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Monitoring change in aquatic invertebrate biodiversity: sample size, faunal elements and analytical methods

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Abstract

Replication is usually regarded as an integral part of biological sampling, yet the cost of extensive within-wetland replication prohibits its use in broad-scale monitoring of trends in aquatic invertebrate biodiversity. In this paper, we report results of testing an alternative protocol, whereby only two samples are collected from a wetland per monitoring event and then analysed using ordination to detect any changes in invertebrate biodiversity over time. Simulated data suggested ordination of combined data from the two samples would detect 20% species turnover and be a cost-effective method of monitoring changes in biodiversity, whereas power analyses showed about 10 samples were required to detect 20% change in species richness using ANOVA. Errors will be higher if years with extreme climatic events (e.g. drought), which often have dramatic short-term effects on invertebrate communities, are included in analyses. We also suggest that protocols for monitoring aquatic invertebrate biodiversity should include microinvertebrates. Almost half the species collected from the wetlands in this study were microinvertebrates and their biodiversity was poorly predicted by macroinvertebrate data.

Introduction

Biodiversity in wetlands is being reduced by agricultural, urban and industrial development through most of the world (Barbault & Sastrapradja, 1995; Ricciardi & Rasmussen, 1999) and the situation is often perceived as a crisis (Savage, 1995). In response, many programs have been initiated to conserve wetland communities and processes at a regional or local scale (e.g. Hails, 1996; Froend et al., 1997). Such programs usually include monitoring of biodiversity. Ideally, any species loss or significant changes in community structure will be detected and appropriate management intervention will occur to recover the species or community.

Developing monitoring protocols that are likely both to be adopted and to provide adequate information about changes in aquatic invertebrate biodiversity is difficult (Fairweather, 1991; Humphrey et al., 1995). As management agencies are faced with an ever greater array of situations that require monitoring, protocols that minimize costs will be preferred. This is achieved most easily by reducing the number of samples collected and processed, although low numbers of samples often prevent important changes being detected (Fairweather, 1991; Streever, 1998).

Most aquatic studies sample relatively small areas or volumes and, therefore, collect only a small proportion of species present at the sampling site (see Rouch & Danielopol, 1997; Turner & Trexler, 1997; Butcher, 1999). There is scope to increase the area of substrate, or volume of water, sampled and collect a greater proportion of the species present. Thus, large-volume samples may be one way of maintaining the ability to detect change while reducing number of samples and associated costs (Andrew & Mapstone, 1987; Kneib, 1991).

Another challenge in monitoring aquatic invertebrate biodiversity is selecting the appropriate organisms to sample. Results will be useful only if the

groups chosen are representative of trends in overall aquatic invertebrate patterns (see Green, 1979, p. 34). Few studies have examined the relationship between overall aquatic invertebrate biodiversity and its components but, working on trees, Azarbayjani & Richardson (1999) showed little correlation between richness within most arthropod orders and overall aboreal arthropod diversity. Results from large-scale wetland surveys also suggest predictions of overall species richness based on the richness of particular orders will frequently be unreliable because various orders exhibit different patterns of occurrence (Halse et al., 2000b; see also Usseglio-Polatera et al., 2000).

Many monitoring studies have used species richness as a measure of biodiversity and analysed the data with ANOVA. Two drawbacks are that comparatively large numbers of samples are required to detect trends and that the method works best when species loss, rather than turnover, is occurring. The earliest changes in biodiversity, however, are usually the replacement of sensitive species by more tolerant ones without a reduction in overall species richness (Growns et al., 1992). Multivariate dissimilarity measures, which take account of turnover, are better indicators of biodiversity and may be analysed using ANOVA (Faith et al., 1995; Edward et al., 2001) or ordination (Gray et al., 1990; Streever, 1998).

This paper reports a protocol for monitoring aquatic invertebrate biodiversity in wetlands, based on large sweep samples, minimal within-wetland replication, identification of micro- and macroinvertebrates, and ordination analysis. It is an extension of the ordination method proposed for wetland monitoring by Froend et al. (1997) and its intended use is examination of long-term temporal changes at individual wetlands. We tested the protocol at five ecologically different wetlands, representing surrogates for some of the changes that might occur at a single wetland over many years as a result of anthropogenic changes in its catchment, to examine whether the protocol was likely to detect changes in the aquatic invertebrate community. It is not our intention to challenge the need for replicated, controlled studies when the causal effects of particular environmental impacts are being examined.

Materials and methods

Five relatively shallow, basin wetlands in the wheat-growing zone of south-western Australia (Figure 1)

Figure 1. Location of the five wetlands in south-west Western Australia. The 300 mm and 600 mm rainfall isohyets correspond approximately to boundaries of the area with most secondary salinisation.

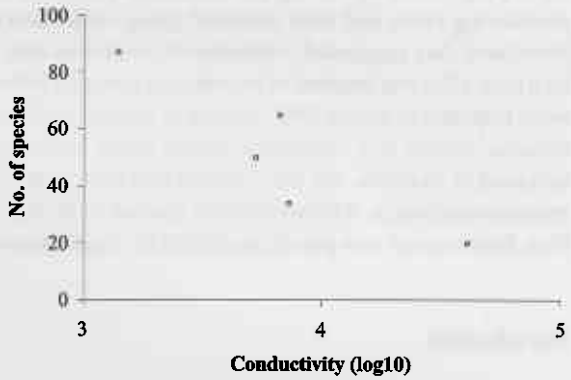

Figure 2. Number of invertebrate species in each wetland compared with log-transformed conductivity values as a measure of salinity ($r^2 = 0.831$, $p < 0.05$).

were sampled in spring (October) 1997. The region has a Mediterranean climate with winter rainfall and hot, dry summers. Annual average rainfall varies from 600 mm at Lakes Towerinning and Wheatfield to 400 mm at Lake Bryde (Figure 1), with 80–90% of rain falling between May and September. Average maximum temperatures in January and February range from 32 °C at Lake Logue to 26 °C at Lake Wheatfield (Bureau of Statistics, 1995).

