Wetland Bioassessment Manual
(Macroinvertebrates)

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1. Introduction

This manual provides a set of standard methods for the assessment and monitoring of Australian wetlands using aquatic invertebrates. The choice of methods will depend upon the objectives of your assessment or monitoring program. The issue of which protocols and which analytical techniques to use is discussed further at the end of this section.

1.1 Why monitor?

In a recent scoping review undertaken for the LWRRDC National Wetlands Research and Development Program Bunn *et al.* (1997) included monitoring as one of seven priority topics for wetland research and development in Australia. They noted that wetlands should be monitored:

- to collect baseline data for inventories
- to record ecological changes that may be occurring
- to measure progress of management programs
- to collect data that will contribute to better understanding of these systems
- to check on the performance of management agencies

Bunn *et al.* (1997) suggested that a close and iterative relationship should exist between monitoring and management programs, such that a program of adaptive management can truly exist. They emphasised that a strong relationship between monitoring and research is a *very potent means of refining and extending scientific knowledge of wetland ecosystems*. For this to happen, rigorous scientific methodology must be employed and the expectations and assumptions of the program must be stated as hypotheses requiring scientific investigation.

1. 2 A monitoring framework

The framework for monitoring (Fig. 1), proposed by Finlayson (1996), recognises problem identification, definition of objectives and formulation of hypotheses as the first three steps to be undertaken in the design and implementation of a wetland monitoring program. However, for a monitoring program to be truly effective not only must the objectives be clearly defined, the possible management responses to the monitoring results must also be articulated in the planning phase.

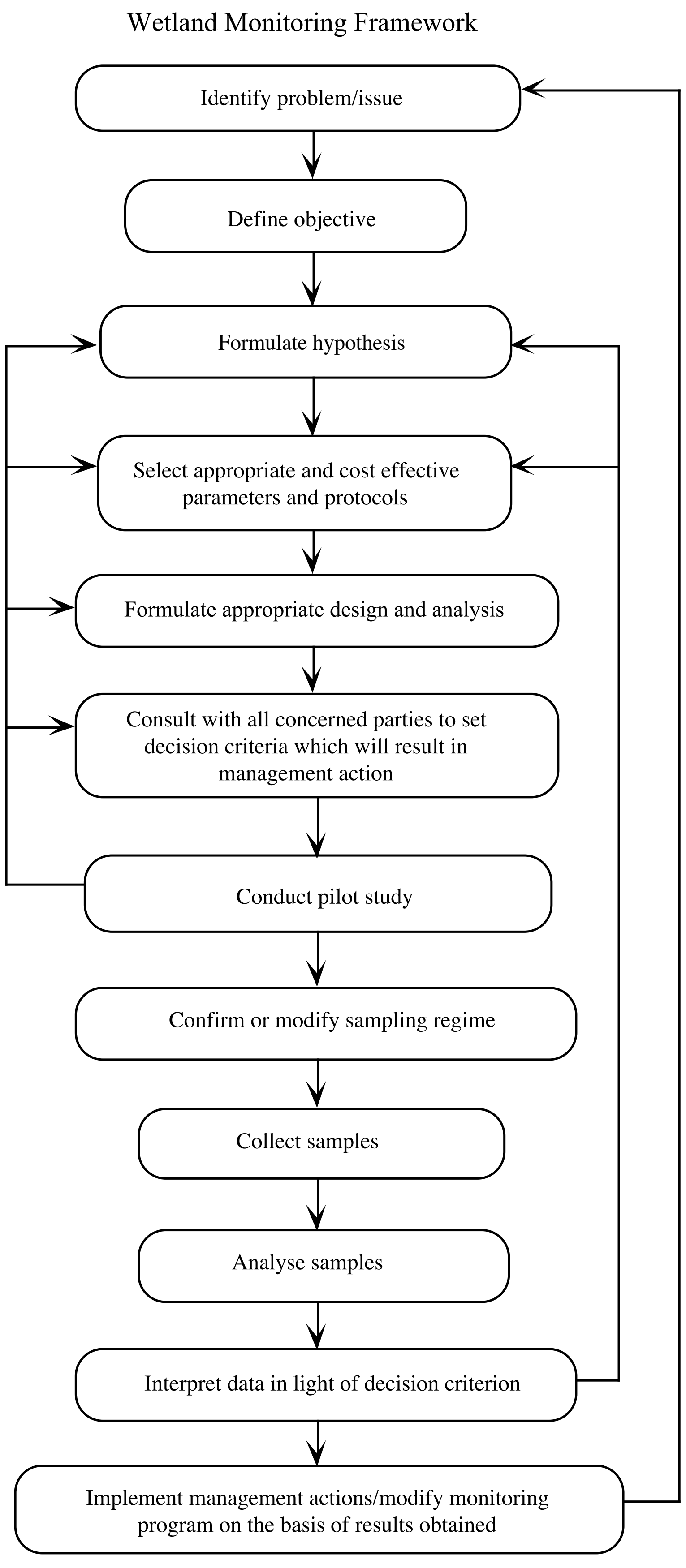

Figure 1: Wetland monitoring framework (after Finlayson, 1996).

