Assessing and monitoring aquatic biodiversity: what have we learnt ?

CRC FOR FRESHWATER ECOLOGY

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Cooperative Research Centre for Freshwater Ecology

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Some useful general links

Department of Environment & Heritage
http://www.deh.gov.au/biodiversity/index.html

The National River Health Program
http://www.deh.gov.au/water/rivers/nrhp/

National Strategy for the Conservation of Australia's Biological Diversity
http://www.deh.gov.au/biodiversity/publications/strategy/index.html

Summary

Setting objectives

When setting objectives for aquatic biodiversity assessment studies it is useful to consider which of three broad issues you wish to address:

1. investigate effects of disturbance on biodiversity,
2. study natural biodiversity patterns or
3. investigate drivers of biodiversity.

It is also necessary to consider the degree of quantification required. Studies with higher levels of accuracy in quantification can provide comprehensive species lists and assess impacts on specific biotic groups. Studies with lower accuracy in quantification can answer questions relating to ecosystem health and can potentially identify areas with high conservation value.

What to measure in aquatic biodiversity studies?

What biotic group or groups to measure?

A biotic group may be deemed appropriate for a study because we as humans value it in some way, or because it has intrinsic value such as performing essential ecosystem functions. When the study objectives do not target particular groups such as these, good indicators will be those that can respond to disturbance or natural gradients within geographic scales or timeframes appropriate to the study.

Particular biotic groups, such as macroinvertebrates, are often favoured in aquatic biodiversity assessment studies, partly because sampling protocols and taxonomic keys for them are well documented. But such groups will not be appropriate unless they are relevant to the study objectives.

Will different biotic groups give similar results?

Biodiversity studies often measure only one biotic group. Can it be inferred that if the diversity of one biotic group is high in one place then the diversity of other groups will also be high in that place? Recent studies of multiple biotic groups have found this is not necessarily the case. For example, a study of wetlands in the Wimmera region of Victoria found that the wetlands with the most macrophyte species did not also have the most macroinvertebrate species. This suggests caution is required when extrapolating results of biodiversity assessment from one biotic group to another.

Can you use higher taxonomic groups as surrogates for species richness?

Family and genus richness of macroinvertebrates can be used as a surrogate for species richness and species-level community structure, in studies of rivers where the objectives require relative comparisons rather than comprehensive species lists. For example, higher taxa surrogates are often appropriate for rapid health assessments and possibly for assessment of conservation value.

Family-level macroinvertebrate community structure can be used as a surrogate for species-level community structure in wetlands, but the sub-samples may need to be larger than those needed for rapid health assessment in rivers.

Can you identify species accurately?

New molecular techniques have shown that, for some fish and aquatic invertebrate groups, animals that were thought to be members of a single 'species' actually belong to several species that look the same. If accurate identification of species from these groups is important, use of genetic techniques should be considered.

Design issues for aquatic biodiversity studies

1. What is the appropriate spatial scale?

The area covered by a biodiversity study, and the scale at which sample replication is focused, will depend on the area over which a disturbance may extend and/or the scale at which natural drivers of biodiversity work.

Patterns in fish and macroinvertebrate diversity have been linked to natural drivers at small (habitat) and large (catchment) scales. Catchment-scale drivers include barriers to natural dispersal, the availability of habitat, and elements of flow regime such as total discharge and flow seasonality.

The implication is that variation in biodiversity at the smaller scale may confound patterns at the larger scale. Studies interested in relative comparisons of biodiversity at a large scale may need to standardise habitats sampled and focus replication at the site scale.

2. What is the appropriate time scale?

From the few long-term studies of aquatic biodiversity, it appears short-term studies (< 3 years) may give an incomplete picture of how aquatic systems respond to disturbance.

3. What habitats to sample?

The range of physical habitats present at a site needs to be sampled to estimate macroinvertebrate and fish diversity accurately (e.g. for inventory types of study).

Studies requiring relative rather than absolute estimates of macroinvertebrate species richness (such as assessment of river health) may be able to use a subset of available habitats. The number of habitats required for a study needs to be tested for individual bioregions.

4. How many samples to take?

Inventory studies requiring accurate estimates of species richness will need more samples than other types of studies such as those for assessing conservation value, where accurate estimates of species richness are not a priority.

The greater the degree of small-scale heterogeneity in physical habitat in a study area, the greater the number of samples likely to be required to assess aquatic biodiversity.

5. When to take samples?

Issues that will determine the most appropriate time to undertake sampling include:

- seasonal variation in the composition and abundance of particular biotic groups,
- time since major human-induced or natural disturbance, and
- logistic considerations related to flow conditions and the life-cycle of the target group.

Sample collection issues

1. Do you sample different groups at the same sites?

Difficulties in site selection can arise in studies using multiple biotic groups if the different groups are responding to disturbance or natural biodiversity drivers at different scales. For example a study in Sydney's water supply catchments showed macroinvertebrates respond to catchment-scale land use while riparian vegetation responds to site-level land-use activities. All relevant scales of such drivers need to be taken into account in site selection.

2. What sampling methods to use?

Studies assessing different fish sampling methods show that all techniques are biased toward particular types of fish.

A range of gear types may be required for inventory types of study, particularly where there is a large range of habitat types present in the study area. Studies such as those assessing river health may be able to use fewer gear types to give standardised but less accurate estimates of fish richness.

Interpretation and evaluation issues

1. How do you use 'number of species' as a biodiversity measure?

Measures that can be used in conjunction with number of species to aid interpretation and provide information about ecosystem function include:

- community structure (incorporates information about composition and abundance).
- grouping species according to criteria such as native vs introduced, feeding characteristics, reproductive or other life history characteristics.

Introduction

Calls to protect aquatic biodiversity have become almost universal in management plans for freshwater systems, and they are supported by legislative requirements to manage biodiversity at State and Federal levels in Australia. The 2001 Fenner Conference on Biodiversity Conservation in Freshwaters recommended the establishment of freshwater reserves, an increased inventory effort for freshwater systems, and additional actions to protect and rehabilitate high value systems (Georges and Cottingham 2002). To act on these recommendations and to effectively implement management plans for freshwater systems, it is necessary to be able to assess aquatic biodiversity.

The purpose of this document is (i) to highlight some of the issues associated with assessing aquatic biodiversity, and (ii) to synthesise what we have learned from a variety of projects done by researchers and students who are part of the CRC for Freshwater Ecology. We have not attempted to formulate a prescriptive checklist describing how to assess aquatic biodiversity in all situations, given the complex array of potential study types and also the knowledge gaps in some areas. For example, we do not discuss groundwater, hyporheic (sub-surface), or cave stream biodiversity (but see Boulton et al. 2003).

What is aquatic biodiversity?