Prior to agricultural clearing, vegetation consisted mostly of open woodland dominated by eucalypts or heathland dominated by Proteaceae and Myrtaceae. Wetlands were usually fringed by the trees *Casuarina*

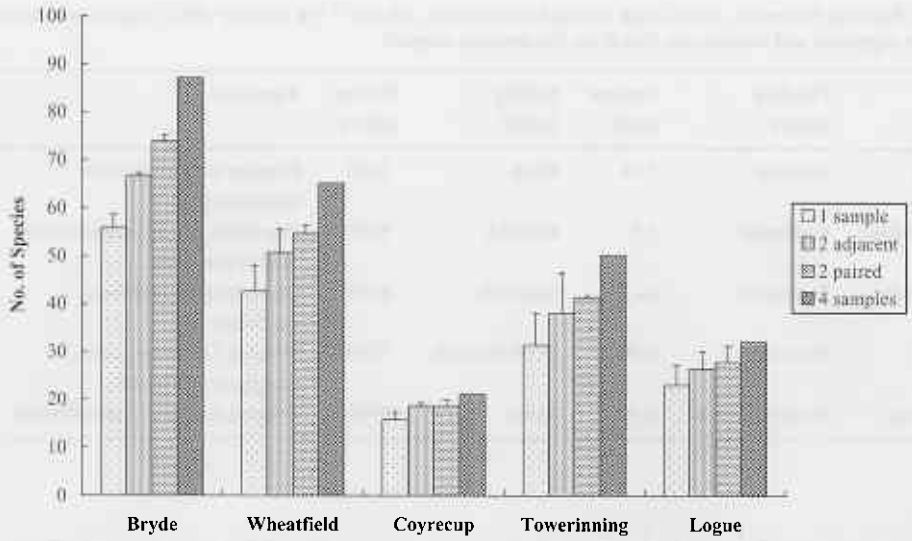

Figure 3. Mean number (± SE) of invertebrate species collected from each wetland by single samples, two (adjacent) samples from the same sector, two (paired) samples from different sectors. Number collected by four samples is also shown (see text for details of sample collecting).

obesa and *Melaleuca* spp., with occasional eucalypts (Halse et al., 1993a). Sedges occurred around the water's edge of most wetlands and across the lakebed of shallow seasonal waterbodies. *Casuarina obesa* and *Melaleuca* spp. sometimes occurred across large parts of the lakebed of wetlands that intermittently flooded to greater depths (ca. 2 m).

Land clearing, and the replacement of deep-rooted perennial vegetation with annual crops, has reduced transpiration rates and resulted in rising saline water tables in much of the agricultural zone, especially between the 600 and 300 mm isohyets (George et al., 1995). The process is often referred to as secondary salinisation and has profoundly affected the ecology of many ecosystems in south-western Australia (see Williams, 1999), including causing the death of much wetland vegetation and its replacement by salt-tolerant samphire. Remnants of dead trees around wetlands are now a common sight (Halse et al., 1993a).

Attributes such as size, depth, type and extent of riparian vegetation, and frequency of flooding varied among the five wetlands but probably the most ecologically important differences related to salinity status and associated changes in riparian vegetation (Table 1). As well as simplifying vegetation communities, secondary salinity reduces the diversity of aquatic invertebrates (Timms, 1981) and alters waterbird communities (Halse et al., 1993b).

In terms of relationships between the wetlands, Lakes Bryde, Towerinning and Coyrecup can be viewed as being positioned along a temporal gradient of secondary salinisation with Lake Bryde still relatively fresh, Lake Towerinning mildly salinised and Coyrecup showing effects of long-term (40 + years) salinisation. Lakes Logue and Wheatfield have similar salinity to Towerinning but more complex submerged and riparian vegetation, respectively, and are displaced from the gradient. Their salinity levels mostly reflect natural processes: an historical marine connection at Wheatfield and evapo-concentration at Logue.

Sampling in each wetland was stratified, with two widely separated areas being sampled (called sectors) and two random samples being collected within each sector. The aim was to obtain measures of within-sector and between-sector variation. Each sample consisted of 'benthic' and 'planktonic' sub-samples. The benthic sub-sample was collected by 50 m of vigorous, discontinuous sweeping over a distance of about 200 m with a 250 μm mesh pond-net on a D-shaped frame (350 mm wide and 250 mm high). All identifiable wetland habitats $\leq$1 m deep between the shore and centre of the wetland were sampled, including water column, submerged vegetation, bottom sediments, along submerged logs and around tree trunks. Contents of the pond-net were emptied into a bucket several times during sampling to reduce resistance of the net in the water. After elutriation and removal of large debris, the sub-sample was preserved in 70% ethanol. The planktonic sub-sample was collected by 50 m of more gentle sweeping with a 50 μm mesh

Table 1. Flooding frequency, water depth (m) and conductivity (μS cm^{-1}) in October 1997, long-term salinity pattern, dominant vegetation and wetland size (ha) at the five wetlands sampled

	Flooding pattern	October depth	Salinity pattern	October salinity	Vegetation	Size
Bryde	Seasonal	1.74	Fresh	1400	Fringing trees (*Melaleuca* spp/eucalypts)	97
Towerinning	Permanent	3.2	Brackish	5300	Vegetation mostly cleared, a few dead or live trees	180
Wheatfield	Permanent	ca. 2	Brackish	6700	Dense fringing *Melaleuca cuticularis*	150
Logue	Seasonal	0.36	Fresh-brackish	7300	Fringing *Casuarina obesa*, canegrass on lakebed	425
Coyrecup	Semi-permanent	0.9	Saline	40900	Fringing dead trees (salt-affected)	448

pond-net on the same-sized frame in the same habitats, other than benthos, and was preserved in 1–2% formaldehyde.