1.3 Why macroinvertebrates ?

Macroinvertebrates, which Rosenberg and Resh (1993) defined as invertebrates retained by mesh sizes between 200 and 500 microns, are an essential component of wetland food webs and responsible for a significant proportion of the secondary production occurring in wetlands. Hellawell (1986) noted from a literature survey that macroinvertebrates, together with algae, were the two groups of organisms most often recommended for water quality assessment. However, Rosenberg and Resh (1993) suggested that macroinvertebrates were the group most often used in practice. The advantages of using macroinvertebrates for the biomonitoring of wetlands are similar to those listed by Rosenberg and Resh (1993) for aquatic biomonitoring in general, and include the following:

a) they are ubiquitous (i.e. are likely to be present in all wetlands studied);
b) the numbers of taxa present offers a spectrum of response to environmental stresses
c) their sedentary nature enables effective spatial analyses of point souce pollutants or other impacts;
d) long life cycles compared to other groups enables elucidation of temporal changes caused by environmental impacts.

However, as noted by Barmuta *et al*. (1997), with respect to the assessment of Australian rivers, macroinvertebrate bioassessment should be implemented as part of an integrated monitoring program that includes physical, chemical and other forms of assessment appropriate to local conditions.

1.4 Which protocol, which analysis ?

This manual partly represents the wetland equivalent of the River Bioassessment Manual produced for the Monitoring River Health Initiative by Anon (1994) and the Australian Rivers Assessment System User Manual (Simpson *et al*. 1996). However, unlike the former, this manual is not restricted to a single protocol based exclusively on predictive modelling. Both a rapid bioassessment sampling protocol and a quantitative sampling protocol are described, in addition to a number of different analytical techniques. The calculation of disturbance indices, to summarise the magnitude of various impacts on a wetland, is also presented to provide a measure against which the validity, or otherwise, of bioassesment techniques can be determined.

A rapid bioassessment approach is commonly used to describe a sampling technique which will provide a 'rapid turnaround of results' (Norris and Norris, 1995). Such an approach costs less than more detailed biological assessments and can potentially be undertaken by non-specialists. A quantitative protocol, which involves the collection of randomly located, replicated samples to determine species presence/absence or abundance per given area, is usually more time-consuming, and correspondingly, more expensive. Resh and Jackson (1993 suggested that the key features of rapid bioassessment which reduce time and costs include: the collection of single rather than replicate samples; the use of semi-quantitative samplers, usually a long-handled net; examination of only a portion of the specimens collected; and the use of a relatively coarse level of taxonomy (often family)

Rosenberg and Resh (1993) noted that "quantitative sampling is difficult because the contagious distribution of benthic macroinvertebrates requires high numbers of samples to achieve desirable precision in estimating population abundance. The resulting sample processing and identification requirements can be costly and time-consuming." The development of rapid bioassessment techniques has occurred largely to overcome these problems of time and cost. Rapid bioassessment is particularly useful where a broad assessment of the 'state' or 'health' of one or more wetlands is required. A quantitative approach is more appropriate when information on the conservation value, or invertebrate biodiversity, of a specific wetland is required, or the impact of a specific event (e.g., discharge of a pollutant) is to be determined.

The following tables have been prepared to assist with the choice of monitoring approach (sampling protocols and analytical methods). These are a guide only, since the particular circumstance for choosing assessment and monitoring techniques will often be site specific. It should be noted that for situations in which an assessment is required for a wetland, any form of corroboration by using more than one technique will improve the likelihood that a correct result has been obtained.

Table 1 Sampling protocols for wetland bioassessment

Protocol	**Application**
Rapid bioassessment protocol	Provides a broad assessment of the 'state' or 'health' of one or more wetlands. Useful for regular or routine monitoring of wetland condition. A rapid, simple and inexpensive methodology.
Quantitative sampling protocol	Provides quantitative information on the composition of wetland invertebrate communities. Useful for assessment of biodiversity and conservation values. A rigorous protocol which requires careful design, replication of samples is essential. May be time-consuming and costly because of the need to accurately identify and count specimens.

Table 2 Analytical techniques for wetland bioassessment

Analysis	**Appropriate situation**
Richness (number of species/families)	Cannot be used as an absolute measure of wetland condition unless a trend over time is recorded for a specific wetland, or baseline studies have been undertaken to establish numbers of taxa associated with undisturbed and degraded conditions for wetlands within a specific region.
Indicator species	Assumes that the occurrence of, or absence of, particular species indicates a specific disturbance regime. Useful for the confirmation of trends found using other tools. Particular applications include the identification of nutrient or organic enrichment, and acidification.
Biotic index (SWAMPS)	Can be used for an absolute assessment of individual wetlands, particularly where nutrient enrichment is a major impact.
ANOVA	Used to distinguish true trends of change over those that have arisen through random chance or sampling error. Compares single variables (eg. richness, total abundance). Often not applicable to datasets collected using a rapid bioassessment protocol because particular assumptions must be met (eg, replicate, random samples required) .
Predictive modelling	Can be used for an absolute assessment of individual wetlands as part of a surveillance operation to assess change over time. Assumes that a test wetland belongs to a reference state, and measures its deviation from that expected condition. Incorporates macroinvertebrate, physical and chemical analyses. Can be used to indicate possible causal mechanisms.
Multivariate analyses - classification and ordination	Used when a suite of wetlands is being assessed to determine differences or similarities amongst them. Provides a grouping or ranking of wetlands relative to each other. May be used to identify 'gradients' of disturbance

2. Rapid Bioassessment Protocol

Rapid bioassessment methodology involves the collection of a set number of invertebrates, from defined habitats, the use of inexpensive field equipment and a relatively coarse level of taxonomy (identification is usually to family). These features enable the methodology to be employed by relatively inexperienced individuals (Somers *et al*. 1998.) with the expectation of a reasonable level of accuracy.

