

Survey Guidelines for Non-Vascular Plants

A report produced under the
NSW Biodiversity Strategy

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Acknowledgments

We are grateful to the Technical Working Group (Emma Pharo, Alison Downing, Tom May, Adrienne Burns and Jason Sonneman) for their input, advice and encouragement, and to the NSW Biodiversity Strategy Implementation Group for guidance and endorsement. We also thank the following for their contributions: Elizabeth Brown, Bette Reese and Andi Cairns.

Cover photograph by Jaime Plaza (Botanic Gardens Trust).

Cover design Helen Stevenson (Botanic Gardens Trust).

Published by:
Botanic Gardens Trust
Sydney

November 2003
© NSW Government
ISBN 0 7347 5449

CNR2003.006

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Executive Summary

Non-vascular plants (sometimes called cryptogams, microphytes or lower plants) are technically those plants and plant-like organisms in which the reproductive structures are hidden or cryptic. They are usually small, inconspicuous and often overlooked. The group include a complex array of lichens, bryophytes (mosses, liverworts and hornworts), algae, cyanobacteria, fungi and bacteria. Together these organisms play important roles in terrestrial and aquatic ecosystems. Specifically the organisms protect the soil against erosion, fix atmospheric nitrogen, phosphorus and carbon and sequester it in the soil, moderate soil moisture, influence the germination and establishment of vascular plants, and provide habitat for soil animals. In some ecosystems they are important indicators of landscape health.

Despite the importance of non-vascular plants, there have been few attempts to include them in routine vegetation surveys. This is due to the fact that the organisms are usually small, often at ground-, not eye-, level, and cryptic, and often require detailed chemical and/or taxonomic procedures for their identification. Identification is complicated by the severe shortage of cryptogamic specialists and a lack of understanding of suitable methodologies for studying them.

The aim of this manual is to produce field- and laboratory-based guidelines and methodologies for surveying and monitoring of non-vascular plants. The manual is based on an assessment of current literature and expert opinion. The manual reviews techniques for surveying algae, fungi, lichens and bryophytes, and examines and discusses techniques for evaluating the organisms at a range of levels from species to genera to morphological groups. The manual should provide sufficient information to allow a range of users from untrained enthusiasts to technical experts to undertake surveys of non-vascular plants in a range of environments at varying scales and intensities.

The manual describes three types of survey which might be considered. The decision about which survey to use depends on the aims, objectives and financial aspects of the particular project. Surveys are either 1) qualitative, opportunistic, generally, rapid, low-input reconnaissance surveys; 2) targeted, non-standardised, quantitative, generally one-off assessment of specific areas which may be ecological hotspots, and; 3) standardised, quantitative surveys which enable compilation of species and site-level diversity. A table is provided to guide the user to the most appropriate type of survey and therefore the appropriate field or laboratory methods to adopt. Suitable methodologies for sampling a range of environments (e.g. soil, trees, rocks, aquatic) and situations (culturing soil cyanobacteria) are provided along with relevant references for those seeking greater detail. Licensing, quality assurance and Occupational Health and Safety issues are also discussed.

1. Introduction

1.1 THE PURPOSE OF THIS MANUAL

Until recently there have been few attempts to include the survey of non-vascular plants in routine vegetation survey. The reasons for this are numerous, but are related to a shortage of cryptogamic specialists, the small size and cryptic nature of the organisms which make them difficult to see, the necessity to use detailed chemical and/or taxonomic tests to identify them, the incomplete description and enumeration of non-vascular organisms in Australia, and the deficiencies in suitable methodologies.

The aim of this manual is to produce field- and laboratory-based guidelines for survey and monitoring of non-vascular plants in non-marine habitats, based on an assessment of current literature and expert opinion. The manual describes techniques for surveying algae, fungi, lichens and bryophytes and examines and discusses techniques for evaluating the organisms at a range of levels from species to morphological groups. Microscopic forms (i.e. those not visible to the naked eye) are discussed briefly, but are largely outside the scope of this manual. The manual should provide sufficient information to allow a range of users from untrained enthusiasts to technical experts to undertake surveys of non-vascular plants in a range of environments at varying scales and intensities.

1.2 WHAT ARE NON-VASCULAR PLANTS AND CRYPTOGRAMS?

Non-vascular plants (sometimes called cryptogams, microphytes or lower order plants) are those plants and plant-like organisms in which the reproductive structures are "hidden from view". This is a grouping of convenience, rather than a natural taxonomic group. In many countries of Europe, Asia and North America, non-vascular plants such as bryophytes, lichens and some fungi are obvious components of the landscape. They sometimes form the major producer organism component in the ecosystem, as in *Sphagnum* bogs throughout much of Britain and Scandinavia, and reindeer moss fields in the Tundra (Longton 1992). In Australia, non-vascular plants can be major and obvious components of ecosystems, for instance the soil crusts so vital to rangeland ecosystems and the moss beds of rainforests.

In Europe, in particular, there has been a long tradition of both taxonomic and ecological investigation of cryptogams. The taxonomy is widely known and accessible, and included in popular publications like *The Oxford Book of Flowerless Plants* (Brightman and Nicholson 1966). Much of the methodology recommended in this manual is derived from work conducted in Europe and North America over the past 75 years (Brown *et al.* 1976, Seaward 1977). Many of the classical studies upon which the mathematical and statistical models for ecosystem investigation are based used bryophytes or lichens (e.g. Alvin (1960).

Throughout this report we use the terms cryptogam and non-vascular plants interchangeably. The main groups of cryptogams or non-vascular plants are the algae, fungi, lichens and bryophytes. These are discussed briefly below.

1.2.1 Macroalgae

Usually encountered as green, blue-green or brown and slimy growths, these organisms include the cyanobacteria (blue-green algae) and simple eukaryotic photosynthetic organisms, either as individuals or in colonial or cooperative forms that are visible to the naked eye. They include gelatinous and other sheet- or colony-forming cyanobacteria, the silkweeds (Zygnemataceae) and blanket weeds (Cladophoraceae) and their relatives, the velvet mosses (*Vaucheria* spp in the Chrysophyta), the visible aggregations of Diatoms, and the stoneworts (Charophyta). Most, but not all, are aquatic. Some are prominent in soil-crusts in arid areas (Appendix V), while others grow sub-aerially on bark and timber, waterfall walls and seepages (Appendix VI).

1.2.2 Macrofungi

Fungi include the basidiomycetes, some ascomycetes (mostly Pezizales), and some larger and persistent slime moulds such as *Fuligo* (Flowers of Tan). With the exception of some of the bracket fungi, the visibility of macrofungi is dependent on the highly seasonal and erratic production of fruiting bodies.

1.2.3 Lichens

Lichens are not single organisms like cyanobacteria or bryophytes; they are a combination of a fungus (the mycobiont) and an alga or cyanobacterium (the photobiont), living in a symbiotic relationship. The fungus which provides the structure, and the alga provides nutrition through photosynthesis. Lichens are divided into four groups based on structure. Crustose lichens are rather shapeless and bound tightly to the substrate. Foliose (leafy) lichens have horizontally spreading lobes and are often anchored using rhizines. Fruticose (shrubby) lichens are erect, pendulous or 'shrub like' or drooping, and consist of tubular or cylindrical branches. Squamulose (warty) lichens look like small lobes on the soil only a millimetre or so across.

The body (thallus) of a typical lichen consists of three main layers: cortex, medulla, and algal layer. The cortex comprises a specialised layer of fungal cells and forms a protective sheath around the top layer of the lichen. Hyphae and rhizines (root-like structures) grow out from the lower cortex and are used for anchorage and to absorb nutrients. The medulla is a layer of loose fungal tissue used for storage of water and food. The algal layer contains either green algae or blue-green algae (known as cyanobacteria). Lichens reproduce sexually using spores, or asexually when small fragments break off and become re-established elsewhere. In sexual reproduction, the spores must come into contact with a suitable algal partner before establishment of a new lichen is successful. Lichens are ubiquitous, but become more and more visible in harsher environments where their tolerance of long periods of dormancy (usually due to lack of moisture) gives them a competitive advantage over other organisms.

1.2.4 Bryophytes

Mosses, liverworts and hornworts are all bryophytes. They are green plants, usually small and they have a two-part life cycle. The green, photosynthetic plants on which male and female reproductive structures occur are the readily recognizable component of bryophyte life cycles. Capsules in which spores are produced, are produced seasonally, usually on slender stems and are not as conspicuous as the green plants. Mosses have obvious stems and leaves, the leaves are usually arranged spirally around the stems. The liverworts occur in two forms, leafy or thallose. "Leafy liverworts", have well developed stems, and the leaves are usually arranged in three rows, one row along each side of the stem, and a third row of smaller leaves on the undersurface of the leaves. "Thallose liverworts" have flattened stems (strap-like, or flattened sheet of tissue) or they may have leaf-like flanges along a main axis and superficially appear to be leafy. The hornworts are thallose, with flattened pads of undifferentiated green tissue, and erect, needle like reproductive structures which look rather like short grass stems. While they flourish in rainforest and other damp areas, mosses, liverworts and hornworts are also found in arid environments (Scott 1982). Appendix IV provides definitions for morphological groups of Bryophytes, with some local examples.

