eg. six-monthly). It is recommended

Figure 5.1: Quality assurance in algal enumeration



that 5% of samples be recounted by a different laboratory as advised by standard quality control procedures (APHA, 1995). In Australia, such external quality assessment is provided within the framework of National Association of Testing Authorities (NATA) registration. In 1996, NATA began a proficiency testing program for laboratories performing algal enumeration. Eighteen laboratories participated in the first round of tests. Since 1998, participation in the interlaboratory proficiency testing has been compulsory for NATA-registered laboratories. NATA's address is given in Appendix A.

5.2 Reporting of data

A standard procedure which is documented in writing is followed for all steps in creating, transferring and storing data. On arrival in the laboratory, each algal sample should be given a unique and permanent identification number which will allow staff to link all records to the relevant sample from its arrival to issue of a final report. This procedure may be facilitated by an electronic laboratory sample management program. Standard field and laboratory recording sheets are essential (see Appendix E).

For every step of transferring data from one medium to another, a person other than the operator needs to verify the step. For example, final count results, if recorded on paper, need to be transferred from the original data sheet to the electronic database for storage and reporting. A person other than the data entry operator needs to verify the correctness of the data entered. Typing errors during data entry of final count results can be minimised by using an algal counting software program (see Appendix D) to directly enter counts into the computer during counting.

The database should be established with the help of an experienced phycologist to ensure an adequate taxonomic system is used and special considerations for algal enumeration are incorporated. The database for the algal counting results should be relational, allow easy transfer of data to other applications (eg. statistical analysis software) and facilitate report writing. An algal coding system (see above) encompassing different levels of taxonomic identification (open system) is essential. While using a code for data storage, (embedded) actual taxonomic names will appear on the database entry form if a relational database is used. Thus, fewer errors will be

made by the operator since names are easier to remember than codes.

Effective data storage depends on continuing database maintenance, including regular back-up, checks for correctness of entries, documentation of changes in procedures for algal counting and data entry, and incorporation of changes in taxonomy. Such maintenance is critical during long-term monitoring programs in particular, in order to keep the data accessible and useful for data analysis. For example, organisms may have been counted as unidentified flagellates at the beginning of the monitoring program then, after full identification, counted in a different taxonomic category and under a different code. Without documentation it is impossible to interpret such data sensibly. Species names may change over the years; for example, *Melosira granulata* (Ehrenb.) Ralfs, one of the more frequent members of riverine phytoplankton in Australia, was renamed *Aulacoseira granulata* (Ehr.) Simonson in 1989. In accordance with NATA requirements, data need to be stored for at least three years (NATA, 1992).

6. Staff

This section discusses occupational health and safety issues for field and laboratory work, in particular with regard to potentially toxic cyanoprokaryotes, as well as staff training needs.

6.1 Occupational health and safety issues

Standard occupational health and safety measures for field work as laid down in the organisation's field sampling manual and laboratory manual need to be implemented. Issues to consider include: sending at least two people into the field; carrying life saving gear; providing a mobile phone in working order; leaving clear information about the intended travel route and the expected time and date of return; and carrying sufficient drinking water and food for emergencies. Staff should have regular first aid training, including resuscitation procedures for field and laboratory, and be aware of dangers in the work place.

When field work involves sampling for potentially toxic cyanoprokaryotes, especially where they have formed scums, certain safety measures need to be followed. Avoid all direct body contact with the water containing the cyanoprokaryotes or cyanoprokaryote scums, since toxins might cause allergic reactions on the skin and in the eyes. Use rubber gloves, waders and protective eye gear when taking and handling the samples. Avoid contact with water spray when travelling in a boat because aerosols from infested water may cause respiratory problems or bring on asthma attacks. Under such conditions, odour masks may be worn. *Wash hands before drinking, eating or smoking.* Ingested toxic cyanoprokaryotes can cause acute allergic reactions as well as low level chronic damage of, for example, the liver. Working gear, such as waders, needs to be washed with clean water and soap after use.

Care needs to be taken with transport of *chemicals in the field* to avoid spillage and the inhalation of fumes while driving. *Formalin and glutaraldehyde are toxic chemicals* (carcinogenic) (see specification sheets from chemical suppliers) which should be stored safely (eg. airtight container) and separate from food items. Use of gloves while handling these chemicals is recommended.

Iodine, although not acutely toxic, is *harmful* and inhalation of vapours (eg. from open sedimentation vessels in the laboratory) should be avoided. Vessels can be placed under a fume hood, temporarily sealed with flexible film or, if resources permit, stoppered measuring cylinders can be employed. Staff should not work in areas of sample storage where the inhalation of built-up fumes poses a health hazard. The usual safety measures for the laboratory apply with respect to the handling of glassware and chemicals and to sample disposal.

Attention should be given to the ergonomics for microscope work. For example, a desk-high bench (lower than normal laboratory benches) and an adjustable chair should be provided to avoid muscle injury to workers, as should an appropriate light source that does not interfere with the illumination for microscopy. Ideally, a microscopy laboratory should have south-facing windows. The usual ergonomic requirements apply for the computer set up.

The maximum daily duration for sitting at the microscope is five hours. A good work routine includes getting up from the microscope or computer at regular intervals to take breaks, and performing a variety of tasks each day. Staff should be encouraged to do appropriate daily exercises to counterpoise the restricted sitting positions.

6.2 Training

Staff involved in algal enumeration need to be trained by an experienced algal counter. It will take several months to train new staff to recognise the more common genera and to produce reliable counting results. Training of a qualified algal taxonomist takes several years. A collection of preserved material and photomicrographs will be of great help. Staff from smaller laboratories often benefit from spending a few days in a larger laboratory with experienced staff.

It is strongly recommended that annual workshops are held at a national level among the algal workers in water resource agencies in Australia, to allow exchange of information on procedures and identification, and an increase in the level of taxonomic expertise. Specialists for particular algal groups may be invited to the workshops. Algal taxonomy is complex and still under

constant review, and there is a constant need for phytoplankton workers to keep abreast of ongoing developments. The first Australian National Algal Workshop was held in Adelaide in February 1999, providing an overview of the characteristics of algae and dealing with the identification of common Australian cyanoprokaryote species and common diatom genera under the guidance of Australian taxonomic specialists. In future, such workshops will be held on a regular basis, dealing with different taxonomic groups. Details will be announced in the relevant newsletters and on the Australian algal discussion list (ALGANTIPOD@latrobe.edu.au). The first two Australian algal keys were produced for this workshop (see Appendix C).

It is essential that staff performing field sampling be thoroughly trained in the required procedures and understand the reasons for (a) taking the sample and (b) the protocol that needs to be followed when taking the sample. It is recommended a refresher course be conducted for field staff annually. Such training will significantly increase sample quality and accuracy of data and therefore justify the additional costs involved.

7. Guidance on introducing a new method

This chapter gives guidance on how to accomplish a change from an existing method for sampling, algal counting and chlorophyll-*a* analysis to a benchmark method recommended in this manual, without losing continuity of data for ongoing monitoring programs.