2.1 Number and location of sampling sites within a wetland

One of the foundations of rapid bioassesment methods for rivers has been the description of both aquatic and riparian habitats (Norris and Norris, 1995). Similarly for wetlands, the dominant habitats must be identified, and the approximate percentage of the wetland occupied by each habitat recorded as a precursor to the implementation of the invertebrate sampling program

As most wetland assessments are undertaken with the objective of describing the 'health' or integrity' of the entire waterbody it is suggested that sampling incorporates a minimum of four sites per wetland – corresponding (approximately) to the four sectors, north, south, east and west (Fig. 2). One site per wetland is unlikely to adequately represent the biotic heterogeneity present in all but the most highly disturbed of systems. Sampling of a minimum of two habitats, from each of four sectors of an approximately circular waterbody, is recommended. Long linear wetlands or extremely heterogeneous waterbodies may need to be divided into several separate sections for assessment. Examples from the Swan Coastal Plain are given in Fig. 2. Yangebup Lake, which is approximately circular, is treated as a single, circular waterbody. Loch McNess is considered to comprise two separate waterbodies, a northern seasonal swamp and a southern permanent waterbody. The bathymetry of the two sections of the lake are quite different, due to dredging undertaken in the southern section many years ago. Lake Joondalup, which is a long, linear wetland, is subdivided into northern, central and southern sectors.