In the laboratory, benthic samples were sieved through a stack of 2 mm, 500 μm, 250 μm and 90 μm mesh sieves and the portion retained in each sieve was sorted under a dissecting microscope. Planktonic samples were sieved through 250 μm, 90 μm and 53 μm mesh sieves. All animals were sorted and identified to the lowest level possible. This was usually species or morphospecies and, for convenience, we use the term species when referring to the number of taxa identified in a wetland. We also use the terms microinvertebrates and macroinvertebrates to break invertebrates into two groups based loosely on size. We define microinvertebrates as species belonging to the Protista, Rotifera, Cladocera, Ostracoda and Copepoda and we refer to all other invertebrates as macroinvertebrates, although many of them are quite small (e.g. some species of Nematoda, Acarina and Chironomidae).

Analysis

Numbers of invertebrate species collected in samples from different wetlands were compared by 1-way ANOVA using PROC ANOVA in the SAS analysis package (SAS Institute 1990), after checking that data were normally distributed and homoscedastic. Number of samples required to be 90% sure of detecting 20% or 30% decline in species richness in a wetland was estimated by the method of Snedecor & Cochran (1980, pp. 102–106), using $\alpha = 0.05$ and $\beta = 0.10$.

The average proportion of species recovered by single samples, two samples from the same sector of a wetland and two samples from different sectors was compared with the total number of species present in the wetland, as estimated by the formula of Karakassis (1995)

$$\overline{S}_{k+1} = a + b\overline{S}_k \quad \text{and} \quad S_\infty = a/1 - b,$$

where S_∞ is the theoretical limit of the asymptote of the species accumulation curve, $\overline{S}_k$ is the mean number of species in all possible combinations of k samples, and a and b define the regression line of $\overline{S}_{k+1}$ on $\overline{S}_k$.

The formula of Kay et al. (1999), in slightly modified form, was used to derive species accumulation curves

$$a_i = a_{i-1}(a_4/a_3),$$

where a_i is the mean number of additional taxa collected in the ith replicate, and a_3 and a_4 are the mean number of additional taxa collected in the third and fourth replicates, respectively. For samples of all invertebrates from Coyrecup, a_2 and a_3 were used to provide an estimate of the rate of change of the species accumulation curve because, owing to chance $a_3 = a_4$, which prevented a_i reaching an asymptote (general case is that $a_i > a_{i+1}$).

Similarity of invertebrate samples from within and between wetlands was examined by ordination. This was done to test whether changes likely to occur within one wetland over time, as a result of human activity, could be detected reliably by single samples. In a formal monitoring program, the ordination would contain invertebrate data from several 'marker' wetlands, typical of the range of wetlands in the region, and a temporal series of samples from the wetland being monitored. The marker wetlands, sampled prior

to the commencement of monitoring, provide a standardised framework for interpreting the magnitude and direction of changes in invertebrate biodiversity at the monitored wetland.

Ordination was based on semi-strong-hybrid multi-dimensional scaling in the PATN analysis package (Belbin, 1993) with presence/absence invertebrate data and the Czekanowski dissimilarity measure. Dissimilarity values >0.95 were re-calculated using the shortest path option (Belbin, 1993). *F*-ratios, calculated by ANOSIM (Clark & Green, 1988), were used as a measure of within-wetland variation relative to separation between wetlands. Information about the overall invertebrate community of each wetland available from micro- or macroinvertebrates alone was examined by comparing ordinations based on micro- or macroinvertebrates with those based on all invertebrates.

Ability of two samples from different sectors to characterise the overall invertebrate community of a wetland was examined using four 'paired' samples, which were derived by combining all possible pairs of samples from the two different wetland sectors. In addition, all four samples from a wetland were combined to provide a 'composite' species list for the wetland. Paired and composite samples were used, together with simulated samples, in two ordinations. The first examined effect of species turnover on position of samples in ordination space and included 20 modified samples, derived by replacing approximately 10%, 20%, 30% and 40% of the species in composite samples of each wetland with species from the other four wetlands. Replacements were random, within the constraint that only biologically realistic changes were allowed. Ubiquitous species known to have wide ecological tolerances were rarely replaced and species that occurred in only one or two samples from a wetland were more likely than those in four all samples (although some four-sample species were replaced, especially in the 30% and 40% steps). Replacements made at the 10% step were carried into the 20% step, unless they were randomly selected to be replaced, etc.

The second ordination examined effect of species loss on position of samples in ordination space and included 15 modified samples, derived by removing 10%, 20% and 30% of species from composite samples, using the same methodology as for replacements.

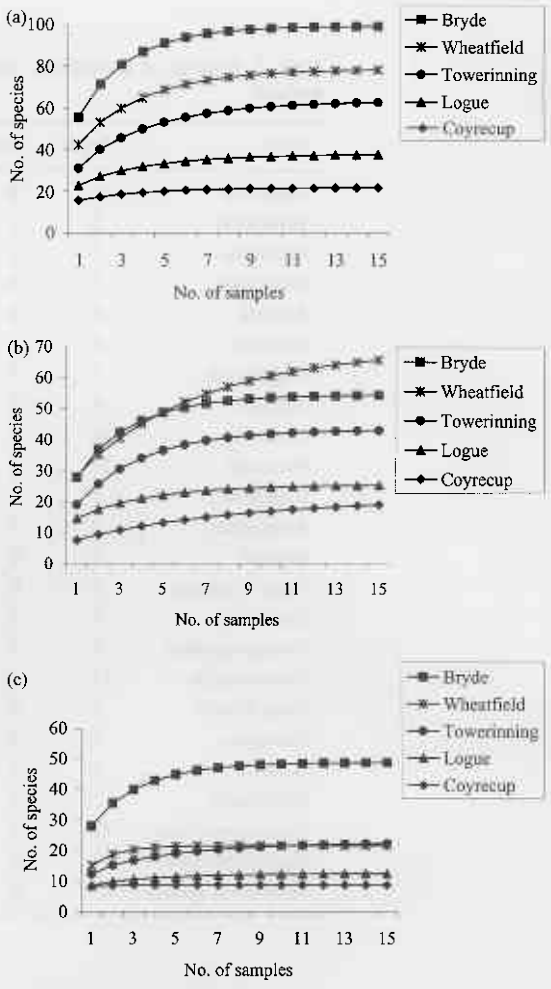

Figure 4. Species accumulation at each wetland with increasing number of samples. (a) all aquatic invertebrates, (b) macroinvertebrates, (c) microinvertebrates.