1.3 WHY ARE NON-VASCULAR PLANTS IMPORTANT?

Non-vascular plants play important roles in terrestrial and aquatic ecosystems (West 1990, Bates and Farmer 1992, Longton 1992, Johansen 1993, Eldridge and Greene 1996, Belnap and Lange 2001). Despite their importance, however, we know relatively little about them, where they are found, and even less about their conservation status. As non-vascular plants are small and often susceptible to disturbance, they play important roles as early warning signs of changes in ecosystem health. Their value as bio-indicators lies in

the fact that they are often present when much of the vascular plant flora has been removed by grazing, flooding or drought. In semi-aquatic environments, non-vascular plants and other cryptogams are even less well understood.

In general, non-vascular plants are important because they:

- protect the soil against both wind and water erosion by aggregating soil particles into larger non-erodible fractions. Many non-vascular plants are pioneering species, and help to protect the soil surface after burning;
- fix atmospheric nitrogen and sequester it in the soil. This nitrogen fixation occurs through cyanobacteria associated with lichens and from free-living cyanobacteria in the soil;
- increase the level of organic carbon in the soil by fixing carbon dioxide from the atmosphere;
- enhance the level of phosphorus in the soil by binding of algal filaments onto fine soil particles;
- moderate the levels of soil moisture. In some cases the organisms may reduce water infiltration and in other cases increase infiltration. Dark-coloured algal crusts may also contribute to a reduction in evaporation;
- influence the germination and establishment of vascular plant by producing 'safe sites', and;
- provide a habitat for soil animals which help to promote invertebrate diversity and are important for promoting healthy soils.

1.4 PROBLEMS WITH USING NON-VASCULAR PLANTS IN FIELD SURVEY

As indicated above, cryptogams have often been excluded from many biological surveys. The organisms are very small and often hidden and, like invertebrates, they are often overlooked and rarely encountered during field survey. Cryptogams and non-vascular plants are strongly influenced by small-scale changes in soils, relief, altitude and microclimate, and therefore fine-scale differences in distribution means that they vary widely over spatial scales which are much smaller than those generally encountered with standard vegetation surveys. These problems are exacerbated by the fact that there are relatively few non-vascular plant experts in Australia.

Despite the fact that there are relatively few non-vascular specialists in Australia, a substantial body of work on cryptogam taxonomy and distribution has been developed over the last decade in Australia. Work has continued on cryptogams of arid rangelands (Downing 1992, Downing and Selkirk 1993, Eldridge 1993, 1996, Rogers 1994, Eldridge and Greene 1996, Eldridge and Tozer 1996, Eldridge and Koen 1998) as well as higher rainfall areas (Downing *et al.* 1995, Kantvilas and Jarman 1993, Fensham and Streimann 1997, Eldridge *et al.* 2000, Pharo and Beattie 1997, 2002). There has also been substantial research on macroalgae (Entwistle 1989, Burns and Ryder 2001).

2. Determining the level of data to collect: species, genera or morphological groups?

2.1 INTRODUCTION

Despite a growing awareness of the importance of non-vascular plants over the past decade, there has been a reluctance to include cryptogams in most field surveys to the level of species due to the many problems with their identification. It is unreasonable to expect cryptogams to be included in all surveys to the level of species identification. Few keys were available until the last decade, and there are not enough experts with sufficient training to undertake what is required.

The standard genus and species approach requires the use of trained professionals and detailed keys, and is highly labour and time intensive. One way to collect information on non-vascular plants is to use para-taxonomists and to identify organisms to the level of morphological groups rather than species or genera. The use of morphological groups in field survey is described below.

2.2 THE MORPHOLOGICAL GROUP APPROACH

Non-vascular plants form natural morphological groupings. Morphological groups are groups of organisms with the same outward appearance or morphology. A knowledge of the morphology provides a useful way of characterising organisms because of the strong relationship between the form of an organisms and its ecological function. Appendix I provides a summary of morphological groups for non-vascular plants.

Morphological groups (describable clumps of mixed cryptogamic organisms) are appropriate for all cryptogams. Fungi, which have a below-soil habitat and ephemeral fruiting bodies, may only be placed into useful morphological groups for small periods of time. Morphological groups can be characterised according to sight (e.g. colour, texture, shape, fruiting structures etc), touch (e.g. slimy, brittle, gelatinous) and feel (e.g. earthy, musty etc.). Morphological types can be photographed, and compared between and among sites. More importantly, morphological groups are measurable.

Morphological groups are applicable to all of the non-vascular plants. Determination of components in morphological groups may require the use of microscopes to distinguish, for example, algae and bacteria which are difficult to distinguish with the naked eye. Other groups are easier to distinguish. For example the bryophytes comprise two distinct morphological groups (mosses and liverworts) which can be distinguished on the basis of appearance (Pharo 2002). Taxonomic complexity can be evaluated with vouchers, away from the field, while the determination of cover or other measure of ecological involvement can be done without knowledge of the exact biological components. The advantages of morphological groups are numerous. These are described in Table 1.

Table 1. Advantages and disadvantages of using morphological groups compared with traditional methods based on identification to species level. No level of importance is attached to their order (from Eldridge and Rosentreter 1999).

Advantages	Disadvantages
<i>Biological considerations</i>	
Communicates an image of the organism	some organisms are difficult to characterise even to a morphological group
Communicates a function	different workers may place species in different morphological groups
Eliminates confusion caused by taxonomic changes	differences in colour may be effected by the abiotic environment
Is independent of continent, region or area	
<i>Efficiency considerations</i>	
easier to measure with less indecision and greater repeatability	changes in species composition occurring within a morphological group may go undetected
Requires less training	
more rapid and statistically powerful data analyses	
no dangerous chemicals required in field	
allows more rapid field measurements	
cheaper to monitor	

3. What type of survey is required?

3.1 INTRODUCTION

Three levels of surveys are commonly required for assessing vegetation, and the appropriate level depends on the aims, objectives and financial aspects of the particular project. These levels are 1) qualitative, opportunistic, generally, rapid, low-input reconnaissance surveys; 2) targeted, non-standardised, quantitative, generally one-off assessment of specific areas which may be ecological hotspots, and; 3) standardised, quantitative surveys which enable compilation of α -diversity and β -diversity, and which may be measured repeatedly over time. The cost, and levels of input and experience required increases dramatically.

To decide which survey to use, it is necessary to consider what expertise and resources are available, and the reason for the survey. Table 2 has been devised to help in that decision making process, not to constrain it. It is up to you to decide which criteria are of most importance. The ✓ corresponds to ‘essential’. That is, under resources required, if you only have access to a low-powered microscope then you can do an Opportunistic Surveys but probably not Targeted or Standardised Surveys. Under rationale, if you need the results for a legal decision you must use a Standardised Survey.

Table 2. A ‘Decision Table’ designed to help the user to decide which survey is the most appropriate

CRITERIA	Opportunistic Survey	Targeted Survey	Standardised Survey
Resources — what do I need?			
Ability to document the survey	✓	✓	✓
Low powered microscope	✓		
High powered (more than X100) microscope	✓	✓	✓
Experts in non-vascular plant taxonomy	✓	✓	✓
General identification guides	✓	✓	
Wide range of scientific literature	✓	✓	✓
Need for collection and long-term storage of samples (vouchers)		✓	✓
Ability to repeated the survey at some later time			✓
Rationale — what is the purpose?			
Results for education or community awareness	✓	✓	✓
Results to be published in refereed journals		✓	✓
Results to be legal/policy decisions			✓

Although the type of data collected can be independent of the type of survey, generally data collection becomes more rigorous as the survey level increases. For example, opportunistic surveys are generally one-off (Table 3) and data are only collected to morphological group or the genus level. However, the opportunity exists to collect information using objectively-based methods such as quadrats and plots even for one-off studies.

Table 3. Types of surveys used for collecting information on species diversity (adapted from NPWS 2001)

Survey type	Description	Characteristics
Opportunistic (e.g. intuitively-controlled) survey	Records collected while doing other things or other surveys; qualitative	can add to species lists (especially rare species); does not provide an objective view of regional biodiversity; statistics unusable
Targeted, non-standardised survey	sites chosen because they are likely to be species-rich; often variable set of field techniques, uneven sampling, quantitative	habitat-specific species likely to be found; generally presence-absence data; useful for gathering species lists; limited objective comparison of regional biodiversity
Standardised survey	Consistent effort and sample selection across the survey area; quantitative	generally cover-abundance data; provides an objective view of regional-scale biodiversity; statistically robust.

3.2 OPPORTUNISTIC SURVEYS

Many studies are qualitative in nature, lack replication (designed so as to be able to be repeated), robustness and representativeness, and collections are not restricted to plots of known size and shape, or collected over fixed periods of time (Rosentreter and Eldridge 2002). These studies are useful for providing an indication of the likelihood of finding a particular species but cannot be used to compare and contrast biodiversity within and among sites, across time, or even across broad geographic regions. These surveys can add to species lists (particularly rare species), but do not provide an objective view of regional biodiversity. Therefore they do not represent a true indication of biodiversity, community structure or ecosystem health (McCune and Lesica 1992).

Table 4. Description of the three levels of intensity for surveying non-vascular plants.