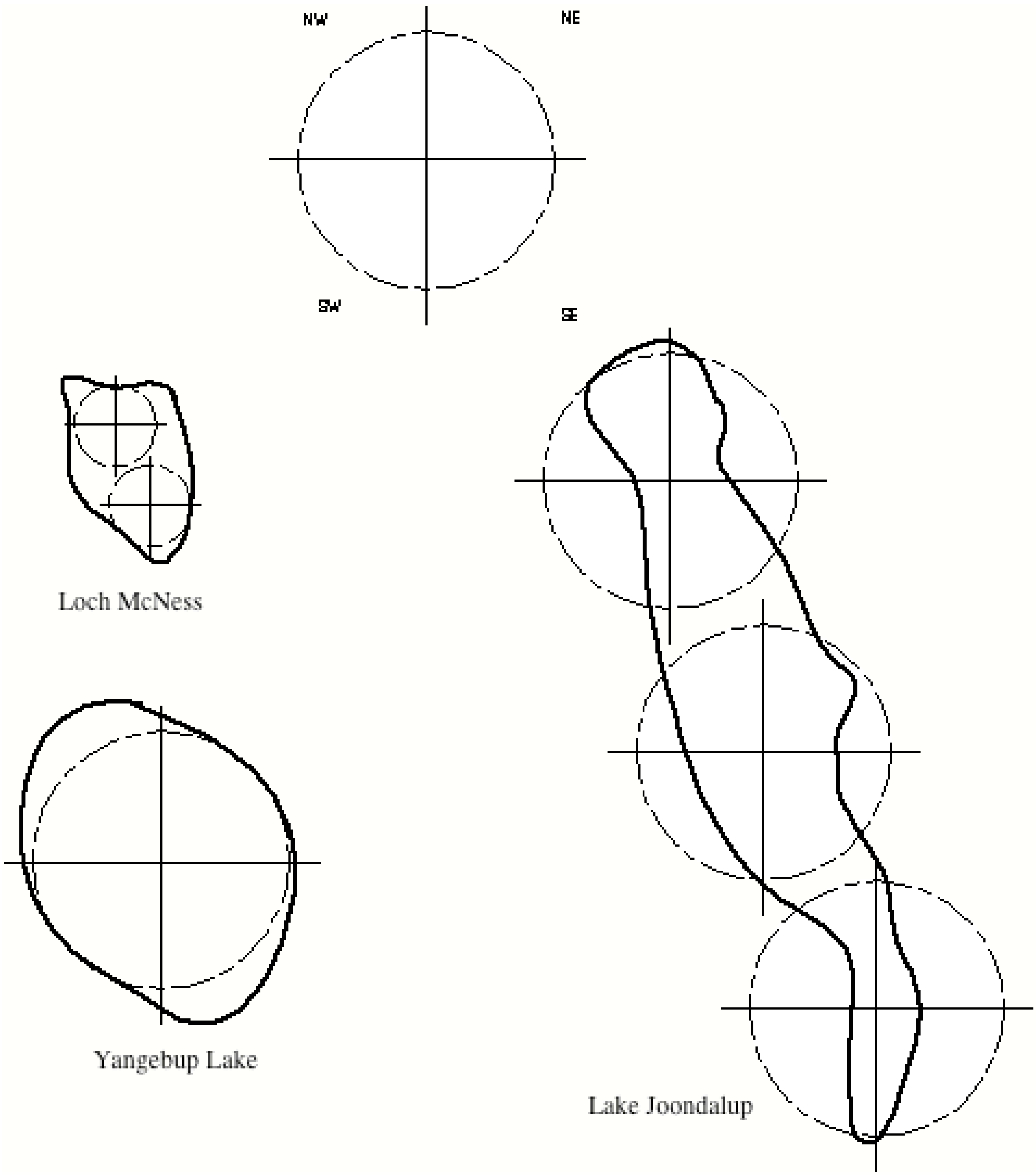

Figure 2: Diagram illustrating the subdivision of wetlands into sectors, and multiple sections, for representative sampling.

The habitats most likely to be present include: open water; submerged macrophytes (eg, *Myriophyllum, Potamogeton*); emergent sedges/rushes (eg, *Baumea, Typha* and *Schoenoplectus*); and fringing or flooded woodland (e.g. *Melaleucas*). For predictive modelling, at least two habitats should be sampled, in each sector, at each wetland. This is necessary because it is difficult to predict in advance how extensive each type of habitat may be, in a particular wetland, in a particular year.

2. 2 Timing and frequency of sampling

The timing and frequency of sampling should be sufficient to ensure that seasonal variation is described. A bimonthly sampling regime will provide considerable information on seasonal variability under most Australian climatic regimes while four-monthly or six- monthly may also be adequate. Realistically, the decision on the timing and number of samples to collect within a 12 month period is likely to be influenced by the amount of funds available. In southwestern Australia (which experiences a Mediterranean climate) the minimum recommended sampling regime is a single sampling period at the time of maximum wetland water level because this occurs at approximately the same time (late spring) for all wetland types (which are predominantly groundwater-dominated) within the region. In contrast, the occurrence of minimum water levels is more variable through time, occurring from summer through to late autumn, depending on wetland size and bathymetry.

In some circumstances, depending on the objectives of the assessment or monitoring program, it may be more appropriate to sample at lower water levels, particularly when the stressses imposed by eutrophication, or salinisation, are usually greater at this time. The most suitable time for sampling floodplain wetlands needs to be established on the basis of data collected over at least one annual cycle.

2.3 Sample collection and processing

Comparisons of different approaches to sample processing for rapid bioassessment are given by Growns *et al*. (1997) and Somers *et al*. (1998). The following protocol is currently used for biomonitoring of wetlands on the Swan Coastal Plain, WA.

Samples are collected for a standardised period of time (two minutes), from each habitat, in each sector, using a long-handled sweep net (250μm mesh).

The technique used is dependent on the habitat sampled. In open water and submerged macrophyte habitats, the sweep net is moved in a zigzag motion from the water surface to the lake bed. In the emergent macrophyte habitat the net is vigorously forced through the macrophytes from the bases of the plants to the water surface.

Samples are sorted whilst the invertebrates are alive and 'on-site' if possible. Two hundred animals are picked from the samples, with no more than ten individuals of each family / morphotype to be collected. Samples are picked for a maximum of 30 minutes. Where there are few invertebrates in any one sweep sample, further samples are to be taken and 'picked' until the 30 minute time period expires. Abundance estimates are made at the time of picking and recorded for each taxonomic group. Occurrence is scored as: 1= rare (<10 animals / sweep); 2=common (11-100 individuals / sweep); 3=abundant (101-1000 individuals / sweep); 4= highly abundant (>1000 individuals / sweep). All invertebrates are preserved in ethanol (with 2% glycerine) prior to identification to the level of family in the laboratory.

All specimens are identified to family with the exception of Oligochaeta (Class), Turbellaria (Order) and Chironomidae (Sub-family). Keys and descriptions of invertebrates recorded from Western Australian wetlands are given in Davis and Christidis (1997). An interactive guide to the Western Australian wetland fauna is also is available on CD-ROM from Dr Jenny Davis, Murdoch University.

2.4 Measurement of environmental variables

Environmental variables should be recorded at the time of sampling or water samples may be taken for subsequent laboratory analysis. The suite of parameters measured will depend upon the objectives of the monitoring program. Commonly recorded variables or descriptors include: latitude, longitude, depth, area of open water, degree of water permanence, sediment characteristics, soil type, temperature, dissolved oxygen, pH, conductivity, colour (gilvin) turbidity, ions, nutrients and chlorophyll *a*.

Where parameters can be measured at the time of sampling (eg depth, pH, conductivity, temperature, dissolved oxygen at bed), these should be recorded at each sampling site. Water samples should be collected for parameters that need to be analysed in the laboratory (eg, gilvin, turbidity). Where parameter measurement is expensive (eg. nutrients, chlorophyll *a*, effluent concentrations) average wetland samples can be obtained by bulking water samples from all sites within the wetland.

2.5 Analyses

All of the analytical methods described in Section 4, with the exception of ANOVA, are applicable to data collected with a rapid bioassessment protocol. However, predictive modelling requires the sampling of a sufficient number of reference wetlands for a model to be constructed. Similarly, multivariate analyses also require the sampling of multiple wetlands. As noted earlier, the use of more than one analytical approach increases the chance that a correct assessment will be made.