The definition of 'biodiversity' is a source of debate among scientists, although legislative definitions are generally similar. The UN convention (http://www.biodiv.org/convention/articles.asp?lg=0&a=cbd-02), for example, defines biological diversity as

> *the variability among living organisms from all sources … and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems.*

Some scientists assert that biodiversity *per se* is more simply about species richness, composition and relative abundance, while recognising that the preservation of biodiversity depends on preserving the processes that maintain ecosystems (Terborgh 1999).

In this document the term 'aquatic biodiversity' refers to the diversity of life in systems other than those found in terrestrial, marine and estuarine areas. In a practical sense such an all-encompassing term can be unwieldy.

In aquatic systems, assessment of biodiversity has tended to focus on species and communities within several groups. So, while recognising the broader scope of biodiversity, this document focuses on the *compositional aspect of aquatic biodiversity at the species and community level*, expressed in terms such as 'fish diversity' and 'algal diversity'.

What do we mean by biodiversity assessment?

A range of different terms is used to describe the quantification of biodiversity in aquatic ecosystems. The different terms reflect differences in study design (see Table 1). For example,

- are a number of measurements taken over time?
- are results compared to a standard or reference?

In this document we use terms modified from Hellawell (1986).

Biodiversity inventory

Biodiversity inventory is the comprehensive collection or collation of core or baseline information on species within one or more biotic group(s). An inventory typically measures the geographic distribution and sometimes the change in distribution of constituents from one time to another. It answers the question 'what is there?', which can be an endpoint in itself or a starting point for specific assessment and monitoring studies.

An example is the inventory of biota done in the waterholes of Cooper Creek, Lake Eyre Basin, Warrego River and Border Rivers. Groups measured included fish, turtles, macroinvertebrates, microinvertebrates, macrophytes, and algae (phytoplankton and benthic diatoms). Data were collected on species composition and the relative abundance of different species in waterholes (e.g. Arthington et al. 2005).

Biodiversity surveillance

Biodiversity surveillance involves the collection of information/data systematically over time. It answers the question 'What is there at different times?'.

An example is a study of macroinvertebrate diversity in two intermittent streams in Victoria during a drought (1982) and following wetter years (1983–1984) to see how biodiversity changed over time and in response to changes in flow and water permanence (Boulton and Lake 1992).

Table 1. Key features defining three types of biodiversity study: inventory, surveillance, monitoring and assessment

Study features	Type of study		
	Inventory	**Surveillance**	**Monitoring & assessment**
Comprehensively measure at least one biotic group	➨		in some circumstances
Make multiple measurements over time		➨	➨
Compare measurements to a reference, control or guidelines			➨

Biodiversity monitoring and assessment

Biodiversity monitoring and assessment involve evaluating biodiversity against a benchmark or previously formulated standard. For aquatic biodiversity studies the most common benchmarks are control sites or a reference condition. Biodiversity assessment can also place local biodiversity patterns in a broader regional context with the aim of identifying and prioritising areas for protection. Assessment can answer the questions: 'Does a site exhibit the expected level or type of biodiversity?' and 'Are biodiversity targets being met?'.

An example is a study that assessed how river regulation affects fish diversity (Humphries et al. 2002). It compared a mildly regulated (or 'control') river (the Broken River) to a heavily regulated river (the Campaspe River), in terms of the richness, composition and abundance of fish larvae in each.

Why assess aquatic biodiversity?

Chiefly, we assess and monitor aquatic biodiversity because we value it, or because we have a legislative obligation to do so. In practice, we often assess aquatic biodiversity to determine whether or not management actions are having the desired outcomes in aquatic ecosystems.

Biodiversity has been recognised as having cultural value, economic value and intrinsic value (Georges & Cottingham 2002, G. Wiegleb preprint) to sections of society with particular philosophical, economic and ecological viewpoints. Also, some people believe it is ethically wrong for any species or generation to deplete the Earth's resources, including its biodiversity, solely for its own benefit.

Cultural

Biodiversity is central to the cultures of Aboriginal and Torres Strait Islander peoples. Cultural value also includes biodiversity's aesthetic value and recreational value.

Economic

Biodiverse systems may be a source of future materials or medicines; and there can be economic spin-offs related to aesthetic and recreational values, particularly with regard to tourism. Biodiversity can also be linked to 'ecosystem services' such as the provision of fresh water, flood mitigation, removal of nutrients and other pollutants, trapping of sediments, moderation of toxic algal blooms, decomposition of organic matter and provision of fish and other aquatic foods.

Intrinsic

Intrinsic value is inherent in the elements of biodiversity, and unrelated to the usefulness of biodiversity to humans. It is derived from properties of the biodiversity element itself, such as its role and function in an ecosystem, or an organism's individuality, etc.

Steps to consider in aquatic biodiversity monitoring and assessment

Six steps can be followed to develop and implement aquatic biodiversity studies (modified from Gaines et al. 1999):

1. Set objectives, define questions or hypotheses.
2. Identify what to measure.
3. Design the sampling program (or pilot study).
4. Collect baseline data. If doing a pilot study, reassess 2 and 3.
5. Collect monitoring data
6. Evaluate — assess the state of biodiversity and the appropriate management steps.

This order remains the same, regardless of the study objective, but not all steps are necessary to address each type of objective.

Setting objectives for aquatic biodiversity studies

Setting objectives is a standard first step in aquatic assessment studies (ANZECC and ARMCANZ 2000, Downes et al. 2002). It is useful to consider the following questions, to ensure that the data collected or collated can answer a specific research question that can provide information for aquatic biodiversity management decisions.

What is the nature of the study?

If we take the broadest definition of aquatic biodiversity (see section 3) then all studies of aquatic biota can be thought of as aquatic biodiversity studies. This range of studies can be classified into three broad categories (Figure 1):

1. investigate effects of disturbance on biodiversity,
2. study natural biodiversity patterns, or
3. investigate drivers of biodiversity.

What precise management and research questions do you seek to answer?

A study objective may be quite general, so it is necessary to formulate specific questions or hypotheses that can be tested and quantified through a sampling program or with existing information.

For example the objective might be: To determine whether environmental flow allocations have improved biodiversity in floodplain X. The specific research question may be: ‘Has the number of native fish species in floodplain X increased significantly since commencement of the environmental flow regime?’.

What approach is appropriate?

The type of information and level of detail required in a study will affect decisions about the approach and study design (Downes et al. 2002).

One key question to consider in biodiversity studies is whether you need an accurate estimate of the number of species, their relative abundances and a comprehensive list of all species present. All sampling, by definition,

produces estimates, but different approaches give different levels of accuracy. For detailed and highly accurate information, the 'inventory' and quantitative 'impact assessment' approaches (Figure 1) need to be used.

If on the other hand you are interested in comparing the relative richness of different sites or the same sites (or systems) over time but the actual identities of species are not important, then a more qualitative approach may be appropriate. For impact assessment, a qualitative approach might constitute a 'health assessment' and in the realm of natural biodiversity studies qualitative approaches might be appropriate to assess conservation value (Figure 1).