Type of survey	Opportunistic surveys	Targeted surveys	Standardised surveys
Surveying intensity	Genus or morphological group; generally one-off	Genus- or species-level survey	Comprehensive, recurrent survey
Type of data collected	presence-absence; morphological group	morphological group; genera, species; cover	genus, species, sub-species, cover-abundance; environmental variables
Type of measurement	time- or area-based, qualitative	quadrat- and time-based; quantitative	rigorous, scientific, quantitative
Level of expertise required	low (amateur, enthusiast)	moderate (semi-professional, para-taxonomist)	high (professional, expert, scientist)
Re-survey interval	nil	sometimes, usually not	various
Statistical rigour	low	low to moderate	high
Degree of vouchering	generally not required	low to moderate level of collection	high level of collection; rigorous
Client(s)	community groups e.g. Landcare, local councils, Streamwatch etc	State-government agencies, policy makers community	various
Relative cost	low	moderate	high

Some opportunistic surveys are time-based (e.g. 20 minute searches). Opportunistic studies do not attempt to control for differences in environmental factors such as the water quality, soil erosion, tree health or the experience of the observer. Thus data may be collected for a site by a number of observers with widely differing levels of expertise (McCune *et al.* 1997).

Opportunistic, qualitative studies typically involve the collection of specimens in a somewhat haphazard sequence, over landscapes which are often of ill-defined size and with widely varying habitat complexity, with no specific plots, with no specific time spent searching at each collection site, and with no comparable degree of sampling effort e.g. Will-Wolf (1998), Downing *et al.* (2001), Rosentreter and Eldridge (2002).

As indicated above, opportunistic surveys allow the collection of data to the level of genus or morphological group (though this will depend on the skills of the observer). As with vascular plant surveys, observers may only record the dominant organism at a site as “moss or fungi” or alternatively, the data may be lumped into a genus (e.g. *Pottia* sp.), or as “unknown lichen sp. #1, 2, 3 or 4” (Brotherson *et al.* 1983). Taxa may also be lumped into morphological groups e.g. “thin rope-like moss”, “yellow foliose lichen” (Rosentreter and Eldridge 2002) or “purple-green patches of a felt-like organism”.

3.3 TARGETED AND NON-STANDARDISED SURVEYS

This type of survey varies from opportunistic or targeted surveys in requiring a finer level of data collection, usually to genus and species, though morphological group data may also be collected. The technique will necessarily involve some microscope use, and the use of chemicals for determining lichen species. The use of a variable set of field techniques such as plot-based and time-based techniques means that sampling is typically uneven across the landscape, precluding the use of rigorous statistical techniques and making it difficult to compare between areas. It does however provide a useful index of diversity for planners and policy makers (Table 4). Sites are often easily accessible, may be near points of interest such as ecotones or areas excluded from grazing, or ecological hotspots (e.g. Neitlich and McCune 1997). In the case of aquatic algae, they may be within unregulated rivers which are accessible to collectors or near major areas of population.

3.4 STANDARDISED SURVEYS

Standardised surveys require the use of techniques that provide reproducible, statistically robust, quantitative data. Techniques for assessing non-vascular plants can range from repeated photography, remote sensing or aerial photography interpretation on large plots of up to several hectares in size down to line intercept or quadrat methods for finer scale measurements.

Plots of various size, within which are placed transects and sub-plots, typically form the basis for data collection. The size of sampling units should be appropriate to the size, density and spatial distribution of the organisms being studied, their habitats, and the nature of the impact being investigated (Rosentreter and Eldridge 2002).

No matter what their size, plots should be stratified according to vegetation community, landform, soil type or substrate. This ensures that the relative contribution of a particular vegetation community or soil type is taken into consideration when the total area is measured (Rosentreter and Eldridge 2002). For example, a study of corticolous bryophytes may be stratified according to stem age, condition or diameter. Similarly, studies of aquatic algae may be stratified by water depth, water condition or stream order. Standardised surveys will necessarily require the collection of voucher specimens, and some culturing of algae and bryophytes may be necessary.

Appendix VIII provides an example of descriptive quantification of the components of an aquatic part of an ecosystem. When combined with the morphological groups in Appendix VII, it may be possible to provide a repeatable and comparative description of the cryptogamic diversity in such systems. Similar methodologies, using scales like the Braun-Blanquet (1927) scale, and morphological groups, may allow the inclusion of cryptogams in all kinds of surveys without undue increase in survey time in the field. The characterising of the organisms that make up the morphological groups can be completed at leisure and at a distance from the field site.

4. Habitat types for sampling cryptogamic organisms

4.1 SAMPLING HABITAT TYPES

The type of survey, and the most appropriate method of assessing non-vascular plant communities depends on the level of information required and the type of substrate to be sampled e.g. water, rock, soil. Four broad habitat types which are useful for sampling vascular plants can be identified for cryptogamic taxa. These are rangelands, woodland-dry forest, wet forest-rainforest, and broadwater and are described briefly below:

4.1.1 Wet Forests and Rainforest

This type is typically referred to as Brush; common remnants in the north of the state, more 'gully vegetation' south of Sydney. Bryophytes and some specialised lichens dominate the understorey depending on light levels. Bracket fungi are usually in evidence, while other forms may be seasonally visible. As the floristics are still incomplete, and individual communities quite complex, the morphological group approach, accompanied by the collection of voucher samples is probably the most realistic means of data gathering.

4.1.2 Woodland and Dry Forest

This extends from the upper western slopes to the coast (and including much of the heathland) and so the mangroves. The vegetation is at least partly dominated by moderately tall perennial evergreen trees, with usually leathery (or otherwise) drought adapted foliage. As rainfall increases the complexity shifts from lichens to bryophytes, with an increasing seasonal visibility of fungi, and contributions by green algae especially in soaks and waterfalls. Morphological groups can provide a rapid overview of cryptogam contribution, but should be followed up with species-based data collection.

4.1.3 Rangelands

The dry country, western woodlands and shrub steppe describe much of the land ecosystems from the lower western slopes to the South Australian border (140°E). Vegetation includes mallee, saltbush, mulga, porcupine grass and samphire flats. The cryptogams most prominent in this category are the soil crust formers, blue-green algae (especially tubular Oscillatoriales), lichens and some mosses and liverworts (Eldridge and Tozer 1997). Rosentreter et al. (2001) provide a well reasoned methodology for rangelands.

4.1.4 Broadwater

Broadwater comprises an open, often shallow body of water still or slow moving, dominated by reedy emergent plants; water meadows and lagoons in coastal riverine systems, tableland, transient swamps and often artificial billabong/lagoon systems of inland rivers. Much the same form classification can be applied to transient waterways in woodland, forest and rainforest. Algae usually dominant forms here, rarely liverworts or mosses. As microscopic examination is almost invariably required to identify complex floating, suspended and attached macroalgal communities, description by reference to morphological form is the appropriate way to collect data in the field. See Appendices VII & VIII for more detail.

4.2 SAMPLING SUBSTRATE TYPES

Given that non-vascular plants are strongly patterned at small spatial scales, they are most strongly distributed in relation to substrate rather than vegetation community or climatic zone. A review of the literature indicates that the appropriate methods for sampling non-vascular plant communities depends on whether the taxa are growing:

- on trees and shrubs (termed corticolous);
- on soil and organic matter (terricolous);
- on rocks and cliffs (saxicolous), or;
- in water (aquatic).

These substrate habitats are discussed in detail below.

4.3 CORTICOLOUS TAXA OF FOREST AND WOODLAND ENVIRONMENTS

Lichens and mosses play an important role in the health and maintenance of forest ecosystems (Knops *et al.* 1991). Cyanobacteria, cyanolichens and some mosses produce nitrogen for forest nutrient cycles (Pike 1978), and intercept and redistribute wetfall and dryfall nutrients and pollutants (Will-Wolf *et al.* 2002a). Non-vascular plants provide a habitat for forest invertebrates, nesting material for vertebrates, and are an important food source for vertebrates such as reindeer, caribou and flying squirrels (Rominger *et al.* 1966, Hayward and Rosentreter 1994).

The distribution of wet and dry forest and woodland species varies markedly in relation to elevation, climatic factors (temperature, moisture status, light levels, precipitation etc) with the result that some areas are species rich whilst others are species poor. The distribution of lichens and bryophytes in forests and woodlands is influenced by many microhabitat variables including: stand age, the size and age of gaps, size, age and decomposition status of course woody debris (Kantvilas and Minchin 1989, Pharo and Vitt 2000, Pharo *et al.* 2000). A large amount of information for the following section has been gleaned from Will-Wolf *et al.* (2002b).

4.3.1 Plotless techniques

Plotless techniques are useful for measuring species abundances. Techniques include determining the number of hits along branches and trunks (e.g. Hilmo 1994), or rating the number of clumps of pendulous lichens or mosses within the canopy in relation to a standard (known) clump (Armleder *et al.* 1992). Sometimes biomass can be determined by estimating or measuring the size of individual thalli (e.g. McCune 1990, Esseen and Renhorn 1998). This technique applies equally well on most terrestrial substrates.