3 . QUANTITATIVE SAMPLING PROTOCOL

Specific management objectives for a wetland, or set of wetlands, may require a more quantitative approach than a rapid bioassessment protocol provides. Quantitative assessment requires the measurement of invertebrate densities (counting of invertebrates per known area of wetland bed or volume of water) and a random, or stratified random sampling design.

3.1 Timing and frequency of sampling

As for the rapid bioassessment protocol, the timing and frequency of sampling should be sufficient to ensure that seasonal variation is described. A pilot study should be undertaken to establish the spatial and temporal variability in macroinvertebrate occurrence in the system to be assessed.

3.2 Number and location of sampling sites within a wetland

The number of samples taken from any wetland will depend on the size of the wetland and variability in habitat. As noted above, a pilot study is recommended to establish the spatial variability in macroinvertebrate occurrence in the system studied. Sufficient samples need to be taken to be representative of the system studied and to enable statistical analysis of the results. Analysis by ANOVA requires the collection of replicate samples, samples must also be random and measurements within and across samples must be independent (Norris and Georges, 1993). *A priori* decisions on mesh size and taxonomic resolution will depend on requirements of the study.

3.3 Sample collection and processing

Specialised samplers are required to ensure that invertebrates are collected from a known area of wetland bed or volume of water. Examples include;

a) a corer, which removes a known area (and depth) of bed;

b) a standpipe, placed on the bed, from which a known volume of water is pumped, with a bilge pump, through a net;

c) a plankton net, towed at mid depth over a known distance; and

d) a long handled net, which can be used to obtain semi-quantitative samples, by moving up and down, through the water column, over a measured distance (often 10m) of wetland bed.

Useful references comparing the performance of different types of samplers for the collection of wetland macroinvertebrates include Cheal *et al*. (1993) and Turner and Trexler (1998).
Samples are preserved in ethanol and stored, under cool conditions. until laboratory processing can occur. All specimens must be enumerated, either by direct counting, or by subsampling. Identification may be to family, as described in Section 2, or species, depending on the objectives of the program.

e) Measurement of environmental variables

As described in section 2.4.

3.5 Analyses

All of the analytical methods described in Section 4 are potentially applicable to data collected with a quantitative sampling protocol. As noted earlier, the use of more than one analytical approach increases the chance that a correct assessment will be made.

4. ANALYSES

4.1 Richness - number of families/species

Collection of baseline information on the number of families (or species) of invertebrates associated with undisturbed and degraded wetlands, within a specified geographical range, will enable some comment to be made on the condition of the wetland with respect to invertebrate richness. For example, studies of wetlands in the Jandakot region on the southern Swan Coastal Plain, using a standardised sampling technique (two minute sweeps from four sites) revealed that degraded wetlands generally supported less than ten families, while undisturbed wetlands contained 20+ families. However, a similar study undertaken in the Gnangara region, on the northern Swan Coastal Plain, found that some highly disturbed (i.e. nutrient-enriched) wetlands contained 20+ families, while relatively undisturbed wetlands supported fewer species. These results highlighted the importance of undertaking baseline studies and the dangers of extrapolating beyond the geographical area encompassed by the baseline. While knowledge of the number of families alone cannot provide all we might need to know about a wetland there is some value in being able to quickly categorise a wetland on the basis of richness, as: low, intermediate or high.

4.2 Indicator Species

A number of species of wetland invertebrates display a positive response to nutrient enrichment. As a result, the presence of these species at a wetland in large numbers (usually hundreds or thousands of individuals per two minute sweep) can be used as 'indicators' of enrichment. Davis and Christidis (1997) found that the following species were indicators of excessive nutrient enrichment in wetlands of the Swan Coastal Plain:

Daphnia carinata (Cladocera), *Candonocypris novaezelandiae* (Ostracoda), *Micronecta robusta, Agraptocorixa hirtifrons* (Hemiptera), *Polypedilum nubifer, Kiefferulus intertinctus* (Diptera).

Although the identification of invertebrates to species, rather than families, is often not a 'rapid' process, with practice all of the species above can be quickly recognised. The scoring of numbers of individuals can also be time-consuming, however, when wetlands are enriched, one or more of these species is usually present in such large numbers that estimates, rather than counts, are all that is needed.

4.3 Biotic Index - SWAMPS (Swan Wetlands Aquatic Macroinvertebrate Pollution Score)

A biotic index, SWAMPS (Swan Wetlands Aquatic Macroinvertebrate Pollution Score) was developed for wetlands on the Swan Coastal Plain by Chessman, Trayler and Davis (in prep.). This index was developed using the objective iterative method of Chessman *et al*. (1997) for macroinvertebrate families of rivers in eastern Australia. Numerical values were assigned to wetland macroinvertebrate families to reflect their sensitivities to nutrient enrichment. SWAMPS indices were obtained by assigning scores to each family recorded at each wetland with the RBA protocol and dividing this total by the number of families present. The grade numbers for common invertebrate families recorded from wetlands of the Swan Coastal Plain is given in Table 3. The probable water quality status associated with SWAMPS values between 1 and 10 is given in Table 4

Table 3: Water quality status associated with SWAMPS values for wetlands of the Swan Coastal Plain. Adapted from Chessman (1995).