What to measure in aquatic biodiversity studies?

Two of the issues that arise when choosing what to measure in a biodiversity study are:

1. What group or groups of biota to look at? and
2. What surrogate of biodiversity to use, e.g. number of higher taxa (such as genera, families), abiotic variables, etc.? See Box 1.

What biotic group or groups to measure?

A number of factors may help determine which biotic groups to measure:

1. *The objective of the study*. The biotic group may be implicit in the objective. For example if the objective of a fish habitat rehabilitation project is to increase the diversity of the native fish community then fish are an obvious choice of biotic group.
2. *The scale of the study*. The life history and distribution of the group need to be appropriate to the objectives of the study. If you are interested in

Box 1: What are surrogates?

Surrogates are entities that we measure to tell us something about other entities (Sarkar 2002). We use surrogates in biodiversity studies to help us deal with the complexity of biodiversity (Gaston 2000). In essence, surrogacy is a relation between an estimator variable and a target variable (Sarkar 2002).

Species richness is a common measure of biodiversity (Gaston 2000) although there are others including species composition, abundance, measures of genetic diversity, etc. Some authors claim these measures are the targets. In other words they *are* biodiversity (e.g. Terborgh 1999). Other authors claim that measures such as species richness for one or several biotic groups are surrogates for *all* the genetic, species and ecosystem diversity in a place (e.g. Sarkar 2002). In other words overall aquatic biodiversity (see Section 3) is the target. For example, estimating the number and composition of fish species in a wetland could be a surrogate for the aquatic biodiversity of the wetland. However, generally we don't know how the diversity of single biotic groups relates to overall aquatic biodiversity.

A commonly used surrogate in aquatic biodiversity studies is number of higher taxa (genus, family, etc). Higher taxon richness of a biotic group is generally used as a surrogate of the species richness of that group. So for example species richness is the target and family richness is the estimator. Other surrogates of species richness include subsets of species composition (e.g. colonially-nesting waterbirds as a subset of all waterbirds) and abiotic variables. Appropriate use of such surrogates requires the relationship between estimator and target to be quantified (Butcher 2003).

Knowledge gap:
Relationships between surrogates and overall aquatic biodiversity

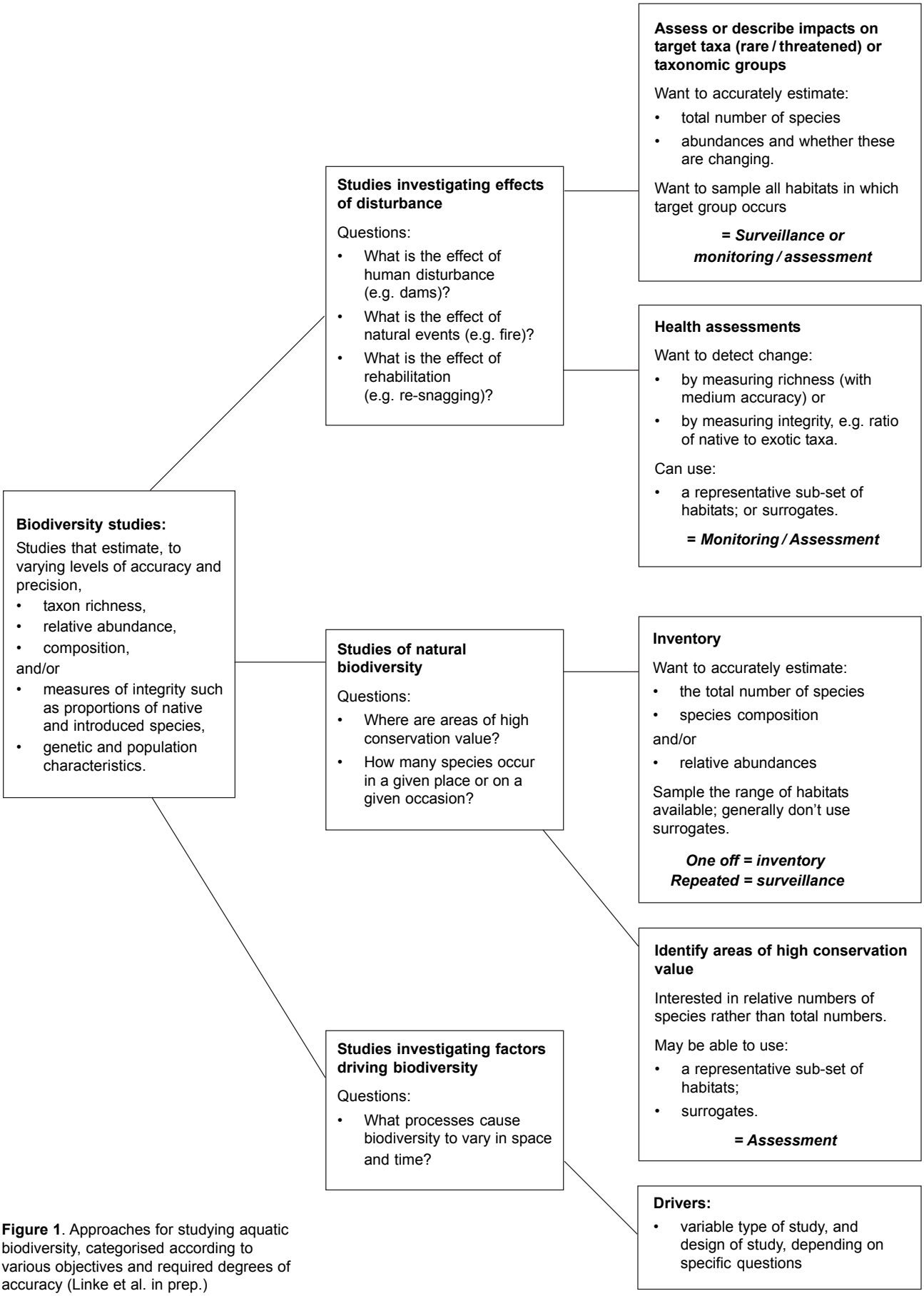

Figure 1. Approaches for studying aquatic biodiversity, categorised according to various objectives and required degrees of accuracy (Linke et al. in prep.)

detecting change within a given period you would need to select a biotic group that has the capacity to respond within this timeframe.

