3 Ecological characterisation of wetlands

Ecological design and survey

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Abstract

The collection of information for use in wetland management needs to be preceded by a series of steps to ensure that time and resources are well spent. This planning phase should include clear identification of the issue(s) to be addressed and what stakeholders perceive to be the values of the wetland. From this base, objectives can be formulated and the appropriate variables for study selected. In wetland studies, variables will generally include those that are intrinsic to management issues (eg pest species or species of economic importance) or some type of ecological indicator. Design of sampling programs will be based on the objectives but may be constrained by logistical factors (suitable control sites, access, human resources and funding). A BACIP monitoring design is described as an example of a statistically rigorous approach that has been used successfully in the Wet-Dry tropics.

1 Introduction

Careful planning and design of wetland sampling programs should allow for the generation of information of direct relevance to managers and stakeholders. To emphasise the importance of the planning and design phase, it has been suggested that the design stage of a long-term monitoring program should account for some 10% of the projected 20-year operating costs, although this would be proportionately less for shorter term programs (Ward et al 1990). The current paper provides a provisional framework for how to approach the planning of ecological surveys, and concentrates on aspects of design as they relate to sampling in tropical wetlands. Statistical considerations are also discussed in relation to sampling designs for wetlands but detailed explanation of methodology is not attempted.

2 A framework for approaching wetland management issues

Effective wetland management and protection requires a range of types of information and knowledge about the constituents and processes that define wetlands. Scientists may be approached to provide this type of information to managers or other stakeholders. Some of the types of information necessary for the ecological characterisation of wetlands have been discussed previously (Finlayson 1996a,b) but before such information is actually gathered we need a framework to ensure the data gathering process is effective (Bunn et al 1997, Finlayson & Mitchell 1999). Such a framework has been adapted from the current revision of the Australian (NWQMS in prep) water quality guidelines section for biological assessment of water quality (fig 1). Whilst not attempting to be prescriptive, the framework provides a sequence of key steps to be considered in the planning phase of wetland studies.

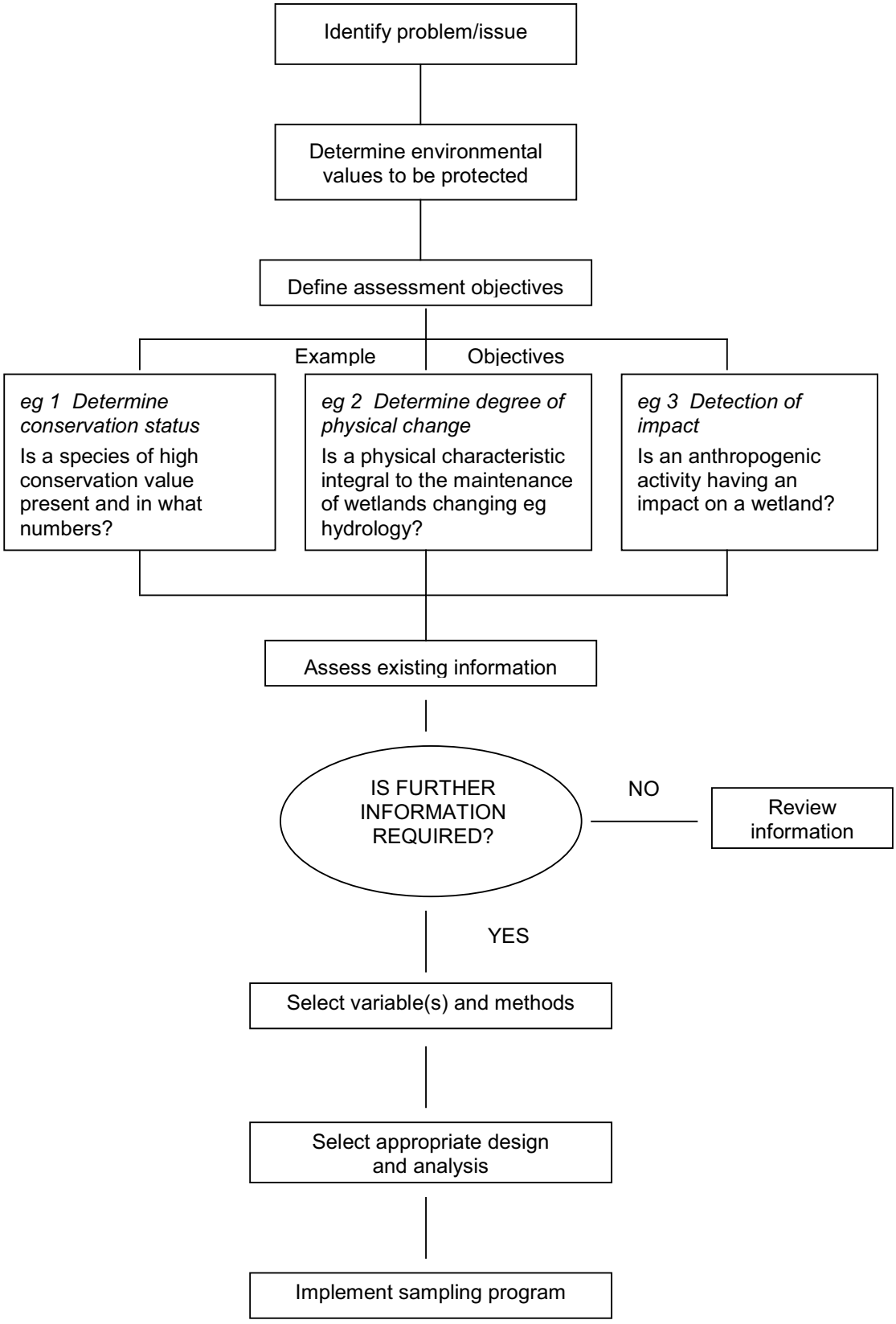

Figure 1 A framework for determining information needs to address wetland management issues

A review and sound knowledge of existing information is a key element in developing data-gathering programs. Information may be found in published research, management, monitoring and policy documents and from local knowledge (Finlayson 1996a,b). Existing information can be used to help formulate objectives and decide on whether a sampling program is necessary or if review and synthesis of current information will suffice. At a basic level researchers need to determine, for the wetland of concern, what government policies pertain to the protection and management and whether guidelines and/or compliance standards are in place for the collection and analysis of variables of interest (eg NWQMS in prep for studies based on water quality).

Linking the scientific aims of any information-gathering exercise (whether it be data collection or review) to the needs of managers and stakeholders of freshwater resources has become an increasingly necessary action (Finlayson 1996a,b); this is well exemplified in the current review of Australia's national water quality guidelines (NWQMS in prep). The current water quality guidelines recognise six environmental values ranging from ecosystem protection to maintenance of water quality for various uses such as human consumption and agriculture. Some of the benefits and values of wetlands that may need to be protected have been classified as functions, products and attributes (Finlayson 1996a,b). Valued functions of wetlands include flood mitigation and retention of nutrients, valued products include wildlife resources, forage resources and water supply, while valued attributes of wetlands include biological diversity and cultural features. The design of sampling programs for wetlands needs to ensure that the resulting information can be used to address whether or not particular values are being protected (fig 1).

Setting of objectives provides the justification for data collection and should also allow the effectiveness of programs to be evaluated (Maher & Norris 1990). Basing sampling programs solely on logistical considerations (ease of access and choice of variables that are easy to measure) rather than to provide information for a defined objective, has resulted in considerable data being collected without a means of converting them into information and contributing to management decisions (Ward et al 1990; Finlayson & Mitchell 1999). Ideal objectives have the following features (from Maher & Norris 1990):

- Are clearly and concisely defined
- Specify what is to be achieved
- Deal only with attainable results and do not express idealistic aspirations
- Indicate when each stage will be completed

3 Selection of variables

The types of variables used in the study of aquatic ecosystems have traditionally been split into two broad categories: 1) physical and chemical variables such as flow, depth, and chemical constituents of water, sediments etc; and 2) biological variables selected from the range of resident flora and fauna. While this paper will focus on the use of these types of variables in wetland sampling programs, it is worth noting that the success of wetland management strategies may also be assessed by socioeconomic indicators where socioeconomic health is linked to ecosystem health. Examples of socioeconomic indicators include: 1) human health; 2) sustainable human use of resources; and 3) favourable public perception of the quality of life and the environment (Cairns et al 1993).

Biological variables are generally used when organisms within wetlands are the primary source of interest or they provide information that physical and chemical variables cannot (table 1). Conversely, physical and chemical variables are most often used in relation to compliance criteria and to explain biological processes (table 1). Assessment of water quality has traditionally used physical and chemical variables (Norris & Georges 1986), although the importance of biological variables in the assessment of ecosystems has been emphasised more recently (eg Metcalfe-Smith 1994, NWQMS in prep). Biological, physical and chemical methods are all relevant for assessing ecosystem health and the problem must dictate the methods to be employed for this assessment, not the reverse.

Table 1 A comparison of objectives for sampling programs using physical and chemical versus biological variables

Objectives of studies using physical and chemical variables	Objectives of studies using biological variables
1. Compliance monitoring eg discharge regulations for industry, human health concerns.	1. To directly determine the effects of contaminants on living organisms.
2. To explain and predict biological processes eg sampling nutrient levels in water to predict algal blooms.	2. To provide an integrated assessment of environmental conditions over time including multiple stresses and cumulative impact.
3. Early warning of impact (where levels known to effect biota can be detected).	3. Early warning of impact (where levels of physical and chemical variables known to effect biota cannot be detected).
	4. To assess the effect of activities that do not result in physical and chemical alterations eg flow alteration, habitat destruction and overharvesting.
	5. To assess conservation status of species.

Assuming biological variables are accepted for use in a sampling program, the next issue is to select the appropriate ones (fig 1). The choice of variables can proceed hierarchically in the manner of the proposed framework (fig 1). Firstly the variable must be intimately related to management goals. Secondly measures of the variable must lie within the appropriate temporal and spatial scales relative to the management goals (ie be able to address the objectives) and thirdly methodologies for their use must have been developed (Cairns et al 1993).

Biological variables intrinsically tied to wetland management goals will include species of economic or conservation significance (eg barramundi or magpie geese). Likewise, studies of pest species such as feral pigs and *Mimosa* will require information on these taxa (and probably variables related to their potential impact). Alternately, selection of *indicators* of ecological health may be appropriate.

Indicators can be defined in a very broad sense as ‘measurable variables for characteristics of an ecosystem’ (Grillas 1996). Unlike the biological variables mentioned above, indicators may not be of interest in themselves (eg macroinvertebrates, algae) but they are able to reflect something about the broader ecosystem. Biological indicators have been defined as ‘a species (or species assemblage) that has particular requirements with regard to a known set of physical or chemical variables such that changes in presence/absence, numbers, morphology, physiology, or behaviour of that species indicate that the given physical or chemical variables are outside its preferred limits’ (Johnson et al 1993). A number of attributes of good biological indicators have been recognised (Johnson et al 1993):

1. Taxonomic soundness (ie species have been confidently separated) and easy recognition by the non-specialist. This ensures consistent identification by a broad range of people, eg fish.
2. Cosmopolitan distribution. This means that comparison between all sampling sites is possible because the indicator occurs in them all. It also means the organism can be affected by a range of environmental perturbations in many different types of aquatic systems and habitats, eg algae and macroinvertebrates.
3. Numerical abundance. Reduces sampling effort and is necessary for statistical purposes, eg macroinvertebrates.
4. Low genetic and ecological variability. Particularly important for impact assessment to ensure differences are due to the impact and not natural variability in the indicator measured.
5. Large size. Easier to sample/observe, eg birds, macrophytes.
6. Limited mobility and relatively long life history. Limited mobility will facilitate collection and reduce avoidance of impacts while the life history needs to be long enough for the organism to be collected/observed with ease and be exposed to potential impacts, eg macrophytes, macroinvertebrates.
7. Ecological characteristics are well known. This allows accurate interpretation of patterns and changes in distribution and abundance of the indicator. Not many indicator groups satisfy this criterion.
8. Suitable for use in laboratory studies. Testing of cause and effect can only theoretically take place in the controlled environment of the laboratory, eg microinvertebrates.

The relative importance of the above attributes in determining indicator selection will vary according to the objectives as described in the following section. The most common aquatic indicators used in impact assessment of freshwater systems are macroinvertebrates, fish, algae and macrophytes. Non-aquatic indicators of interest in tropical wetlands include birds, mammals, amphibia and reptiles.