4.3.2 Plot techniques

Plot techniques range from time- or area-based ocular searches of forest plots (e.g. Selva 1994, McCune and Lessica 1992) to sub-sampling using transects and microplots (Will-Wolf *et al.* 2002a,b). Time-based sampling generally requires a standard period of sampling on sites of similar size with operators of comparable experience (Will-Wolf *et al.* 2002b).

Plot techniques range from one or a few large plots to complex sub-sampling of microplots within a macroplot based on stratification (number of distinguishable layers) of the forest environment (Will-Wolf *et al.* 2002b). Generally the macroplot captures a homogeneous area in terms of forest or woodland while

the microplots capture the variability of habitats (e.g. trunk, upper branches, roots) within a forest. McCune and Lessica (1992) found that whole-plot ocular searches generally capture a greater number of species than microplots or even belt transects. However, microplots were more efficient for determining cover but measurements took longer to complete. Clearly the combination of large plots for determining diversity and a large number of small sub- or micro-plots for determining cover and abundance provides the best measure of bryophyte and lichens.

The single tree is the most widely employed discrete 'microplot' sampling unit used for epiphytic species (Will-Wolf *et al.* 2002b). However, the number of microplots employed will depend on the variability of the habitat. For studies of epiphytic lichens and bryophytes for example, 10-25 replicates microplots of 0.01-0.2 m² are generally used. Microplot branch lengths of 0.2-1m with 25-100 replicates are also typical (Will-Wolf *et al.* 2002b).

Different approaches are required for different elements of the forest and woodland ecosystems. Monitoring of soils within woodlands and forests should be undertaken according to protocols described in Box 3. Much of the methodology for examination of non-vascular organisms on bark can be transferred to soil, rock or other terrestrial habitats.

4.3.2.1 Trunk and branch habitats

Microplots are typically used to sample various microenvironments on the trunk e.g. branches, smooth bark, rough bark, wood type, lower bole etc (Will-Wolf *et al.* 2002b). These microplots may or may not be permanent depending on the study. A ladder quadrat (Figure 1) is useful for recording bryophytes and lichens growing on trunks.

4.3.2.2 Canopy habitats

Direct sampling of the canopy is a useful and important method of sampling corticolous communities. Canopies can be accessed by: using canopy cranes or climbing into the canopy, felling trees, or using trees felled by loggers. Fallen limbs only provide an estimate of species abundance but they are clearly easier to sample (Will-Wolf *et al.* 2002b).

BOX 1: LICHENS AND BRYOPHYTES OF EUCALYPTUS FORESTS

Equipment:

50 m and 10 m tapes, 0.25 m² quadrats

Methods:

- establish plots of 50 x 10 m.
- search all twigs, branches and trunk for mosses, lichens and liverworts to about 2 m above the ground.
- for tree boles only, collect a separate inventory of all species within five 10 by 10 m subplots.
- for soil species, record all species within ten 0.25 x 0.25 m quadrats at 5 m intervals along the 50 m transect.
- identify organisms in situ to species, genus or morphological group level and collect voucher specimens for determination to species level where possible using standard identification techniques

Analyses:

- analyse data separately by substrate type e.g. epiphytes on trees and shrubs (smooth bark, rough bark, papery bark and leaves), non-epiphytes and forest floor (logs, stumps, rocks etc)

References:

- Jarman and Kantvilas (1995, 2001), Kantvilas and Jarman (2002)

4.4 ROCKY AND SEASHORE ENVIRONMENTS

Lichens, algae, cyanobacteria and fungi, and to a lesser extent bryophytes, are able to survive on a range of nutrient-poor substrates. Some do not take many of their nutrients from the substrate, but rely on nutrients from water and the air to survive.

Rocks, pebbles, boulder fields, masonry, stone buildings and gravestones and provide a rich source of substrate for non-vascular plants because there is little competition from vascular plants. Different rock types often support unique assemblages of taxa, with for example sandstone surfaces supporting different organisms to basalt or granitic substrates. Some lichen species are restricted entirely to human-made surfaces (Aptroot and James 2002), whilst mosses and lichens growing on limestone substrates (limestone, caliche, kunkar) show strong affinities with species growing on calcareous soils (Downing 1992, Downing *et al.* 1995, 2001).

Like corticolous habitats, rocky habitats also have a wide range of microhabitats due to rock size, shape, slope and aspect which create slight differences in temperature, moisture, relative humidity and microtopography (Pentecost 1980, John and Dale 1989, 1990).

BOX 2: LICHENS AND BRYOPHYTES OF ROCK FACES**Equipment:**

50 m or 100 m tapes, device for measuring distance, 0.25m² quadrat with 10 cm cross-hairs

Methods:

- Establish large plots running up the slope or rock face.
- Place between three and five transects along the long edge of the plots.
- Select at least 10 stations along each transect for detailed assessment of lichens and bryophytes.
- At each station select the three nearest rock faces suitable for sampling i.e. flat, not covered by trees and shrubs, and > 1 m² in area
- Use a sampling quadrat consisting of a grid of points 10-20 cm apart.
- Record all organisms under each grid point in situ either to species, genus or morphological group level.
- Collect voucher specimens for determination to species level where possible using standard identification techniques.
- Where appropriate, collect environmental data at each quadrat (e.g. slope, rock type, aspect etc).

Analyses:

- analyse data separately by rock type, aspect etc.

References:

- John and Dale (1989, 1990), Pentecost (1980)

4.5 SOIL SURFACE ENVIRONMENTS

Many of the techniques for assessing non-vascular plants on soil have been developed for arid and semi-arid shrub-steppe (also known as rangeland). These techniques are equally applicable to grasslands and shrublands in more mesic areas. The standard method for measuring non-vascular plants on soils in rangeland environments is to make all measurements within large plots stratified according to vegetation community or landscape type (Belnap *et al.* 2001, Rosentreter and Eldridge 2002). Within these plots non-vascular plants are sampled using quadrats placed at regular intervals along the transects, or alternatively, using the line-intercept method along the transects (Hilty *et al.* 2003, 2004). Quadrat or line-intercept measurements are often supplemented with site-specific photographs.

As with studies of vascular plants, sampling is often stratified according to soil type, landform or vegetation community. For example in grasslands and shrublands most of the unvegetated soil occurs in the interspaces, hence most of the non-vascular plants are found here. Measurements must therefore reflect this spatial patchiness. The amount of sampling (and subsequent laboratory identification of taxa) is highly depended on resources (time and money). In general, one day of field sampling generates about 5-7 days in the laboratory (excluding preparation of voucher specimens).

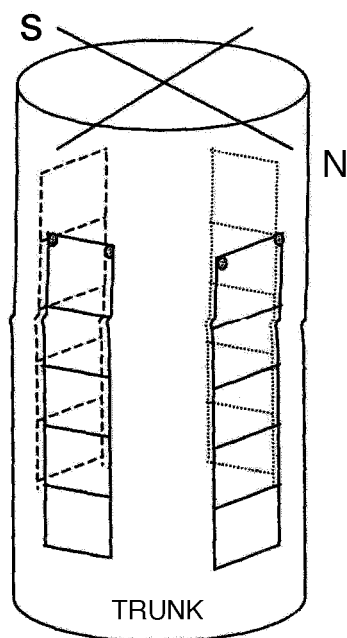


Figure 1: Ladder-sampling device for tree trunks (from Scheidegger *et al.* 2002)

4.5.1 Quadrat methods

On sparsely-vegetated sites, a condition which is common in low rainfall areas or in sites west of the Great Dividing Range, non-vascular plants are generally recorded in quadrats of variable size (20–50 m) within larger (generally 20 x 20 m or 20 x 50 m) plots. Like vascular plants in dryland environments, non-vascular plants tend to be distributed in patches. Therefore the methods of assessing them must take into account this patchiness.

A range of quadrat sizes has been used to sample non-vascular plants (see Rosentreter and Eldridge 2002). In general, quadrats need to be small enough so that the whole area under consideration is easily visible without the site being trampling when the taxa are being measured. Typically quadrats are less than 50 cm (Daubenmire 1959, Rosentreter 1986, Eversman 1995, Ponzetti *et al.* 1998), and long narrow or small rectangular (20 x 50 cm) quadrats are generally easier to evaluate than larger square plots (Elzinga *et al.* 1998). As with vascular plants, plots may be nested.

Lichen and bryophyte cover and frequency have been measured in Australian rangelands using square quadrats ranging from 70 x 70 cm (Eldridge and Bradstock 1994, Eldridge 1996, Eldridge 1999, Eldridge and Tozer 1996) to smaller sizes of 15 x 15 cm where finer-scale changes are required in relation to changes in grazing out from a watering point (Rogers and Lange 1971, Rogers 1972). In some situations where diversity and cover levels are high and the cover of bare soil is poor, non-vascular plants may be measured in very small (< 5 cm²) circular quadrats which are observable under a dissecting microscope and enable measurements of cover-abundance to be made on a species basis. Ultimately the choice of quadrat size will depend on: the spatial clumping of the organisms, the amount of bare soil and the intensity of sampling required.

Quadrat sizes for macrofungi are generally similar in size to those of vascular plants, and it may be appropriate to include fungal records in those where fungal fruiting bodies are encountered in surveys so that appropriate numbers and cover can be established.