Before a change in methodology is undertaken, prepare a document containing the following information:

- reasons for changing method
- improvements expected from method change
- documentation of existing and new method
- outline for introduction of new method
- comparison between existing and new method.

For the process of introducing a new method where continuity of data is needed, the steps described below may serve as a guide.

Reasons for changing method

The reasons for changing the method are documented. They will vary from laboratory to laboratory but may include the following: the existing method is outdated or semi-quantitative in nature; there is a desire to change to a benchmark method; a new methodology has become available; or, perhaps, the purpose of algal monitoring within the organisation has changed and requires a different methodological approach.

Improvements expected from method change

Detail the qualitative and quantitative improvements in results the new method will bring. Explain how results from the new method will be more representative of the true value of what is being measured than those obtained with the existing method. For example, a change of method from the concentration of live algae using sand filtration to sedimentation of Lugol's-preserved samples would represent a change from a semi-quantitative method to a quantitative method. As a consequence, different species may be found and/or their cell concentrations may increase or decrease compared to results gained from the existing method.

Documentation of existing and new method

A step-by-step description of each method should be prepared in the format usual for the laboratory's quality control manual. *The theoretical principles of the old and new method should be entirely understood and documented and the differences between the two clearly spelled out.* In some instances, the two methods would be based on different principles so that a direct comparison would yield no conclusive results. Considering these principles, decide on whether a comparison between the two methods is meaningful and how it can be accomplished following the considerations outlined below.

Outline for introduction of new method

Prepare an outline of how the new method will be introduced, including the individual steps to be taken, the time frame for the changeover process, staff and funding required, new equipment to be purchased and implications for quality assurance and quality control.

1. Specify the *outcomes* expected from the comparison and a *time frame* for the process of changeover, including the validation of the new method against the existing one.

If the comparison is undertaken by experiment, and if all the necessary samples have been collected beforehand (preserved samples only), the experiment can be run as one block. Thus, it can be performed in a relatively short time (say, within a few months) depending on staffing and the time made available outside the routine counting. If, however, fresh samples are needed, the experiment can be run whenever suitable samples occur and the data from different samples collated in due course.

If the comparison is undertaken by running the two methods in parallel, *a period of one year is recommended to include samples from all seasons and samples representing different environmental conditions in the waterbody that occur during the course of the year* (Bartram and Ballance, 1996).

2. Perform the comparison, either by experiment or by concurrently running the methods, and clearly document the differences in results gained from the two methods.

3. Completeness and representativeness of comparison

Regardless of whether the comparison is made by experiment or by a parallel running of the methods, *it is important to select a representative subset of samples.*

Samples should:

- originate from different types of waterbodies (universal application of results);
- cover all flow conditions experienced;
- represent the different seasons normally monitored;
- contain the whole range of species routinely encountered in the laboratory; and
- represent the complete range of cell concentrations routinely encountered.

Different algal species may be differently affected by sampling or counting with an alternative method. Therefore, it is essential to choose the subset of samples for the comparison in such a way that it contains all species which are routinely encountered in the laboratory.

4. Document the expertise of staff with the two methods and the taxonomic keys used before and after the changeover as this might impact on the outcome of the comparison.

5. Quality assurance and quality control matters

Clearly flag in your database documentation the change of method, indicating the date for each waterbody monitored and how it might affect future analysis of the data set.

Provide a reference to where in the laboratory's quality assurance and quality control documentation the changeover is documented.

6. Review in detail the outcome of the comparison (eg. advantages and disadvantages) of the new method against the expected outcomes under 1. Decide on how the new method will be introduced.

Comparison between existing and new method

The major component of the changeover will be the execution and documentation of the comparison between the two methods. Such comparison may be

achieved either by an experiment or by running the two methods in parallel for a defined period of time.

Experiment: chlorophyll-a method

To test for equivalence of the two methods in the analysis of a single factor such as chlorophyll-*a*, a minimum of three different concentrations is tested using a minimum of five subsamples for each method. If a large range of concentrations is normally encountered, test more than three concentrations. The results are then compared applying the statistical analysis outlined below, according to APHA (1995).

1. The data are tested for normal distribution and transformed if necessary.
2. The standard deviation is calculated and a sufficient sample size is chosen accordingly.
3. Using the F-ratio statistic the variance of each method is tested.
4. Finally the average values from each method are tested for differences using the Student t-test.

If the results from the two methods are significantly different, plot the data and calculate the two regression equations. A conversion factor can then be calculated expressing how data obtained by the new method relate to those obtained by the existing method according to

$$f = \frac{R_{old}}{R_{new}} \quad (11)$$

where R_{old} is a test result obtained by the existing method, R_{new} is a result obtained with the new method and f is the factor. References regarding details of statistical analysis can be found in APHA (1995).

Experiment: sampling

If the change concerns the sampling methodology, take samples employing both methods at the same time. Take a minimum of 10 subsamples, but if possible 20 subsamples, from the respective sample and count as usual. Repeat the experiment on several occasions, but at least 10 times, so that samples represent the range of conditions normally encountered for the waterbody in question (see 'representativeness of samples' above). Review the results as described under 'Experiment: algal enumeration' below.

Experiment: algal enumeration

Obtain a large enough sample volume to be able to take the necessary number of subsamples. From a water sample, take a minimum of 10 subsamples, but if

possible 20 subsamples, for each method and proceed with the algal identification and enumeration as per described methods. In each sample, identify all occurring algal taxa and count the 7 to 10 most abundant. Also count any large (but not necessarily abundant) taxa such as *Ceratium*, and any taxa that form large colonies. Additionally, determine the total cell count for each subsample. Repeat the same procedure several times (at least 10 times) with different samples chosen according to the criteria for representativeness set out above.

The results should give a clear indication as to whether there is, between the two methods, (a) a difference in the number and type of species identified (qualitative) and/or (b) an increase or decrease in cell concentrations (quantitative). In the documentation of the comparison, state in detail what the differences were; for example, which species were detected by one method but not by the other, or which species had a higher or lower cell count in the new method as compared to the existing method. Through this detailed descriptive data analysis a picture will emerge as to where the differences lie between the two methods in question.

For multifactor analyses such as algal counts, a direct statistical comparison for a full count from a natural sample is intrinsically very complicated. There is no proven model for such a situation available in Australia. The matter is further complicated by the fact that, within a sample, different levels of accuracy exist for each taxon according to its concentration or the number of algal units found in the sample. Thus, for algal counts, the calculation of a single conversion factor is not possible. However, it may be estimated by the percentage of the results gained with the new method that are in general higher or lower than those gained with the old method.

Parallel use of methods

As an alternative to the experimental approach, the two methods may be used in parallel for a fixed period of time. This period is generally one year in order to capture samples representing the usual extent of variation experienced by the waterbody. This applies to algal enumeration, sampling methodology and chlorophyll-*a* analysis.

If the two methods are used in parallel, it is advisable to use a subset of the samples processed by the laboratory in its routine work. The subset is chosen according to seasonal difference in species composition, cell concentration, source of water sample, type of

waterbody, type of sample and occurrence of problem species as the dominant algae.