3.1 Indicator organisms, populations and communities

Biological indicators can be studied at a number of levels to tell us something about the health of wetlands. At an organism level, a variety of measures from biochemical to life-history changes and bioaccumulation may be measured in individuals. For example, in the Alligator Rivers Region (ARR), freshwater mussels (*Velesunio angasi*) have been used as indicators through bioaccumulation and growth and mortality studies (Humphrey et al 1990). Bioaccumulative indicators are a 'special kind of indicator organism that accumulate pollutants from their surroundings and/or food so that an analysis of their tissues provides an estimate of environmental concentrations of pollutants' (Johnson et al 1993). In the ARR, mussels have been analysed for their accumulation of stable and radioactive metals that may be waste products from mining (Humphrey et al 1990).

The use of biological indicators at the population (one species) or community (more than one species) level is concerned less with directly measuring the physiological responses of individuals than it is with the ultimate, integrated expression of a response to the environment, ie the presence, absence and abundance of species. Population studies are often concerned with variables such as density, age structure and sex ratio, while community studies often deal with changes in community structure. Changes in community structure can

be in the form of simple changes in biomass, changes in the relative abundance of species, disappearance of species and combinations of these (Hellawell 1986). Structural changes often reflect changes in how the community is functioning. For example, discharge of domestic sewage to streams can cause dramatic changes in macroinvertebrate fauna – some taxa sensitive to chemical changes will disappear and some that can feed on the organic material will dramatically increase in numbers (Campbell 1978).

Deciding upon indicator populations versus communities should not be viewed as competing tasks, but rather as complementary tasks with each addressing different objectives. Indicator species appear to be most effective at: 1) directly measuring progress towards the restoration and maintenance of populations that possess commercial and/or social value, and 2) tracking progress towards remediation of specific forms of environmental impact by identifying species known to be especially sensitive to individual stressors. In contrast, a community level approach to wetland monitoring provides a more robust assessment of ecosystem health in a region as it is impacted by the cumulative effects of many stressors ranging from persistent contaminants to the introduction of exotic species (Cairns et al 1993). Use of communities also has inherent statistical advantages (Humphrey et al 1995).

4 Study design

The three main study types undertaken to address wetland management issues ie survey, surveillance and monitoring, are detailed in Finlayson 1996a. Design will be different for each of the study types reflecting differences in their scope and objectives. System understanding programs (survey and surveillance) will have broad objectives while monitoring will have narrowly defined or limited objectives (Maher & Norris 1990). An implicit feature of different study types and objectives is their differing requirements for statistical inference. In statistical terms, a study design will have inferential power if a change can be linked to an identified source of impact. This often relates to separating natural variability in the biological indicator from variability caused by the impact, eg being able to separate changes in a wetland plant community caused by the removal of buffalo from seasonal or interannual changes in plant communities. Another aspect of statistical inference relates to the ability to state the magnitude and ecological significance of the change, eg a change has been detected but changes of such magnitude and for this duration are not uncommon in such ecosystems (Humphrey et al 1995). Therefore, in the spectrum of study types, surveys would be expected to have little or no need for statistical inference while at the other extreme, monitoring programs would have a strong need for statistical inference.

4.1 Where to sample

Study design in the statistical sense involves decisions on the spatial distribution of sampling stations and decisions on the frequency of sampling at those stations. In deciding where to take samples (selection of sampling sites), the objectives as well as ecological, statistical and logistical factors need to be considered. The objectives of a study will generally dictate the broad or macro level of site selection such as different classes of wetland or types of river. For example, in the ARR you may distinguish between different billabong types such as backflow billabongs at creek junctions versus floodplain billabongs. Ecological factors influence site selection at the micro level and define the exact places to be sampled, eg where in the backflow billabong will the samples be taken – in open water, at the edge, at a particular depth? Consideration of site selection at the micro level is determined by the need to obtain a ‘representative’ sample within the macro location (Ward et al 1990).

One of the most important statistical aspects of site selection is the provision of control sites. Controls provide a benchmark against which changes in the indicator in the impacted areas can be judged, and are thus a necessary component of studies where statistical inference of ecological change is required. Such controls need to be biophysically similar to the site(s) or area(s) subject to the putative impact and also need to be free of other highly localised disturbances unrelated to the putative impact (NWQMS in prep). The nature of tropical wetlands is such that finding control sites can be problematic, eg wetlands can form contiguous systems or large interconnected complexes (Storrs & Finlayson 1997) which can make finding control sites that are independent of the impacted sites difficult. This illustrates another statistical consideration of site selection, namely independence with respect to the indicator being used.

Many statistical methods also require samples to be taken at random. In simple random sampling, every sampling unit in the population (of your indicator organisms) has an equal chance of selection. In practice, many studies in tropical wetlands are designed to sample different micro locations or strata (as described previously). Random selection of sites within these strata then constitutes stratified random sampling. From a statistical viewpoint these 'strata' should be more homogeneous than the whole system and should be well-defined areas of known size (Elliott 1977). For example, in sampling macroinvertebrates, zones of flowing waters in the ARR have been split into different substrate types (cobble, sand and vegetated) which are quite distinct and support different communities.

Logistical considerations such as access, funds and human resources may influence the location of study sites and how many sites are used within the constraints of the previous factors. A major logistical constraint in the location of sampling sites in tropical wetlands is access. If access is required during the Wet season, roads may be cut off by floodwaters or impassable if unsealed. Sites may need to be located where there is easy access by boat in the Wet or near helicopter landing areas. The number of sample sites to be employed is usually a function of the budget available for sampling, the size of the system and the variability of the target indicators (a consideration in the statistical power of the design – described in section 5).

4.2 How many samples to take

In the study design phase, the number of samples to be taken on a given occasion and how many sampling occasions there should be must be considered. There are two reasons for replication in a study: 1) to estimate the value of a given measure, such as mean density of individuals, with a desired degree of precision and risk of error (common in surveys and population studies at single sites); and 2) for statistical inference about differences, as mentioned previously (Resh & McElravy 1993). Environmental variability is a fundamental problem facing those concerned with assessing changes in space and time. Conclusions that a given environmental measure actually differs at particular sites or times can only be made when observed differences in means between sites/times are greater than would be expected based on observed variation within sites or times (Norris & Georges 1986).

There are a number of mathematical procedures for estimating the number of replicates required for a study (Elliott 1977). The actual number will depend on: 1) the size of the mean; 2) the degree of aggregation; and 3) the degree of precision and statistical power required, ie using a small-sized quadrat is useful in increasing sample replicates but not if mean values are low and zero counts of abundance become common. The greater degree of aggregation the more sample replicates required (if mean density is constant). The higher the degree of precision required, the more sample replicates required (Resh & McElravy 1993). Generally a

compromise needs to be made between statistical accuracy and the labour required to collect and process samples.

4.3 When to sample

As with other aspects of sampling design, the timing of sampling will be intricately linked to the study objectives (fig 1). A particular event of importance may be targeted and this will dictate sample timing. For example, fish migration studies in the ARR occur at the end of the Wet season as fish move from spawning grounds on the floodplain to permanent waters in the upper reaches of streams (Humphrey et al 1990). Time of day may also be important for surveying some animals eg fish.

In a broader context, timing of sampling in tropical wetlands is often related to the strong and predictable seasonal variation in water regime and species' response to this. Seasonal variation in distribution, abundance or behaviour of the selected indicator may all influence the timing of sampling. For example, sampling of macroinvertebrate communities in streams and billabongs in the ARR for monitoring purposes occurs at the end of the Wet season when abundance and diversity are at a peak (eg Outridge 1988). Conversely, mapping of vegetation in billabongs may occur during the Wet season when aquatic plants flower and reach their peak biomass (Finlayson et al 1989, Finlayson et al 1994).

Statistical considerations in the timing of sampling relate to studies that seek to identify changes in some particular variable over time. The most rigorous of sampling designs will have samples taken before and after a change or impact (NWQMS in prep). In many cases, however, pre-impact data may not be available such as for uncontrolled or unforseen impacts. In some instances historical information collected for other purposes may serve as a suitable reference for other issues (again emphasising the importance of a thorough knowledge of existing data). For example, plant surveys conducted in the ARR to determine potential effects of mining may be useful for assessing the effect of the introduction of weeds such as *Salvinia* which is present now but was not present at the time of the original survey. Another alternative for dealing with a lack of pre-impact data is comparison of sites currently impacted with a range of biophysically similar control or reference sites (NWQMS in prep).

5 The BACIP study designs as an example of statistically rigorous design

BACIP is an acronym for Before, After, Control, Impact, Paired differences, which summarises the design structure. A BACIP design involves sampling closely matched, but independent, areas simultaneously at several times before the impact occurs, and for several times after the impact in both impact and control areas. Each time period is summarised not by the individual observations at the control and disturbed sites, but rather by some measure of the difference between the two sites at that time. In a BACIP design employing a number of control locations, if the size of these difference values changes after the impact, the putative impact is inferred to have been responsible for that change. The design assumes that the difference value in the indicator between control and impact areas would have remained the same if the impact had not occurred (Faith et al 1995). For example, BACIP designs have been tested on data from the South Alligator River catchment and have shown a high sensitivity in detecting impacts from a disused mine on the macroinvertebrate community structure of the adjacent creek (Faith et al 1995).

Statistically rigorous designs such as BACIP are appropriate in highly valued environments, such as the Alligator Rivers Region, where even small impacts are of interest to the stakeholders (as is the case with uranium mining). In cases such as these, monitoring study designs are required that allow application of statistical tests with high power. Statistical power refers to the level of confidence that a Type II error has not occurred. Type II errors occur when it is concluded that means are from the same sample population when they are not, eg it is concluded that macroinvertebrate communities were not affected by mine waste water releases when they were. Type I errors, on the other hand, are when it is concluded means are from different sample populations when they are not, eg it is concluded that macroinvertebrate communities were affected by the release of mine waste waters when they were not. Study designs with high statistical power, therefore, are able to guarantee that an impact no greater than a prescribed amount has gone undetected.

The appropriate indicators for BACIP designs are those that are proven to be tightly linked to the potential impact and unlikely to be affected by extraneous natural factors. Pilot studies would generally be required to determine these factors prior to the commencement of the monitoring program itself. It is worth noting, however, that one needs to be cautious about over-reliance on statistical procedures – sound design also requires an understanding of underlying biological processes and careful planning (Humphrey et al 1995).

6 Conclusion

Successful and effective ecological data gathering requires a planning phase that includes determination of the issues, values of the ecosystem to stakeholders and clear formulation of objectives. Decisions regarding what variables to study and how they should be described or quantified will primarily be a function of these objectives and their inherent statistical requirements, within the limitations of logistical considerations. Tropical wetlands provide a number of unique challenges for ecological study which need to be recognised in the planning and design stage of information gathering.

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Survey techniques for invertebrates

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Abstract

Benthic macroinvertebrates are a popular biological indicator group for assessing the health of aquatic ecosystems. Use of macroinvertebrates requires decisions regarding both sampling and processing strategies for which quantitative, semi-quantitative or qualitative approaches can be employed. Selection of the appropriate techniques is dictated by the objectives of the study, the nature of the habitat to be sampled and available resources for processing. Qualitative rapid assessment techniques have become popular recently because of the large effort often required to process quantitative samples.

1 Introduction

The validity of any ecological investigation depends crucially upon the sampling technique and strategy adopted at the outset. Inadequate sampling effort cannot be compensated for by sophisticated analytical techniques. Invertebrates, unlike some other indicators such as fish and vegetation, can only be identified and quantified via collection; visual surveys are not possible. The use of invertebrates in a study also requires consideration of how to process or 'sort' samples given that many sampling techniques result in a mixture of sediment, detritus and animals (Hellawell 1986, Abel 1989, Rosenberg & Resh 1993).

Benthic macroinvertebrates are the most commonly sampled of the invertebrates and refer to those organisms that inhabit the bottom substrates (sediments, debris, logs, macrophytes, filamentous algae, etc) of freshwater habitats for at least part of their life cycle. Macroinvertebrates are those retained by mesh sizes ≥200–500 μm (Rosenberg & Resh 1993). Several reviews have found that macroinvertebrates are the most commonly used group for assessing water quality (Rosenberg & Resh 1993). They have also been used in a wide range of biological sampling programs and for a variety of reasons including: monitoring changes in genetic composition, bioaccumulation of toxicants, toxicological testing in the laboratory and field, and measurement of changes in population numbers, community composition, or ecosystem functioning.

Benthic macroinvertebrates have many of the characteristics for good biological indicators and this explains to a large extent their popularity in water quality studies. As a group they have a large number of species which offer a spectrum of responses to environmental disturbance and can be sampled using simple inexpensive equipment. Their relatively long life cycles and relative immobility mean that benthic macroinvertebrates act as continuous monitors of the water they inhabit, enabling long-term analysis of both regular and intermittent discharges, variable concentrations of pollutants, single or multiple pollutants and even synergistic or antagonistic effects (Rosenberg & Resh 1993).

