Technical Memorandum 43

Requirements for effective biological monitoring of freshwater ecosystems

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Supervising Scientist for
the Alligator Rivers Region

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Contents

Abstract

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A comprehensive program of biological monitoring can provide assurance that management goals for water quality are being achieved. A monitoring program should include elements to provide both early detection of adverse effects, and assessment of impact in terms of ecological significance; it should aim to permit testing of hypotheses with statistical tests which have sufficient power to detect effects at some prescribed 'acceptable' level of impact. The key elements of a biological monitoring program in place to assess the effects of uranium mining in the Alligator Rivers Region in northern Australia are discussed – here the emphasis is on avoidance of detrimental change in unmodified aquatic ecosystems. Recommendations are made for a biological monitoring program for aquatic environments that have been substantially modified by agricultural practices -- here the emphasis is on amelioration of existing impacts.

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Requirements for effective biological monitoring of freshwater ecosystems

Introduction

The Alligator Rivers Region Research Institute (ARRRI) was asked by the Centre for Environmental Toxicology to contribute its expertise in biological monitoring to a discussion workshop on the effects of agricultural pesticides on aquatic ecosystems in the cotton growing districts of northern New South Wales and southern Queensland. This paper briefly reviews the rationale and practice of biological monitoring of aquatic ecosystems in the Alligator Rivers Region (ARR) of the Northern Territory, and concludes with recommendations and suggestions that may be pertinent for biological monitoring of aquatic environments in cotton growing districts.

Biological assessment of the impact of mining activity in the Alligator Rivers Region

Uranium mining and processing operations in the ARR are carried out under a regime of controls designed to protect the environment from harmful effects arising from the dispersion of mine wastes.

A major environmental concern associated with present and proposed mining developments in the ARR is the management of excess water that accumulates each year within mine sites as a consequence of the seasonal monsoonal rainfall. Such waste waters pose an environmental risk if allowed to drain uncontrolled from a site. Research at the ARRRI has focussed on the development of biological methods to provide assurance of the absence of observable detriment to aquatic organisms inhabiting streams that may receive mine waste waters. The objective of this impact assessment program is unusual, by Australian standards at least, in that it is focussed on the *avoidance* of detrimental change in what are effectively pristine ecosystems, rather than the assessment and rehabilitation of systems already degraded (Humphrey et al 1990).

The ARRRI has developed a two-fold approach to the biological assessment of effects that could result from dispersion of mine waste waters to the environment. The components of the approach are:

1 Pre-release laboratory determination of the toxicity of waste waters

Controlled release of excess mine waste water, at dilutions at or below which no observable toxic effects will occur, has been recommended as a solution to management of excess mine waste waters in the ARR. The rationale and methodology for this approach have been discussed by Brown (1986): in short, testing aquatic organisms is essential because the effects of complex and variable wastes cannot be predicted from their individual constituents. The principal features of the approach are (i) direct measurement of the change in toxicological responses of local organisms selected from widely different taxa and trophic levels to the actual waste water as it is diluted with receiving water; and (ii) the dilution ratio of the whole waste water required to render it harmless can be used as a control parameter to regulate its discharge.

2 Biological monitoring of aquatic ecosystems

When mine waste waters are dispersed into surface waters, it is essential that a comprehensive and sensitive program of biological monitoring is in place to identify, quantify and assess possible effects on organisms in the receiving waters. In the case of controlled releases of waste waters, such a program is required to confirm the adequacy of predictions derived from laboratory determinations of the toxicity of waste waters (CSIRO 1992, Humphrey et al 1990). The elements of the program of biological monitoring under development at ARRRI are briefly discussed in the following sections. Much of this material has been summarised from Humphrey et al (1990), Humphrey and Dostine (1993) and Faith et al (1991).

Choice of methods for biological monitoring

The methods to be used in a biological monitoring program depend largely on the nature of the problem. In developing a comprehensive biological monitoring program, it is important to examine both short-term (eg resulting from 'pulse' disturbances) and longer-term (consequential or cumulative) effects. The dispersive characteristics of aqueous wastes to aquatic ecosystems must be considered here and thus the program should be sensitive to effects resulting from increased concentrations in the water column and those arising from build-up of constituents in sediment. For either short-term or longer-term effects, it is important in any monitoring program that there are elements to provide both early *detection* of impact and *assessment* of impact in terms of ecological significance.