Results

At least 150 aquatic invertebrate species were collected from the five wetlands, with Lake Bryde yielding most species (87) and Coyrecup fewest (20) (Table 2, Appendix). The proportion of microinvertebrates varied from 48% in Bryde to 31% in Wheatfield. At order level, differences in proportion of Cladocera among wetlands were especially pronounced (23% of species in Bryde, < 2% in Wheatfield).

Mean numbers of species collected per invertebrate sample differed significantly between wetlands (Table 3), and there was a significant negative relationship between species richness and salinity (Figure 2), confirming that species richness will change if the salinity of a wetland changes substantially over time (see also

Table 2. Number of invertebrate species of various taxonomic groups in the five wetlands

Taxa	Bryde	Towerinning	Wheatfield	Logue	Coyrecup
Hydrozoa	0	0	1	0	0
Turbellaria	1	1	1	1	1
Nematoda	1	1	1	1	1
Tardigrada	1	0	0	0	0
Rotifera	6	0	4	0	1
Mollusca	0	0	1	0	1
Oligochaeta	3	3	2	1	0
Acarina	0	1	3	0	1
Cladocera	20	3	1	2	1
Ostracoda	11	8	8	6	4
Copepoda	5	6	7	3	3
Amphipoda	1	1	2	1	1
Isopoda	0	0	1	0	1
Other Crustacea	2	0	1	0	0
Coleoptera	7	5	8	3	2
Ceratopogonidae	2	4	1	3	1
Chironomidae	15	6	9	4	2
Other Diptera	4	4	4	3	0
Hemiptera	3	3	3	4	0
Odonata	3	1	3	1	0
Trichoptera	2	3	4	0	0
Microinvertebrates	42	17	20	11	9
Macroinvertebrates	45	33	45	22	11
Total no. of species	87	50	65	33	20

Table 3. Mean ± SE numbers of micro-, macro- and all invertebrate species collected in four samples and estimated number of samples (N^*) needed to detect a 20% decline in species richness with 90% confidence. Numbers of micro-, macro- and all invertebrates differed significantly between wetlands ($F = 71.1$, $F = 38.3$, $F = 51.1$, respectively, $p < 0.0001$). Means with different letters were significantly different ($p < 0.05$)

Wetland	Microinvertebrates		Macroinvertebrates		All species	
	Mean	N^*	Mean	N^*	Mean	N^*
Bryde	26.8±0.5 A	2	29.5±0.9 A	3	56.2±1.1 A	2
Towerinning	12.2±1.4 B	23	19.0±2.1 B	23	31.2±3.4 C	21
Wheatfield	14.0±1.3 B	16	28.2±1.7 A	7	42.2±2.9 B	9
Logue	8.2±0.6 C	11	14.8±1.5 B	19	23.0±2.0 D	15
Coyrecup	7.2±0.2 C	3	8.2±0.6 C	11	15.5±0.6 E	4

Table 4. Estimated number of samples (N^*) before an additional sample collected < 1 extra species, the estimated numbers of species present in each wetland using formulae of Kay et al. (1999) and Karakassis (1995), and the number of species collected in four samples

Wetland	N^*	Species estimated		Species collected
		Kay et al.	Karakassis	in four samples
Bryde	8	99	97	87
Towerinning	9	63	58	50
Wheatfield	10	78	75	64
Logue	6	38	36	34
Coyrecup	5	23	23	20

Timms 1981, 1998). Using ANOVA, the estimated number of samples needed to be 90% sure of detecting a 20% decrease in species richness at individual wetlands varied from 2 at Bryde to 21 at Towerinning (Table 3). Variation in these estimates reflects stochastic error in estimates of variance because of small sample sizes in our study and about 10 samples would probably produce the statistical power required at most wetlands. Estimated number of samples needed to detect a 30% reduction varied from 2–10.

Differences between wetlands were less clear-cut when micro- or macroinvertebrates alone were examined; for example Wheatfield was similar to Bryde in terms of macroinvertebrate richness but more like Towerinning in number of microinvertebrates (Table 3). There was a tendency for fewer samples to be needed to detect a 20% decline in microinvertebrate than macroinvertebrate richness, although the opposite applied at Wheatfield (Table 3).

Estimates of species richness in each wetland suggested a single sample usually collects about 60% of species present at the time of sampling (range 43–81%), two samples from different sectors 75%, and four samples 89% (Table 4, Figure 3). Between-sector variation in species composition was usually greater than within-sector variation so that, except at Coyrecup where there was no difference, about 7% more taxa were collected by two samples from different sectors than by two from the same sector. Depending on wetland, 6–10 samples were required to collect all species present (Figure 4a). Rate of accumulation of microinvertebrates was greater than macroinvertebrates and fewer samples were needed to collect all micronvertebrate species (Figures 4b and 4c).

All samples contained sufficient information about the invertebrate community of their source wetland to separate them in ordination space from samples collected in the other wetlands (Figures 5a and 5b). Furthermore, all samples from a wetland were close in ordination space to the composite sample for that wetland and, thus, probably contained most of the information signal of the full invertebrate community. While these results are, at least partly, a consequence of the ordination containing wetlands with very different community composition (the spread of samples from the same wetland would be greater and between-wetland separation less clear if all wetlands had similar communities), it strongly implies that ordinating a temporal series of single samples from a wetland within a framework of marker wetlands will detect any substantial ecological changes that have occurred.

Relative positions of samples from different wetlands changed somewhat in ordinations based on microinvertebrates or macroinvertebrates alone, with the microinvertebrate pattern being more similar to that of the whole invertebrate community than to macroinvertebrates (Figures 5c–e). Samples from the same wetland were usually closest to each other in ordination space, relative to the distance between wetlands, in the microinvertebrate ordination, which was reflected by the highest F-ratio from ANOSIM analysis (all invertebrates, $F = 3.301$, macroinvertebrates, $F = 2.393$, microinvertebrates, $F = 4.109$, composite samples excluded).