2 Qualitative versus quantitative sampling

Quantitative sampling allows the absolute abundance of organisms within a designated area to be estimated while qualitative sampling aims to recover a representative range of organisms present in the community. Semi-quantitative sampling is halfway between the two as it provides an indication of the relative abundance of the components of the community but does not enable one to relate them in absolute terms to a defined area or volume of the habitat (Hellawell 1986).

Quantitative sampling is often useful in monitoring studies rather than surveys and surveillance (Abel 1989). This is partly because the data is potentially suitable for analysis by almost any method (within other design constraints). Changes in the abundance of species can also be determined by quantitative sampling, eg knowing that the abundance of a species has halved or doubled over time, or that the ratio of abundance of two species has altered, may be of interest in a monitoring program. Problems with attempting quantitative sampling is that estimation of absolute population density is extremely difficult. The pattern of distribution of benthic invertebrates seems to be such that very large numbers of samples are required for reliable estimates of population density. Even to estimate population densities to within ± 20% or 40% of their true values may require several hundred samples (Abel 1989). Another problem is that most commonly used samplers only sample the top few centimetres of substratum, which can lead to serious errors where significant proportions of animals live deeper within the substratum.

An example of the use of quantitative sampling by ***eriss*** has been in long term monitoring of macroinvertebrate communities of the South Alligator River. Sampling of this river was initiated when mining at nearby Coronation Hill was proposed. Data that was comparable over time, able to detect changes in abundance and could be analysed to give a high degree of statistical inference was required, so a Surber sampler (quantitative technique) was used (Dostine et al 1992).

Qualitative and semi-quantitative sampling techniques are generally most useful in survey and surveillance types of study. The advantages of qualitative sampling techniques are that: 1) they generally do not require elaborate apparatus; 2) they usually catch a high proportion of the total species present at each station; and 3) they often provide fairly comparable figures, especially when the habitat and collector are the same for all samples. Disadvantages are: 1) they cannot be used in deep water; and 2) the samples do not provide estimates of numbers per unit area (Elliott 1977).

An example of qualitative sampling done by ***eriss*** is billabong surveillance carried out over the last two years. In this instance the spatial and temporal range of species present in billabongs was of interest rather than making statistical inference regarding differences in community structure. For this study a dip net (qualitative technique) was used.

3 Description of sampling techniques

3.1 Qualitative techniques

Hand nets (or dip nets) are one of the most versatile implements for qualitative sampling. Essentially a mesh bag attached to a rectangular or triangular metal frame is fitted to a handle or pole. In running water it is held vertically upon the stream bed and an area of the substratum immediately upstream of the net mouth is disturbed by hand or foot. In vegetated areas with no flow the net is swept through the vegetation and may also be used to disturb the

underlying substratum. Although the method is qualitative in that the area (and depth) disturbed are not fixed, some operators attempt to minimise this source of variability by sampling for a definite period or by sampling for a fixed distance (Hellawell 1986).

Drift sampling is a passive technique for collecting macroinvertebrates that move into the water column actively or passively and then drift in the current – a mechanism for dispersal and avoidance of disturbance. Drifting animals are sampled by attaching a net to the substrate and leaving it to collect animals. This technique is only appropriate for use in flowing waters and is employed in studies of species that are more easily located in the drift, or more commonly, for looking at sources of colonisation and environmental change. Increased turbidity, for example may trigger drift (Hellawell 1986).

3.2 Quantitative sampling

Surber samplers combine a rectangular quadrat to delineate the area of bed to be sampled and a net into which the disturbed benthic invertebrates are swept by the current. Because Surber samplers rely on flow to wash the disturbed invertebrates into the receiving net they are generally only used in flowing water habitats. As the area of bed to be sampled is fixed, these samplers are quantitative, although the depth to which the operator disturbs the substrate may vary. This means that even these methods may not provide absolute measures of population densities (Hellawell 1986).

Grab samplers are designed to remove a portion of substrate and animals by a biting action. Originally designed for marine work they are used in freshwater situations where there is deep water and fine substratum. A number of different designs of grab samplers exist but they are generally inefficient with particle sizes exceeding 16 mm and where animals are buried more than 3 cm below the surface (Abel 1989). This is because coarse material can get lodged in the mouth of the sampler preventing its closure and thus resulting in loss of sample material.

Air lift or suction samplers use compressed air to scour substrate and raise water, lighter substrate material and fauna as the air ascends within a delivery pipe. The material is then discharged into a net where the animals and debris are retained while the water, air and very fine substrate escape. These techniques are used over a set area, eg the area of a cylinder that is placed on the substratum, to ensure they are quantitative. They are generally used in fine substrates within shallow static or slow-moving water where flow-dependent samplers such as the Surber sampler become inefficient.

Colonisation samplers provide an artificial substrate for invertebrates to colonise. Many patterns of colonisation sampler have been described but most are designed to provide interstices. Some designs simulate a natural gravel bed, while others are intended to mimic water weeds. Samplers may be embedded in the substratum, rested upon it or suspended in mid-water. The rationale behind the development of artificial colonisers was to 1) provide a uniform sampling area and thus improve the scope for statistical inference from data; and 2) overcome differences found with active sampling techniques attributable to the varying abilities of operators. However, the representativeness of communities that colonise artificial substrates to those found in natural substrates and the ecological significance of these communities has been debated (Hellawell 1986). For example, ***eriss*** has successfully used gravel-filled mesh baskets in a rocky stream (Rockhole Mine Creek) to standardise sample size. Use of similar colonisation samplers in the sand beds of Magela Creek were unsuccessful because colonising fauna was unrepresentative of that found in sand beds (C Humphrey, pers comm).

4 Factors that influence the choice of sampling technique

As is apparent in the previous description of sampling techniques, two of the crucial factors in deciding which technique is to be used for collection of macroinvertebrates are whether qualitative or quantitative samples are required (which will be related to objectives) and what type of habitat is to be sampled. In this instance important features of the habitat include whether it is a flowing (lotic) or non-flowing (lentic) environment, what the substratum consists of (particle size, amount of vegetation) and depth. Another consideration may be the efficacy of different methods, ie the extent to which they may introduce bias by recovering certain organisms more readily than others and the degree to which fauna is separated from habitat material.

Tropical wetland environments can be difficult to sample quantitatively. This is particularly so for deeper, highly vegetated areas such as those found in billabongs and floodplains. Extracting animals from a defined area of these complex habitats is difficult without also collecting large amounts of vegetation (and thus increasing sample processing time) and preventing escape of more mobile taxa. Another difficulty with quantitative sampling is that techniques usually only cover a small area of substratum so the problem becomes how many sampling units are necessary to ensure that the sample includes most of the species present (Elliott 1977).

5 Qualitative versus quantitative sample processing

In this instance sample processing is defined as procedures used to extract animals from other material such as organic debris and sediment that may be collected in sampling. Most sampling techniques (with the possible exception of drift nets in certain environments) recover a range of material in addition to the desired invertebrates. The amount of excess material will depend on the habitat and the technique. If a quantitative sampling technique is used it is likely that quantitative processing will take place. Quantitative processing of semi-quantitative samples will allow comparison of rank abundances of species (ie abundance of species relative to each other will be known but absolute abundance for a defined area will not). Qualitative sampling probably only justifies qualitative processing.

Samples that are to be quantitatively processed require preservation after collection. Samples are then sorted through systematically at an appropriate level of magnification (generally 10x using a stereo microscope) to pick out the invertebrates for later identification. This procedure is necessary to ensure accurate enumeration and identification when there are large quantities of detritus in the sample. Samples from habitats with small amounts of detritus (eg sand habitats) may not require processing. For samples with high numbers of animals a sub-sampling technique is often used to reduce the time and effort required for processing. A range of subsamplers exist, but one needs to be chosen that will give subsamples representative of the whole sample. Results from subsamples can then be multiplied to give whole sample estimates.

A common approach to qualitative sample processing is live sorting. This procedure involves picking invertebrates out of the sample while they are still alive and only preserving those specimens. This procedure generally takes place in the field where samples are placed in a tray for sorting without any magnification aid. Standardisation of effort can be via setting a finite time period for live sorting (generally 30–60 min) or by defining the number of animals to be retrieved. These techniques result in greater time spent during sampling but less time spent in the laboratory. Problems in using qualitative processing techniques in tropical

wetland systems include inclement weather and remote sampling locations which may result in a delay between collection and processing.

The relative merits of qualitative versus quantitative sample processing are similar to those described previously for sampling techniques (section 2). Qualitative processing has become more popular in recent times as part of efforts to reduce time and cost in assessing environmental conditions at a site and for use in broad scale studies where sampling of many sites is required for quick information turnover to managers (Resh & Jackson 1993). Quantitative processing techniques for samples from vegetated tropical wetland habitats can be very time consuming with recovery of 200 animals being known to take over a day (personal observation). Work done recently at ***eriss***, however, suggests that there can be high variability in recovery of rank order abundance using live sort techniques (Thurtell 1996, unpub). Further work may be required in tropical wetland habitats to determine processing methods that give consistent but timely results.

6 'Rapid Biological Assessment Techniques' – an example of qualitative sampling and processing

There are two objectives to rapid assessment: 1) reduced cost and effort (relative to quantitative sampling; and 2) to summarise the results of site surveys in a way that can be understood by nonspecialists such as managers, other decision-makers and the concerned public. Efforts to reduce costs must not be carried to the point that information used in the analysis does not adequately represent the site examined. Likewise the analysis and summarisation should not be so simplified that impact-related conditions are not detected. (Resh & Jackson 1993).

In Australia a nationwide biological monitoring program (the Monitoring River Health Initiative) is currently underway, based primarily on rapid assessment of benthic macroinvertebrates. Models derived from this program will be used to assess biological responses to water quality and/or habitat changes in rivers. Qualitative sampling techniques in a variety of habitats are followed by picking of live organisms on site. Sampling is standardised by area sampled, eg 10 m sweep along river edge, while sorting is time standardised: live sort for 30 min. Animals are identified in the laboratory to family level (Davies 1994).

7 Conclusion

Invertebrate survey involves the collection of specimens (sampling) and separation of specimens from associated detritus prior to counting and identification (sorting). A range of techniques from quantitative to qualitative exist for both sampling and sorting. The choice of technique will depend on the objectives of the study, the nature of the system to be sampled and available resources. Highly vegetated tropical wetland systems can be difficult to sample quantitatively.

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Survey techniques for fish

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Abstract

Fish survey may be used to assess and manage biodiversity, to study the ecology and ecosystem of the fish species, and/or to manage harvesting. The information obtained from a sampling program provides measures of fish abundance that may be qualitative, semi-quantitative and sometimes quantitative. A measure of fish abundance may be added to data on size and age distribution, fecundity and fishing effort, in order to model population dynamics and evaluate effects of harvest. There are many fish sampling techniques, and a method should be chosen according to the objectives of the study and the effectiveness of the technique in the environment concerned. Here a number of fish survey techniques are described, with comments upon their effectiveness in the Alligator Rivers Region, Northern Territory.

1 Introduction

Surveys of fish are generally directed at one of three distinct objectives: assessment and management of biodiversity, studies of the ecology of fish and their ecosystem, or management of harvesting. Whilst similar capture procedures may be used for all, the different objectives, by and large, require different information from the surveys. There is, of course, a wide range of techniques used for fish capture and it is important to understand their advantages and limitations to judge which are appropriate. The very mobile nature of fish poses some different problems to invertebrates in the design of surveys. For example, when fish may range over a large area each day, how far apart do sites need to be to be considered as independent replicates and, indeed can sites ever be considered truly independent? Nevertheless, the large size and relatively low diversity of freshwater fish makes them easier to identify by comparison to invertebrates. This makes it possible to obtain biodiversity data rapidly so that fish are potentially useful for environmental monitoring as well as their other values. On the downside, representative samples of fish communities are much more expensive in terms of field-time than invertebrate samples.

2 Biodiversity survey

In biodiversity surveys the task is usually to establish the species present in an area or specific habitat type and, often, to quantify their abundance in a manner that can be easily repeated in the future in a monitoring program. Thus, the information obtained can vary from qualitative to quantitative. The measure of abundance of each species captured can relate to either the number of fish or their biomass, and often both.

For some purposes the presence or absence of different species is sufficient information. This can be quantified as frequency of occurrence data to indicate the rarity or commonness of a species. Simple ranks of abundance may be recorded if semi-quantitative data are appropriate.

These strategies are often used in the comparison of community structure although quantitative data are also appropriate.

3 Ecological studies

In studies of ecological processes more quantitative data are usually necessary and the data recorded then may be the number and biomass of fish of each species per sample. Preferably the sample unit should relate to some measured quantity of habitat. In many surveys the length and weight of each individual is measured and samples of scales, otoliths or spines may be removed for age analysis if required. When very large numbers of fish are involved such measurements are only made on a sub-sample.