With the test objectives described above, the ARRRI has developed a variety of techniques for biological monitoring in the ARR. These studies are outlined in Humphrey et al (1990) and in Humphrey and Dostine (1993) fig 2; the methods yield data over a range of spatial and temporal scales. The broad classes of techniques used in this program may be summarised as follows.

Early detection of short-term effects

The early detection of possible effects arising over the short term, ie within the duration of a release or shortly thereafter, is important to allow adjustment of a release, or subsequent releases, within a Wet season. Early detection systems employed by ARRRI measure the whole-body responses of captive organisms to enhanced concentrations of constituents in surface waters during and after release periods. Organisms are held either in (i) containers positioned under a shelter on the creek bank and through which a flow of appropriately diluted water pumped from Magela Creek takes place ('creekside monitoring'); or (ii) containers positioned in the creek ('*in situ* monitoring'). Creekside monitoring systems have the advantage of enabling continuous observations to be made; this is particularly important during releases when associated high water levels make access to *in situ* containers difficult. Viable creekside tests have been developed for two species of freshwater snail, *Amerianna cumingii* and *A. carinata* (reproduction, early development and juvenile mortality), and two species of fish, *Mogurnda mogurnda* and *Melanotaenia nigrans* (larval mortality and growth). Humphrey and Dostine (1993) list desirable attributes of organisms used in creekside tests. Not least of these attributes is sensitivity to the toxicant of concern, representation from different phyla and/or trophic levels, choice of organisms that can be readily cultured and a good understanding of the biology of the test organism. The most successful *in situ* technique employed to date is the reproductive responses of freshwater mussels (*Velesunio angasi)* held in mesh-covered containers buried along the creek edges (Humphrey et al 1990).

The other class of studies outlined in the schema of Humphrey et al (1990) aims to detect effects which may be evident over the longer term. These studies are of two main types: (i) studies of natural communities and populations; and (ii) chemical monitoring of the biota.

Studies of natural communities and populations

Karr (1987) argues that assessment efforts 'must include ecological insight from studies of the structure and dynamics of populations, communities and ecosystems'. Thus whilst not necessarily providing prompt detection – if only for the lag in sample processing and data analysis – assessment of mining impact must ultimately refer to investigations of natural populations and communities. To this end, measurement of the attributes of communities and populations of organisms, coupled with appropriate design and analysis, provides direct evidence of the extent to which the ARRRI management objective is being achieved, ie no observable detriment to the biotic integrity of ecosystems.

There are good arguments for the need for studies on more or less discrete assemblages of organisms or 'communities' for biological monitoring (as opposed to reliance on population studies of single species). Community study offers a potentially wide range of sensitivities to toxicants; further, where there is no *a priori* basis for selecting 'indicator' species, or for considering one group of taxa more important than another, consideration of the entire community is appropriate. Of course, wherever there is concern for biological diversity, studies of communities of organisms are essential. There are other theoretical advantages in adopting a community approach to monitoring. Thus, Smith et al (1988) argued that different species (of the marine benthos) 'act as "replicates" of each other's responses enhancing the strength and detectability of the response' (to a pollution gradient). Analysis of community-level attributes such as functional organisation also offers a powerful interpretive tool when considering spatial and temporal community changes (eg Bunn 1986, Pedersen & Perkins 1986).

Community studies of benthic macroinvertebrates and fish in the ARR have been ongoing for several years. Other than study of fish community structure in billabongs, analysis of differences in fish migration patterns between years may also offer an ecosystem-level response to mining-related change. Migration counts made by the end of each Wet season (for the past 7 consecutive Wet seasons) represent an index of recruitment success of fish in breeding habitats downstream of Ranger Uranium Mine.

Chemical monitoring of the biota

The second class of study designed to detect long-term effects addresses the potential hazard to the biota resulting from the accumulation of metals in depositional areas downstream of mining activities. The approach is to determine baseline concentrations of chemical elements in tissues and organs of long-lived organisms, including fish and freshwater mussels, to allow comparisons of trends over time. Should increases occur in future, assessment of potential risks may be made by appropriate laboratory experiments.