3. Availability of expertise and methods.

The biotic groups commonly used in aquatic biodiversity studies have been macroinvertebrates, fish, macrophytes, algae and waterbirds. The diversities of macrophytes (aquatic plants) and waterbirds have been most commonly measured in wetlands, whereas macroinvertebrates and fish have most commonly been measured in rivers. Other biota that have been studied include turtles, platypus, microinvertebrates, riparian vegetation, bacteria and amphibians. Ecosystem processes are now starting to be measured in river health assessment programs (e.g. in the Healthy Waterways Program in South-east Queensland). However, there is still little knowledge about how ecosystem processes relate to species richness or overall aquatic biodiversity.

http://www.ehmp.org/ehmp/

Aquatic macroinvertebrates (Box 2) and riparian vegetation (Box 3) are two biotic groups that the CRCFE has used when developing methods for assessing biodiversity. In aquatic systems, invertebrates as a group offer the advantages of being present in high abundance, biomass and species richness (Rosenberg and Resh 1993). In Australia, much work has been done over the last decade or so to develop sampling methods, taxonomic keys and data analysis techniques for macroinvertebrates (partly through the National River Health Program). However, identification to species can still be a time-consuming and skilled job (see section on use of 'higher taxonomic groups', p. 14).

Riparian vegetation, as well as having intrinsic importance, partially determines the physical conditions in the riparian zone and river channel, and is an important habitat for flora and fauna (Werren & Arthington 2002, Land & Water Australia factsheet: http://www.lwa.gov.au/downloads/publications_pdf/PF020253.pdf). Riparian vegetation is a challenge to sample and interpret because it grows in linear and mosaic patterns, in response to hydrology and geomorphology (Williams and Roberts 2005). It has been used less often than macroinvertebrates for assessing aquatic biodiversity. Werren and Arthington (2002) developed a protocol for rapid riparian condition assessment with specific reference to the impacts of water resource development and flow regulation ('health assessment' — Figure 1). This protocol is being applied in a range of natural resource management contexts in Queensland.

To assess diversity in riparian vegetation communities takes stratified sampling, to deal with the mosaic pattern. The changing width of riparian zones in relation to stream order also needs to be taken into account when designing sampling (Williams and Roberts 2005).

Will different biotic groups give similar results?

When different biotic groups show similar spatial patterns in species richness it is referred to as 'cross taxon congruence'. For example, if the wetland with the highest fish diversity in a catchment also has the highest macrophyte diversity, there is cross taxon congruence.

Some aquatic biodiversity studies have found cross taxon congruence and others have not (Heino 2002). The Wimmera wetlands study (Butcher 2003), for example, collected data on the species richness of invertebrates, plants and birds, but did not find cross taxon congruence; that is, the wetland with the most

Box 2: Use of techniques developed for health assessment to assess conservation value

AUSRIVAS (Australian River Assessment System: see http://ausrivas.canberra.edu.au/) is a rapid prediction system that was developed for assessing river health using aquatic macro-invertebrate communities (see 'health assessment' approach, Figure 1).

This approach was adapted for use in identifying sites of high conservation value in Sydney's water supply catchments (see 'assess areas of high conservation value', Figure 1), as follows.

(i) The sampling sites were selected with the overall aim of maximising the biological and habitat variability between sites so that the study would sample the widest possible range of taxa. Composite samples (combined from all the habitats available) were used to test how many habitats were required in addition to the edge and riffle habitats that are standard for AUSRIVAS sampling.

(ii) Although AUSRIVAS predictive models normally work at family level, the Sydney study identified four insect groups (Plecoptera, Odonata, Ephemeroptera and Trichoptera) to species level where possible; other groups were identified to family.

(iii) Data analysis for assessing conservation value was based on all species found, rather than excluding species that occur at less than 10% of sampled sites, as in the standard application of AUSRIVAS. The study calculated O/E for each site, **O**bserved being the number of taxa (of those expected to be found) observed at a site, and **E**xpected being the number of taxa expected to occur at a site in the absence of impact. Sites assessed as not having lost taxa were then analysed for conservation value by calculating O/E(BIODIV).

O/E(BIODIV) is calculated using taxa with less than 50% probability of occurrence ('rare taxa'). The standard application for river health uses the taxa most commonly found, i.e. that have >50% probability of occurring (O/E 50). A high O/E(BIODIV) value indicates a site is notably richer in taxa that are not often present at similar sites.

This adaptation of AUSRIVAS enabled macroinvertebrate diversity between sites to be interpreted in a standardised way in the context of natural habitat characteristics. Sites of high conservation status, where there are naturally few taxa but many of those are uncommon (low diversity but high rarity) were readily identified using the adapted O/E.

This approach is not designed to accurately estimate all species present at a site and their relative abundances (that is, it is not appropriate for inventory — see Figure 1). Also, like AUSRIVAS, it only applies to flowing waters, at present.

(Sims et al. 2001, Linke & Norris 2003)

plant species did not have the most invertebrate species. Lack of cross-taxon congruence has also been noted in Western Australian wetlands (e.g. Davis et al. 2001).

Lack of congruence among biotic groups prevents the extrapolation of observed patterns of species richness of one biotic group to another. However, it is likely that cross taxon congruence varies depending on the biotic groups and the scale at which they are being measured (Heino 2002). Also, responses of different biotic groups to disturbance or rehabilitation, and patterns of change over time, may differ, further confounding extrapolation.

Box 3: An inventory of riparian vegetation in Sydney's water supply catchments

The study measured the diversity of riparian vegetation within and between sampling sites. It also investigated the characteristics of the environment related to that diversity, for potential use in a predictive model.

The study sites were the same as those used for assessing conservation value using macroinvertebrates (see Box 2). They included as many orders or types of stream, and as many different types of upstream catchment characteristics related to land use (reference, agricultural and urban categories) as possible, across the catchments of the study area.

Within sites, transects were laid at right angles to the stream channel, for sampling differences in site morphology (littoral, bank, terrace, swamp, etc.). Within transects, plots were located to sample the range of vegetation types.

The study recorded:

1. species composition, including number of species, their distribution patterns and lifeforms (e.g. herb, woody, climber, fern);
2. vegetation structure, including vegetation height, canopy cover, number of dominants and proportions of lifeforms;
3. the number and proportion of native and alien (non-Australian) species at each site (its 'integrity').

Major findings

1. Even the most common species were only found at a few sites. In other words, there was high beta-diversity (variation in species composition from place to place).
2. Small-scale factors such as specific environmental characteristics of plots and sites were important in determining species richness.
3. Even at sites categorised as being in reference condition, there were numerous alien species.

Implications

Modelled relationships with predictor variables are likely to be simpler for single species than for overall species richness. Therefore, species-specific models may have better predictive capacity than species-richness models.

However, the sample size would need to be larger for species-specific models than for species-richness models in this study area, because most individual species were only found in a few sites.

Using a monitoring program for riparian vegetation designed around a reference system concept (as is used in AUSRIVAS) will require special effort to generate conceptual but quantitative descriptions of reference status. Use of catchment characteristics such as land use to predict reference status may not be suitable for predicting reference condition for vegetation, because of the ubiquitous presence of alien species.

(Williams & Roberts 2005)

What measures to use?