When it is not important to return fish alive, much other information can be obtained from fish specimens. Common variables measured include gut contents for diet analysis, gonads for development stage analysis and fecundity estimates, liver condition, fat deposit condition. Blood samples and indicators of the incidence of disease and parasite attack may also be examined to assess the health status of fish.

In all surveys measurements of environmental correlates and appropriate spatial data should be recorded.

4 Harvest management

In fisheries management research the data on the fish in question are always in some quantitative form, usually semi-quantitative. Where there is a fishery (commercial, subsistence or recreational) there is the possibility of obtaining data on catch per unit fishing effort (CPUE) from fishermen. This data can provide a measure of fish abundance and, combined with information on size and age distribution and fecundity obtained by biologists, and other information on fishing effort, provides the information required for modelling population dynamics and evaluating effects of harvesting. In many cases only a few, or often only one, species are examined in a study. Where multiple species catches are involved the population dynamics of each species may have to be examined separately.

Measurement of fish age is a specialised task requiring lots of tedious work. Also, the mathematics of population dynamics is complex and is a task more appropriate for specialists in this field.

When a source of fish catch data from fishermen is either not available or unsuitable for various reasons, it is necessary for biologists to obtain their own measures of fish population size and specimens from other data. Many methods of fish sampling have been developed for different situations and species. Again it is useful to separate the methods into semi-quantitative and quantitative measures of abundance. Semi-quantitative measures are those that can only yield catch per unit effort data while quantitative methods yield data on density (numbers per unit area, or volume, of habitat). The advantage of quantitative measures is that data obtained by different procedures can easily be compared with one another by adjusting to common units. *Semi-quantitative data can only be validly compared with data obtained by the same, preferably identical, procedure*.

In general, catches of fish made by passive procedures, such as traps and gill nets, provide only CPUE data. Catches made by active procedures, such as seine nets and electrofishing, can provide absolute density measures if a known area is fished. Mark and recapture methods

provide estimates of total population size that could also be expressed as a density measure if the total area of water inhabited by the population is known.

5 Appropriate sampling procedures

Very often in freshwaters, the waterbodies being examined may not be very large and the resident fish population may be similarly small, especially if it is a large species. Killing of the catch, therefore, could have a significant effect on the size of that local population. This may have unfortunate effects on your inferences from repeat sampling if this was not already considered. In that situation it is appropriate to return as many fish to the water unharmed and the method chosen should be one that makes this possible.

Some of the more common sampling methods are outlined here.

It should also be noted that survey/research work on fish may require approval of animal experimentation ethics committees associated with different research organisations. The guidelines provided by these committees should be consulted when planning sampling and fish handling procedures.

5.1 Gill nets

These comprise a wall of netting (usually nylon) suspended by floats and weighted on the bottom. The nets must be anchored in place – an interesting exercise in strong currents. Fish catch themselves by swimming into the net and entangling their spines and operculae with the mesh. The size of the mesh dictates the size of the fish caught. Consequently, for research purposes a range of mesh sizes is used to capture the range of fish sizes of interest. Note that commercial fishermen are usually highly restricted in the mesh sizes they can use and this must be recognised when using their catch data. Commercial barramundi fishermen use gill nets.

Gill nets work well for larger and scaly fish. They work less well for smooth scale-less fish and very poorly for very small fish.

This passive procedure provides only CPUE data. However, if a large area is enclosed by fine mesh and subjected to repeated fishing with records of catch taken at fixed intervals, gill nets can provide an estimate of total abundance in the enclosure area from the rate of decline in the catch. This is termed a trap-out procedure.

A major disadvantage of gill nets is that the fish will die if not released soon after entanglement. This can be minimised by ‘running’ the nets continuously.

A gill net catch decline procedure, with continuous net running to minimise mortality, is used by NT DPIF for their barramundi survey work on Corroboree Billabong on the Mary River and on Yellow Water in Kakadu National Park.

5.2 Seine nets

These are sometimes called haul nets or beach seines. A seine comprises a length of netting (usually multi-filament) with a float line and a weighted bottom ‘lead’ line attached. The net is set so as to encircle an area containing fish and is then ‘beached’ by hauling it to shore by both ends. The fish are then gathered by hand.

Mesh size is important. Larger mesh sizes can be moved through the water faster and hence are more effective for catching larger and faster fish. However, small fish may pass through the larger mesh. Two or more nets with different mesh sizes may be used to overcome this

problem if all sizes of fish are required. A compromise single mesh size is often used in survey work and stretched mesh of 5–10 mm is quite useful for this. Very fine mesh of 1–2 mm may be necessary to catch very small sizes of fish but these filter water very slowly and most large fish escape.

Seine nets are difficult to use on a rough bottom with logs and boulders and in dense vegetation they will roll up and allow all fish to escape. They are therefore best suited to open water with a clear bottom but a necessary tool in any fish survey.

5.3 Fish traps

These generally comprise a square or round box of mesh with a funnel entrance at one or both ends. They are placed on the bottom and fish swim into them either by accident or attracted by bait inside. Once inside, the funnel makes it more difficult for them to get out.

The composition of the catch in baited traps is obviously influenced by the type of bait used. Unbaited traps avoid this problem, but all traps are somewhat selective in the species that will use them. Often wings of netting are attached to the front of traps to direct fish towards their entrance. Fyke nets are a design commonly used in fish research. These have at least two sets of funnels inside the cage to reduce escapement.

5.4 Electrofishing

This is done with devices that pass electric currents through the water to either stun the fish temporarily or direct them to swim towards a net. The cathode is usually left in the water and the anode is usually a collecting net.

In AC operation the electric pulse usually stuns the fish which must then be collected quickly in the net before they sink or revive and disappear. This shocking procedure (electronarcosis) requires good visibility and preferably shallow water. The pulse strength and frequency affects the size of fish stunned.

In DC operation the fish are induced swim towards the anode by the pulsed current (galvanotaxis). This method is useful in muddy water and in vegetation. DC units use a much stronger current than AC and are potentially more dangerous.

Experiments with the use of electrofishers in wetlands in the Alligator Rivers Region many years ago found them to be ineffective in the very low conductivity waters present much of the year. However, NTDPIF use an AC unit on a boat for collecting specimens of barramundi in billabongs for special purposes but not for population size estimation.

5.5 Pop-net traps

Popnets are traps designed to obtain quantitative measurement of fish density in dense vegetation. They are very much a research/monitoring tool although I saw a report of a similar procedure being used to catch fish in reed swamps on the Nile River in Africa.

Pop-nets are essentially a square enclosure of netting with a float frame at the top and a weighted frame at the bottom. The trap is set by inserting the net into the vegetation so that it rests on the bottom and there is a narrow passage through the vegetation for the net to extend to the surface. The top and bottom are then bound together by a trigger-strap so it all sits on the bottom. In this folded condition the trap is left for some time to allow fish to re-establish themselves in the area after the disturbance. The net is then triggered by pulling a rope, it rises to the surface enclosing the fish and then the work of extracting the fish begins. The

vegetation is removed by hand and then the area is fished with a small seine net to catch all the fish enclosed. Seining is repeated until no more fish are captured.

The disadvantages are exposure to leeches and crocodiles. The former can be overcome, but the latter is more problematic!

eriss uses this procedure in shallow lowland billabongs that were sampled in the past, prior to the removal of buffalo, by gill netting and seine netting. The vegetation is now too dense for seining and the area of open water for gill netting greatly reduced.

A related technique is called a Drop net. These devices quickly drop down through vegetation to enclose the fish that are then obtained in similar manner. A variation of the traditional cast net.

5.6 Visual census

Where water clarity is good enough, visual counting of fish from either above the water or, where crocodiles allow, within the water is a very useful sampling technique that does no damage to the fish at all. It is widely used in the sea for reef fish surveys. In freshwaters, fish observation from above the water is greatly facilitated by polarised sunglasses (preferably amber colour). It is possible to make both semi-quantitative and quantitative density estimates by variations of technique. As with bird watching, learning to recognise different fish species requires knowledge of the fauna and experience in the procedure before sampling. It is probably less biased than other procedures in terms of fish size and species detected (table 1) but differences between observers can be a problem.

In the Top End visual techniques are most appropriate in the clear headwater reaches. In the lowland reaches they are only suitable very early in the Dry season when water clarity is greatest. At ***eriss*** we routinely use visual techniques for monitoring. These include counting migrating fish from the bank and counting fish in channel billabongs from a clear-fronted canoe. We have also used visual counts of fish in fixed quadrats or along measured lengths of stream from the bank in small shallow streams.

5.7 Video

Like the visual census outlined above, opportunities for use of video are limited by water clarity that is very poor in freshwaters by comparison with the sea.

5.8 Sonar

Sonar devices are used extensively in large lakes and marine waters for locating schools and individual large fish. Such information requires a lot of calibration to be of use in survey or population studies.

They are commonly used for counting migrating salmon.

5.9 Poisons

Plant toxins are widely used in Aboriginal fishing (Bishop et al 1982). Rotenone, a derivative from derris roots used in Polynesia, has been widely used by scientists for sampling fish. It has the disadvantage of killing everything but it can be neutralised with potassium permanganate to restrict its area of action. Its use is more common in marine situations (rock/reef pools etc).

Table 1 Comparison of fish numbers detected by gill-netting, seine-netting, and visual, bank-side count methods in the upper reaches of Jim Jim Creek, Kakadu National Park in 1996

Scientific Name	Gill-netting	Seine-netting	Visual count*
Neosilurus ater	92	0	25
Nematalosa erebi	76	0	6
Syncomistes butleri	24	0	35
Megalops cyprinoides	29	0	0
Scleropages jardinii	27	0	0
Anodontiglanis dahli	25	0	44
Neosilurus hyrtlii	22	0	5
Hephaestus fuliginosus	5	0	38
Arius leptaspis	4	0	0
Lates calcarifer	3	0	11
Toxotes chatareus	4	0	0
Arius midgleyi	1	0	0
Pingalla midgleyi	64	2	45
Leiopotherapon unicolor	45	5	28
Amniataba percoides	122	17	45
Strongylura kreffti	23	1	2
Ambassis macleayi	8	5	0
Glossamia aprion	5	1	1
Melanotaenia splendida inornata	62	375	289
Craterocephalus marianae	0	2367	439
Melanotaenia nigrans	0	343	106
Craterocephalus stercusmuscarum	0	253	267
Ambassis agrammus	0	34	24
Glossogobius giuris	0	18	0
Mogurnda mogurnda	0	3	1
Pseudomugil gertrudae	0	3	7
Denariusa bandata	0	0	1
Total no of species	**19**	**14**	**20**

*only made before road opened

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Appendix 1 Field exercise with fish

Four sampling techniques will be demonstrated: visual census, gill nets, minnow traps and pop-net traps.

The visual census will be undertaken at Barramundi Falls. An instructor will issue students with polaroid glasses and then show students the different fish that can be seen by mooching along the bank. They will then be shown a quadrat method of estimating fish abundance. If time permits a bankside transect will be done and students can try snorkelling to get a better view of the fish. Students should keep records of the species detected and their abundance for comparison with catches made at different location by nets and traps.

Gill nets and pop nets will be demonstrated at Corndorl Billabong. The pop nets will be set early in the morning. On arrival at the billabong after the morning lectures a gill net will be set and then the pop nets triggered. Clearance of the pop nets and 'running' the gill nets will then be undertaken. Students should record the numbers of each fish species captured by the two methods. Comparison of the list of species obtained by the three methods should show very different assemblages. Students will be asked to consider what inferences can be made from this.

Vegetation survey methods

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1 Introduction

Most general discourses on vegetation survey and design deal primarily with terrestrial habitats. Wetland environments present a particular set of challenges to surveying and sampling vegetation communities. In the Alligator Rivers Region, the dynamics of wetland systems are also heavily influenced by seasonal factors characteristic of the Wet-Dry tropics. Hydrological processes associated with the seasonal inundation of the wetlands are a major determinant of vegetation community composition (Finlayson 1993, Finlayson et al 1989). At a given point plant communities change with seasonal wetting and drying cycles, as well as along some environmental gradients such as water depth. In describing vegetation patterns associated with such regimes, plant communities need to be defined temporally as well as spatially. This has implications for sampling methodology and design employed in addressing questions pertinent to the aims and objectives of wetland management.

In the ARR the objective of many vegetation surveys has been to characterise and map the wetland vegetation communities, particularly those associated with the Magela Creek floodplain, in order to assess the impact of mining disturbances (Sanderson et al 1983, Finlayson et al 1989, 1994). The overall approach has involved broad scale characterisation of the major vegetation communities by a variety of descriptive methods. These surveys have then provided a basis for and framework within which more detailed studies have been established using quantitative vegetation parameters.