SWAMPS	Water quality status
Greater then 6	Good water quality
5-6	Doubtful quality, possible mild nutrient enrichment or pollution
4-5	Probable moderate enrichment or pollution
Less than 4	Probable severe enrichment or pollution

Table 4: Interim nutrient or pollution sensitivity grade numbers for common invertebrate taxa recorded from Swan Coastal Plain wetlands

Taxa	Grade	Taxa	Grade
MOLLUSCA		**INSECTA**	
Bivalva		**Ephemeroptera**	
Sphaeriidae	6	Caenidae	7
Gastropoda		Baetidae	7
Physidae	5	**Anisoptera**	
Planorbidae	7	Coenagrionidae	5
Ancylidae	7	Magapodagronidae	4
Lymnaeidae	6	Lestidae	6
Pomatiopsidae	6	Aeshnidae	7
Hydrobiidae	8	Corduliidae	7
Succineidae	6	Libellulidae	8
		Zygoptera juveniles	7
CRUSTACEA		Anisoptera juveniles	7
Cladocera		**Hemiptera**	
Daphniidae	1	Notonectidae	5
Sididae	6	Corixidae	1
Chydoridae	4	Pleidae	7
Macrothricidae	8	Veliidae	6
Moinidae	5	Mesoveliidae	6
Bosminidae	7	**Coleoptera**	
Ostracoda		Haliplidae	7
Cyprididae	3	Dytiscidae	5
Cypridopsidae	1	Hydrophilidae	6
Limnocytheridae	7	Chrysomelidae	7
Candonidae	7	Helodidae	7
Conchostraca	7	Ptilodactylidae	6
Copepoda		Noteridae	4
Cyclopidae	3	**Diptera**	
Harpactacoida	6	Chironominae	5
Centropagidae	10	Tanypodinae	7
Amphipoda		Orthocladinae	5
Ceinidae	5	Ceratopogonidae	7
Perthidae	8	Stratiomyidae	5
Isopoda		Tabanidae	6
Amphisopidae	4	Culicidae	7
Decapoda		Ephydridae	6
Palaemonidae	8	Thaumauleidae	7
Parastacidae	7	Tipulidae	4
		Simuliidae	7
ARACHNIDA		**Lepidoptera**	
Limnocharidae	7	Pyralidae	7
Limnesiidae	7	**Trichoptera**	
Unionicolidae	6	Ecnomidae	6
Eylaidae	6	Leptoceridae	5
Pionidae	4	Hydroptilidae	7
Hydrachnidae	3		
Arrenuridae	6	**CNIDARIA**	
Oxidae	7	Hydrozoa	7
Oribatidae	7		
Hydrodromidae	7	**PLATYHELMITHES**	
Halicaridae	7	Turbellaria	7
Pezidae	8		
Hydracarina juveniles	5	**ANNELIDA**	
		Oligochaeta	6
NEMATODA	5	**Hirudinea**	
		Glossiphoniidae	4

4.4 ANOVA

Analysis of variance is used to distinguish true trends of change over those that have arisen through random chance or sampling error. Univariate ANOVA is used to compare single variables (eg. richness, total abundance). Multivariate ANOVA (MANOVA) will enable multispecies abundance data to be used rather than total abundance. It must be noted that there are particular assumptions associated with the data that must be met prior to undertaking either ANOVA or MANOVA. For these reasons, univariate analyses are often not applicable to datasets collected using a rapid bioassessment protocol.

4.4 Predictive Modelling -

Predictive modelling for wetland assessment uses a suite of reference sites to predict the expected composition of families of invertebrates at a test site; if the test site has fewer families than expected based on the distribution of reference site values, then it is considered to be degraded or affected in some way. This approach involves the identification of a large number of wetlands of high environmental quality across a specified geographical range. At each wetland the macroinvertebrate communities are sampled and the habitats characterised by a standard set of physical and chemical variables that are largely unrelated to likely pollutants, or human impacts. This set of reference sites is then classified according to their biota to produce groups of sites containing similar fauna. A numerical analysis is used to identify the environmental attributes which best describe each group of reference sites. The environmental attributes of a test site are compared with those of the reference sites to determine which group or groups of reference sites it most closely resembles. The fauna of these corresponding reference sites is compared with the test site: if the test site supports fewer taxa than are predicted by the reference sites, it is judged to be degraded. The predicted taxa list also provides a 'target' invertebrate community which can be used to measure the success of remediation and restoration activities. The steps involved in the construction and development of these types of predictive models are illustrated in Figure 3 from Simpson *et al*. (1997).