Paired samples from different sectors of a wetland were closer in ordination space to both each other and composite samples than were single samples (Figures 5a, 5b and 6). Paired samples also showed better discrimination between wetlands ($F = 5.586$ vs 3.301, composite samples excluded). Thus, a pair of samples from different sectors provided more precise information about the invertebrate fauna of a wetland than a single sample and enable finer-scale differentiation of ecological character.

Ordination of modified sample data, representing different levels of species turnover and loss at the five wetlands, showed species turnover caused larger shifts in ordination space than species loss (Figure 7). Shifts in sample position caused by turnover of $\geq 20\%$ in community composition were about twice the variation exhibited by paired samples at all five wetlands (Figures 7a and 7b) and suggested that changes of $\geq 20\%$ in community composition would be reliably detected by ordination of a temporal series of paired samples from a wetland ($p < 0.2$).

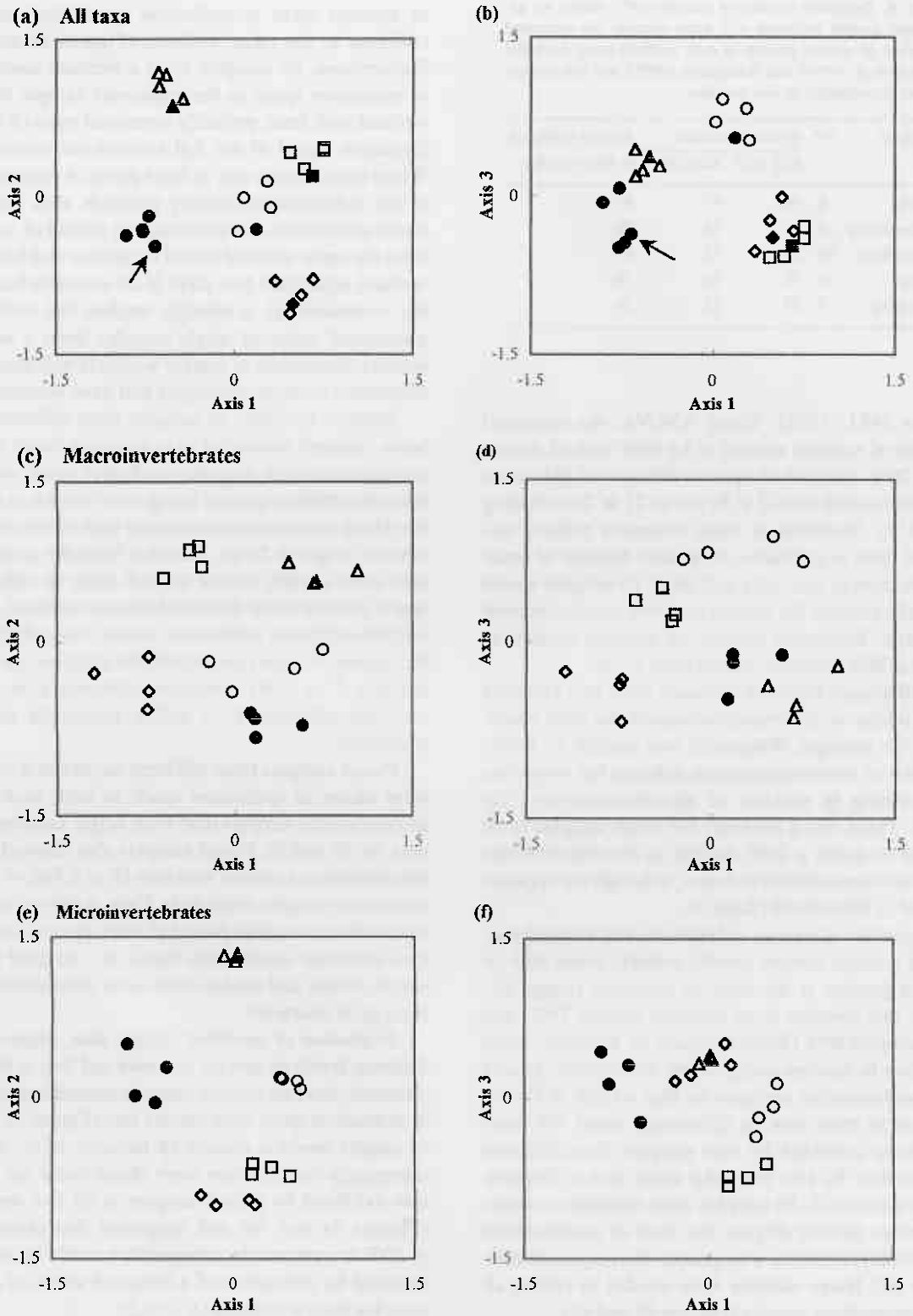

Figure 5. Three-dimensional ordination of samples from five wetlands. ◊ Bryde, ○ Towerinning, ● Wheatfield, □ Logue, △ Coyrecup. (a, b) All invertebrate data, with composite samples (closed symbol or arrowed), stress = 0.11; (c, d) macroinvertebrate data, stress = 0.13; (e, f) microinvertebrate data, stress = 0.06.