2 Approaching the survey

Any vegetation study or survey is based on the description and examination of identifiable entities such as plant communities. Invariably, analyses of data about these entities has to be derived from some kind of representative subsampling.

The manner and methods by which entities are recognised, defined and sampled depends on a number of things:

- the aims and objectives of the survey;
- the scale of the survey;
- the type of vegetation being studied;
- the type of analyses that will be applied to the data;
- compromises and trade-offs that must be made for logistic and financial reasons.

Approaches to arranging sampling according to recognised vegetation entities vary in their objectivity. Subjective approaches may be expedient, but rely on adequate reconnaissance and familiarity with the vegetation. When use of some kind of probability statistic is anticipated, more objective approaches involving random sampling regimes are necessary. However, the

level of sampling intensity required by these approaches to reveal the more obvious vegetation entities apparent from subjective reconnaissance may not warrant their use (Mueller-Dombois & Ellenberg 1974). It is important to acknowledge that for any given method there will be some level of subjective judgement involved. For large scale or reconnaissance vegetation surveys the purpose is often to detect and obtain a description of vegetation pattern and classification. This differs from the usual purpose of smaller scale statistical surveys, which is to determine an unbiased estimate of the mean for some variable of the population or community as a whole.

2.1 Non-parametric methods for vegetation classification

Clustering and ordination are the non-parametric techniques most frequently used in vegetation community classification. Large data sets can be simplified and condensed to reveal underlying pattern.

Cluster analysis aims to find natural groups, such that samples within a group are more similar than those in different groups. However, the clusters are artificial and may be unrealistic in situations where communities intergrade with one another.

In a study of the Magela floodplain communities, Sanderson et al (1983) produced a classification of the herbaceous aquatic vegetation, the detailed categories of which were not reproducible in subsequent Wet seasons. However, discriminating at a higher order of dissimilarity resulted in fewer, broader, but more realistic vegetation categories that appeared to be consistent over several Wet seasons.

Ordination techniques preserve continuity and intergradation between samples by arranging sampling units along one or more axes that represent the effects of combinations of variables. The relative positions of the sampling units in the ordination space indicate ecological similarities and differences. By correlating environmental data with the ordination coordinates, it is often possible to identify correlated (and possibly causal) factors, eg community groups may be identified through a correlation with water depth or period of inundation.

3 Vegetation community sampling

In general, four major steps need to be considered when approaching vegetation sampling:

1 stratification or recognition of vegetation entities or communities

2 selection of a sample plot within these communities

3 size, shape and number of sample sites

4 selection of estimate parameter/s to record from the sample sites.

3.1 Homogeneity

Homogeneity is held to be an important consideration in selecting an area as being representative of a vegetation community in which sample plots are to be located, ie:

- The sample plot should be large enough to contain all species belonging to the plant community;
- the habitat represented should be uniform within the sampling plot;
- plant cover should be as homogeneous as possible.

Lack of homogeneity compromises the validity of a recorded vegetation parameter or statistic as a meaningful average for the sampled area. Attempts have been made to objectively identify whether an area is homogeneous or not, but it often remains a matter of subjective judgement through observation and familiarity with the vegetation.

3.2 Defining sample plot area

An area or plot, within which subsampling is to occur, thus needs to be defined with respect to its adequate representation of a given community species composition. Here the minimal area concept may be a useful guide, ie the smallest area in which the species composition of a given community is adequately represented. Basically, the larger the area the more species will be encountered, up to a point where no further new species are encountered.

In ideal circumstances this can be determined by a series of nested quadrats – within each of these quadrats, species presence is recorded. The resultant plot of species number against quadrat size produces a curve characteristic for a given community. At some point along the curve a decision is made with respect to the effective representation of species composition for that community against manageable plot size, which ideally should never be smaller than the minimal area of the quadrat sizes used in the analysis. In reality, plot sizes are often set intuitively through previous experience with community types, ie tree dominated communities require much larger sample plots than small annual herb communities. Often plot dimensions for broad vegetation structure classes have been established through other studies and serve as a useful guide.

3.3 Quantitative vegetation parameters

The quantitative vegetation estimate parameters most often used in community sampling are:

- Frequency (presence/absence) – number of times a given species is recorded as present in a given number of quadrats or at given number of sample points within a sample plot;
- cover (crown, foliage, shoot, basal area) – usually expressed as percentage of the sample quadrat area;
- density – number of individuals of each species per unit area;
- biomass – the amount of living matter per unit area.

Frequency, in contrast to the other parameters, is an objective but non-absolute measure – results are influenced by sampling frame or quadrat size and shape, and as such have most meaning in relation to that particular size and shape selected. No counting is involved, frequency being a recording of species presence only. This has the advantage of being potentially quick and easy to record. Frequency is, however, affected by the spatial distribution of plant species, ie the degree of dispersion or clumping, and this makes optimum quadrat size difficult to determine. Low frequency values may arise from patchy concentrations of individuals with evenly spread individuals giving high values. This effect of non-randomness has implications when relating frequency to abundance values such as density and biomass. Density estimates require that an individual plant can be identified, which may be difficult for many wetland species as these often form clonal entities of indeterminate origin. Biomass may be quantified indirectly (eg by cover and basal area) for terrestrial phases and some emergent phases of wetland plant species; otherwise, sampling is usually destructive, ie the sample is physically separated and removed from the sample site. Destructive sampling also requires care in its application so as not to constitute a disturbance in itself that may influence subsequent sampling. Generally a destructively sampled site can

only be sampled once (unless, perhaps, in an experimental situation where vegetation removal is the variable being studied). Thus nested quadrat sampling methods which are less affected by quadrat size and spatial dispersion, and produce closer approximations to density values can be useful, in that they are expedient, do not require individuals to be defined, and are non-destructive (Morrison et al 1995).

However, surface or emergent vegetation in flooded habitats will not always be a reliable estimate of the submergent fraction. Studies such as those that aim to relate to fish habitat may demand characterisation of vegetation in the water column. This is usually done through destructive biomass harvests. These are time consuming and physically demanding, as well as presenting a challenge to identifying and quantifying the more tangled masses of unattached submergents. Bailey et al (1983), in a study on seasonal distribution of aquatic macrophytes biomass in Corndorl billabong on the Magela Creek, took total biomass estimates from water column samples and calculated dry weights from dried subsamples of the quadrat mass wet weight. No attempt, however, was made to differentiate species. Sampling difficulties increase further when below-ground biomass estimates are required (Finlayson 1991).

3.4 Sample quadrat dimensions

There is some argument over methods for determining optimum quadrat size within a sampling plot. Usefulness of the minimal area/species area curve principle has been debated (Mueller-Dombois & Ellenberg 1974), but its applicability is dependent on what is considered to represent the most important function of plant distribution. In some cases this may be the distribution of the quantity of plant material (ie biomass) rather than the distribution of individuals. It is argued that this has little to do with the value of the species area curve as an indication of the representative species composition of the community. Where species diversity is considered the most important aspect of plant distribution, the species area curve is a useful tool for determining the smallest sample area with a maximum number of species for a community.

In practice, a number of quadrat sizes (usually nested) are laid out and replicated within a vegetation community plot, and examined for some index of variability. Sanderson et al (1983) determined by this method, over a number of different vegetation types, that 4 m^2 was a suitable minimum area for sampling cover estimates from random and transect orientated quadrats. However, for the clumped distribution of large floating leaved species, 10 m^2 was considered more appropriate to use.

In Sanderson's study, biomass harvests were conducted separately, with sampling sites based on communities defined in the earlier cover estimate survey. Macrophytes were cleared from 0.84 m^2 quadrats and sorted into species. Wet weights were taken for each species, and average dry weights of all species combined and expressed per m^2 for each vegetation type.

Bailey et al (1983) combined both visual observations of species composition and direct sampling of biomass along a fixed transect. Quadrat size was somewhat loosely defined in terms of visual observations of relative species composition (approximate percentage contribution by each species to the biomass of the community), made every 5 m along the transect. Biomass samples were collected every 20–50 m using 0.25 m^2 or 4 m^2 quadrats, depending on water depth and plant communities. The former quadrat size was considered more appropriate to floodplain areas of depth less than 30 cm, whereas the larger quadrat size was chosen primarily to address the edge effects associated with the difficulty in cutting thick matting submergent grasses on the quadrat perimeter. In effect, combining visual and physical biomass estimates may provide an opportunity to assess the congruency of the two measures,

such that the visual method could represent an acceptable and more expedient approximation of the latter, given the relationship is consistent. While such sample estimate relationships may be cost effective, save time and allow greater numbers of samples to be obtained more readily, they may not hold where the nature of the sampled community changes and thus need to be checked and reviewed appropriately.

Knerr (1998) and Finlayson et al (1989), in comparable studies on Magela floodplain vegetation communities, combined a variety of broad descriptive methods with specific transect sampling methods. Knerr sampled quadrats located both opportunistically and along fixed transects established by previous sediment seedbank sampling studies. In both cases, a concentrically nested quadrat method, as outlined by Morrison et al (1995), was used for recording frequency estimates in four major grassland communities. Quadrat dimensions are shown in figure 1.

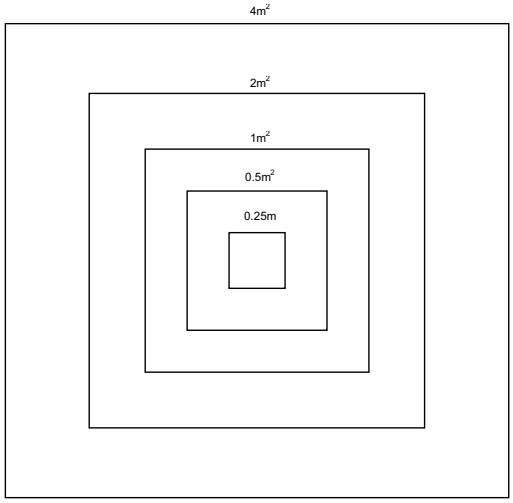

Figure 1 Arrangement of sub-quadrats in each nested quadrat used to survey *Brachiaria* grassland, *Hymenachne* grassland, *Oryza* grassland and *Pseudoraphis* grassland communities on the Magela floodplain (1995–96)

Knerr also used the same nested quadrat layout to determine optimum dimensions for flowering culm counts (essentially a density estimate) of dominant species in major grassland communities for seed production estimates.

By calculating the coefficient of variation of the total number of flowering culms within all nests of each species, optimum quadrat size was determined. Plotting the coefficient of variation against cumulative area suggested that quadrats larger than 1 m^2 were associated with little difference in coefficient of variance (fig 2), ie counting culms in quadrats larger 1 m^2 would be a waste of time and effort.

In general, a number of practical issues are associated with the use and selection of sample quadrat dimensions in wetland vegetation:

Defining an individual plant – amorphous indeterminate clonal masses are difficult to define as being in or out of a quadrat for density estimates (cover and biomass may be more appropriate parameters in this situation).

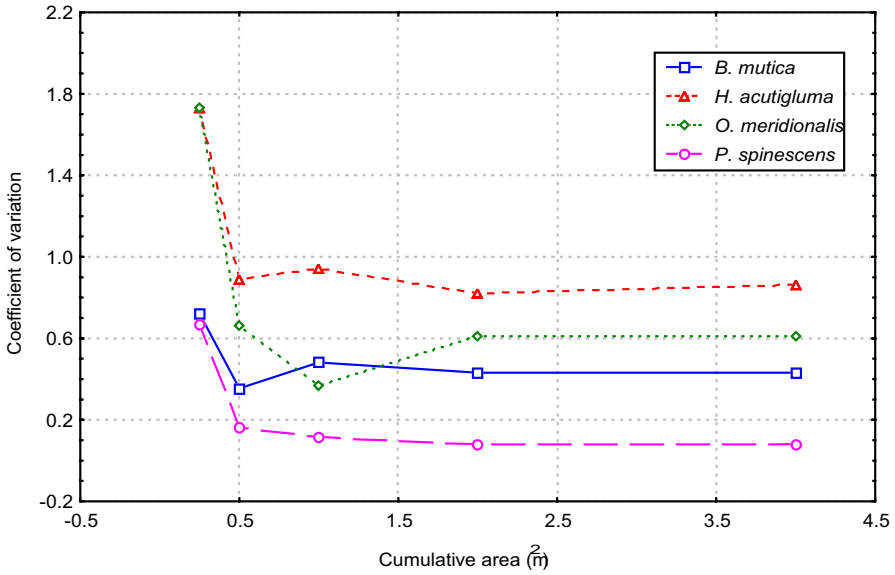

Figure 2 Coefficient of variation (standard deviation/mean) of the number of flowering culms recorded with increasing sample area during peak biomass production in May 1996 for: *Brachiaria mutica*, *Hymenachne acutigluma*, *Oryza meridionalis* and *Pseudoraphis spinescens*

Edge effects – smaller quadrats, by virtue of having a larger perimeter to area ratio, may be subject to greater edge effects. Increasing the size of the quadrat will ameliorate this but the time and effort involved in data collection will be proportionately greater. Bailey et al (1983) found that in harvesting thick matting submergent grasses, the relative error due to peripheral omission or commission of material decreased with increasing quadrat size. The 4 m^2 sample size chosen represented a compromise between a manageable volume of plant material and an acceptable sampling error.