For a comparison of chlorophyll-*a* analyses or sampling methodology, use the same guidelines with respect to the number of occasions and number of replicate samples as for 'Experiment: chlorophyll-*a*'. For algal enumeration, process five subsamples for each method on each occasion. A minimum of 20 different samples should be analysed for this test. Tabulate results as per (a) and (b) under 'Experiment: algal enumeration'.

References

- APHA (American Public Health Association) 1995, *Standard methods for the examination of water and waste water*, 19th edn, APHA, Washington D.C.
- Baker, P. 1986, *Enumeration of phytoplankton*, Progress report No. 1, State Water Laboratory, Engineering and Water Supply Department, South Australia, Library reference number 86/48.
- Bartram, J. and Ballance, R. 1996, *Water quality monitoring UNEP*, E & FN Spon, London.
- Chorus, I and Bartram, J. (eds) 1999, *Toxic cyanobacteria in water, a guide to their public health consequences, monitoring and management*, E & FN Spon, London.
- Clark, H. and Lidston, J. 1993, *Photometric chlorophyll-a determination method*, State Water Laboratory, Victoria, Report no. WQ 57.
- Fisher, R.A., Thornton, H.G. and MacKenzie, W.A. 1922, 'The accuracy of the plating method of estimating the density of bacterial populations', *Annals of Applied Biology*, 9, 325–359.
- Furet, J.E. and Benson-Evans, K. 1982, 'An evaluation of the time required to obtain complete sedimentation of fixed algal particles prior to enumeration', *British Phycology Journal*, 17, 235–258.
- Gilbert, J.Y. 1942, 'The errors of the Sedgwick-Rafter counting chamber on the enumeration of phytoplankton', *Transactions of the American Microscopy Society*, 61, 217–226.
- Harris, G. 1978, 'Photosynthesis, productivity and growth: The physiological ecology of phytoplankton', *Arch. Hydrobiol. Beih. Ergeb. Limnol.*, 10, 1–171.
- Hasle, G.R. 1969, 'An analysis of the phytoplankton of the Pacific Southern Ocean', *Hvalraad ets Skrifter*, 52, 1–168.
- Hasle, G.R. 1978, 'The inverted microscope method', in Sournia, A. (ed) *Phytoplankton manual*, UNESCO, Paris.
- Hötzl, G. and Croome, R. 1994, 'Long-term phytoplankton monitoring of the Darling River at Burtundy, New South Wales: evidence and significance of cyanobacterial blooms', *Australian Journal of Marine and Freshwater Research*, 45, 747–759.
- 1996, 'Population dynamics of *Aulacoseira granulata* (Ehr.) Simonson (Bacillariophyceae, Centrales), the dominant alga in the Murray River, Australia', *Archiv für Hydrobiologie*, 136/2, 191–215.
- ISO (International Standards Organization) 1980, *Water quality – Sampling – Part 1: Guidance on the design of sampling programmes*, ISO 5667-1.
- 1994, *Quality management and quality assurance standards*, ISO 9000.
- Jones, G. 1997a, 'Limnological Study of cyanobacterial growth in three South-East Queensland reservoirs', in Davis, J.R. (ed) *Managing algal blooms*, CSIRO Land and Water, Canberra.
- Jones, G. 1997b, *National protocol for the monitoring of Cyanobacteria and their toxins in surface waters*, Draft, CSIRO Land and Water in association with the Agriculture and Resource Management Council of Australia and New Zealand.
- Klee, O. 1993, *Wasser untersuchen, Biologische Arbeitsbücher* (The examination of water, Biological Workbooks), Quelle & Meyer Verlag, Heidelberg.
- Laslett, G.M., Clark, R.M., Jones, G.J. 1997, 'Estimating the precision of filamentous blue-green algae cell concentration from a single sample', *Envirometrics*, 8(4), 313–339.
- Lhotsky, O., Rosa, K. and Hindak, F. 1974, *Supis Sinic a Rias Slovenska*, Bratislava, Czechoslovakia.
- Lund, J.W.G. 1959, 'A simple counting chamber for nanoplankton', *Limnology and Oceanography*, 1, 57–65.
- Lund, J.W.G., Kipling, C. and Le Cren, E.D. 1958, 'The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting', *Hydrobiologia*, 11, 143–170.

- McAlice, B. 1971, 'Phytoplankton sampling with the Sedgwick-Rafter cell', *Limnology and Oceanography*, 16, 19–28.
- Margalef, R. 1969, 'Counting', in: R.A. Vollenwieder, J.F. Taling and D.F. Westlake (eds) *A manual of methods for measuring primary production in aquatic environments including a chapter on bacteria*, International Biological Programme/Oxford, Blackwell Scientific Publ. (IBP Handbook 12), London, 7–14.
- Marker, A.F.H., Nusch, E.A., Rai, H. and Riemann, B. 1980, 'The measurement of photosynthetic pigments in freshwaters and standardization of methods: conclusion and recommendations', *Ergebnisse der Limnologie*, 14, 91–106.
- May, V. 1988, The effect of drought and position in the dam on the algal composition of two dams in New South Wales. *Cunninghamia*, 2(1), 75–84.
- Moss, A.J. and Hunter, H. 1992, 'Why, where, when, what and how to sample', in G.F. Rayment and W.A. Poplawski (eds) *Proceedings of the Training workshop on sampling for water quality monitoring*, Condamin-Balonne Water Committee, 27–28 May 1992, Dalby, Queensland, Dalby No 1992-05, pp. 6–20.
- NATA (National Association of Testing Authorities) 1992, *General requirements for registration*, NATA.
- 1993, *Supplementary requirements for registration – biological testing*, NATA.
- Nauwerk, A. 1963, 'Die Beziehungen zwischen Zooplankton und Phytoplankton im See Erken' (The relationship between zooplankton and phytoplankton in Lake Erken), *Symbolae Botanicae Upsalienses*, 17(5), 1–163.
- Nusch, E.A. 1980, 'Comparison of different methods for chlorophyll and phaeopigment determination', *Archiv für Hydrobiologie Beihefte Ergebnisse der Limnologie*, 14, 91–106.
- 1984, Results from an interlaboratory trial concerning the determination of Chlorophyll-*a*, *Zeitschrift für Wasser und Abwasser Forschung*, 17, 189–194.
- O'Farrel, A.V. and Lombardo, R.J. 1998, 'Cross-channel and vertical variation in diversity and abundance of phytoplankton in the Lower Paraná River, Argentina', Large Rivers Vol. 11/2, *Arch. Hydrobiol. Suppl.* 115/2, 103–123.
- Paasche, E. 1960, 'On the relationship between primary production and standing crop of phytoplankton', *J. Cons. CIEM*, 26(1), 33–48.
- Reynolds, C.S. 1984, *The ecology of freshwater phytoplankton*, Cambridge University Press, Cambridge.
- 1988, 'Potamoplankton: paradigms, paradoxes and prognoses', in F.E. Round (ed) *Algae and the aquatic environment*, Biopress, Bristol, 285–311.
- Reynolds, C.S. and Jaworski, G.H. 1978, 'Enumeration of natural *Microcystis* populations', *British Phycology Journal*, 13, 269–277.
- Schwoerbel, J. 1970, *Methods of hydrology: freshwater biology*, Pergamon Press.
- Sherman, B.S. and Webster, I.T. 1994, 'A model for the light-limited growth of buoyant phytoplankton in a shallow, turbid waterbody', *Australian Journal of Marine and Freshwater Research*, 44, 847–862.
- Sherman, B.S., Whittington, J. and Oliver, R. 1999, 'The impact of destratification on water quality in Chaffey Dam', Proceedings of the Kinneret Symposium on Limnology and Lake Management 2000+, *Arch. Hydrobiol.* (in press).
- Thronsdon, J. 1978, 'Preservation and storage', in A. Sournia (ed) *Phytoplankton manual*, UNESCO, Paris.
- Tubbing, M.J., Admiraal, E., Backhaus, D., Friedrich, G., de Ruyter van Steveninck, E.D., Mueller, D. and Keller, I. 1994, 'Results of an international plankton investigation on the River Rhine', *Water Science and Technology*, 29/3, 9–19.
- UNESCO 1974, *A review of methods used for quantitative phytoplankton studies*, Final report of SCOR Working Group 33, UNESCO Technical Papers in Marine Science, 18, Paris.