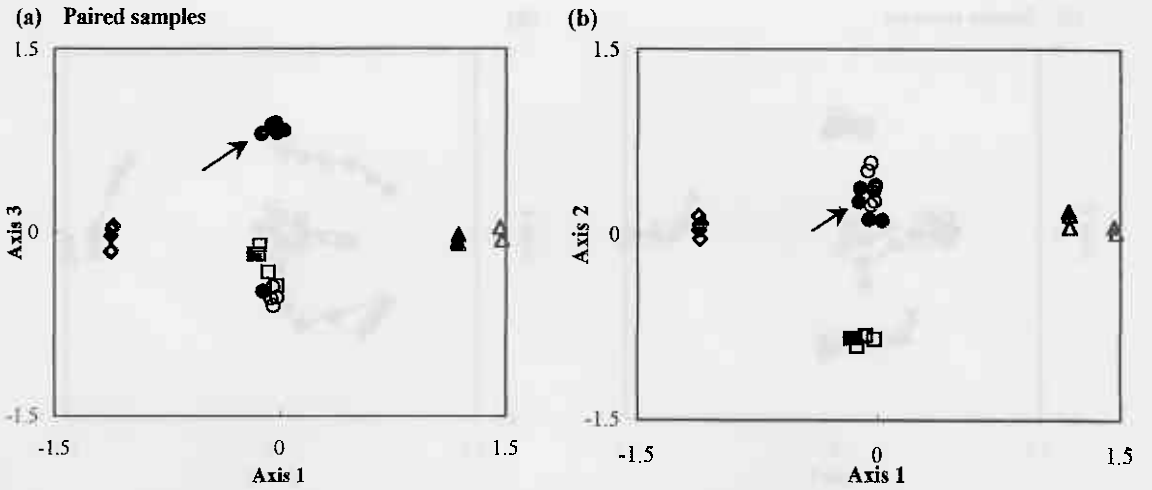

Figure 6. Three-dimensional ordination of paired and composite (closed symbol or arrowed) samples from each wetland. ◇ Bryde, ○ Towerinning, ● Wheatfield, □ Logue, △ Coyrecup, stress = 0.09.

Discussion

Forty-five percent of species collected during this study were microinvertebrates, which is similar to proportions recorded in other studies and reinforces that microinvertebrates form a major component of wetland biodiversity (Dole-Olivier et al., 2000; Halse et al., 2000b). The patterns of micro- and macroinvertebrate biodiversity in the five wetlands differed (Table 3, Figure 5) and it was clear that monitoring based on one group alone would have been misleading in terms of overall invertebrate biodiversity. Despite this, most studies of wetland ecology and aquatic invertebrate biodiversity sample only macroinvertebrates (Resh & McElravy, 1993).

The estimate of 6–10 samples to collect all species in the five wetlands (Table 4) was similar to the 4–6 samples needed at Toolibin and Walbyring Lakes in south-western Australia using similar sampling methods (Halse et al., 2000a). Four factors contributed to species accumulating faster in these studies than in most others reported in the literature (e.g. Rouch & Danielopol, 1997; Butcher, 1999): (1) sweep sampling, which collects species more efficiently than most techniques (Cheal et al., 1993; Turner & Texler, 1997), (2) large effort per sample, (3) employing both small and intermediate mesh sizes to increase likelihood of collecting micro- and macroinvertebrates, respectively, and (4) sampling a range of microhabitats. Sweep samples of micro- and macroinvertebrates, collected over large areas and incorporating as many microhabitats as possible, yield more information on community composition than small samples at little extra cost. Our experience has shown that processing costs are more related to number and type of samples than volume of those samples.

Despite recovering species more efficiently than most sampling protocols (Rouch & Danielopol, 1997; Butcher, 1999), our single sweep samples collected only about 60% of invertebrate species present in a wetland. Even when several samples were taken, some patchily distributed or low-abundance species were missed. Yet, when documenting changes in biodiversity is the objective, these rare species may be of most interest. We have no solution to this conundrum other than increasing sample size. We suggest that taking paired samples, which on average collect 75% of species present, represents a reasonable trade-off between cost and completeness of the species list but species accumulation curves can be used to determine the number of samples required for a higher recovery rate (Moreno & Halffter, 2000).

This study confirmed that large numbers of samples are usually required to demonstrate changes in species richness at a wetland, unless expected changes are large (about 10 samples per wetland sampling occasion were needed to detect 20% decline in species richness and five to detect 30% decline). Examining change in species composition using ordination is a more efficient way of detecting changes in biodiversity (Gray et al., 1990; Warwick, 1993), particularly because the first changes in invertebrate biodiversity at wetlands subject to environmental change usually in-