3.5 Determination of sample size

The number of sample points or quadrats that are required from a given sample plot needs to be determined; this is important where an estimate of the mean for some variable of a plant community, such as density, is required. There are a number of methods used to help define the optimum number of samples. One frequently used in vegetation sampling involves calculating the running or cumulative mean for some parameter of measurement for key species in a sample plot. As more quadrats are sampled, the variability in the running mean decreases to a point determined as an acceptable trade-off between accuracy and feasibility.

The number of plot replicates within a vegetation community also requires consideration. The configuration and number of sample plot replicates, as well as sample size, will influence the assessment of statistical variance and thus the outcome of subsequent analyses. How this issue is dealt with depends on the objective of the survey, particularly where environmental variability and changes in space and time are of concern.

Sampling intensity and precision need to be appropriate to the aims of the survey (Austin 1991). For instance, a study examining the effects of a fire event on a vegetation community may involve replicate sample plots within each of burnt and unburnt areas of the community, where each plot is characterised by a number of sample quadrats. Intensive sampling of many quadrats within few replicated sample plots may be statistically inadequate and ultimately a waste of effort. Similarly, if species richness (the number of species per unit area) is the

relevant parameter for the aims of the survey, then the analytical advantages of larger sample sizes and sample plot replicates afforded by more easily collected nested frequency data may be greater than that of fewer, but more intensively collected, biomass harvest data. If, however, some precise measure of abundance, eg biomass, is more relevant to the aims of the survey, then the whole issue of sample size and replication has to be reappraised. Again there is a compromise between accuracy, acceptable error and feasibility.

3.6 Sample timing

As noted earlier, the seasonality of the wetland environment in the Wet-Dry tropics is a major controlling factor in the dynamics and composition of vegetation communities. In attempting to characterise vegetation communities over several years, Finlayson et al (1989) determined that those communities defined by sampling during peak biomass production periods appeared to be the most consistently represented between wet seasons.

3.7 Transects

Line transects have featured prominently in most of the ARR wetland studies cited. The transect is a particularly appropriate way of configuring sample points when monitoring the dynamics of wetland vegetation communities over spatial and temporal gradients, such as water depth, floodwater dispersal and regression, as well as movement of free floating vegetation. The transect could be viewed as a long narrow sample plot, except that it may traverse vegetation community boundaries. As mentioned previously, Bailey et al (1983) exploited this aspect where community boundaries could not be easily defined. Transect length is usually a matter of subjective judgement based on inferred or discernible gradients, ie from the edge of a flood high-water-level through to a zone of permanent inundation. Assessment of the number, size and distribution of quadrats along the transect, as well as statistical arguments associated with replication, follows similar principles to those discussed previously. For temporal surveys, relocation of permanent transects is essential. Most simply, transect end points can be prominently marked (to be still visible above peak flood levels) or at least one end point (usually terrestrial) marked, from which an accurate compass bearing can be taken with which to lay out some kind of marked line (tape measure, rope with floats placed at measured intervals). Transect end points should always be georeferenced as accurately as possible. Physical markers can be inadvertently or deliberately removed or burnt, pushed over by animals, or simply the location forgotten.

3.8 Remote sensing

Finlayson et al (1989), Knerr (1998) and Sanderson et al (1983) all used remote sensing (aerial photography) as a basis for a regional definition of broad vegetation categories, and as an aid to preliminary stratification of sites for more detailed community sampling. The generation of vegetation maps in this way is often an iterative process, where initial interpretation is modified by subsequent surveys, the results of which, in turn, provide an increasingly accurate basis for further study.

Knerr (1998) also carried out a visual assessment of the dominant plant species at 1048 locations georeferenced during airboat reconnaissance. These points were used as a guide in identifying vegetation types defined in previous surveys by Finlayson et al (1989), as well as assist in air photo interpretation of major grassland communities.

Georeferencing sample data points is essential in any vegetation survey, particularly where permanent sites must be revisited to monitor temporal changes. In addition such data has the

potential to be entered into a GIS, providing opportunities for overlaying other data sets and attaching attributes.

4 Summary

Many of the vegetation surveys carried out over the ARR wetland systems have taken broadly similar approaches (Bailey et al 1983, Sanderson et al 1983, Finlayson et al 1989, Knerr 1998). All begin with a descriptive overview and attempt to establish a consistent description of principal vegetation communities. These then provide the basis for more detailed studies employing quantitative parameters.

Whether a survey is an essentially descriptive exercise, such as mapping vegetation communities, or interrogative and attempting to address some kind of hypothesis concerning causal relationships with environmental variables, in a less than ideal world, the challenge is to find an equitable trade-off between optimum sampling methodology and technical feasibility, while still addressing the fundamental aims and objectives of the survey.

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Survey techniques for hydrology, water quality and sediments

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Abstract

Survey techniques, whether for water or sediment quality, require that the objectives of the study be determined before sampling commences. If this is not done then the probability is high that useful information will not be obtained from the data, and the implied objectives of the project will not be met. Other planning requirements for successful project execution include: a comparative assessment of available resources and proposed needs; the establishment of data quality objectives; a benchmark to determine whether the objectives of the project have been met; and a detailed description of how the technical aspects of the project will be carried out. All these requirements are incorporated into a sampling protocol. These notes describe how a sampling protocol can be developed, and are tested using a pilot study, which also serves to allow an estimate to be made of variability of indicator concentrations within the project area. The notes also describe quality control and assurance methods for sample collection, transport and analysis; and how the final numerical database can be compared with an 'attainment benchmark' or 'success criterion', preceded, if necessary, by mathematical manipulation of the data.

1 Water sampling

This paper will provide an introduction to the design of water-sampling protocols, the collection of samples and the subsequent manipulation of data. For illustrative purposes, it will address the issues with particular reference to providing advice to users of the *Guidelines for fresh and marine water quality* (GWQ). These guidelines are largely based on toxicological data and are heavily oriented towards the ecological protection of wetlands. One focus of the guidelines is to provide guidance to users on consistent and uniformly applied surveying principles for the complete series of steps from project design to statistical evaluation.

1.1 Steps to acquiring data to compare with guideline values

The task of acquiring data for comparison with the GWQ can be divided into eight discrete steps:

1 Assess the resources that you have available.

2 Define the temporal and spatial boundaries of the sampling problem.

3 Establish an attainment benchmark, which broadly means the proportion of measured values which must not exceed the guideline.

4 Design a sampling protocol that maximises the representivity of samples, and minimises that component of variance that is not relevant to the environmental value.

5 Collect the samples, with due regard to technical aspects of quality control and quality assurance.

6 Analyse the samples, with equal regard to quality control and quality assurance.

7 Determine the biologically available component of the physico-chemical indicator, either by using a speciation-specific method of analysis (eg ASV or some chromatographic techniques), or by submitting broad analytical data to a thermodynamic speciation model (such as MINTEQ or HARPHRQ).

8 Perform a statistical analysis on the data and compare with the attainment benchmark.

1.2 Definition of the sampling problem and its relationship to available resources

1.2.1 General issues in the design of protocols

The most important issues in the design of a sampling protocol for physico-chemical indicators are:

- to carefully determine the specific objectives of the study and the resources available
- to collect representative samples
- to manage variance

These issues are closely related. A careful assessment of the purpose of the study will usually suggest temporal and/or spatial constraints on sample acquisition, which will reduce the number of samples required, increase their representivity for the objectives defined, and minimise that component of variance that has little relevance to the relevant problem. These general issues are graphically illustrated by the decision tree in figure 1.

1.3 Establishment of an 'attainment benchmark' and calculation of sampling intensity

1.3.1 General principles in assessing sampling intensity

Conceptually, more samples are required when:

- variability is greater
- measured values are closer to the guideline value
- the environmental value being protected is more important

These concepts can be expressed by the inequality:

$$G > \{X + [(s/\sqrt{n}) \times t_{1-\alpha}] + z_{\beta} \times s\}$$

where G is the guideline value, X is the sample mean, *s* the sample standard deviation, *n* the number of samples, $t_{1-\alpha}$ the value of the *t* distribution for (n-1) degrees of freedom and (one-tailed) degree of confidence required, and z_{β} the (one-tailed) value of the normal distribution for the frequency required.

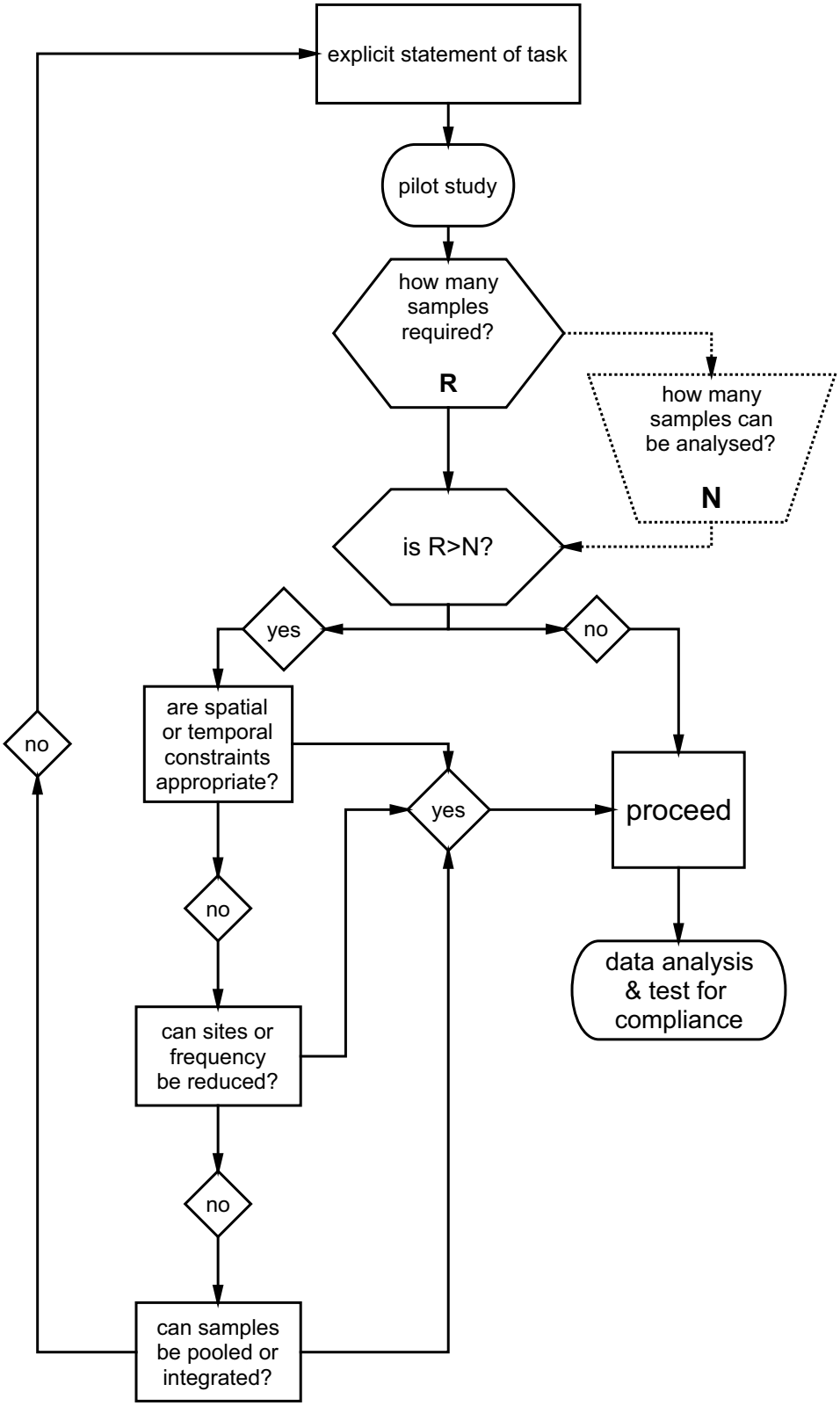

Figure 1 Decision tree for determining temporal and spatial constraints on sampling

Therefore, if the benchmark for comparison with a guideline value of 100 µg/L was 95% confidence that 95% of measured concentrations were less than this value, a sample dataset with X = 70 µg/L, s = 14 µg/L and n = 10 would yield a value for the right hand side of the expression of:

$$70+[(14/3.16) \times 1.833] + 1.645 \times 14$$

which equals 101.2, and the benchmark is not achieved. The expression is a sensitive function of n (assuming that $s \sim \sigma$) and this feature reduces the need for the guidelines to specify the number of samples required. A small number of samples can only satisfy this criterion when either:

- measured values are much smaller than the guideline value (in which case extensive sampling would be tedious and unproductive in any case)
- variance (and hence *s*) is small
- the environmental value has less importance, in which case the confidence with which a certain proportion of values are less than G could be relaxed, for example 50% confidence that 95% of values were less than the guideline value.