Choice of organisms for monitoring

Hellawell (1986) presents a comprehensive appraisal of the relative merits of different groups of organisms for use in biological surveillance programs. The foremost consideration here is to identify in advance possible hazards and to select groups potentially at risk from these hazards. Humphrey and Dostine (1993) discuss the potential of the biota for monitoring in the ARR. While gill-breathing aquatic organisms (or life stages of organisms) have always been regarded by the OSS as being most at risk from water-borne contaminants in the ARR, terrestrial and semi-aquatic vertebrates with links to aquatic food chains were not regarded by Humphrey and Dostine (1993) as being at risk from dietary metal contamination.

Benthic macroinvertebrates and fish communities have been selected as the most practical and useful choices for monitoring in the ARR (Humphrey et al 1990). Macroinvertebrate communities are diverse and abundant, their taxonomy is reasonably well known, sampling methodologies are well developed, they are relatively sedentary and respond rapidly to changes in water quality. On the other hand, characteristics of fish communities may reflect the overall water quality throughout the stream rather than that pertaining at a particular site.

Design and analysis for components of monitoring programs

Any effective biological monitoring procedure used to assess potential impacts on ecosystems will require the testing of appropriate hypotheses on the effects of the purported impacts (Fairweather 1991, Humphrey & Dostine 1993). Indeed, up until recently very few monitoring studies had been conducted in Australia that incorporated practical statistical tests to detect possible human impact upon aquatic ecosystems. (See papers presented at the workshop 'Detecting Human Impacts on Aquatic Environments', University of Melbourne, November 1990, *Australian Journal of Marine Freshwater Resources* 42, and the conference on 'Environmental Biometrics', University of Sydney, December 1992, *Environmetrics* [in press].) Norris and Georges (1986) and later Maher and Norris (1990) reviewed at length the subject of design and analysis for (biological) assessment of water quality (specifically freshwaters). These authors, however, although addressing requirements for sample replication within sites, neglected to consider the framework or design in which such information could be validly applied for a test of impact.

The basic design requirements of proper control and independent replication are particularly difficult to establish in lotic systems (Faith et al 1991). Sites arranged longitudinally within a stream are linked by biological and physical processes – upstream processes may affect downstream results hence the notion of an upstream control site independent of sites downstream is problematic. A common design in stream experiments (and in monitoring exercises to detect effects arising from point source discharges in streams) uses an upstream undisturbed area as the control and areas downstream as experimental sites. In such a design replicate samples taken from each site are spatially segregated and constitute 'pseudoreplicate' – a test may reveal differences between the two sites but it cannot implicate the treatment (or effluent) as the causal factor. For the specific case of impact assessment, the two factor ANOVA model regarded as 'optimal' by Green (1979) has been criticised by several authors (eg Hurlbert 1984, Stewart-Oaten et al 1986, Underwood 1993). In this design, control and impact sites are sampled before and after an impact begins – an interaction between 'times' and 'sites' according to Green indicated that the difference between the two sites had changed between times; however, as pointed out by the aforementioned workers, the change in differences may or may not be due to an impact effect.

A design utilising a form of temporal replication has been proposed as a solution to the problem of testing for the effects of an unreplicated impact (Bernstein & Zalinski 1983, Stewart-Oaten et al 1986; Stewart-Oaten et al 1992). In the so-called BACIP design (Stewart-Oaten et al 1992) (Before, After, Control, Impact, Paired differences) samples are collected simultaneously from single impact and control sites before and after the perturbation has occurred. The difference between sampled abundances at impact and control areas at any one time is regarded as a replicate observation. The means of sets of differences between the two areas before and after are compared by a t-test or the equivalent (Stewart-Oaten et al 1992). The test assumes that the data satisfy a number of assumptions, most important of which is independence of the observed differences in a time series. Stewart-Oaten et al (1992) provide tests for checking these assumptions. The control site may be selected non-randomly such that the probability of 'large, local and long-lasting' (Stewart-Oaten et al 1986) influences which affect only one site is minimized. Selection of an upstream control site for a pollutant impact in streams would seem to be relatively straightforward – selection of control sites in more open systems (eg coastal sewage outfalls) less so.