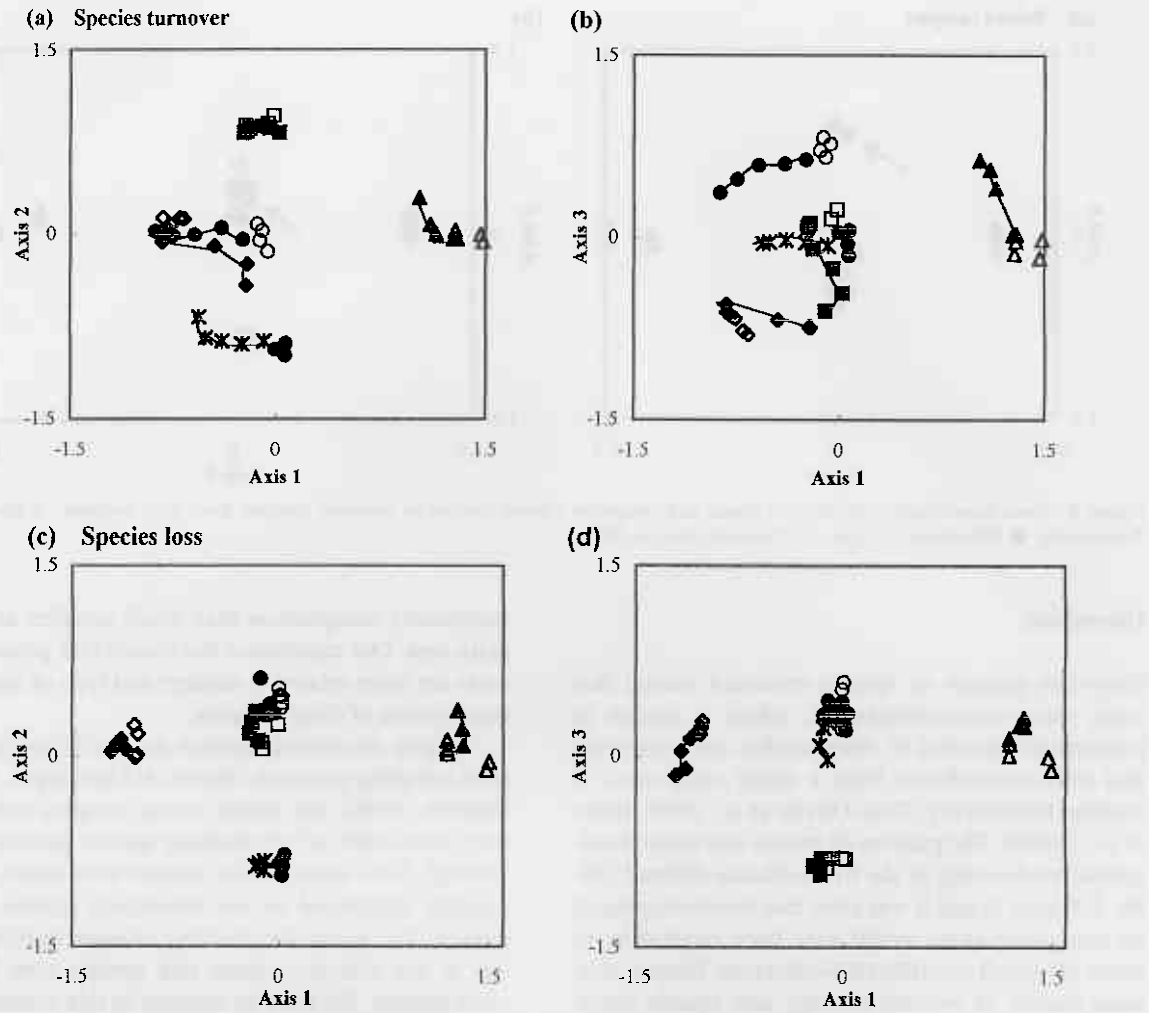

Figure 7. Three-dimensional ordination of paired samples from each wetland. ◊ Bryde, ○ Towerinning, ● Wheatfield, □ Logue, △ Coyrecup. (a, b) effect of 10–40% species turnover on position of composite sample (composite and simulated samples shown by closed symbols and connected by line), stress = 0.11, (c, d) effect of 10–30% species loss on position of composite sample, stress = 0.10.

volve species replacement rather than reduced richness (Growns et al., 1992).

Streever (1998) showed significant pattern among the invertebrate communities of different sites by ordinating combined data from two small core samples per site, although patterns became more stable as number of samples increased. Halse et al. (2000a) showed that single large samples could distinguish between different types of wetland. This study confirmed earlier results: ordination of data from large paired samples produced stable patterns and characterised the wetland community from which they were taken (Figure 6).

Ordination of modified sample data, simulating types of change likely to occur over time as a result of anthropogenic change, suggested $\geq$ 20% species turnover could be detected reliably (Figures 7a and 7b). The relative insensitivity of ordinations to changes in species richness itself, which is characteristic of multivariate techniques (Clarke et al., 1996), is beneficial in a long-term monitoring program in that small variations in sampling effort over time (or among operators) have minimal effects on results.

We have not tested the discriminatory power of paired-sample monitoring in a formal way because visual assessment of effect size can be an effective method of evaluation (see Anderson et al., 2000). More rigorous testing could be achieved by visual assessment of simulated data from a larger number of wetlands or by ANOVA of dissimilarity measures (see Edward et al., 2001). However, the magnitude of

changes that are biologically significant is yet to be determined and this should precede the development of formal statistical tests.

The proposed monitoring protocol is not intended to detect disturbance-induced changes that are smaller than the natural fluctuations between most years. These can only be detected using complex designs and, even then, are difficult to demonstrate (Underwood, 1993). However, in strongly seasonal and semi-arid climates, such as occur in much of Australia (Gentilli, 1972), drought and flood events may cause much larger changes in community composition, at intervals of a decade or so, than are likely to be caused by anthropogenic factors (Halse et al., 1998; Timms, 1998). Unusual natural events complicate the monitoring of trends in wetland biodiversity and we suggest that the most appropriate action is to exclude extreme years (as defined by water levels or other criteria) from analysis, although in some situations change in the frequency of extreme events, and the occurrence of their associated invertebrate communities, will be important. Exclusion of years with extreme events reduces both the likelihood of Type I error (false evidence of anthropogenic change) and the cost of achieving an acceptable level of Type II error.

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Appendix. Species recorded in the wetlands. 1 Bryde, 2 Towerinning, 3 Wheatfield, 4 Logue, 5 Coyrecup.

Taxa	Wetland				
	1	3	5	2	4
HYDROZOA					
Cordylophora sp.			1		
TURBELLARIA	1	1	1	1	1
NEMATODA	1	1	1	1	1
TARDIGRADA	1				
ROTIFERA					
Macrotrachela sp. A			1		
Philodina sp. A	1				
Hexarthra fennica (Levander)			1		1
Hexarthra mira (Hudson)	1		1		
Testudinella patina (Hermann)	1		1		
Brachionus quadridentatus Hermann	1				
Brachionus rotundiformis (Tschugunoff)			1		
Euchlanis dilatata Ehrenberg	1				
Trichocerca rattus carinata (Muller)	1				
GASTROPODA					
Coxiella sp.			1		1
OLIGOCHAETA					
Ainudrilus nharna Pinder&Brinkhurst	1				
Tubificidae			1	1	
Dero digitata (Muller)	1	1			
Paranais litoralis (Muller)		1			
Enchytraeidae	1	1	1		
ARTHROPDA					
ACARIFORMES					
Hydrachnidae			1		
Eylais sp.		1			
Halacaridae					1
Oribatida			1		
Mesostigmata			1		
ANOSTRACA					
Branchinella lyrifera Linder	1				
CONCHOSTRACA					
Caenestheria sp. nov. A (nr *lutraria*)	1				
CLADOCERA					
Alona diaphana King	1				
Alona rectangula novaezealandiae Sars	1				
Alona diaphana vermiculata Smirvov&Timms	1				
Alona sp. nov. A (Bryde)	1				
Alona cf. *crassicauda*	1				
Biapertura rigidicaudis s.l. Smirnov	1				
Biapertura cf. *longiqua* Smirnov	1				
Leydigia cf. *ciliata* Gauthier	1				
Monospilus diporus Smirnov & Timms	1				
Monospilus elongatus Smirnov & Timms	1				