One advantage of this comparison benchmark is that progressive concentration data can be evaluated, and the monitoring exercise terminated or scaled down when the criterion is satisfied. This course of action must still accord with any temporal sampling requirements eg a need to monitor over an annual cycle may need to be complied with, even if guideline values were not exceeded using a three-month data set. However, sampling frequency may legitimately be able to be reduced.

1.3.2 Additional comments on sampling intensity

The value of the *t* statistic converges to that of a normal distribution for large *n*, and is only about 5% larger at n = 20. The collection of twenty samples would normally be regarded as an absolute minimum, regardless of apparent compliance with a smaller number. Note also that the statistical treatment described assumes an approximately normal distribution of data. Where data are markedly non-normal, approaches discussed later may be considered.

In a few cases guideline values are expressed in terms of an increase from a reference or otherwise specified value. In this case, the number of samples required is expressed by the equation:

$$n = 2s^2(z_\alpha + z_\beta)^2/d^2$$

Where z_α and z_β are the upper critical points (ie one-tailed) of the normal distribution for the % confidence and frequency specified (for example 95%, 95%). The pronumeral *d* is the difference required to be observed. For example, if a 10 µg/L increase in an indicator concentration is the maximum allowable increase, with non-compliance judged by 95% confidence that 95% of observations exceed the critical value, and s = 14 µg/L, then the number of samples required is:

$$n = 2\times14^2\times(1.96+1.96)^2/10^2$$

$$\sim 60$$

The use of *z* rather than *t* assumes a sample size large enough (>~20) to render the difference between the statistics insignificant, and this would typically be the case for this form of compliance monitoring. The equation above requires a value for *s*. This value can be determined either from an historical database (assuming that data acquisition methods and

quality control are sufficiently consistent to permit valid comparison), or from a pilot study that is extensive and representative enough to give a good estimate of σ (the population standard deviation). Note that *d* can be expressed as a percentage if *s* is replaced by the relative standard deviation (coefficient of variation).

1.4 Representivity and indicator variance

A few hypothetical examples will help to clarify the general issue of representative sample collection, and its relationship to the problem of 'irrelevant variance'. In each case they involve the exercise of judgement in sampling, and an implied abandonment of a strictly random sampling protocol.

1.4.1 A stratified reservoir with outlet below the thermocline

In this case there would be little point in extensive sampling of surface water, except to determine the nature and extent of the stratification. Surface samples would not be representative of the outlet water, and would inappropriately increase the variance of sample concentrations. In addition, if for example water was drawn off once a week, there may be little point in sampling more frequently than this.

1.4.2 Water sampling in association with stream or lake macroinvertebrate monitoring

Where physico-chemical indicators are determined together with benthic macroinvertebrate sampling, water samples should be drawn from as close to the bed of the water body as possible, especially in water deeper than one metre, unless it was demonstrated through a pilot study that the water body has vertical homogeneity for the indicators being measured. Surface samples would not necessarily be representative, and collection from a variety of depths may increase variance in a way that would not assist the objectives of the study.

1.4.3 Compliance monitoring of waste releases, or natural increases with disruptive events

Deterioration in water quality is often associated with predictable events, such as waste releases from industrial sites, or increases in indicator concentrations during storms. In these cases, sampling must be timed to coincide with the spatially and temporally relevant event.

1.5 Estimating the variability of the sampling area and optimising sample collection

Where a reliable historical database does not exist, an initial estimation of variance must be made using a pilot study. This is particularly important where a random sampling protocol is necessary, either because of prescribed requirements or because of an initial assumption that all potential sites are equivalent.

Where an entire catchment must be monitored, the location of sampling sites can best be optimised using graphical methods or the minimisation of a mathematical function, such as simulated annealing. Simulated annealing belongs to family of techniques (Dixon & Chiswell 1996) that 'spatially optimise' the selection of sites. This method requires a digital elevation map, and operates by minimising a cost function. For example, if sampling sites are required to be located at points of equal upstream drainage area, the cost function to be minimised would be the standard deviation of subcatchment areas. Similarly areas of equal discharge, total stream length, or some other parameter may be required, and the standard deviation of these would be minimised with the cost function.

At each site it may be possible to reduce the number of samples collected by integrating the collection or by pooling individual samples. Pooling or integration is usually performed in the dimension of least variance. For example, if a pilot study shows little vertical stratification but considerable variability along a transect, either depth integration or pooling may be considered. The latter technique allows the flexibility of retaining individual aliquots for later determination if data analysis indicates the necessity of performing additional analyses.

1.6 Other strategies to minimise unwanted variance

1.6.1 Filtration

Variance can be minimised by filtering the sample, assuming that this can be done without contamination, and that the indicator that you are measuring is not altered in the process. Filtration reduces variance because particulate components of natural waters are, in general, more heterogeneously distributed than soluble ones. Particulate phase components usually have low bioavailability and therefore have little relevance to the philosophy and mechanisms underlying the establishment of guideline values for the protection of ecosystems. This is because guideline values are usually established using soluble, highly bioavailable forms of toxicants. Unfiltered samples have the advantage of providing a 'safety margin' (that is, providing an upper limit of bioavailability). However, the magnitude of this safety margin cannot be quantified and in extreme cases can be several orders of magnitude. In any case, a safety margin is built into the derivation of guideline values. It should also be noted that thermodynamic speciation models have the greatest predictive power when solution-phase components only are included.

1.6.2 Mixing zones

Where industrial effluent is the issue of environmental concern, a mixing zone immediately adjacent to the site of release will usually be observed. This zone typically displays a high degree of spatial heterogeneity, which greatly increases the variance of concentration measurements. Such intense sampling rapidly consumes overall resources available for the project without necessarily increasing knowledge of the system to any meaningful extent. Unless sampling of the mixing zone is explicitly required under the terms of the monitoring, it should be avoided. In this latter case, an abridged suite of indicators which can be measured rapidly, such as those measured using specific ion electrodes (including pH), electrical conductivity and those amenable to in-field colorimetric determination (some nutrients) should be considered.

1.7 Quality control and quality assurance in the field setting

Quality control and quality assurance are different but related concepts. In the context of the guidelines:

> *Quality control means devising and implementing safeguards to minimise the corruption of data integrity.* These safeguards must be installed at every step of the process that leads from project definition to the decision on whether measured concentrations are in compliance with the guidelines.
>
> *Quality assurance means devising tests of whether the safeguards have been effective.*

Quality control in the acquisition of field samples has been comparatively neglected until recent times, and quality assurance arguably more so. This is probably the most important single reason why historical data sets should be viewed with caution.

Explicitly:

> The overall objective of quality control in the measurement of physico-chemical variables is the determination of the indicator concentration that existed at a specifically defined location and time immediately before the sample was taken. In most cases this requirement extends to the chemical speciation of the indicator.

Field activities are widely viewed as being relatively uncomplicated, and field operatives are frequently selected on the basis of qualities (for example vehicle-handling or bush skills) that have little relevance to the assurance of sample integrity. Field procedures are frequently regarded as being matters of common sense, and specific training in preparation and execution of sampling protocols as a misallocation of resources. In fact, the sequence of events from project conceptualisation and design, to the return of samples from the field is so complicated as to require:

- detailed planning and preparation
- elaborate safeguards
- rigorous training
- extensive acquisition of quality assurance samples.

The discussion below raises issues in planning and execution of a sampling protocol that may not be able to be resolved without implementing a 'dry run'. As noted above, the inclusion of a pilot study into the overall project plan not only yields crucial initial data, but also permits the identification and resolution of logistical and quality control deficiencies which could otherwise undermine the viability of the definitive project. The preparation of a sampling protocol should therefore be viewed as an iterative process. Many of the ideas below are summarised and paraphrased from Keith (1991).

1.7.1 Planning and preparation

The first step in any planning exercise for physico-chemical sampling is to determine any logistical and administrative constraints that may be placed on the exercise. These may include the availability of specialised sampling equipment, transport, staff who are appropriately trained, access to possible sites in the case of unusual meteorological events, and permission that may be required to visit sites. Evidence that these issues have been considered and resolved should be fully documented and incorporated in the formal sampling protocol.

Concurrently, or immediately afterwards, a complete list of analytes should be decided. This in turn mandates consideration of a number of inter-related quality control issues such as:

- Is analytical equipment of sufficient sensitivity, and are support services and appropriately trained staff available?
- What type of collection vessels and preservation techniques are required for the analytes? For multi-indicator studies this will require the construction of a matrix in most cases. For example, samples for heavy metal and organic carbon determination require plastic and glass containers respectively. Samples for heavy metals are usually preserved with acid, which is inappropriate for nutrients, whereas biocides are inappropriate preservatives for heavy metals. Preservatives may interfere with the analytical method for indicators even if the preservative does not affect the analyte *per se*. In practice, this dislocation of sampling and preservation requirements may be the effective equivalent of collecting many more samples than a simple calculation would suggest, with potentially severe implications for sampling intensity.

- How will sampling equipment, including collection vessels, be cleaned and transported in an uncontaminated condition to and from sampling locations?
- What strategies will minimise contamination at the time of collection?
- To what extent can the integrity of samples be compromised and still satisfy the objectives of the project, and what can be done if the degree of compromise is too great? This consideration usually takes the form of formal 'data quality objectives' which are a type of continual quality assessment throughout the project, using field quality assurance samples (which are discussed in more detail below). Data quality objectives *must* be specified before a project starts, and describe what actions are to be taken if a failure of quality control is detected. Data quality objectives must be supported by sufficient quality assurance samples to allow the diagnosis of the source of quality failure.
- are there any resource or logistical bottlenecks at the laboratory that will cause processing delays that will undermine sample integrity?

Decisions on all these points need to be completely specified, and form part of the written sampling protocol.

Once sampling sites have been decided, their location must be accurately specified, preferably using a global positioning system. Where transects are sampled, the location range should be specified if this is within the precision of the positioning instrument. The exact location of sampling sites and any subsites must be recorded in the sampling protocol. Taking note of the time when samples are taken is an obvious but frequently overlooked requirement of rigorous sample definition. Where automatic sampling devices are used, their timing mechanism must be calibrated to ensure that samples are acquired at the specified intervals. This is especially critical where hydrological or other conditions result in significant short-term concentration variations.

1.7.2 Some practical safeguards

- Sample containers and their caps should be soaked in at least 5% v/v acid for 24 hours unless special circumstances explicitly make this inappropriate. They should then be thoroughly rinsed with water, with the final rinse being with laboratory quality water. Soaking in a detergent solution is optional for most applications but is probably unwise for containers used for sampling organic compounds or nutrients. Glass containers should be heated in a muffle furnace at ~500°C for 20 minutes and stored dry. Plastic containers should be stored completely filled with high purity water.
- Sample containers should be transported to the field in sealed plastic bags, with a separate bag for each container type.
- Reagents for use in the field should likewise be stored in decontaminated containers and transported in separate sealed bags.
- All field equipment, such as filtration apparatus (including membranes), measuring devices and sampling equipment such as depth samplers must be cleaned before being transported in their separate receptacles. Elements of field equipment that will come in contact with samples after collection must be cleaned to the same standard as containers, as must other components which may contaminate contact elements during transport. The internal components of depth samplers, hand aspirators and tubing through which samples flow are often neglected in this regard.
- Containers filled with water should be emptied away from the immediate site of sampling, downstream if possible. Recap before submersion and thoroughly rinse with sample before taking the final volume.

- If taking samples from a flowing stream from a standing position, collection should be accomplished facing upstream. Similarly, if taken from a boat, collection should be from the bow with the boat facing upstream. Ideally, the sampling site should be approached from downstream.
- In still water, collect the sample away from the direction of approach.
- If taking samples by hand from beneath the surface, wear disposable gloves.
- 'Surface' samples should be taken from a few centimetres below the surface, unless you deliberately wish to bias your sample with the surface film. The container cap should not be removed until the container is submerged.
- 'Bottom' samples should be taken a few centimetres above the bed to avoid sediment contamination. A conscious attempt should be made to avoid disturbance of the bed during approach.
- Fill containers completely with sample and recap while submerged.
- Leave containers uncapped and out of their transport bags for the minimum time consistent with the recommendations above.
- Ensure that the chain of custody is fully documented. This means that the person responsible for each step in the sampling process is recorded.
- A field record of unusual meteorological or hydrological conditions, particular difficulties encountered during sampling, unexpected delays or other departures from normal circumstances should be made for possible later evaluation, together with the original records of any field measurement devices.