Underwood (1993) argues that the BACIP design is inadequate to infer a causal relationship between a purported impact and observations of change in natural systems, and that multiple control sites are mandatory for such a purpose. Underwood argues that time courses of abundance of a population are rarely the same from one place to another and that therefore, there is no valid reason to suppose that any pattern of difference between two populations evident before the disturbance would continue unchanged over time if the disturbance had no effect. Stewart-Oaten et al (1986) acknowledge the possibility of a confounding effect due to a large unpredictable event or perturbation coincident with the onset of the potential impact. The present authors consider that the arguments of Underwood have less force when the BACIP design is applied to 'standardised' end-points derived from creekside tests and for data from multivariate description of community structure. Creekside testing data are constrained by the well-controlled, experimental procedure to reflect variation in water quality – any change in the temporal pattern of differences between sites can be inferred to be a pollutant effect, particularly where there are simultaneous water chemistry measurements and laboratory studies have quantified the relationship between pollutant concentration and organism response. For the multivariate case, multi-species 'replicates' would presumably cancel effects of 'meandering' time courses that could arise between sites at the population level.

It is appropriate to raise, at this stage, the issue of the use of controls for statistical design and analysis in monitoring studies. A serious disadvantage in the use of multiple controls in a particular monitoring design is the possibility of introducing a 'poorly behaved' control that serves to mask and confound a small but (ecologically) significant impact (eg Faith & Wood 1992). Power in the test may also be reduced for this reason. No doubt, this issue (use of controls) is likely to generate considerable debate by researchers in future. Thus, it can be equally (and easily) argued that there are also risks associated with reliance on single controls in monitoring studies. Additional controls increase the strength of the inference of impact – then, alternative explanations for a change in difference values require an unlikely coincidence of confounding effects. Where it is prudent to consider and incorporate additional controls, a resolution insofar as BACIP designs are concerned might be to exclude from analysis those control sites which have been shown in the pre-disturbance period to differ from potential impact sites; additional control sites would be used solely as a check against possible 'misbehaviour' of the designated control site at a critical period.

There will always be problems in selection of suitable control sites for monitoring environmental impact in lotic systems, as discussed above. Because of lack of independence amongst sites in a single stream, discrete locations upstream of the disturbance (pedantically) still represent no more than 'pseudo-controls' in any design. Selection of undisturbed sites in tributaries wherever study of non-mobile organisms is concerned, may be less problematic. Selection of true controls in separate streams, however, will raise the probability of one site, at least, behaving differently from others, hence the appeal in our recommendations for not including these in data analysis.

Other than the potential problems arising from the use of multiple controls, ANOVA designs such as that recently proposed by Underwood (1993) have other inherent problems for use as a statistical test of impact. These include the difficulty in determining the power of the test, potential problems in developing multivariate analogues and the potential for serial correlation in the time-series of raw (or transformed) abundance data.

The application of the BACIP design to creekside testing and to community-level data will be discussed below.

1 Application of the BACIP design to creekside monitoring

Creekside monitoring in Magela Creek provides for early detection of adverse effects arising from mining by evaluating lethal and sub-lethal responses of captive organisms exposed to mine waste waters. Tests are conducted at two sites on the margins of the creek: at one site upstream of Ranger where control, uncontaminated waters are drawn, and at the second site located 5 kilometres downstream, where, in the event of waste water release, 'receiving' (fully mixed) waters are drawn. As noted above, ARRRI has developed a number of viable creekside monitoring tests using two species of freshwater snails and two species of fish. The following discussion will refer to one of these tests, ie egg production in the freshwater snail *Amerianna cumingii*.

Egg production of *Amerianna cumingii* has been shown from laboratory toxicity tests to be as sensitive a response as any in other test organisms used at ARRRI, to Ranger mine waste waters (Lewis 1992). Trials under controlled laboratory conditions suggest that egg production in this species (ie mean egg numbers per pair of snails produced over a set period) has a relatively low coefficient of variation compared with six other species of snail studied. Further, the test organisms exhibit low natural mortality under field conditions – particularly during periods of high flow and associated enhanced acidity of creek waters under these conditions (cf. larval fish *Melanotaenia splendida inornata* [Humphrey et al 1990]).

Egg production at each site is measured as the mean number of eggs laid by sixteen replicate pairs of snails of a specified size over a four day period. Data from non-release periods during 1991/92 and 1992/93 Wet seasons are presented in fig 1.