4.5.2 Line-intercept techniques

The line- intercept method (Canfield 1944) is used to measure the proportion of the transect line comprising various species or groups of species including vascular and non-vascular plants. It is used widely in the western United States (Belnap *et al.* 2001) and has the advantage that all cover components (non-vascular plants, vascular plants, litter, dung, bare ground etc) can be measured simultaneously, allowing comparisons between various components. It is best used where the cover of non-vascular plants is high (Kaltenecker 1997). Transect lengths typically range from 10-50 m (Kaltenecker *et al.* 1999, Ponzetti and McCune 2000, Rosentreter and Eldridge 2002). Larger (100 m) transects (Johansen *et al.* 1984) are rarely used. The efficiency of the technique can be increased by using fixed transects by having a number of small transects (say five 10 m transects) rather than one big (50 m) transect. The soil surface is often misted with water prior to measurement to increase the chance that cryptic organisms are recorded (Kaltenecker 1997). This technique has not to our knowledge been used for soils in Australia.

BOX 3: SOIL LICHENS AND BRYOPHYTES

Equipment:

50 m tape, 0.25 m² quadrats, dissecting microscope

Methods:

- establish a 50 x 20 m plot with a central 50 m transect. Align the long dimension of the plot up and down the slope to allow the taking of other measurements (e.g. landscape function) using the same transect.
- stratify the transect into different patch types where the vegetation is strongly patterned into vegetated and non-vegetated patches (e.g. grasslands and shrublands).
- place ten 0.5 by 0.5 m quadrats at 5 m intervals along the transect
- record the total cover of non-vascular plants and the relative contribution by moss, liverwort, lichen and algae-cyanobacteria within each quadrat. Other measurements such as soil surface condition (Tongway 1994, 1995) may be collected from the same quadrats (Eldridge and Tozer 1996, Ponzetti and McCune 2000)
- collect samples of each cryptogam species within each (or every other) quadrat (depending on time resources) and place in a suitably labeled paper bag. The amount taken should reflect the relative abundance of each species. If no species are evident within a quadrat, a soil sample should be taken to check for the presence of cryptic species
- collect additional samples from the general areas of the plot but from outside the quadrats in order to build up a comprehensive picture of the total diversity of organisms at a site and for making voucher specimen collections.
- gently sieve samples through a 2 mm sieve to remove excess soil, and record all species using a 10x dissecting microscope in the laboratory.
- identify organisms to genus, species or morphological group level using standard techniques.

Data recording and analyses:

- record data as either presence-absence or cover-abundance of each species/ genus/ morphological group from each quadrat, or;
- assign an abundance score to each species using an appropriate cover-abundance scale e.g. 1= cover <25% and < 5 individuals, 2=cover <25% and 6-25 individuals, 3=cover <25% and 25-50 individuals, 4= cover <25% and >50 individuals, 5= cover 25-50%, 6= cover 50-75%, 7= cover >75% (Cuddy 2001), or;
- assign a weighted abundance score to each species e.g. 1 = < 5 individuals (or segments in the case of the foliose and fruticose lichens), 2 = 5-20 individuals, 3 = 20-100 individuals, 4 = 100-1000 individuals, 5 = > 1000 individuals.
- derive frequency or abundance scores for each site by calculating the proportion of quadrats containing a given species.

References:

- Eldridge (1996), Eldridge and Tozer (1996), Rosentreter and Eldridge (2002)

Keys:

- Filson and Rogers (1979), Filson (1988, 1992) and McCarthy (1991) and more recent generic revisions for lichens, Catcheside (1980), Scott and Stone (1976), Streimann and Curnow (1989) for mosses; Scott (1985) for liverworts.

BOX 4: CYANOBACTERIA AND ALGAE FOUND ON SOILS

Equipment:

50 m or 100 m tape and quadrats (0.25 m²), dissecting microscope with graticule

Methods:

- establish standard plots of 50 x 10 m collect an intact core of soil from the top 1 cm of the soil from within standard quadrats (see Soil Lichens and Bryophytes).
- look at the total piece of soil under a dissecting microscope and use the graticule as a transect, recording species, groups (e.g. morphological groups) or whatever classification you want per segment of the mini-transect. For example 0-12.1 = brown scum, 12.1-14.7 = *Collema*, 14.7-23.6 = bare soil etc. You use the graticule as if it were a very small transect.
- alternatively, view the core under a dissecting microscope and use the graticule to select a large number of random locations at which to record the species occurring under each point. This is similar to a point-quadrat technique. This will give a measure of cover by species but will underestimate the rarer species and you will need to record more than 200 points per sample.
- Appendix V will provide recommended terms to describe morphological groups encountered.

BOX 5: CULTURING SOIL CYANOBACTERIA AND ALGAE

Equipment:

50 m or 100 m transect, 30 cm plastic ruler

Methods:

- collect soil samples from the top 1 cm of the soil from within standard quadrats (see Soil Lichens and Bryophytes). However, for algae and cyanobacteria it may be preferable to sample from small sections of the transect using a 30 cm plastic ruler.
- place soil in sterile containers for transportation to the laboratory.
- in the laboratory place 10 g soil into a flask with 30 g sterile acid washed silica sand and 25 ml Kratz and Myers (1955) medium D.
- incubate at 25°C in continuous light (3,000 lux) for 2 months.
- maintain fluid levels with distilled water.

Analyses:

- analyse data separately by substrate type e.g. bare soil, below vegetation, on scalded soil etc.

References:

- Rogers (1989)

Keys:

- Use standard keys e.g. Komarek and Anagnostidis (1999, 2001), Ettl and Gärtner (1995).

4.6 AQUATIC AND OPEN WATER ENVIRONMENTS

There are three components of aquatic environments: epiphytic (growing on aquatic vegetation), benthic (on submerged rock, snags or the bottom), and surface or suspended (either as surface rafts of unattached suspended masses). Suitable methods for algae in seepages and on soil are described above (Box 4).

BOX 6: CYANOBACTERIA AND ALGAE IN AQUATIC ENVIRONMENTS

Equipment:

- white plastic disc 5cm radius on a handle marked at 10 cm above the disc surface
- waterproof light meter
- 30 cm ruler.

Methods:

- record cover and colour on inspection
- determine density using a light reading taken at 10 cm below the water surface and/or on bottom. An improvised Secchi Disc (as described above) can be submerged through the aquatic vegetation.. An alternative method may be to use small quadrats (50 cm square) and collect all the floating or suspended algae within quadrat. Place in graduated measuring cylinder, apply plunger until no liquid appears through holes, read off the 'wet volume' and record.

Analyses:

- analyse data separately by substrate type: see Appendice VII & VIII for cover indices.
- convert the 'wet volume' figures to dry wt (g /unit area) using the recommended coefficient of regression (see Robbins and Boese 2002).

References:

- Robbins and Boese (2002) is a useful reference for assessment of swamp algae.

Keys:

- Komárek and Anagnostidis (1999, 2002) for Chroococcales and Oscillatoriales.
- Prescott (1962), Entwisle et al. (1997) for general references.

4.7 USING PHOTOGRAPHS

Photographs are very useful for to allow confirmation about structural components, and even some taxa at a site as long as the resolution is sufficiently clear and the location of the photograph is known. Photographs should be included in all types of survey, and are a normal part of a Standardised Survey. Photographs of individual plants can often be helpful, and in the case of some groups such as fungi, are crucial to identification. Photographs are unless accompanied by site information. At the least, a photograph should be dated and the location noted. Other information, such as direction of photography, in the case of a photopoint (a peg or other fixed land mark from which photographs are taken at regular intervals) is vital.

BOX 7: MACROFUNGI

Equipment:

- 50 m tapes; quadrats

Methods:

- establish replicate plots of about 1000 m² e.g. 30 m by 30 m (larger plots are likely to yield more species)
- stratify the site according to vegetation community, fire history, soil type, microsite (e.g. decomposing logs, soil, tree trunk etc)
- search the habitat thoroughly for a fixed time period e.g. 30 min
- record the abundance of fruiting bodies on a scale of 1=1, 2=2-10, 3=11-100 etc
- repeat the study in different years as fungi are seasonal. Long-term surveying is essential.
- collect voucher specimens

Analyses:

- analyse data separately by substrate type
- multivariate analyses may be useful as species will not be found at all sites at all times.

References:

- Arnolds, E. (1992), Watling (1995), Burns and Conran (1997), May (2002a&b), McMullin-Fisher *et al.* (2002), Packam *et al.* (2002).

Keys:

5. Collecting and processing voucher specimens

Collecting of voucher specimens is essential for a number of reasons. Firstly it provides a record of the taxa collected at a given site and location and can be lodged at an appropriate herbarium for later study. Voucher specimens provide valuable and reliable material for research into a particular species and are invaluable for studying how species vary between sites. Secondly, a voucher specimen is a record that what was recorded at a site has been correctly identified. If the material has not been correctly identified then a voucher specimen (if correctly sampled) will enable the proper identification of the material. Thirdly, voucher specimens enable ecologist to build up a picture of the distribution of the organisms. Finally, most field samples, especially from woodland, dry forest and rainforest will be mixtures of, rather than individual species. It is often not until the specimens are examined under a microscope that the unusual or uncommon species are detected.