- Utermöhl, H. 1958, 'Zur Vervollkommung der quantitativen Phytoplankton Methodik' (Towards a perfection of quantitative phytoplankton methodology), *Mitteilungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie*, 9, 1–38.
- van den Hoek, C., Mann, D.G. and Jahns, H.M. 1995, *Algae: an introduction to phycology*, Cambridge University Press, Cambridge.
- Vollenweider, R.A. (ed) 1969, *A manual on methods for measuring primary production in aquatic environments*, IBP Handbook No. 12, International Biological Programme, Blackwell Scientific Publications, Oxford.
- Whitton, B.A. 1991, 'Use of coding systems for algal taxa', in B.A. Whitton, E. Rott and G. Friedrich (eds) 1991, *Use of algae for monitoring rivers*, Proceedings of an International Symposium, 26–28 May 1991, Innsbruck.
- Whitton, B.A., Holmes, N.T.H. and Sinclair, C. 1978, *A coded list of 1000 freshwater algae of the British Isles*, Water Archive Manual Series, No. 3, Water Data Unit, Department of the Environment, Reading.
- Whitton, B.A., John, D.M., Johnson, L.R., Boulton, P.N.G., Kelly, M.G. and Haworth, E.Y. 1999, *A coded list of freshwater algae of the British Isles*, Institute of Hydrology, Wallingford, Oxon. Online: http://www.nwl.ac.uk/~loissys/algals_coded_list.htm.
- Willen, E. 1976, 'A simplified method of phytoplankton counting', *British Phycology Journal*, 11, 262–278.
- Williams, D.M., Hartley, B., Ross, R., Munro, M.A.R., Juggins, S. and Battarbee, R.W. 1988, *A coded list of British diatoms*, ENSIS Publishing, London.

Appendices

The appendices contain more useful and practical information. An extensive list of taxonomic algal literature including relevant Australian material is presented together with field and laboratory standard data sheets and listings of the equipment required. Detailed descriptions of different samplers are given as well as information on software for algal counting.

Appendix A: List of agencies involved in development of this manual, including contact officers

Agency name

Ecowise Environmental–ACTEW
Environment Protection Authority, South Australia
Australian Water Quality Centre (South Australian Water Corporation)
Department of Lands, Planning & Environment, Northern Territory
Environment Protection Authority, Victoria
Water Ecoscience
Natural Resources and Environment, Victoria
Goulburn Murray Water

Melbourne Water Commission
Parks Victoria
Gippsland Water
Environment Protection Authority New South Wales
NSW Department of Land and Water Resources
Australian Water Technologies Ensignt
Hunter Water Corporation
Department of Primary Industry & Fisheries, Tasmania
Water and Rivers Commission, Western Australia

Water Authority, Western Australia
Queensland Health Scientific Services
Queensland Department of Environment and Heritage
Queensland Department of Natural Resources, Water Monitoring
Brisbane City Council Brisbane Water
Murray–Darling Basin Commission
ARMCANZ Algal Manager
National Association of Testing Authorities

Liaison officer

Frederick Bouckaert
Peter Goonan
Peter Baker
Armando Padovan/Simon Townsend
Leon Metzeling/Peter Newall
Kumar Eliezer/Frances Cannon
Ross Perry/Warren Wealands
Pat Feehan/Anne Graesser/
Graeme Wilkenson
Mike Chapman
Peter Kemp
Jenny Jelbart
Sean Hardiman/Penny Ajani
Lee Bowling
Peter Hawkins/Jennie Thompson
Bruce Cole
Chris Bobbi
Wasele Hosja/Verity Klemm/
Jane Latchford/Petrina Riatt
Jeff Kite
Maree Smith
Munro Mortimer
Wojciech Poplawski/Glenn McGregor
Graham Baxter
Bob Banens/Martin Shafron
Gary Jones/Mike Burch
Tanya Orlova/Neil Shepperd

Address of NATA for interlaboratory comparisons of algal enumeration:

Proficiency testing for algal counts (interlaboratory comparisons)
Proficiency testing
National Association of Testing Authorities
7 Leeds Street, Rhodes NSW 2138, Australia

Appendix B: Lugol's solution

Mix 20 g of KI (potassium iodide) with 200 mL distilled water and dissolve 10 g pure iodine in this solution. Add 20 g of glacial acetic acid a few days before use (Schwoerbel, 1970). The solution must be stored in the dark (Vollenweider, 1969).

Appendix C: Algal taxonomic literature

Works marked with an asterisk (*) are basic keys. Works marked with a dagger (†) are needed for diatom identification. An English translation of the keys in Krammer and Lange-Bertalot (1986) is being prepared at the EPA Victoria.

*Baker, P. 1991, *Identification of common noxious cyanobacteria. Part I – Nostocales*, Urban Water Research Association of Australia, Research report no. 29, June 1991, Australian Centre for Water Treatment and Water Quality Research, Melbourne and Metropolitan Board of Works, Melbourne.

*— 1992, *Identification of Common Noxious Cyanobacteria, Part II – Chroococcales Oscillatoriales*, Research report no. 46, October 1992, Australian Centre for Water Treatment and Water Quality Research, Urban Research Association of Australia, Melbourne.

Baker, P.D. and Fabbro, L.D. 1999, *A guide to the identification of common blue-green algae (Cyanoprokaryotes) in Australian freshwaters*, Cooperative Research Centre for Freshwater Ecology, Identification Guide No. 25, Thurgooona.

*Bellinger, E.G. 1992, *A key to common algae freshwater estuarine and some coastal species*, 4th edn, the Institution of Water and Environmental Management, London.

Bourrelly, P. 1966, *Les Algues D'Eau Douce. Tome 1: Les Algues vertes*, Editions N. Boubée & Cie, Paris.

— 1968, *Les Algues D'Eau Douce. Tome 2: Les Algues jaunes et brunes. Chrysophycees, Xanthophycees et Diatomees*, Editions N. Boubée & Cie, Paris.