Knowledge gap: Structural and functional aspects of aquatic biodiversity and how they relate to composition

The species is a fundamental biological unit, and the basis of much conservation legislation. As mentioned (Box 1), species richness is a common measure of biodiversity. What does the number of species tell us? For example, does a stream with greater species richness have greater resilience to disturbance such as drought? What does it imply when we say one lake has more species than another? This comes back to the question of relationships between composition and function, of which we have only a limited understanding. Number of species may be an intuitively simple measure of biodiversity but it doesn't tell us much about underlying processes.

Community structure is an alternative biodiversity measure that can tell us about composition as well as ecosystem structure and function. Measures of community structure incorporate species composition and abundances, changes in communities through time, and relationships between species in a community. The structure of a community is an indicator of how it is functioning; that is, processing energy and nutrients (Krebs 1985).

Grouping of species according to functional or life history characteristics is another biodiversity measure. The extent of functional diversity among species in a community is an important determinant of ecosystem processes (Chapin et al. 2000). For example, macroinvertebrates can be grouped into 'functional feeding groups' such as scrapers, filterers, etc. (Cummins 1974), which can provide information about primary food sources. Likewise trophic guilds (groups of species, or particular sizes of species, that feed on the same things) are used in a range of fish studies. The NSW Rivers Survey, for example, found an increasing number of trophic guilds occurred with increasing distance downstream (Gehrke and Harris 2000). Fish also can be grouped by the habitats they occupy. For example, river health has been assessed in some cases on the basis of numbers of benthic fish taxa versus pelagic fish taxa (MDBC 2004).

Wetland plants have been categorised according to their response to water level changes. The three main groups are terrestrial (do not tolerate flooding), amphibious (tolerate flooding and drying), and submerged (do not tolerate drying) (http://www.lwa.gov.au/downloads/publications_pdf/PF000026.pdf ; Brock and Casanova 2000; http://www.lwa.gov.au/downloads/publications_pdf/PF000027.pdf). These groups can be used to monitor changes in wetland habitat over time that are linked to water regime. Such insights may not be apparent from tracking number of species alone.

Both community structure and functional feeding groups were used as measures of aquatic biodiversity in the Wimmera wetlands study (Butcher 2003). Wetlands with different hydrological regimes could not be differentiated on the basis of the species richness of macroinvertebrates or plants. They could, however be differentiated on the basis of invertebrate community structure (both 1 and 3 months after filling) and plant community structure (3 months after filling). In other words, species richness did not differ between wetland types, but abundance and composition of macroinvertebrates and plants did (Figure 2).

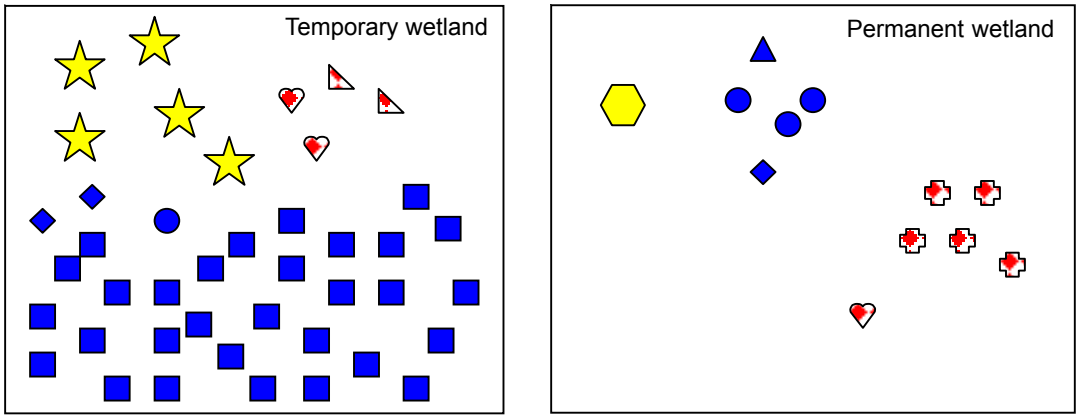

Figure 2. Conceptual representation of the patterns in species richness and relative abundance of macroinvertebrates in the Wimmera wetland study.

In each wetland, species richness = 6, and there are three functional groups, consisting of 1 filterer species (pale), 3 predator species (dark), and 2 collector species (patterned).

Many Australian stream invertebrates cannot be readily assigned to functional feeding groups because they are generalist or opportunistic feeders (Boulton and Brock 1999). Another problem is the arbitrary scale at which differences between species qualify as functionally significant (Petchey and Gaston 2002).

Can you use higher taxonomic groups as surrogates for species richness?

There has been substantial debate in the bioassessment (health) literature on the appropriate level of taxonomic resolution required, particularly when using macroinvertebrates (Bailey et al. 2001).

Identification of biota to species level is critical for studying 'impacts on target taxa' or 'inventory' (Figure 1). Species-level identification is also necessary in cases when *listed* (as opposed to *statistically*) rare, threatened or endangered species are of interest.

However, species-level identification of some groups can be time-consuming. For example, identification and enumeration of 29 macro-invertebrate samples just to family level from the Condamine-Balonne river system took approximately two to three person-weeks' effort by operators with a moderate level of skill in macroinvertebrate taxonomy. Processing the same samples to species level involved more than ten expert taxonomists from various institutions throughout Australia, and the turn-around time was six to seven person-months (J. Marshall, pers. comm.).

Several analyses of benthic macroinvertebrate data have shown little change in the multivariate description of community variation at taxonomic levels from genus to order (Bailey et al. 2001). However, the taxonomic resolution required to develop predictive models of acceptable sensitivity may vary between bioregions (Hawkins and Norris 2000). More biodiverse regions may require lower resolution (genus or species) than less biodiverse regions (Bailey et al. 2001). Initial work on wetlands suggests that if macroinvertebrate species patterns are to be predicted using family-level surrogates, then more specimens may have to be identified than are generally used in rapid assessment of river health (Butcher 2003).

Work is currently underway to determine whether family level identification of benthic macroinvertebrates can be used to assess conservation value of rivers. Initial results suggest it is appropriate in some situations (Box 2, Linke et al. 2004).

Can you identify species accurately?

Even after a lot of training, there can be more misidentifications in some biotic groups at species level than at higher taxonomic levels. Experienced practitioners have been found to have a 7.5% error rate with macroinvertebrate species and 0% with families (Metzeling et al. 2002). However, as much as 50% misidentification has been recorded in quality assurance processes for species-level identification (Sims et al. 2001).