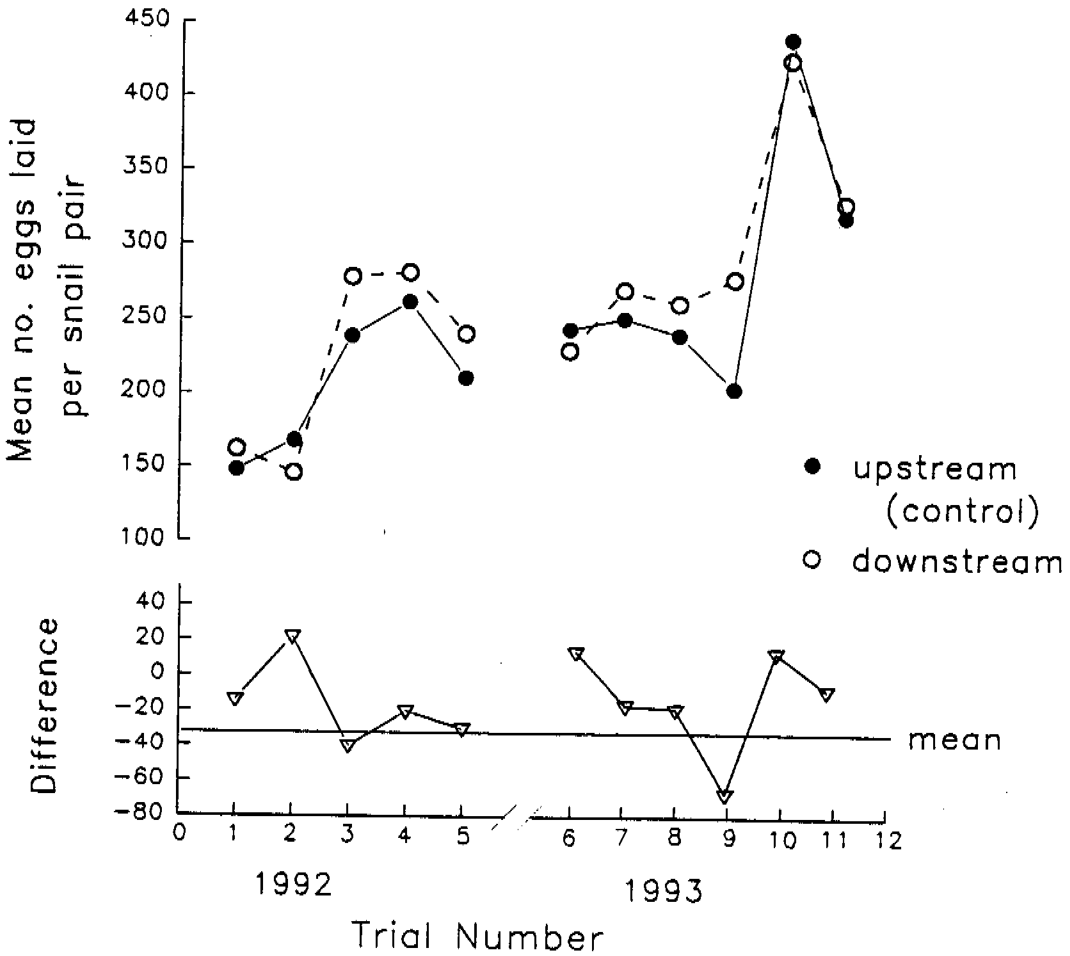

Figure 1 Egg production data for creekside monitoring trials at two sites in Magela Creek during non-release periods for Wet seasons of 1991/92 and 1992/93