— 1970, *Les Algues D'Eau Douce. Tome 3: Les Algues bleues et rouges. Les Eugleniens, Peridiniens et Cryptomonadines*, Editions N. Boubée & Cie, Paris.

Brook, A.J. 1981, *The biology of desmids*, Blackwell, London.

Canter-Lund, H. and Lund, J.W.G. 1996, *Freshwater algae: their microscopic world explored*, Biopress Ltd, Bristol, England.

Cox, E.J. 1996, *Identification of freshwater diatoms from live material*, Chapman & Hall, London.

Day, S.A., Wickham, R.P., Entwisle, T.J. and Tyler, P.A. 1995, *Bibliographic checklist of non-marine algae in Australia*, Australian Biological Resources Study, Canberra.

Entwisle, T.J., Sonneman, J.A. and Lewis, S.H. 1997, *Freshwater algae in Australia*, Sainty and Associates Pty Ltd, Potts Point, Australia.

Foged, N. 1978, 'Diatoms in eastern Australia', *Bibliotheca Phycologica*, 47, pp. 1–255.

Gell, P.A., Sonneman, J.A., Reid, M.A., Illman, M.A. and Sincock, A.J. 1999, *An illustrated key to common diatom genera from Southern Australia*, Cooperative Research Centre for Freshwater Ecology, Identification Guide No. 26, Thurgooona.

Hallegraeff, G.M. 1991, *Aquaculture's guide to harmful Australian microalgae*, Fishing Industry Training Board of Tasmania, Hobart.

Hodgson, D., Vyverman, W. and Tyler, P. 1997, 'Diatoms of meromictic lakes adjacent to the Gordon River, and of the Gordon River estuary in south-west Tasmania', *Bibliotheca Diatomologica*, Band 35, J. Cramer in der Gebrüder Bornträger Verlagsbuchhandlung, Berlin, Stuttgart.

Holland, J. and Clark, R.L. 1989, *Diatoms of the Burrunjuck Reservoir, New South Wales, Australia*, Divisional report 89/1, CSIRO, Institute of Natural Resources and Environment, Division of Water Resources, Canberra.

Huber-Pestalozzi, G. 1983, 'Das Phytoplankton des Suesswassers, Systematik und Biologie', *Die Binnengewässer*, Band XVI, Teil 1–8. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.

Hustedt, F. 1930, 'Bacillariophyta (Diatomeae)', in A. Pascher 1930, *Süßwasserflora von Mitteleuropas*, Heft 10, Gustav Fischer, Jena.

Komarek, J. and Anagnostidis, K. 1998, 'Cyanoprokaryota I. Chroococcales', in *Süßwasserflora von Mitteleuropa*, Band 19, Koeltz Scientific Books, Koenigstein.

- †Krammer, K. and Lange-Bertalot, H. 1986, *Süßwasserflora von Mitteleuropa Band 2/1, Bacillariophyceae (Naviculaceae)*, Gustav Fischer Verlag, Stuttgart, Jena.
- †— 1988, *Süßwasserflora von Mitteleuropa Band 2/2, Bacillariophyceae (Bacillariae, Epithemiaceae, Surirellaceae)*, Gustav Fischer Verlag, Stuttgart, Jena.
- †— 1991, *Süßwasserflora von Mitteleuropa Band 2/3, Bacillariophyceae (Centrales, Fragilariaceae, Eunotiaceae)*, Gustav Fischer Verlag, Stuttgart, Jena.
- †— 1991, *Süßwasserflora von Mitteleuropa Band 2/4, Bacillariophyceae (Achnantheaceae, Literaturverzeichnis)*, Gustav Fischer Verlag, Stuttgart, Jena.
- Leedale, G.F. 1967, *Euglenoid flagellates*, Prentice-Hall Biological Science series, Englewood Cliffs, New Jersey.
- Ling, H.U. and Tyler, P.A. 1986, *A limnological survey of the Alligator Rivers Region II. Freshwater algae, exclusive of diatoms*, Supervising Scientist of the Alligator Rivers Region, Research report 3, AGPS, Canberra.
- 1986, *A limnological survey of the Alligator Rivers Region. Part 2: Freshwater algae, exclusive of diatoms*, Supervising Scientist of the Alligator Rivers Region, AGPS, Canberra.
- McLeod, J. 1975, The freshwater algae of South-Eastern Queensland, PhD thesis, University of Queensland.
- Patrick, R. and Reimer, C.W. 1966, *The diatoms of the USA*, Vol. I, Monographs of the Academy Natural Science, Philadelphia.
- 1975, *The diatoms of the USA*, Vol II, Monographs of the Academy Natural Science, Philadelphia.
- Prescott, G.W. 1951, *Algae of the Western Great Lakes Area* (1982), rev. O. Koeltz Science Publishers, Germany ed. Wm. C. Brown Company Publishers, Dubuque, Iowa.
- *— 1978, *How to know the freshwater algae*, Wm. C. Brown Company Publishers, Dubuque, Iowa.
- Prescott, G.W. and Scott, A.M. 1952, 'Some South Australian desmids', *Transactions of the Royal Society of South Australia*, 75, pp. 55–69.
- *Streble, H. and Krauter, D. 1988, *Das Leben im Wassertropfen*, 8th edn, Franckh'sche Verlagsbuchhandlung, Stuttgart.
- †Thomas, D.P. 1983, *A limnological survey of the Alligator Rivers Region. Part 1: Diatoms (Bacillariophyceae) of the Region*, AGPS, Canberra.
- Vyverman, W., Vyverman, R., Hodgson, D. and Tyler, P. 1995, Diatoms from Tasmanian mountain lakes: a reference data-set (TASDIAT) for environmental reconstruction and a systematic and autecological study, *Bibliotheca Diatomologica*, Band 33, J. Cramer in der Gebrüder Bornträger Verlagsbuchhandlung, Berlin, Stuttgart.
- West, G.S. 1909, 'Algae of the Yan Yean Reservoir, Victoria, A biological and oecological approach', *Journal of the Linnean Society (Botany)*, 39, 1–88.
- Wetzel, R.G. 1975, *Limnology*, W.B. Saunders Company, Philadelphia.
- The publications by Day et al. (1995) and Ling and Tyler (1986) contain the most comprehensive records on papers published about Australian freshwater algae. Among the earliest papers to describe Australian algae which are still useful today are those of West (1909), Prescott and Scott (1952) and Playfair (listed in Day et al., 1995).
- A new taxonomic series called *Algae of Australia* will be published by the Australian Biological Resources Study (Environment Australia) including descriptions of freshwater and marine microalgae and macroalgae for this continent. The first volume is due to be published in 1999.