Traditionally we have based species identifications on morphology — that is, appearance, colour, shape, etc. Recent genetic work has shown that, for some aquatic invertebrate groups, animals thought to be members of a single species actually were from a group of species that look the same. These are called cryptic species. For example, genetic analysis of freshwater mussels collected from central Queensland showed that animals classed as one

species by their physical features actually came from four different species (Baker et al. 2003). Many groundwater invertebrates are difficult to separate on purely morphological grounds and so genetic analysis to differentiate cryptic species is now becoming routine (A. Boulton, pers. comm.). Well-studied groups such as fish also have cryptic species, e.g. mountain galaxias *Galaxias olidus* (Raadik 2001).

Quantifying cryptic biodiversity is important for accurately calculating species richness, but there is still debate about its ecological significance. Some cryptic species have been observed performing different ecological roles (see e.g. Peckarsky et al. 2005). However, multivariate analysis of aquatic macroinvertebrate family-level data can give the same resolution as species-level data so it has been suggested that species within a single family respond coherently to an environmental gradient (Marchant et al. 1995). Thus, closely related aquatic species (particularly the larval and nymphal stages of aquatic insects) may all perform the same ecological role.

Knowledge gap:
Frequency of cryptic species in aquatic systems and their ecological significance

Design issues for aquatic biodiversity studies

Designing a study involves deciding where, when and how many observations or sampling units to make or take to address each objective (Downes et al. 2002). A number of publications deal with designing monitoring, assessing and reporting studies for aquatic ecosystems (Norris et al. 1992, ANZECC & ARMCANZ 2000, Downes et al. 2002). The general principles of design described in these publications also apply to biodiversity studies and should be referred to for guidance in this area.

In aquatic biodiversity studies, decisions are needed about: scale, habitats, sampling intensity and timing. All these decisions depend on the approach taken (Figure 1), as well as on the study's goal and research questions.

What is the appropriate spatial scale?

Decisions on the scale of a study may be dictated by logistic considerations such as the timeframe and boundaries set for reporting and the biotic group(s) under consideration. In environmental investigations it is also important to consider explicitly the spatial and temporal scales relevant to the driving processes (such as disturbance) and responses of biota (Downes et al. 2002). Natural drivers may be of inherent interest (studies of biodiversity drivers), may define environmental gradients along which differences in biodiversity may occur (natural biodiversity studies) or overlay/confound effects of disturbance (disturbance studies). They may also change over time.

Recent studies have shown that biodiversity can be affected by both small and large-scale drivers (Boxes 4 and 5). Patterns of macroinvertebrate diversity in dryland rivers, for example, were related to both small-scale habitat differences and regional differences related to waterhole position in the channel network (J. Marshall pers. comm.).

The decision about where to focus sampling effort needs to take these different sources of natural variability into account. For example, design of an inventory study of aquatic biota at a multiple catchment scale would need to consider bioregional differences related to habitat availability and the frequency and magnitude of environmental variability (such as flow regime — Box 5) and small-scale variability arising from habitat heterogeneity (see later in this section under 'how many samples to take').

Box 4: Case study. Wimmera wetlands — spatial variability in wetland biodiversity

In a biodiversity study of 16 wetlands in the Wimmera region in western Victoria, wetlands from four categories of permanence were examined. Variability in macroinvertebrate and plant species richness at the individual wetland level was very high, which is a common feature of wetlands.

The macroinvertebrate communities differed significantly between wetlands, even within a single wetland category, at both one and three months after they filled with water. For example, each of the four permanent wetlands sampled had different macroinvertebrate communities present (based on a comparison of similarity matrices created from abundance data). This suggests that the variability in diversity lies at the wetland level, and that looking at the invertebrate fauna of a single wetland will not be representative for that hydrological category of wetlands.

It may also be that wetland biodiversity responds to environmental gradients at a broader scale i.e. if the objective of a study is to investigate how wetland biodiversity responds to environmental gradients it may be better to sample more wetlands than to take more samples within wetlands.

(Butcher 2003)

Box 5: Broad-scale drivers of fish diversity in rivers

At large (catchment) scale, fish diversity is influenced by:

1. habitat availability and diversity and
2. the frequency and magnitude of disturbance or environmental variability.

Both of these (1 and 2) can vary at large scales with subsequent effects on fish diversity.

Example 1: New South Wales rivers (Gehrke and Harris 2000)

An inventory of fish in rivers across NSW found different fish communities in four regions (made up of up to 11 river basins per region): north coast, south coast, Murray, and Darling regions. Species richness increased with distance downstream in the two coastal regions but the opposite trend was seen in the two inland regions. Increased richness with distance downstream in coastal regions was thought to be driven by increased habitat diversity, including access to the sea. Reduced richness with distance downstream in inland regions was thought to either be from reduced habitat diversity or human degradation of rivers.

Within regions it was also found that montane types of river supported similar fish communities, regardless of the region. This suggests habitat characteristics associated with high altitudes had an overriding effect on the composition of fish communities.

Example 2: North-eastern Australian rivers (Pusey et al. 2004)

A study of 37 rivers in north-eastern Australia looked at the importance of certain aspects of the flow regime, and their interactions with the riverine landscape in determining fish diversity. Highly seasonal rivers (e.g. in eastern Cape York Peninsula) contained fewer species than more perennial rivers of the same size (e.g. in the Wet Tropics Region). The mechanisms by which total discharge and the pattern of delivery of discharge through time determine species richness is unclear but may relate to habitat availability, energy availability or extinction dynamics, or all three factors acting in concert.

What is the appropriate time scale?

Few long-term aquatic biodiversity studies have been carried out in Australia. It may be that systems that have been subject to drastic change may take many years to reach a new equilibrium in biodiversity (Box 6). Short-term studies may, therefore, give an incomplete picture of how aquatic systems respond to disturbance or restoration. The definition of 'short-term' will depend on the life history of the biotic group(s) under consideration. For example the response of red gums to disturbance is likely to be slower than algae.

Knowledge gaps: Long-term trends in natural biodiversity and the factors driving them, and which long-term trends are natural vs human-induced

Long-term studies of river macroinvertebrates in Victoria (Metzeling et al. 2002) show turnover at the species level over 20 years. During this period species will have gone through at least 20 generations and some species will have gone through many more, demonstrating the powerful potential for change.

How many habitats to sample?

The physical structure of aquatic ecosystems is often spatially variable on a small scale. In rivers, for example, depth, flow, structural features (macrophytes, woody debris, etc.) and the nature of the substratum (sand, gravel, cobble, etc.) can all combine to create different habitats for biota (Boulton and Brock 1999). The definitions of habitats in rivers may also depend on the biotic group in question. For example macroinvertebrate habitats in rivers can be defined by flow and substratum (riffles, edges, pool rocks, macrophytes and wood; Davies 1994). In wadeable streams, fish habitats may be defined hydraulically as runs, pools, and riffles (Kennard et al. 2001). It is important to consider what habitats to sample in aquatic biodiversity studies because different species and different communities can be found in different habitats (Parsons and Norris 1996).