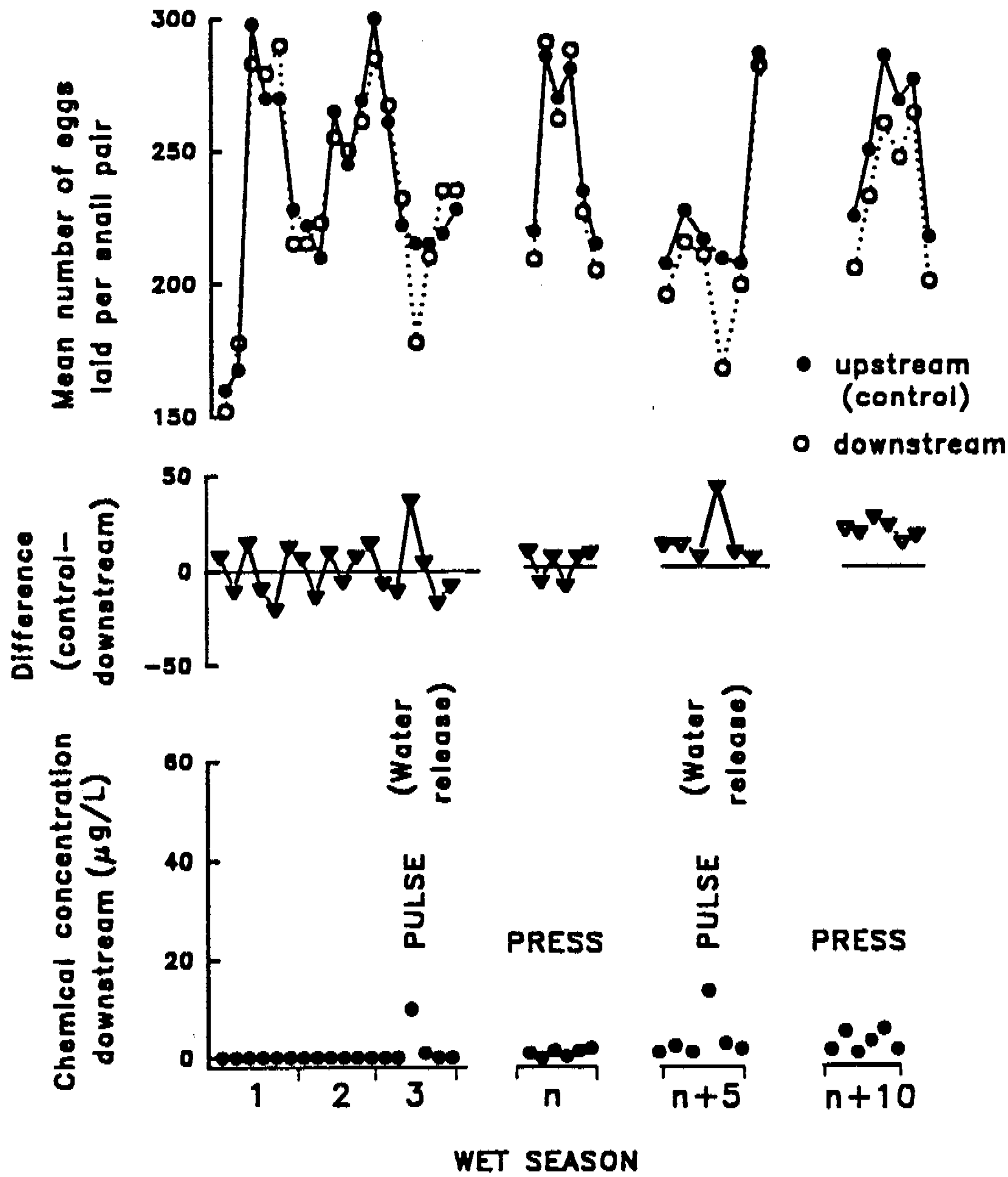

Figure 2 Application of the BACIP design to creekside monitoring in Magela Creek; hypothetical data for egg production of freshwater snails

Egg production between consecutive trials is variable; this variability may reflect changes in the properties of creek waters at different flow stages. Statistical comparison of the paired differences derived before and after impact offers a potentially powerful test for the early detection of effects due to waste substances in the water column. Figure 2 illustrates the application of the BACIP design with hypothetical data for snail egg production. An effect is detected by comparison of sets of differences for tests conducted prior to releases and during periods of sustained releases of mine waste waters. Simultaneous measurement of the concentration of the likely toxicant in downstream waters, in this case uranium, permits ready interpretation of the results.

There are two elements to the question of appropriate sampling effort. Sampling must adequately estimate mean values within both sites, and secondly, allow for the collection of sufficient replicates over time to provide for adequate power in the statistical test for impact. Sample size required at each sampling occasion to give a constant arithmetic mean can be estimated using the method described by Elliot (1977). Power analysis can be used to determine the number of temporal replicates necessary to provide acceptably high power (ie high probability of correctly detecting an effect) (Peterman 1990). Power curves describe the relationship between effect size and the sample size required to detect an effect for a given level of power. Power curves calculated for egg production data with an α value of 0.05 are presented in fig 3. To detect small increases in mean site difference in egg production a relatively large number of temporal replicates are required, eg 19 trials are required to detect an increase in mean site difference of 30 eggs per snail pair with a power of 0.9.