Appendix D: Recommended field and laboratory equipment

Field gear

10 L bucket
Clipboard
Eskies or refrigerator for live samples
Standardised phytoplankton field sampling sheet
Ice
Liquid nitrogen
Lugol's solution and/or formalin
Pen and waterproof labels
Phytoplankton net – mesh size around 20 µm
Safety equipment
Sampling bottles
Waders
Water sampler

Laboratory equipment

(See also items mentioned in section 'Microscope equipment')

Algal counting software
Bench space for sedimentation, preferably under a fume hood
Computer for counting program
Counting chambers, calibrated if necessary – chambers are available from commercial microscopy suppliers
Dark storage space
Eyepiece graticule
Light microscope, either an upright instrument or an inverted microscope, depending on the counting method employed
Measuring cylinders of varying sizes (10 mL to 1,000 mL)
Mechanical counting bank or computer with counting software
Microscope slides and coverslips
Pasteur pipettes
Planimeter for Sedgwick-Rafter calibration
Stage micrometer
Standardised algal counting sheet
Taxonomic literature
Vacuum pump
Vaseline, hot plate and pointed tweezers if home-made Utermöhl chambers are used
Vibration-free microscope bench
Whipple graticule

Suppliers of specialised items

Precision-built Utermöhl chambers:

Phycotech
Suite 100, 620 Broad St, St Joseph MI 49085, USA
Phone +616 983 3654, Fax +616 983 3653
Email phycotech@parrett.net

Hydro-Bios Apparatebau GmbH
P.O. Box 8008, D-24154 Kiel-Holtenau, Germany
Phone +0431 36960 0, Fax +0431 36960 21
Email hydrobios@t-online.de

Micrometers and Whipple graticules:

Graticules Ltd
Morley Rd, Tonbridge, Kent, TN9 1RN
Phone +0732 359061, Fax +0732 770217

Utermöhl chamber building instructions

A slide 3 mm thick to fit the inverted microscope at hand is cut out of perspex and a hole of 1 cm diameter cut in the centre position (bottom slide). The bottom edge of this slide needs to sit on the microscope stage to coincide with the optical plane. A second slide of the same dimensions, also with a hole but 4 mm thick, is cut and a tube with an inner diameter of 1 cm and a height of 5.5 cm is glued permanently to the slide (top slide). These two slides are assembled into a chamber by applying a thin film of vaseline on facing surfaces and pressing together. A thin film of vaseline is applied to the other side of the bottom slide around the hole and a coverslip preheated on a hot plate is attached. The algal cells will sediment onto this coverslip and can thus be viewed directly through the objectives. If concentration of the sample is required, such small chambers necessitate an additional step of subsampling.

Larger Utermöhl chambers, allowing the direct sedimentation of 5 mL, 10 mL, 50 mL and 100 mL without an additional subsampling step, are commercially available from the suppliers given above.

Lund cell building instructions

Onto the long edges of a thoroughly cleaned and dried microscope slide, glue two pieces of brass shim 54 mm long, 2.4 mm wide and 0.5 mm high with epoxy glue. To cure the glue, place in an oven at 16°C for at least two hours. Any excess glue on the insides of the shims is removed with a scalpel. Place a rectangular coverslip onto the shims (see Figure 4.3), large enough to cover the area between them. The above method follows that used by the Water Research Association in the United

Kingdom. In the original method (Lund, 1959), thin glass is used instead of brass shims.

Electronic software for algal counting

There are a number of software programs available which have been developed by algal researchers. The list below is by no means exhaustive and the programs listed are included as examples only. Inquiries should be made to the authors.

<i>Program</i>	<i>Author/contact person</i>
Species Count	Dr S. Cooper, Duke Wetland Centre, Box 90333, Durham NC 27708, USA.
Algal counting program	Dr Paul Hamilton, Canadian program, Museum of Nature, Canada. Email: phamilton@mus-nature.ca Free. Useful for all types of chambers. Measurements for cell dimension can be entered and biovolumes calculated. An improved version of 'Algal counting program' is available from: Dr Veronique Gosselain, Laboratory of Freshwater Ecology, Facultés Universitaires Nôtre-Dame de la Paix, Rue de Bruxelles 61, B-5000 Namur, Belgium. Email: veronique.gosselain@fundp.ac.be
Countem	Dr R. Oliver, Murray–Darling Freshwater Research Centre, PO Box 921, Albury NSW 2640, Australia. \$50.00. Useful for Lund cell and Sedgwick-Rafter chambers only.
Biocount	Dr Gary Jones, CSIRO Land and Water, Science Centre Block B, Meiers Rd, Indooroopilly Qld 4068. \$350.00 single copy, discounts available. Useful for Lund cell and Sedgwick-Rafter chamber.

Appendix E: Examples of 'Phytoplankton field sampling sheet' and 'Algal counting sheet'

PHYTOPLANKTON FIELD SAMPLING SHEET	
Site name:	
Site code:	Sample ID:
Date:	Time: am pm
Weather:	
Water:	
<i>Field Measurements to take</i>	
Temperature	Conductivity
pH	Dissol. O ₂
Secchi Transp.	Velocity
Water Depth	Gauge height
<i>Particulars of water samples</i>	
Depth taken at: m	Method of sampling:
Type of sample: surface	integrated 0 – m
Location sampled: left side	right side main current
<i>Water samples to take for</i>	
Phytoplankton (fixed)	
Turbidity	
Phosphorus	
Nitrogen	
SiO ₂	
Chl- <i>a</i> (algal biomass)	
Phytoplankton net sample (unpreserved)	
Comments:	
Check all measurements and sampling completed	
Entered into d/b by:	on date:
Verified by:	on date:

Algal Counting Sheet

Sample ID: Site Code: Sampling Date:

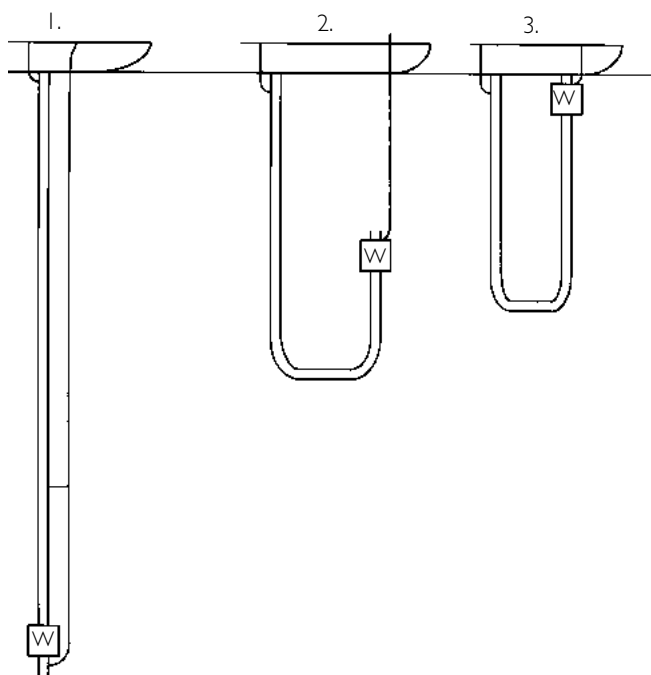
		Units counted
Factor: on date: on date:	<i>Aulacoseira granulata</i> (fil.)	
	<i>Aulacoseira granulata</i> (cells/fil.)	
	<i>Melosira varians</i> (fil.)	
	<i>Melosira varians</i> (cells/fil.)	
	Centric diatoms < 5 µm	
	Centric diatoms > 5 µm & < 10 µm	
	<i>Fragilaria</i>	
	<i>Navicula</i>	
	<i>Synedra</i>	
	<i>Carteria</i>	
	<i>Chlamydomonas</i>	
	<i>Pandorina</i>	
Area counted: Verified by: Verified by:	<i>Actinastrum</i>	
	<i>Ankistrodesmus</i>	
	<i>Crucigenia</i>	
	<i>Dictyosphaerium</i>	
	<i>Elakatothrix</i>	
	<i>Golenkinia</i>	
	<i>Oocystis</i>	
	<i>Pediastrum</i>	
	<i>Scenedesmus</i>	
	<i>Sphaerocystis</i>	
	<i>Ulothrix</i> (fil.)	
	<i>Ulothrix</i> (cells/fil.)	
Volume sed.: on date: on date:	<i>Closterium</i>	
	<i>Euastrum</i>	
	<i>Staurastrum</i>	
	<i>Dinobryon</i>	
	<i>Mallomonas</i>	
	<i>Synura</i>	
	<i>Chroomonas</i>	
	<i>Cryptomonas</i>	
	<i>Rhodomonas</i>	
	<i>Ceratium</i>	
	<i>Euglena</i>	
	<i>Trachelomonas</i>	
Slide No.: Identified by: Entered into d/b by:	<i>Microcystis</i>	
	<i>Lyngbya</i>	
	<i>Anabaena</i>	
Comments:		