Where a target biotic group is found in more than one habitat a decision needs to be made about which of the habitats to sample. Studies assessing river health tend to focus on a small range of common habitats so that

Box 6: Case study. Lake Pedder — the importance of sampling at an appropriate temporal scale

Biodiversity assessment of the Huon-Serpentine impoundment in Tasmania (originally Lake Pedder) has been undertaken since 1975. The nature of the study, as per Figure 1, was 'describing effects of disturbance' — in this instance describing the effects of creating a new impoundment on target biotic groups (primarily macro-invertebrates, with fish also).

From 1975 to 1977 the littoral (edge) invertebrate fauna underwent a huge increase in abundance to reach a peak. This was followed by a steady decline to reach a very low abundance by 1999. The mean number of macroinvertebrate species per site peaked in both 1977 and 1986 and then fell gradually to 1996. There was also a progressive shift in dominance in the macroinvertebrate community from insects to a single crustacean species (*Austrochiltonia australis*).

Two endemic fish species peaked in abundance in 1977 then drastically declined in abundance. *Galaxias pedderensis* is now probably Australia's most endangered species (Boulton and Brock 1999).

It is well known that there is often a boom in abundance and productivity in newly-created impoundments. In this case it was probably due to provision of abundant detritus. The subsequent decline in faunal abundance was likely due to a drastic decline in detritus and nutrient levels and simplification of habitat structure. In this situation a two-year study may have mistakenly concluded that creation of the impoundment increased macroinvertebrate diversity.

(Lake 1998)

standard comparisons can be made between sites over time (e.g. AUSRIVAS protocols). In contrast, inventory types of study should sample the range of habitats available.

The applicability of using single habitats for assessment of conservation value was tested using a predictive modelling approach with macroinvertebrate data from Sydney's water supply catchments (Box 2). In this study, sites identified as having a high proportion of rare macroinvertebrate taxa (and thus high conservation value) were the same regardless of whether results from a single habitat were used or results from composite habitats were used (Linke and Norris 2003). However, bioregional differences in habitat heterogeneity mean the applicability of using single habitats for assessment of conservation value should be tested before application in other areas.

The sampling effort required to assess fish diversity in wadeable streams of south-east Queensland has also been assessed (Box 7). The pilot work looked at both how many hydraulic habitat types or mesohabitats (runs, riffles, pools) needed to be sampled and the intensity at which those habitats needed to be sampled (Box 7).

How many samples to take?

The number of samples necessary to take in a study depends on the precision required relative to the effect size to be detected and the confidence desired in the result. Studies seeking a high degree of precision to detect small effects with high confidence will generally need to take more samples. For example, in the approaches to studying biodiversity presented in Figure 1 'impacts on target taxa' and 'inventory' types of studies are likely to require more samples per habitat than 'health assessment' and 'conservation value' studies. It is usually best to determine these aspects of study design through pilot studies.

One example of a study that determined appropriate numbers of samples was the inventory (and drivers) of biodiversity study on the Wimmera wetlands (Box 4).

Box 7: Sampling effort required for assessing health using fish diversity — south-east Queensland

The sampling effort required for fish in the Ecosystem Health Monitoring Program (http://www.ehmp.org/ehmp/) was assessed. The sampling methods that were used are designed for wadeable streams in south-east Queensland. Within discrete mesohabitat types (riffles, runs, pools) it was found that multiple-pass electrofishing plus supplementary seine-netting was required to accurately estimate fish species richness, species composition and relative abundances. Equivalent sampling efficiency was observed among mesohabitat types.

At the reach scale, intensive sampling of two or three individual mesohabitats (equivalent to 80–120 m stream length) was required for estimates of species richness and assemblage structure to stabilise (i.e. 80–90% similarity to fish assemblage data obtained for more extensive sampling over stream reaches up to 250 m in length). Sampling of additional mesohabitats added little new information. Less intensive single-pass electrofishing, even over long stream distances, produced inaccurate estimates of fish species richness and assemblage structure (due to species-specific differences in susceptibility to capture by electrofishing).

If a research question only requires species presence/absence (richness) information, two electrofishing passes are adequate for accurate estimate calculation, however, if species abundance estimates are required, three or four electrofishing passes are necessary.

(Kennard et al. 2001, M. Kennard pers. comm.)

A pilot study showed that to reach a stable estimate of macroinvertebrate species richness, six minutes of sweep net sampling across a range of wetland microhabitats was required (Butcher 2003). Extra sampling was not cost effective, as few additional taxa would be collected for a considerable increase in the time required for identifications and sorting animals from detritus. Trials were also done on appropriate levels of sub-sampling. These found that to record roughly half the species present, 2000 individuals needed to be identified. This illustrates that inventory types of biodiversity study which seek stable estimates of species richness require greater sampling effort than comparative studies (Figure 1) concerned primarily with relative richness levels.

http://ausrivas.canberra.edu.au/

A number of studies have looked at sampling requirements for river health assessment using the AUSRIVAS protocols. Hose et al. (2004) found that four replicate samples in riffle and edge habitats from reference sites was optimal. This assessment was based on the number of replicate samples required to record a high proportion of macroinvertebrate taxa present and provide consistency in the allocation of sites to categories of biological quality while trying to minimise effort. However, another study focusing on riffle habitats found a single collection of benthic macroinvertebrates was sufficient for river health assessment when taken from a site in good condition that had a large area of nearly uniform substrate (S. Nichols pers. comm.).

The studies described here indicate spatial heterogeneity of habitats at a small scale might be an important factor influencing the number of samples required. Studies with lower accuracy requirements (health and conservation value assessment – Figure 1) may require fewer samples but this needs to be tested for different study areas. The question of at what scale to focus sampling effort is also important. It has been recommended that regional or land-use scale health studies should maximise replicate sites and site-scale assessments should maximise replication within sites (S. Nichols pers. comm.).

When to take the samples?

The timing of sampling in aquatic biodiversity studies needs to be considered from a number of perspectives:

1. Seasonal variation in the presence and abundance of species. Seasonal changes in temperature, flow, etc. may influence the richness and abundance of aquatic taxa. For inventory studies, sampling in a number of seasons may be required to record the range of taxa that occupy a particular site over time. For comparative studies, survey data need to be collected at the same time of year as reference site data.
2. Time since a natural or human-induced event may affect biodiversity at particular times. This may be an extreme episodic event like flood, fire or drought. More ‘regular’ events such as wetland inundation may also need to be taken into account. For example successional patterns are seen in temporary wetlands following inundation including short term proliferation of some crustacean species (Butcher 2003).
3. Logistic considerations. You may want to sample at a time when the target biotic group is likely to be mature and easy to identify. For riparian vegetation it may be useful for sampling to coincide with the main flowering season so identification of species is possible. For riverine flora and fauna, sampling during times of high discharge may lead to inefficient (or dangerous!) sampling.