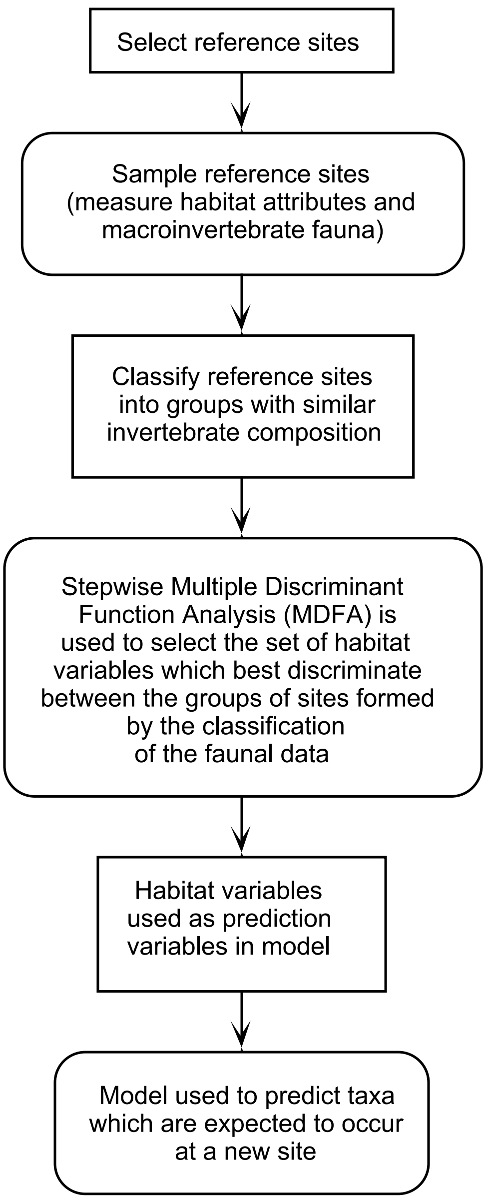

Figure 3: Steps used in model construction (from Simpson *et al*., 1997).

Construction of the model for the Swan Coastal Plain, Western Australia, was undertaken by Richard Norris and Justen Simpson at the CRC for Freshwater Ecology, University of Canberra. The model was run using AusRivAS software available at:
http://ausrivas.canberra.edu.au/ausrivas

New and separate models will need to be constructed for wetlands occurring in different regions of Australia. It is also intended that the model constructed for wetlands of the Swan Coastal Plain will be updated by sampling of a larger suite of reference wetlands. Further information regarding the construction of predictive model can be obtained from Dr Richard Norris at the CRC for Freshwater Ecology, Canberra.

To simplify interpretation and assist managers, AUSRIVAS provides a set of bands representing different levels of biological condition (Barmuta, Chessman and Hart, 1997). The width of the bands is based on the distribution of observed to expected ratios (OE50) for the reference sites in a particular model. The width of the reference band labelled A, in Table 2, is centred on the value 1 and includes the central 80% of reference sites. A site whose O/E index is greater than the 90th percentile of the reference sites is considered to be richer than the reference condition and is allocated to Band X. A site whose index falls below the lower 10th percentile of the reference sites is considered to have fewer families than expected and is allocated a lower band according to its value. The bands together with simple interpretations and likely causes are given in Table 2.

Table 2: Banding scheme used by AUSRIVAS and AUSWAMPS

Band	Definition
X	More families found than expected, potential biodiversity "hot-spot" Mild organic enrichment
A	Index value within range of central 80% of reference sites
B	Fewer families than expected. Potential impact either on water quality or habitat quality or both resulting in a loss of families.
C	Many fewer families than expected. Loss of families due to substantial impacts on water and/or habitat quality
D	Few of the expected families remain. Severe impact.

4.5 Classification and ordination (multivariate analysis)

Classification and ordination can be used to simplify large data sets, to show overall trends and allow the definition of indicator species or assemblages which best represent differences between sites or times. Norris and Georges (1993) suggested that multivariate techniques show greater promise than univariate comparisons for detecting and understanding spatial and temporal trends in macroinvertebrate data, because each taxon is considered rather than contributing to a reduced dataset comprising a single value for a site. Although most multivariate techniques are largely in the category of data exploration or hypothesis generating approaches, some techniques are available for hypothesis testing. For example, ANOSIM developed by Clarke & Warwick (1994). can be used to test the significance of groupings identified *a-priori*. Discriminant analyses can be used to explore the relationship between biological and environmental data so that the change in environmental variables most closely associated with the change in biological ones can be identified.

Multivariate analysis of wetland invertebrate datasets cannot be regarded as a 'rapid' form of analysis, nor can it be employed with a small number of wetlands, however useful information can be obtained from ordination and classification of data collected using either a rapid or a quantitative protocol.

Gradients of disturbance are often revealed by ordination. For example, ordination of wetlands on the Swan Coastal Plain, on the basis of their invertebrate communities, revealed a gradient from less to more enriched wetlands (Growns *et al*. 1992). Similarly classification will often separate groups of less disturbed wetlands from degraded wetlands.

5. MEASUREMENT OF ENVIRONMENTAL VARIABLES

The suite of parameters measured will depend upon the objectives of the monitoring program. Where parameters are measured at the time of sampling (eg depth, pH, conductivity, temperature, dissolved oxygen at bed or profiles in deeper systems), these should be recorded at each sampling site. Where parameters need to be analysed in the laboratory (nutrients and chlorophyll *a*), water samples should be collected and kept cool, or frozen, until analysis is undertaken. Where analysis is expensive (for example, nutrients, chlorophyll *a*, effluent concentrations) average system-wide samples can be obtained by bulking water samples from each site.