Many non-vascular plants have limited distributions, and others may be locally or regionally rare. Many bryophytes are widely distributed, not only in Australia, but throughout much of the southern hemisphere. Lichens in particular are slow-growing, and indiscriminate collecting, even for scientific purposes, may cause considerable damage to a colony which will take many years to recover. Therefore when collecting vouchers, **DO NOT** take large amounts, as small, representative samples are generally more than adequate and cause minimum disturbance. However numerous samples, where their forms change slightly in the field, may be vital to an accurate survey for both biodiversity and community structure. As microscopic examination of vouchers for taxonomic scrutiny is an integral part of the process, numerous small samples, even when they turn out to be much the same floristically, are better than a few random samples. For organisms which are seasonally variable (e.g. fungi) collections need to be made at varying times of the year.

Information must be collected with the specimens, and a specimen without site information is useless. Specimens should be accompanied in the bag with a label or tag. At a minimum, this label should include a description of where the specimen was found, including brief description of the vegetation, soil, substrate (e.g. rock, soil, log), date and name of the collector and any other special features. Alternatively, if many specimens are being collected, the tag should show a collecting number which refers to a standard field data book where the remaining notes will be found.

Best practice, especially when professional help with determinations is to be sought, or some of the specimens are intended for inclusion in an herbarium, involves both a field data book record and a label completed as far as possible. The locality data needs to include the **geographic locality**, the **latitude** and **longitude** (in minutes and seconds if possible), the **state** and if known the botanical region. The collector's **name** (in a clear hand), **collection number** and **date** are also vital, as are the **habitat** notes, **substrate** notes and **host** notes. It should be understood that institutions will not always keep all specimens, but will select for useful ones, but they do always keep distribution data.

Fungi may require more specialised information. Fungal specimens should be accompanied by information on colour and condition of the reproductive receptacle and surfaces, presence or absence of a ring or other features of the stalk, smell and taste. Taste, which is mentioned in some field guides, is not recommended as a necessary test, and should be avoided. So a voucher label for *Pisolithus* sp might read:

‘Disturbed ground (road edge), open tall woodland, light yellow sandy soil over sandstone...[date, location, latitude, longitude, altitude, collector]...two or three in close packed clump, dirty yellow and black, marbled, torn open; spores blackish staining yellow ochre on fingers; smell unpleasant, bit like old socks; not tasted.’

The Australian National Herbarium and some of the State Herbaria have qualified staff to assist in identifying specimens, which they may wish to retain within the herbarium.

There are some differences in the way non-vascular communities should be sampled in terms of voucher specimens. These are described briefly below.

5.1 DRY AREA SPECIES

Most dry area lichens and bryophytes, and all but the more fragile rainforest or moist area species are generally hardy and easy to curate. Samples from trees can be prised off with a short, stout knife while samples from soil can usually be obtained with a small knife or a paint scraper. Rock-inhabiting species are generally more difficult. Mosses such as *Grimmia* which grow in small cushions can generally be prised off the rock in one piece. Crustose lichens however will need to be removed with a piece of the rock attached. A chisel and hammer is necessary for the task. When choosing specimens for collection it is preferable to choose pieces with fruiting bodies (apothecia, capsules) as these will make identification easier.

Specimens should always be transported in dry paper bags or folded newsprint envelopes. Plastic, with few exceptions, should never be used as this tends to encourage the growth of fungi or mildew which will quickly contaminate the samples. For fragile soil-borne specimens, it is often useful to wrap the specimen in paper before placing it in a bag. Samples dominated by mosses, or leafy liverworts should be collected with a minimum of soil, but still sufficient to retain rhizoids and or protonema, and placed between folded newsprint or in paper bags (not in plastic) and stacked upright with top turned down but not pressed. Thallose liverworts or hornworts, especially when reproductive, may be placed in small screw cap plastic bottles or cardboard boxes, to protect fragile sporophytes when found and with sufficient soil to preserve rhizoids.

5.2 WET AREA AND FRAGILE SPECIES

Species such as *Riccardia* and *Aneura* which inhabit rainforest and wet, shaded gullies, are both fragile and susceptible to collapse of cell structure upon drying out. Oil bodies which often occur in leaf cells of some species of leafy liverworts can be essential for identification of the specimen, and these disappear when the specimen dries out. These should therefore be kept moist if possible. This will involve placing the specimens in wet plastic satchels or in screw top containers and kept cool, but be careful to take them out of the container as soon as possible. Whilst in the field, a quick sketch of the form and branching pattern may be helpful for later identification. Samples of aquatic bryophytes such as *Sphagnum*, *Sanionia* and *Blindia* can be collected and transported in paper as can the aquatic mosses *Fontinalis* and *Drepanocladus*, and the liverworts *Riccia fluitans* and *Ricciocarpus natans*.

5.3 ALGAL SAMPLES

Dry algal crusts may be kept in newspaper envelopes or brown paper bags. They may sweat and become mildewed in sealed plastic bags. They do not require to be fixed at this time. Store in a cool dry place. Moist or waterlogged algal mats may be placed, drip dry, in plastic snap-seal 'lunch bags' or plastic specimen bottles. They should be kept cool but not frozen. If they are to be kept for more than a couple of days before laboratory examination they should be fixed with 70% alcohol (containing 7% Formalin if permitted under OH&S rules), or 'a tincture' of Lugol's Iodine.

Aquatic samples, either floating mats and blanket weeds, or epiphytes should be treated similarly to *moist mat samples, with excess fluid removed but not squeezed.

5.4 FUNGAL SAMPLES

Fungi, even woody brackets and punks, should be collected in paper bags and kept from becoming either too dry or sweating. Try to have fungi identified as soon as possible after collection. Drying is something of a skill, and requires the right equipment. To get more information here contact Fungimap Australia at <http://fungimap.rbg.vic.gov.au> . Extensive field notes and photographs are often invaluable.

When including fungi in surveys it is often not possible to do valid 'morphological group and cover' data gathering because of the ephemeral nature of fruiting body production. However, it is worthwhile to a) complete a list (with vouchers) of all macrofungi encountered in a survey, with number of individuals where relevant and b) include scoring for fungal fruiting bodies (sporomes or basidiomes in some texts) in any transects or quadrats. Hopefully that would mean looking up tree trunks for bracket fungi, and noting things like the husks of scleroderm puffballs (*Battarraea* and *Tulostoma* husks, in mallee, can last for years). As noted above, the quadrat size for fungi is in the same range as for vascular plants. Where a long term monitoring program is established more specific methods for fungi may be found in Arnolds (1992).

6. Supplementing field data with laboratory or glasshouse data

Two techniques are widely used to enhance the likelihood that all taxa are described for a particular site. These typically involve growing or ‘plating out’ taxa in the laboratory (algae) and germinating spores of organisms found in the soil (bryophytes)

6.1 PLATING OUT ALGAL COMMUNITIES

There is much talk in papers where blue-green algae are the main crust formers about the requirement to set-up *dilution plate cultures* (e.g. Grondin and Johansen 1993). To set up a dilution plate culture, a subsample is taken from the cyanobacterial crust, measured, and then suspended in distilled water in a measured series of more and more dilute aliquots of subsample. The most dilute aliquot is then mixed with an agar suspension and poured into petri dishes. Following incubation in a suitable growth cabinet, the number of new algal colonies which appear is used to estimate the population numbers and taxonomic complexity of the crust. The method is time consuming and prone to error if not performed on a regular basis. Except in special circumstances this method is well beyond the requirement of surveyors. Where plating out is done for floristic concerns, then it may be justified, especially when the crust has been subject to environmental disruption and no simpler way is available to reach taxonomic determinations. If it is for productivity or organism activity /vitality concerns then it is rather beyond the present scope.

An adaptation of Warcup (1959), where subsamples of soil and crust are submerged in agar, may also be applicable. It is especially designed for fungi, which generally get overlooked, but may also elicit particularly blue-green algae.

BOX 8: PROCEDURES FOR PLATING OUT ALGAL COMMUNITIES

Equipment:

- petrie dishes, flask shaker table; top balance; Erlenmeyer flasks; graduated pipettes, and measuring cylinders; single-sided razor blades
- Chu No. 10; Bold’s Basal Medium; Starr’s soil extract.

Methods:

- make up a 8% agar solution.
- set up a dilution plate culture. A sub-sample is taken from the cyanobacterial crust, weighed, and then suspended in distilled water in a measured series of more and more dilute aliquots of subsample. The most dilute aliquot is then mixed with an agar suspension and poured into petri dishes. Incubate at 14o C for 8-16 days in a light regime of 12 hrs on 12 hrs off (or other regime).
- count colonies as for bacteria, especially if only cyanobacteria present.

Analyses:

- analyse data separately by species

References:

- Grondin and Johansen (1993), Johansen (1993), Johansen et al. (1984), Warcup (1959), Warcup and Talbot (1962).