Sample collection issues for aquatic biodiversity studies

Do you sample different biotic groups at the same sites?

For studies using more than one biotic group, it may be advantageous in terms of cost-savings, working on contextual data, and comparison of final results, to use common sites. This is mainly an issue for studies seeking to make generalisations at a large scale rather than studies concerned with biodiversity at specific sites. For example, identifying sub-catchments with high conservation value in Sydney's water-supply catchments did not necessarily require the same specific sites to be sampled for all biotic groups (Sims et al. 2001, Williams and Roberts 2005).

In that Sydney study, macroinvertebrates and riparian vegetation *were* sampled at the same sites (Williams and Roberts 2005, Box 3). Site selection was based on broad-scale characteristics such as stream order and upstream land use. Sites selected in this way did not perfectly correspond with adjacent land use, a criterion important in determining vegetation condition. For riparian vegetation, sampling protocols need to accommodate both macro-scale and local characteristics.

The Sydney study highlighted that different types of aquatic biota are affected differently by various environmental factors and disturbance regimes. Such differences between biotic groups are likely where the groups are found in different habitats (e.g. in-stream vs riparian), or have different size ranges and life spans (e.g. riparian vegetation species can have a much greater size range and longer life span than macroinvertebrate species). Recognition of these differences and ground-truthing of sites prior to final selection may optimise selection of sites suitable for all the biotic groups of interest.

What sampling methods to use?

Sampling methods will need to suit objectives and be appropriate to the habitat types involved. In the inventory of fish in NSW rivers, for example, five methods were used to sample fish: electrofishing from a boat, back-pack electrofishing, fyke netting, panel netting and Gee trapping (Gehrke and Harris 2000). Such a range of techniques was necessary because of the diversity in river sizes and habitats to be sampled. Also, information was required on the full range of fish species, sizes and habitat preferences at each site. A study with similar objectives but conducted in highly turbid waterholes on the floodplains of Cooper Creek used four sampling methods (fyke net, beach seine net, small purse-shaped drag net, zooplankton net). Most sampling effort related to the use of replicate fyke nets and beach seining (Arthington et al. 2005).

In contrast, health assessment studies in wadeable streams in south-eastern Queensland used back-pack electrofishers and seine nets only. These methods were effective for sampling these generally shallow streams (i.e. less than 1.5 m depth) (Pusey et al. 1998, Kennard et al. 2001, Box 7).

A range of sampling devices exist for sampling freshwater macro-invertebrates, again often suited to different habitats (Merrit and Cummins 1996). Most stream invertebrates are readily sampled with kick nets, but these devices are unlikely to catch large species such as freshwater crayfish or mussels.

Evaluation and interpretation issues

Interpretation of results from aquatic biodiversity assessment studies needs to reflect the approach adopted at the outset (Figure 1). There is a danger of misinterpretation where data collected for one purpose are used for another purpose; e.g. using health data for site-specific assessment of impacts on target taxa may not be appropriate because of low sampling intensity and restricted number of habitats sampled. Likewise, inventory data may not be appropriate for regional health assessment where the suite of habitats sampled at individual sites is inconsistent.

How to interpret 'number of species' as a biodiversity measure?

A major flaw when using number of species or species richness as a measure of biodiversity is the assumption that sites with higher numbers of resident species are more worthy of conservation effort than sites with fewer species. This assumes that all species are equal. However, locations that are taxa-poor may make important contributions to biodiversity if they harbour rare, endangered, threatened or endemic organisms.

An example of an area with low numbers of species but high value is the original Lake Pedder in Tasmania, especially the quartzite beach. Prior to construction of the dam this area had a low species richness but six endemic species (five invertebrates and one fish) and nine species (eight invertebrates and one fish) whose limited range centred on the lake (Lake 1998). Upland rivers in the Murray-Darling Basin are another example. Some of these rivers have low numbers of fish species but some of these are rare or endangered (e.g. Macquarie Perch; two-spined blackfish). These locations and populations are now largely isolated because of unsuitable or degraded downstream habitats (Lintermans 2002).

At the other extreme, a site with high species richness that consists of many introduced species may not be considered of highest conservation value. For example, in the study of riparian vegetation in Sydney's water supply catchments, sites with the same plant communities differed primarily in their number of weeds rather than in the number of lost native species (Williams and Roberts 2005). Likewise, montane streams in NSW showed unique fish assemblages due to the predominance of two introduced (trout) species (Gehrke and Harris 2000). This may not be a good reason to give montane streams high conservation status.

When using species richness as a measure, therefore, it is critical that concepts such as rarity and representativeness (e.g naturally low diversity areas) are considered. One way is to use measures that take the different values associated with species into account. *What* species, as well as how many? The ratio of native to introduced species is one commonly used approach when assessing the integrity of a system (i.e. 'health assessment' — Figure 1) (e.g. MDBC 2004, Healthy Waterways Program). In fact the abundance and proportion of introduced fish relative to native fish are seen as valuable indicators of river health (Kennard et al. 2005). It is common practice for biodiversity assessment studies to use more than one measure to aid interpretation of results.

http://www.ehmp.org/ehmp/

The predictive modelling approach outlined in Box 2 may be one option for identifying sites with a high proportion of rare species. In terrestrial systems irreplaceability indices are used (the statistical likelihood of an area being reserved if all biodiversity targets are to be achieved e.g. Pressey et al. 1994).

Conclusion

Biodiversity is a complex concept and that is one reason why there is no standard 'cookbook' for how to do aquatic biodiversity assessment. Different definitions of biodiversity and what it encompasses mean that formulating research questions to address objectives can be difficult, but it is a crucial step. One way of categorising different approaches to biodiversity assessment was presented in Figure 1, and this could be used to ensure the assessment approach adopted is suited to answering your research question and thus informing biodiversity management.

Aquatic biodiversity assessment to date has tended to focus on the compositional diversity of species and communities. Most of the work developing sampling protocols and data analysis techniques has been done for riverine fish and macroinvertebrates. This work has provided a good basis for us to answer questions related to fish or macroinvertebrate diversity or health. It cannot be assumed, however that the patterns of diversity evident in these groups will be the same for other biotic groups such as algae or turtles.

Natural drivers of aquatic biodiversity have been described operating at both small (habitat) and large (catchment) spatial scales which has implications for study design. We know less about temporal variability particularly over the longer term (>10 years) although response to natural disturbance (such as wetting and drying) has been described for some groups and ecosystem types.

A number of studies have shown that forming conclusions about disturbance or conservation value based on species richness alone can be dangerous. This is because exotic species are a common part of in-stream and riparian communities and because species richness doesn't tell us about how rare or unique a species may be. Studies of community structure (in which composition and relative abundance can be incorporated) and various groupings of biota that tell us something about how ecosystems are functioning, have proved to be useful measures of biodiversity to complement species richness.

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