Explicit safeguards form part of the planning exercise and should be formally incorporated into the written sampling protocol.

1.7.3 Training

Training for field sampling has two aspects: competence in the technical requirements of the various tasks and a detailed knowledge of the requirements detailed in the sampling protocol. The first are generic skills that are in principle transferable between projects, and include the operation of equipment, field safety procedures and a general knowledge of quality control in the field setting. The second involve specific training in the particular requirements of the project. These may be largely issues of logistics, coordination, communication and project-specific aspects of quality control. Training to produce an intimate knowledge of a specific sampling protocol will clearly be facilitated by involving all those involved in the project in the detailed development of the protocol. This will also make it a more inclusive and authoritative document, as well as far more likely to be adhered to.

1.7.4 Quality assurance in the field

The inclusion of quality assurance samples in a sampling protocol is the only means of warranting that quality control procedures have been effective, and thereby satisfying data quality objectives. There are three main categories of quality assurance samples:

- field and trip blanks
- field and trip reference samples (samples of known concentration)
- replicate samples.

Field and trip blanks are samples that nominally contain none of the relevant indicator (though they may contain matrix species) that are taken to the field. They differ in that trip blanks stay in their transport container(s) (usually plastic bags) for the whole journey while field blanks are removed from their bags, usually opened at the sampling site, then returned. In principle, field blanks experience all manipulations that authentic samples do, except the physical removal of sample. They are therefore unable to detect contamination during the sampling process. Trip blanks do not directly experience the field environment, and are therefore useful as a diagnostic aid when contamination is detected in field blanks.

Field and trip reference samples are analogous to the corresponding blanks, except that they contain the analyte(s) at a known concentration. They convey more information than blanks because they can detect analyte loss (for example to the container walls) as well as contamination, but are less able to detect minor contamination.

Replicate samples are those that are taken, as far as possible, at exactly the same place and time as one another, which should result in them having nearly identical analyte concentrations (presumed variance of zero). Unless there are unusual circumstances no more than duplicate samples need to be taken. Their real value, apart from a direct indication of departure from consistency lies in their matrix equivalence with one another, something that can rarely be achieved with blanks or reference samples. They are also the only way that contamination or loss can be detected at the exact moment of sampling, and so perform the function of a diagnostic aid.

Quality assurance samples can detect both random and systematic errors. In practice, the latter can usually be detected with far fewer samples. This is because the effects of common systematic errors, such as inadequate container preparation, inappropriate containers and deficient transport arrangements will often be evident in most samples determined. In many cases, the magnitude of random errors can only be estimated by analysing a large number of quality assurance samples, more than can be accommodated within resource constraints. The best compromise to probably to take far more quality assurance samples than will likely be required to be analysed. The purpose of many quality assurance samples is diagnostic, that is, they sample a subset of all possible sources of failure of quality control. They are not analysed unless a problem is detected in samples that integrate a range of possible failures, or unless a more detailed investigation of random errors is required.

1.7.5 Recommended numbers of field quality assurance samples

At least duplicate trip blanks and reference samples should be obtained. These would usually not be determined unless quality control failure was detected in field blanks or reference samples, or in duplicate samples.

At least one, preferably duplicate, field blanks and reference samples should be prepared at each sampling site. Normally one of each would be determined.

Replicate samples should be taken for at least one in five unique samples; if logistics permit, one in three is preferable. Unique samples include all subsampling sites, including depth and transect samples. A minimum requirement for analysis in the absence of a demonstrated failure of quality control would be one replicate determined for every ten unique samples.

Although these recommendations may seem excessive and may reduce the number of unique samples that can be determined, there is no other way to provide evidence of the integrity of the samples collected, and hence the success of the sampling exercise.

1.8 Quality control and quality assurance in the laboratory setting

The importance of strict adherence to analytical protocols, and an appreciation of the critical relevance of rigorous quality control and assurance in the laboratory is far more thoroughly appreciated than in the field. Proper laboratory practice is codified in the requirements of registration authorities (such as the National Association of Testing Authorities) and any laboratory holding registration from these organisations will be familiar with the effort required to ensure a credibly performing facility.

Quality control in the laboratory depends on similar precepts to those applying in the field. These are:

- adherence to validated and clearly explained written methods
- sound training on a continuing basis
- proper documentation of all procedures.

Laboratory quality assurance relies on what can broadly be termed independent analytical comparisons, which include:

1.8.1 Blanks

Blanks should be incorporated at every step of sample processing and analysis. However, only those blanks which have been exposed to the complete sequence of steps within the laboratory will usually be determined unless contamination is detected in these. That is, blanks incorporated at intermediate steps are retained for diagnostic purposes only.

Blanks suffer the deficiency of being able to detect contamination only, not indicator loss. In this sense they are inferior to samples of known concentration. They are useful to detect minor contamination, where the superimposition of a small additional signal on a sample of known concentration may not be evident in the statistical evaluation of analytical data.

1.8.2 Samples of known analyte contents

Samples of known concentration may be placed into three main categories: reference samples that have been certified by a rigorous interlaboratory comparison and data analysis; control samples, which are defined here as materials that have been characterised in-house (and perhaps by a small number of additional laboratories); and unknown samples spiked with a known quantity of analyte.

1.8.3 Interlaboratory comparisons

Interlaboratory comparison of unknown samples is mostly useful for testing instrument calibration, performance and operator skills, and these programs are frequently sponsored by testing authorities. Generally, only a modest degree of sample preparation is required, presumably to restrict the range of sources of variance between laboratories.

A more thorough interlaboratory comparison can sometimes be arranged when more than one organisation is involved in the project concerned. In this case the comparison can encompass every step of the sampling, preparative and analysis exercise.

1.8.4 Use of alternative analytical methods

Where a laboratory has access to alternative means of determining a specified analyte, many of the uncertainties regarding the speciation of analytes, speciation behaviour of spikes and the rate of approach to equilibrium can be resolved. This presupposes that at least some of the methods give specific information on the chemical environment of the analyte.

1.9 The relevance of chemical speciation to environmental values

Chemical speciation (the form in which the chemical indicator is present) assumes critical importance where the environmental value concerns ecosystem protection or human health. One problem is determining the chemical form of indicators. Another is deciding which species make a contribution to effects on the environmental value.

In the past, total (that is, unfiltered) concentrations were measured and compared with guideline values, on the understanding that this approach probably overestimates the amount of indicator available to cause detriment to the environmental value. A refinement to this approach is to measure total filtered concentrations. This is a conservative approach (though less so than using unfiltered samples) because the diversity of chemical forms in the solution may have different detrimental effects.

There are two approaches to resolving the 'speciation problem':

- determination of the indicator using an analytical method that is species specific
- use of 'thermodynamic speciation modelling'.

1.10 Statistical evaluation of data

After all analyses have been completed and validated, the product of the sampling and analysis project is an accumulation of multivariate physico-chemical concentration data. The possible sources of variability in these data are

- sampling error
- analytical error
- long range (that is, between site) variability
- short range (that is, subsite for example transect or depth) variability
- temporal variability

The ultimate task is to mathematically process these data in a way that will allow comparison with the guideline recommendation or other appropriate benchmark, which is usually in the form of a single concentration value, less commonly a range.

However, an assumption underlying the use of quantitative tools to compare data with benchmark values is that the data are normally distributed. As it happens, the probability calculation derived from the use of the *t* distribution is not very sensitive to departures from normality (Natrella 1963). However, an attempt should be made to normalise the data to the extent possible before an attempt is made to test for compliance with comparison values. The following manipulations may assist in normalising data.

1 Generally speaking, replicate values should be averaged. This assumes that sampling and analytical errors are small compared with between-site variability. This will usually be the case in the absence of a serious failure of quality control.

2 A test of normality can be made if desired using the reduced temporally and spatially distributed data set for each sampling site. Most spreadsheet programs will analyse data distribution in terms of departure from normality. If the data are satisfactorily distributed, a benchmarking comparison can be made. If not, data transformation will be necessary.

3 The most straightforward method of normalisation is data averaging (Natrella 1963). In the first instance, means should be taken of data in the dimension of smallest variance.

For example, if transect and temporal data were acquired for a sampling site, but variance in the transect data was less than in the temporal data then the mean should be taken of transect data. This results in a reduced data set with a single concentration value for each sampling site for each sampling occasion. Normality could then be tested again, and if satisfactory a test for compliance performed.

4 If large departures from normality were still observed, then mathematical transformation of data is required. For environmental data, the most common transformation is logarithmic, which means that the logarithm of each concentration value is calculated (any base is appropriate, but base 10 and *e* are most commonly used). Other transformations that may be used include square root transformation and transformations using various trigonometric and hyperbolic functions. The transformed data are then tested for normality and if satisfactory, compliance tested, after the transformed mean and standard deviation are converted back to linear form.

2 Sediment sampling

When planning a sampling program for sediments, it is important to remember these are usually highly heterogeneous materials, with the indicators of interest usually present in a number of chemical forms. This typically means that the sediment, once dried, fractionated by size and homogenised must be subjected to several chemical manipulations, called sequential extractions. Sampling and preservation must take into account the requirements of each of these steps, as well as the normal requirements of avoiding contamination and loss. Sediments usually have pronounced vertical gradients for most indicators of interest, so a program must take into account this three-dimensional sampling requirement.

2.1 Selection of sampling sites

It is important to remember that in most cases it will not be feasible to select many individual (that is, unique) sampling sites. This is because, as suggested above, the number of water samples for ultimate analysis proliferates quickly as a result of the interaction of vertical sampling and various sequential extractions. It is self-evident that if many subsamples are generated for each unique sample, preparative and analytical resources will be rapidly expended. Logistical calculations of this type are absolutely essential before a sediment sampling program is initiated.

Given the resource constraints that inevitably accompany sediment sampling, sites must be selected far more judiciously than is the case for water sampling. Even the process of acquisition of samples, whether they are analysed or not, is far more time consuming than for water sampling. The practical consequence is that usually only sites that are suspected to be impacted can be sampled, along with a small number of matched control sites. For lentic wetlands, sampling activities may concentrate on alluvial fans, or other well characterised sites of deposition. For lotic wetlands, the areal distribution should probably first be assessed using total (that is, unfiltered) water samples collected during and immediately after a discharge event.

It should also be remembered that very short range spatial variations may be significant in the case of sediment indicator values, and this is exacerbated by variation in the deposition patterns of plant degradation products.

2.2 Measurement of pore-water indicators

Where sediment contamination by toxicants is suspected to contribute to wetland degradation, it is advisable to determine pore-water concentrations of the relevant indicators. For many toxicants, 'sediment' toxicity is closely related to pore-water concentrations. The most convenient means to examine pore-water is by using 'peepers' which comprise a number of compartments arranged vertically on a rigid support. The compartments are filled with high-purity water and sealed with a dialysis membrane. The peeper is driven into the sediment and over several days the pore-water solution concentrations equilibrate with the water inside the peeper compartments at the various depths.

Other methods of sampling pore-water include displacement from a sediment core with an immiscible solvent such as chloroform, and pressure displacement (squeezing) using an inert gas (such as argon) to drive a piston.

2.3 The vertical dimension

When sampling sediments, it is usually advisable to acquire a core. Surface scrapings are sometimes acceptable, but more so with soils, where the site to be sampled can be directly observed. The risk with surface scrapings in wetlands is that they will be dominated by partly decomposed plant material rather than sediment *per se*. There is little likelihood that such samples would be representative of the indicators of interest.

A sediment core allows an assessment to be made of the vertical distribution of the relevant indicators, that is, how far the species have penetrated into the sediment. In the absence of evidence to the contrary, a core would usually not be sampled deeper than 20 cm. How this is divided depends on the number of subsamples that can be feasibly analysed from each core. If two subsamples are taken, these may be 0–10 cm and 10–20 cm; for three subsamples, 0–5 cm, 5–10 cm and 10–20 cm; and for four subsamples, 0–5 cm, 5–10 cm, 10–15 cm and 15–20 cm. Each subsample is then dried and sieved to the required size fraction (usually 2 mm). Sieving is normally sufficient to homogenise the sample.

2.4 Quality control in sediment sampling

Quality control is far more difficult with sediments than with water samples, primarily because of the awkwardness of sample acquisition, transport and storage, and the opportunities that this presents for compromise of the sample. Quality assurance is also more onerous, partly because of the additional resource requirements to analyse QA samples, but also because the acquisition of comparable replicates and reference samples is difficult.

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