Appendix F: Hosepipe sampler

Use a clear PVC pipe (25 mm diameter) of 2 m to 10 m length, depending on depth of river or lake, with a weight (W) and a rope attached to one end.

1. The weighted end of the pipe is lowered into the water until the whole pipe is in the water. Make sure the boat does not move so that the hosepipe enters the water vertically.
2. Close top end with a rubber stopper and pull up bottom end until a U-shape is formed. Place the weighted end in a container and remove the stopper from the other end. Allow sample to empty into the container.
3. Take required subsamples. The hosepipe should be rinsed with clean water or with the water to be sampled between sites.

Figure F.1: Diagram of hosepipe sampler



Appendix G: Taylor sphere sampler (TASS)

The Taylor sphere sampler is an inexpensive sampler which allows an uncontaminated near-surface sample to be taken at the recommended depth of 0.5 m to 0.7 m (APHA, 1995) from a river by a single operator from the bank or a bridge. It has been developed at La Trobe University Albury–Wodonga to improve the quality of near-surface samples taken from river banks for routine monitoring or research programs under circumstances where a boat is not available to reach the well-mixed zone of the stream. It is suitable for taking samples for phytoplankton as well as other water quality parameters.

The sampler is constructed from inexpensive parts readily available in irrigation supply shops. The materials used in the sampler construction were chosen to avoid chemical contamination of the sample by leaching substances.

The sampler consists of a 0.2 m diameter polypropylene sphere (by Philmac) holding a volume of just over 4 L (Figure G.1). An upper (0.15 m) and a lower (0.45 m) riser tube are connected to the sphere with bushes. The lower tube is weighted with a sleeve of stainless steel tubing. A modified plastic foot valve (by Philmac) at the end of the lower riser tube regulates the water inflow. The upper riser tube has a polystyrene float placed around it to keep the sampler upright in the water. The elbow fixed to the end of the upper riser tube allows for the water to be poured out. The sampler is held by a rope (approximately 10 m long).

To take a sample, the sampler is thrown into the river as far as possible from the bank or bridge. The sampler sinks slowly while filling (approximately 40 s) with the sample taken at a depth of 0.5 m to 0.7 m. The sampler is then pulled into the shore to retrieve the sample. Since the sampler is completely closed the sample cannot be contaminated while retrieving it from the river. The water sample is drained into a sample container through the plastic pipe attached to the elbow on the upper riser tube, preventing the formation of air bubbles. Thus the sample taken is also suitable to measure oxygen concentration.

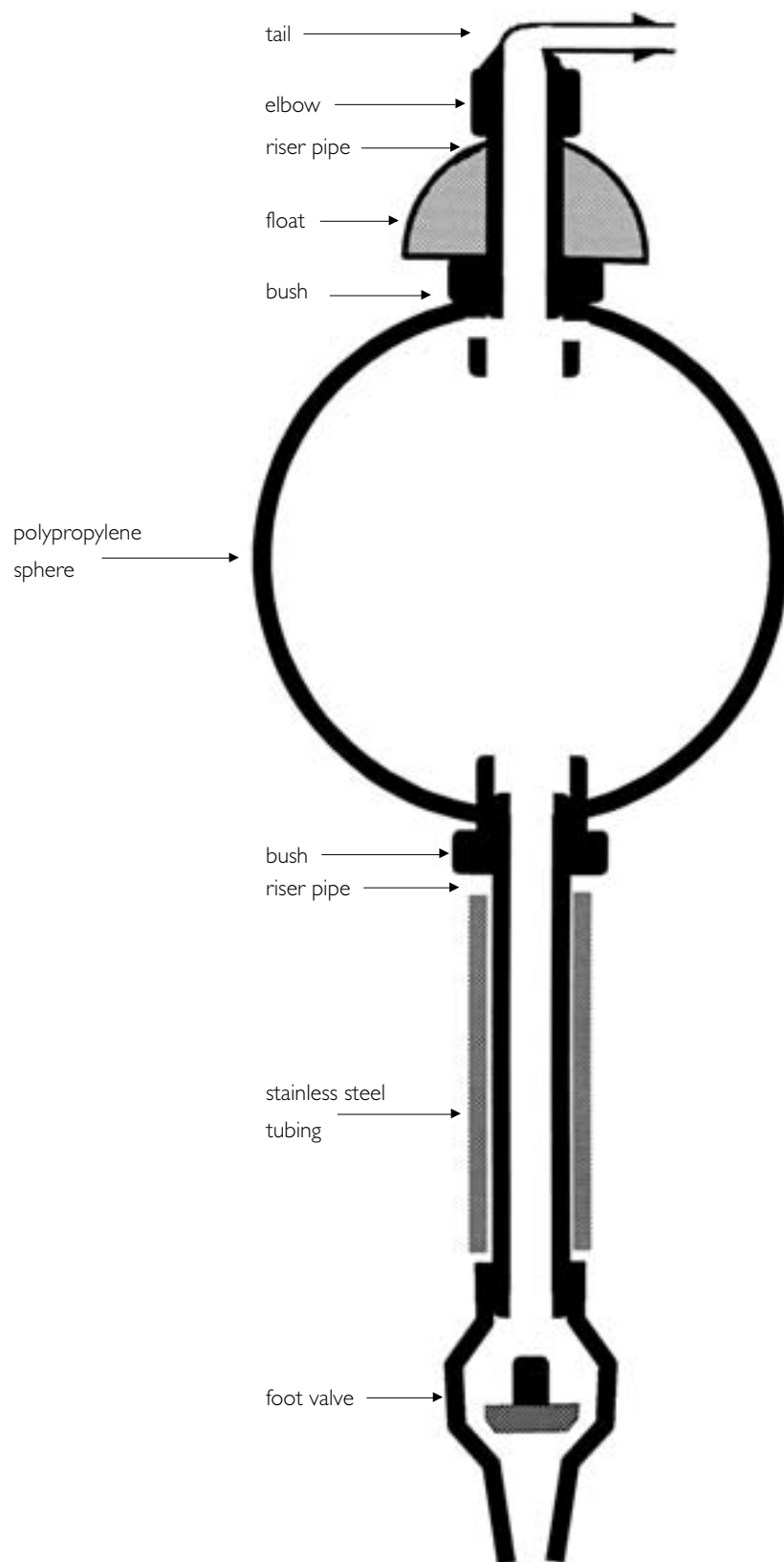
The sampler has been used in a number of different rivers under a variety of flow conditions in a phytoplankton and water quality research project and has performed satisfactorily. Advantageous features of the sampler include:

- it is made from durable and readily available materials;
- it is relatively small, with a sample volume of 4 L;
- it is easy to handle from either shore or bridge; and
- the sample is taken at the point of first contact with the water.