6.2 GERMINATION OF SPORES IN THE GLASSHOUSE

Non-vascular plants can frequently be studied by assessing the spores stored within the soil. This allows investigators to obtain a full list of species for a site even at a time when not most non-vascular plants are absent from the site. Samples of the topsoil are usually crushed and sprinkled onto shallow trays containing sand which has either been sterilised with steam or autoclaved. The soil is then wetted from above and kept in a glasshouse, until moss or liverwort appears. Sheets of glass, perspex or plastic should be placed over the soils to prevent the spores of common glasshouse species such as *Philonotis tenuis*, *Leptobryum pyriforme* or *Funaria hygrometrica* from invading the trays. Specimens can be identified and quantified once they have sufficiently developed.

6.3 ALGAL BIOMASS TO ASSESS THE CONTRIBUTION OF ALGAE

It is possible to use biomass as a measure of assessing the contribution made by macroalgae in the aquatic parts of an ecosystem or within a designated survey area. This approach reduces the need for complete listing of all species present in raft-forming and suspended macroalgal communities. This may be beneficial with filamentous taxa such as *Cladophora* and *Spirogyra*. A recent investigation of estuarine *Chaetomorpha* and *Enteromorpha* has shown that direct measurement of wet volume of filamentous green algae may be linearly related to dry weight (Robbins and Boese 2002). The coefficient of regression for filamentous algae from that study is $R^2 = 0.78$. This method provides an *in situ* estimate; all that is required is a suitable sized quadrat and a measuring cylinder fitted with a perforated plunger and with graduations.

7. Legislative issues: OHS and quality assurance

7.1 OCCUPATIONAL HEALTH AND SAFETY

Fieldwork does not come without risks. Most of these can be understood in terms of common sense. Work in pairs, even if undertaking specific individual tasks. Wear suitable clothing and carry safety equipment appropriate for the area you are surveying. Be prepared for sudden weather changes. Plan the tasks to fit the time allocation. Don't put yourself or colleagues in unnecessarily dangerous situations. Take appropriate food and drink. Notify someone suitable of where you are going, how long you expect to be there and when to expect you back. In the field you become your own OH&S officer.

7.2 QUALITY ASSURANCE

Quality assurance is there to help you do the best job you can and avoid unconstructive criticism. Don't think of it as a watchdog, think of it as insurance. Today it is almost impossible to conduct any research in the public domain without it having, intentionally or not, commercial and political aspects.

- Have a carefully prepared manual/outline of best practice, and stick to it. Review it with colleagues annually.
- Line up someone independent to do some quality control tests on your field practice (if possible) and assessment. Have someone in the office repeat at least 10% of your calculations, prior to each report.
- Participate in a training day/refresher course at least every second year. These are often available through the Australian Network for Plant Conservation, or through the New South Wales Biodiversity Research Network, and similar bodies.
- Never compromise your own standards to suit the customer.
- Where possible store and archive your raw data, you may find it useful for later comparisons or of use to colleagues.

7.3 LICENCING REQUIREMENTS

Non-vascular plants are protected under law in many states, and already a lichen community and a fungal community have been listed as Threatened Ecological Communities under the Threatened Species Conservation Act 1995. A licence to collect from NSW National Parks and Nature Reserves is required, and is obtainable from the National Parks and Wildlife Service.

All collectors should be aware of scientific licencing under the *Threatened Species Conservation Act 1995* (Section 91) for collecting threatened or protected flora. Information on licencing is available in NSW *National Parks and Wildlife Service Threatened Species Management Circular No. 3, Scientific Licences for Threatened Flora and Fauna, November 1999*, and from the Wildlife Licensing Unit, National Parks and Wildlife Service, P.O. Box 1967, Hurstville, NSW, 2220 [<http://www.npws.gov.au/wildlife/licence/scientific.html>].

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9. Appendices

APPENDIX I. MORPHOLOGICAL GROUPS OF CRYPTOGAMS IN TERRESTRIAL ECOSYSTEMS.

Category: H = horizontal (terrestrial), A = aquatic, V = vertical (terrestrial).

Morphological Group	Description	Category
Sheet-like (dry) skin (wet)	Flat on the surface or forming a thin, more or less featureless skin. Mostly algal.	H,A. V uncommon
Pebbly or powdery (dry) globules or irregular masses (wet)	Made up of many small lumps, globules or small irregular masses. Mostly algal.	H, V & A.
Carpet of waves or hummocks (dry) undulating felt (wet)	Thick carpet-like undulating sheets on the growing surface. Mostly algal.	H, A.
Discontinuous overlapping, upward pointing carpet.	Thick but broken up cover, with crests like moused hair. Mostly algal.	H, A.
Crustose	Lichen forming a close-fitting crust on the substrate.	H, V, A.
Squamulose	Lichen of small individual pieces like wood shavings	H, V.
Foliose	Lichen, sheet- or leaf-like with distinct, often upturned margins and may be easily peeled from surface.	H, V.
Fruticose	Lichen, shrub-like composed of numerous erect, and usually branched, processes, mostly topped with sporangia.	H, V.
Turfs	Systems with parallel upright shoots like carpet pile, mostly Mosses.	H, V, A.
Cushions	Systems with erect shoots radiating to form compact dome-shaped group, mostly mosses.	H, V, A.
Mats	System forming a two dimensional mat, extending horizontally, closely appressed to substrate, moss, liverwort, hornwort.	H, V, occasionally A.
Interwoven mats	System with loose, intertwining, three dimensional wefts, of straggling shoots and branches, often ascending and luxuriant, mosses and liverworts.	V, occasionally H, rarely A.
Canopy formers	System with umbrella-like arrangement of branches, producing a raised leafy canopy, mosses, and some liverworts. [do not confuse with Filmy Ferns]	H, V.
Pendulous	Systems with bases attached to substrate, pendulous stems and branches.	V

APPENDIX II. FIELD SCORING AND LINE-POINT SHEETS

Site Number:

Reader + recorder:

Site locality

Latitude: longitude, altitude:

Date/time:

Weather:

Length of transect:

Slope:

Morphological Group (+ sample ID if collected)	Substrate (soil, rock, bark, wet)	From: (cm mark)	To: (cm mark)	Other characteristics

Samples identified by:

Data entry:

Where:

When:

APPENDIX III. QUADRAT AND POINT-QUADRAT FORM

Site Number: _____ Site locality (& lat/long; alt.): _____
 Reader + recorder: _____ Date/time: _____
 Weather: _____ Quadrat size: _____

Fungi	Morphological group	Other characteristics	Cover: isolated = _;
Bryophyte	(+ identification if		< 5% =1; 5—25% =
Lichen	sampled)		2; 25—50% =3;
Algae			50—75% = 4; > 75%
		= 5. (or %)	

Samples identified, if required, by:

Data entry: _____ Where: _____ When: _____

APPENDIX IV. SOME EXAMPLES OF BRYOPHYTES AND THEIR COMMON MORPHOLOGICAL GROUPS

Bryophytes by substrate	Ground	Logs	Rocks	Trees
Turfs: systems with parallel upright shoots like carpet pile.	<i>Bryum</i> <i>Campylopus</i> ; <i>Dawsonia</i> <i>Polytrichum</i>	<i>Campylopus</i> <i>Dicranoloma</i> <i>Pyrrhobryum</i>	<i>Andreaea</i> <i>Syntrichia</i> <i>Encalypta</i>	<i>Syntrichia</i> <i>Zygodon</i>
Cushions: systems with erect shoots radiating in more or less compact dome-shaped groups (see previous notes)	<i>Ditrichum</i>	<i>Leucobryum</i> <i>Campylopus</i> <i>Dicranoloma</i>	<i>Grimmia</i> <i>Ptyrhomtirim</i> <i>Orthotrichum</i>	<i>Leptostomum</i> <i>Ulota</i> <i>Orthotrichum</i>
Mats: systems forming a generally dense and interwoven mat, extending horizontally over substrate (see previous notes)	<i>Triquetrella</i> <i>Marchantia</i> <i>Phaeoceros</i> <i>Achrophyllum</i>	<i>Frullania</i> <i>Lophocolea</i> <i>Lejeunea</i> <i>Achrophyllum</i>	<i>Sclerodontium</i> <i>Rhacocarpus</i> <i>Frullania</i> <i>Pseudolesk</i> <i>-eaopsis</i>	<i>Frullania</i> <i>Radula buccinifera</i> <i>Fabronia</i> <i>Metzgeria</i>
Interwoven: systems developed as a result of the loose intertwining of straggling shoots and branches, often ascending and luxuriant. (see previous notes)	<i>Acrocladium</i> <i>Wijkia</i> <i>Brachythecium</i>	<i>Thuidiopsis spasa</i> <i>Hypnum</i> <i>Sematophyllum</i> <i>Trichocolea</i>	<i>Thuidiopsis</i> <i>Hypnum</i> <i>Wijkia</i>	<i>Thuidiopsis</i> <i>Hypnum</i> <i>Plagiochila</i> <i>Sematophyllum</i>
Canopy formers: systems producing a raised leafy canopy. Dendroid (see previous notes)	<i>Hypnodendron</i> <i>Hymenophyton</i> <i>Hypopterygium</i> <i>Philonotis</i> <i>scabrifolia</i>	<i>Hypnodendron</i> <i>Hypopterygium</i> <i>Hymenophyton</i>	<i>Hymenophyton</i> <i>flabellatum</i> <i>Hypnodendron</i>	<i>Hypnodendron</i> <i>Camptochaete</i> <i>Trachyloma</i>
Pendulous: systems with bases attached to substrate, pendulous stems and branches.		<i>Papillaria spp.</i>	<i>Papillaria spp.</i>	<i>Papillaria spp.</i> <i>Weymouthia</i> <i>Hampeella</i>