Continued

Taxa	Wetland				
	1	3	5	2	4
Plurispina cf. *chauliodis* Frey	1				
Pleuroxus cf. *foveatus* Frey	1			1	
Rak sp. nov. B (Venemores)	1				
Ceriodaphnia laticaudata Mueller		1			
Daphnia cephalata King	1				
Daphnia carinata s.l. King	1	1	1	1	
Daphniopsis pusilla Serventy					1
Daphniopsis queenslandensis Sergeev	1				
Simocephalus exspinosus De Greer	1				
Simocephalus victoriensis Smirnov&Timms	1				
Macrothrix breviseta Smirnov	1	1			
Neothrix cf. *armata* Gurney	1				
OSTRACODA					
Limnocythere mowbrayensis Chapman	1				
Cyprideis australiensis Hartmann			1		
Ilyocypris australiensis Sars	1	1			
Ilyocypris sp. nov. A				1	
Alboa worooa De Deckker			1	1	
Australocypris insularis (Chapman)					1
Bennelongia australis (Brady)	1	1		1	
Candonocypris novaezelandiae (Baird)	1			1	
Cypretta baylyi McKenzie	1				
Cyprinotus edwardi McKenzie		1			
Diacypris spinosa De Deckker		1	1		1
Eucypris virens Jurine		1			
Heterocypris vatia De Deckker	1				
Mytilocypris ambiguosa De Deckker	1	1			
Mytilocypris tasmanica chapmani McKenzie		1	1		1
Reticypris clava De Deckker	1		1		
Ilyodromus cf. *candonites* De Deckker	1				
Cypericercus sp. 442	1				
Cabonocypris nunkeri De Deckker				1	
Platycypris baueri Herbst					1
Sarscypridopsis aculeata (Costa)	1	1	1	1	
Leptocythere lacustris De Deckker			1		
Kennethia cristata De Deckker			1		
COPEPODA					
Boeckella triarticulata (Thomson)	1	1		1	
Calamoecia ampulla (Searle)	1			1	
Calamoecia clitellata Bayly					1
Gladioferens imparipens Thomson			1		
Metacyclops sp. 462	1	1		1	
Metacyclops arnaudi (sensu Sars)		1			
Australocyclops australis (Sars)	1				
Halicyclops cf. *ambiguus* Kiefer			1		
Mesocyclops brooksi De Laurentiis et al.		1			

Continued

Taxa	Wetland				
	1	3	5	2	4
Eucyclops australiensis Morton	1				
Apocyclops dengizicus (Lepechkine)					1
Mesochra baylyi Hamond			1		
Mesochra ? flava Lang					1
Nitocra sp. A		1	1		
Cletocamptus deitersi (Richard)			1		
Onychocamptus bengalensis (Sewell)		1	1		
Schizopera clandestina (Klie)			1		
AMPHIPODA					
Austrochiltonia subtenuis (Sayce)	1	1	1	1	1
Antipodeus sp.			1		
ISOPODA					
Exosphaeroma sp.			1		
Haloniscus searlei (Chilton)					1
DECAPODA					
Palaemonetes australis Dakin			1		
HEMIPTERA					
Saldula nr *brevicornis* Rimes		1	1		
Saldidae				1	
Agraptocorixa parvipunctata (Hale)	1				
Agraptocorixa hirtifrons (Hale)				1	
Micronecta robusta Hale		1	1		
Micronecta gracilis Hale	1				
Anisops gratus Hale				1	
Anisops occipitalis Breddin	1		1	1	
Anisops sp.		1			
ODONATA					
Austrolestes annulosus (Selys)	1	1	1	1	
Hemianax papuensis (Burmeister)	1		1		
Hemicordulia tau Selys	1		1		
TRICHOPTERA					
Notalina spira St Clair		1	1		
Oecetis sp.	1	1	1		
Symphitoneuria wheeleri Banks			1		
Triplectides australis Navas		1	1		
Leptoceridae sp.	1				
DIPTERA					
Tipulidae group C	1				
Anopheles annulipes Walker				1	
Culicoides sp. A	1	1	1	1	
Monohelea sp. A		1			1
Nilobezzia sp. A		1		1	
Nilobezzia sp. B				1	
Ceratopogonidae sp. A		1			
Ceratopogonidae sp. B	1				
Psychodidae		1	1		
Stratiomyidae	1	1	1		
Dolichopodidae	1	1	1	1	

Continued

Taxa	Wetland				
	1	3	5	2	4
Ephydridae	1	1	1	1	
Procladius paludicola Skuse	1	1	1	1	1
Procladius villosimanus Kieffer	1	1	1		
Ablabesmyia notabilis Skuse	1				
Paramerina levidensis (Skuse)	1				
Cricotopus albitarsus Walker	1				
Paralimnophyes pullulus (Skuse)	1				
Orthocladiinae sp. A	1				
Tanytarsus sp. A (nr K10 sensu Cranston)	1	1	1	1	1
Chironomus occidentalis Skuse	1	1	1	1	
Chironomus tepperi Skuse	1				
Chironomus cf. *alternans* Walker	1		1		
Dicrotendipes pseudoconjunctus Epler	1	1	1		
Dicrotendipes sp. A			1		
Polypedilum nubifer (Skuse)	1	1			
Polypedilum convexum Johannsen			1		
Cryptochironomus griseidorsum Kieffer	1				
Cladopelma curtivalva Kieffer	1		1	1	
Parachironomus sp. A	1				
COLEOPTERA					
Allodessus bistrigatus (Clark)	1			1	
Antiporus gilberti (Clark)	1	1		1	
Sternopriscus multimaculatus (Clark)	1	1	1		
Necterosoma penicillatus (Clark)		1			1
Necterosoma sp.	1		1		
Rhantus suturalis W.S Macleay			1		
Lancetes lanceolatus (Clark)			1		
Berosus discolor Blackburn	1	1			1
Berosus munitipennis Blackburn		1		1	
Berosus sp.			1		
Hydrophilidae			1		
Ochthebius sp.			1		
Heteroceridae	1		1		
Curculionidae	1				