Enquiries can be addressed to Peter Taylor at the Department of Environmental Management and Ecology, La Trobe University, Albury–Wodonga campus, phone 02 6058 3885 or email ptaylor@awcnet.aw.latrobe.edu.au

A technical note on this sampler is in preparation.

Figure G.1: Diagram of Taylor sphere sampler



Appendix H: Taylor integrated sampler (TISA)

The Taylor integrated sampler allows an integrated sample to be taken in a river from the surface to a depth of 3 m. It is assembled by one person and operated remotely from the bank so that a sample is taken from the main current without using a boat.

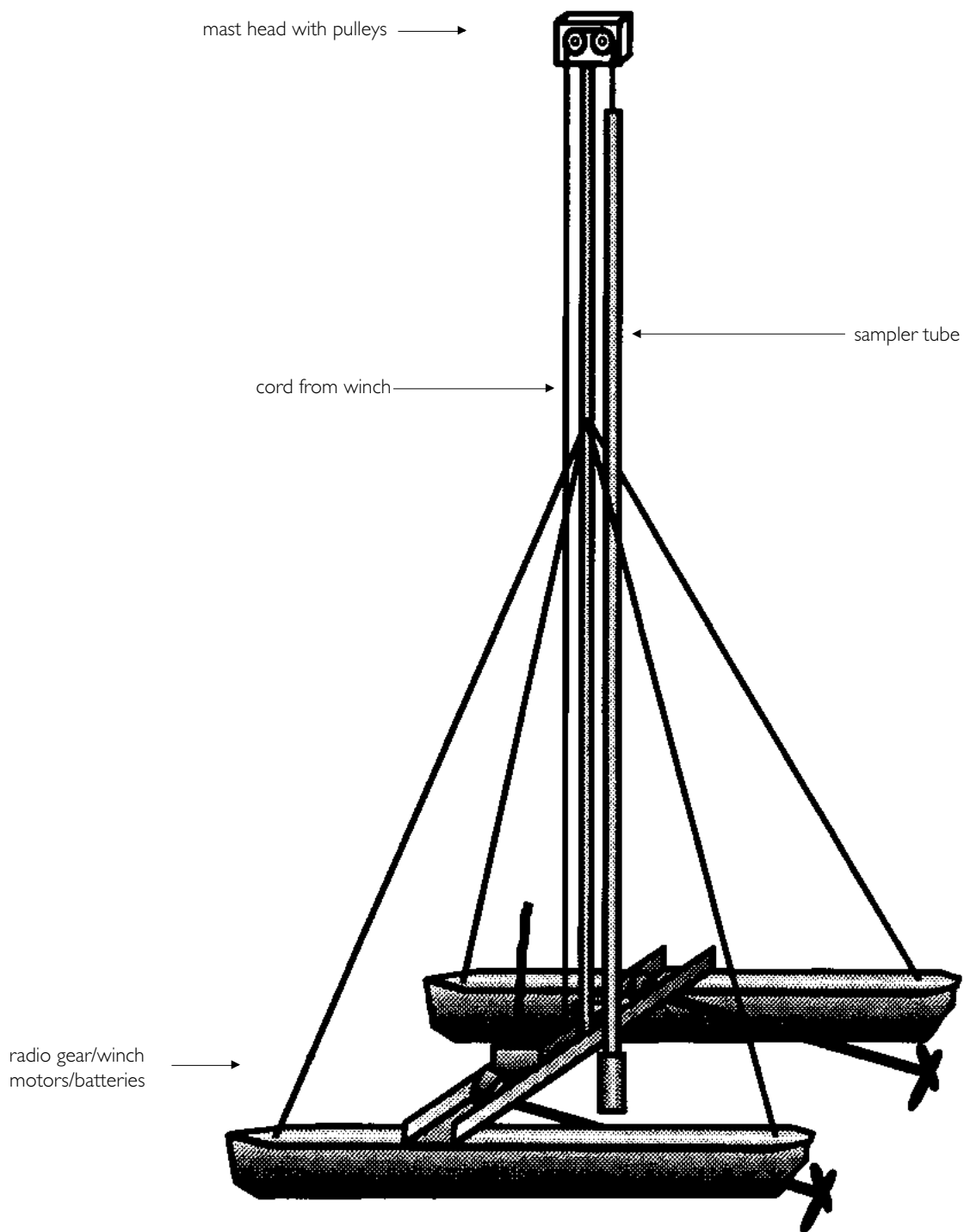
The integrated sampler consists of a radio controlled catamaran (1.2 m long) which carries the sampling pipe (3 m long) vertically. An aluminium beam which joins the two catamaran floats carries the radio equipment, motors, batteries, winch and a mast (see Figure H.1). Two pulleys are fixed to the top of the mast. The sampling tube is attached to a cord which runs over the pulleys to the winch, allowing the sampling tube to be vertically raised out of and lowered into the water by means of the electric winch. The winch is operated by remote control. The catamaran is driven by two propellers run by electric motors and directed by a shorebound operator via radio control. The sampling tube is made of transparent acrylic plastic with a modified self closing foot valve fixed to its lower end to retain the water.

The catamaran and sampler are assembled at shore. With the sampling tube in its raised position, the catamaran is sailed into the main current. The sampling tube is then lowered into the water at constant speed while the catamaran is floating downstream with the current. In this way, one complete water column is sampled. Once the sampler is filled, the tube is lifted out of the water and the catamaran sailed back to shore to retrieve the sample. The sample is drained into a container through a tap at the base of the sampling tube.

The sampling tube can be taken apart into three 1 m pieces for ease of transport. The catamaran also disassembles into a series of parts. It takes approximately half-an-hour to assemble the whole apparatus, take the sample and pack it up again. The catamaran can be launched from any gently sloping river bank or a jetty. The sampler is currently being validated against a hose pipe sampler.

For enquiries, please contact Peter Taylor at the Department of Environmental Management and Ecology, La Trobe University, Albury–Wodonga campus, phone 02 6058 3885 or email ptaylor@awcnet.aw.latrobe.edu.au
A technical note on this sampler is in preparation.

Figure H.1: Diagram of Taylor integrated sampler



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