For all parameters utilising field meters it is essential that meters are maintained in a calibrated working condition, and are cleaned between habitats and between wetlands. Profiles or duplicate measurements from the surface of the water column (5 cm below the surface) and from the bottom of the water column (5 cm above the sediment water interface) will enable the presence or absence of stratification to be identified. In shallow, fully mixed wetlands samples should be collected from mid-depth. Measurements should be performed before sampling for fauna, central to the site chosen.

Latitude and longitude
Can be recorded using a hand-held GPS or calculated from maps.

Size, shape and bathymetry
Can be estimated from aerial photographs, maps and depth profiles.

Depth
The water depth of a wetland can be recorded in two ways. The Australian Height Datum (AHD) for the wetland should be recorded (this might be best performed by automatic datalogging from a surveyed point, or alternatively from a fixed water level gauge at a surveyed point). An estimate of water depth for each site/habitat sampled should be obtained using a graduated rod. This can be achieved by taking 5 measurements over a ten metre transect.

pH

pH should be measured in the field, with a portable meter, because it is readily altered by biological activity and temperature if stored at room temperature.

Conductivity

Conductivity can be measured in the field with a potable meter or from water samples taken back to the laboratory. Measurements are usually recorded in mS/cm or μS/cm.

Dissolved oxygen/temperature profiles

Dissolved oxygen/temperature profiles should be recorded with a portable calibrated field meter. If water samples are collected, care must be taken not to entrain oxygen during bottling, samples should be kept dark and cold until fixed, and a Winkler titration can be performed on return to the laboratory

Colour

Gilvin is measured by determining the absorbance of filtered (0.2 micron filter paper) water at 440nm using a spectophotometer. This figure is multiplied by 2.303 x100 to give the absorption coefficient (for a cuvette 1cm in width). The units of absorbance are $g440m^{-1}$

Turbidity

Turbidity is measured on unfiltered water spectrophotometrically by comparing the amount of light scattered and absorbed by the test solution compared with that passing through a reference solution (i.e. de-ionized water). Purpose-built turbidimeters are available for both laboratory and field use. The units of turbidity are NTU (Nephrelometric Turbidity Units).

Major Ions

Major ions (Na^+, K^+, Ca^{++}, Mg^{++}, $SO_4^{=}$, CO_3^{--}, Cl^-) can be determined by atomic absorption spectrophotometry.

Nutrients

Phosphorus is of great importance in wetland ecosystems because it is often the nutrient most influencing primary production. Total phosphorus and orthophosphate are the forms of P usually measured. Measurement of Total P indicates the amount of phosphorus present within a water sample in both inorganic and organic forms. Measurement of orthophosphate indicates the amount of inorganic P readily available for uptake by aquatic plants. Measurement of total nitrogen indicates the amount of nitrogen present in both inorganic and organic forms within a water sample. Ammonium is the form of nitrogen produced mainly by the breakdown of organic material and urea. Ammonia is readily oxidised to nitrite and then to nitrate. Ammonium and nitrate are commonly utilised by aquatic plants for growth. For accuracy and sensitivity nutrients are best analysed by a professional laboratory although field kits do exist for rapid assessment of inorganic forms of P and N. Filtered (for orthophosphate, nitrite/nitrate and ammonia) and unfiltered samples (for TN and TP) should be obtained from the sample.

Chlorophyll *a*

Chlorophyll *a* content is a useful index of phytoplankton productivity in a waterbody. Water samples for chlorophyll *a* extraction should be filtered as soon as possible after collection. The volume filtered must be recorded. Samples may be stored frozen, in the dark, until analysis. Chlorophyll *a* can be determined either fluorometrically or spectrophotometrically after concentration by centrifugation and extraction with acetone.

6. DISTURBANCE INDICES

Simple indices of wetland disturbance can be developed as an additional means of wetland assessment. Three categories (corresponding to low, medium and high levels) of disturbance have been recognised for the various forms of human impacts that have affected wetlands in southwestern Australia.

Various categories of wetland disturbance have been defined as follows:

Hydrology = change from seasonal to permanent water regime (eg. 1 = original regime of summer dry- winter wet still present, 2 = wetland no longer dries most years, 3 = water present in wetland throughout entire year)

Enrichment = degree of nutrient enrichment (1= not enriched, 2= moderately enriched, 3 = highly enriched)

Contaminants = degree of contamination of sediments with pesticides and heavy metals (1 = no contamination recorded, 2 = some contamination, 3 = highly contaminated)

Introduced fish = presence of *Gambusia holbrooki* (1= fish absent, 2 = small numbers present, 3 = densities very high during spring and summer)

Fringing vegetation = amount (%) of undisturbed vegetation remaining within 100m of edge of wetland (1 = > 80% of undisturbed littoral vegetation present, 2 = 80% - 20% present, 3 = < 20% of littoral vegetation remains)

Scores can be considered individually or averaged to provide an overall index of disturbance.

Other scores of disturbance can be developed depending upon the type of impacts that are important for a specific wetland or group of wetlands. For example further indices may include scoring the number of drains entering a wetland, the extent of grazing pressure evident, the extent of soil erosion, the weediness of fringing vegetation, the percentage of the catchment converted to urban land or farmland, etc.

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