APPENDIX V. ALGAE OF DRY TERRESTRIAL HABITATS

Form	Characteristics	Examples
1. Sheet-like, flat on the growing surface		
• brittle	shiny	<i>Microcoleus</i>
	matt	<i>Lyngbya</i>
• flaky or ropy	regular	<i>Oscillatoriales; Ulothrix</i> & relatives
	irregular	<i>Lungbya; Scytonema; Nostoc flagelliforme</i>
• interwoven	colour fast	<i>Scytonema, Stigonema</i>
	bleached	<i>Zygnemales; Klebsormidiales; Xanthophyceae</i>
• powdery	granular	<i>Chroococcales</i>
	dusty or paint-like	<i>Scytonema; Anabaena</i>
2. Pebbly, made up of many small lumps		
• brittle		<i>Nostoc</i>
• mealy or jelly-like		<i>Chroococcales; Gloeocystis</i> & relatives.
• globular		<i>Botrydium</i>
3. Small waves or hummocks, forming a continuous carpet		
• cushion forming	separating like velvet	<i>Scytonema hofmann-bangii; Vaucheria</i>
	conjoined balls	<i>Nostoc; Gloeocystis; Chroococcus</i>
	warty	<i>Stigonema</i>
• tuft forming	clearly branched	<i>Stigonema</i>
	wavelets	<i>Schizothrix; Rhizoclonium; Zygnemales; Klebsormidiales</i>
4. discontinuous or overlapping, upwardly pointed carpet		
• wavelets		<i>Vaucheria</i>
• turnings, 'wood-shavings'	<i>Nostoc commune</i>	

These are, in most cases, the organisms found in New South Wales, and are best guesses or possibilities more than determinations. With a little experience a limited number can be identified with confidence, including Snot (*Nostoc commune*) which lives up to its Latin and English name, some chocolate stiff bristle-like growth forms of *Scytonema hofmann-bangii*, and the golden green balls of *Botrydium* on mud.

Identifications can be checked against illustrations in literature or those on the Web. Entwisle *et al.* (1997) *Freshwater Algae in Australia* has a good local selection. On the web, search for name in your favourite browser, or see ALGKEY, a key to freshwater algae soon to be available at www.rbgsyd.nsw.gov.au and compare with what you have in your sample.

APPENDIX VI. ALGAE OF SEEPAGES, WATERFALLS AND WET CONCRETE SURFACES

FORM	Characteristics	Examples
1. forming a thin skin over growing surface		
• felt-like	brown or blue-green	Oscillatoriales; Scytonemaceae s.lat.
	green or yellow-green threads	<i>Ulothrix</i> & rels.; <i>Rhizoclonium</i>
	green or yellow-green branched threads	<i>Cladophora</i>
• greasy	purplish or blue-grey	<i>Compsopogon</i> ; <i>Batrachospermum</i> ; <i>Parallela</i> ; <i>Hyalotheca</i>
	Khaki or blue-green	<i>Lyngbya</i> ; <i>Nostoc verrucosum</i>
• mealy		<i>desmids</i>
• fibrous		<i>Cladophora</i> or <i>Pithophora</i> spp.
• gelatinous	crumbles, rusty	<i>diatoms</i>
	blue-green or khaki	<i>Nostocales</i> (esp. <i>Cylindrospermum</i>)
	pinkish or purple	<i>Batrachospermum</i>
	green, often glassy	<i>Gloeocystis</i> & rels; Tetrasporales; <i>Mougeotia</i> sp.
2. forming individual globules or small irregular masses on the growing surface		
• firmly attached	coarse bottle green	<i>Rivularia</i>
	coarse blackish	<i>Stigonema</i>
	jelly-like aquamarine	<i>Nostochopsis lobatus</i>
	jelly-like blue-green to black	<i>Chroococcales</i>
	jelly-like yellow to brown	<i>diatoms</i>
	jelly-like green and glassy	<i>Chaetophora</i>
• easily dislodged	brassy or rusty	<i>diatoms</i>
	green, often glassy	<i>Tetrasporales</i>
	black or purple	<i>Nostoc</i>
• dark tufts		<i>Audouinella</i>
3. forming thick, carpet-like, usually undulating , sheets on growing surface		
fibrous		<i>Cladophora</i> ; <i>Rhizoclonium</i>
• Coated with clear sticky gel		<i>Stigeoclonium</i> spp; <i>Draparnaldia</i> ; <i>Sirogonium</i>
• silky		<i>Spirogyra</i> ; <i>Zygnema</i> ; <i>Oedogonium</i>

FORM	Characteristics	Examples
• gritty	prostrate	<i>Cladophora</i>
	erect	<i>Charaphyceae</i>
4. forming an overlapping or discontinuous carpet with firm, erect fragments		
• wavy		<i>Rhizoclonium</i>
• silky		<i>Zygnemataceae</i>
• velvety		<i>Vaucheria</i>
• gelatinous	khaki, in sheets	<i>Nostoc commune</i>
	reddish or grey-green	<i>Batrachospermum</i>
• bushy	green, crisp	<i>Charaphyceae</i>
	reddish or grey-green	<i>Batrachospermum</i>
	Green, flaccid	<i>Stigeoclonium</i>

Although drawn up for what were specified as the **Aquatic** category of terrestrial systems above, this key can be helpful in discriminating some soil algae when wet. Again it is important to remember the key is an indication, not a definitive determination. See the notes under Appendix II concerning checking identifications against illustrations.

APPENDIX VII. MORPHOLOGICAL GROUPS OF CRYPTOGAMS IN BROADWATER ENVIRONMENTS

E = epiphytic, on aquatic vegetation, B = benthic (on submerged rock, snag or bottom) , S = surface or suspended (either as surface rafts or unattached suspended masses). As it is usually difficult to physically place quadrats or transects in broadwater and stream environments, it may be useful to follow the 'biomass by wet volume' method of Robbins and Boese (2002) or to set up a simple index.

Morphological Group	Description	Category
Gel	Regular or irregular gelatinous mass of algal cells, attached to substrate	E.
Skin	System of algal material covering and close fitting to surface.	E, B.
Fur	More or less uniform furry coating on live or static substrate, less than 10 mm high.	E, rarely B.
Tuft	Clumped and usually branched filamentous algal masses, streaming in the water.	B, more infrequently E.
Shrubbery	Massed individual erect plants, mostly stoneworts.	B.
Globules	Rubbery attached hemispherical or cumulous bodies	B, rarely E.
Streamers	Skeins of filamentous, gelatinous or reticulate matter, sometimes of long indeterminate form	S, B.
Rafts	Irregular (10–30 mm), often entangled masses, both algal and liverwort.	S
Flocs	Small (1–10 mm), more or less uniform, separate flakes or fragments, floating or suspended.	S
Flor	Powdery or otherwise hydrophobic skin on liquid surface.	S

APPENDIX VIII. SCALES FOR RATING MACROALGAE AND BLOOMS

Scales have been developed to assess four features of macroalgae and blooms, i.e. cover, density, patchiness and colour.

The Braun-Blanquet (1927) scale is used to rate cover and density of macroalgae and algal blooms. Cover assessed the proportion of the surface of the water body supporting rafts. Density is calculated either as an actual light meter reading, or a white plastic disk on a 10 cm handle viewed through the water and assessed on a six point scale. Patchiness refers to the average size and packing of the flakes, rafts or blankets of algae.

Colour is often a useful indicator. Sky blue to aeruginose usually indicates the presence of *Microcystis/Aphanozomenon/Anabaena* bloom formers; purple or grey flaky rafts going red when stirred indicates *Oscillatoria princeps*; bright red or orange, forming a skin on glass or plastic indicates *Euglena* spp; bright apple green suggests *Zygnematales*; washed out green or yellow suggests *Cladophora*, and light green to brown *Oedogonium*. This is only an indication, and needs microscopic confirmation.

Rating	Cover	Density	Patchiness
+	sparse cover, occasional scattered rafts	few scattered flocks, or almost none	widely scattered
1	many small pieces, especially if just at margins, cover < 1%	plenty, but the disc is clearly visible	scattered
2	numerous scattered rafts, or covering at least 5% of the area;	covering at least 5% of disc	widely spaced
3	any number of rafts, covering 25—50% of area;	up to half the disc hard to see	closely packed
4	any number of rafts, covering 50—75% of area;	up to 75% of disc obscured	moderately dense
5	covering more than 75% of area	covering more than 75% of disc, at 10cm deep.	very dense

Thus a farm dam with a marginal ring of mixed algae among the emergent water plants may be recorded as: Cover 1; Density 1; Patchiness 2cm, widely spaced; Colour dirty brown. A farm dam with a summer bloom: Cover 4; Density 3; Patchiness < 0.5 cm, close-packed, streaming; sky-blue to pale green.

Useful pictures are given in Mitrovic (1997) *What Scum is That?* More diagnostic for cyanobacteria are the keys and illustrations in Baker and Fabbro (1999) and McGregor and Fabbro (2001).