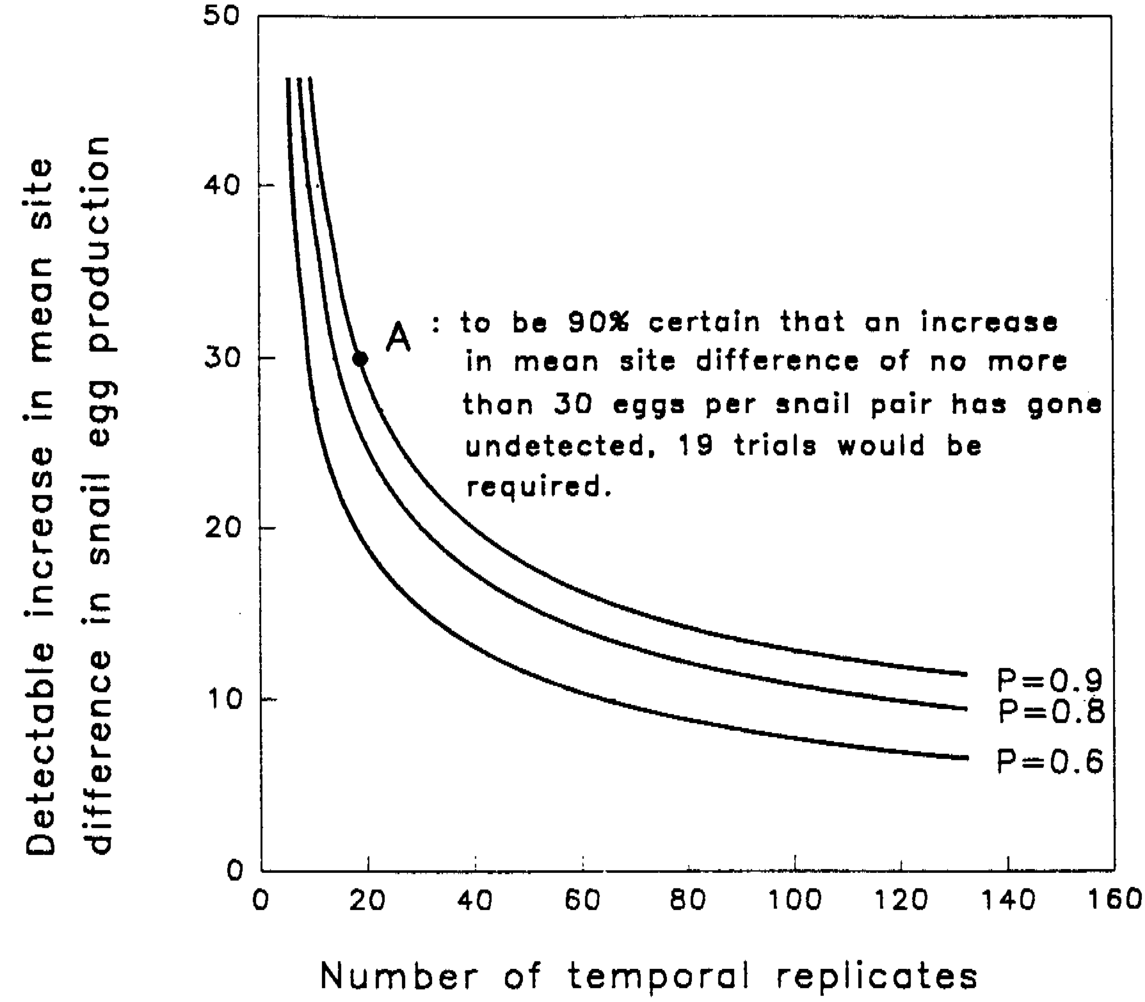

Figure 3 Power curves for snail egg production data, showing the relationship between detectable increase in mean site difference in egg production and the number of temporal replicates

2 Application of the BACIP design to community-level data

Studies of benthic macroinvertebrate and fish communities have been ongoing for several years in the ARR to provide baseline data to assist in the detection and assessment of mining-related change which may manifest over the long term. For example, benthic macroinvertebrate samples are collected from stream substrates at several sites in Magela Creek annually during the Wet/Dry transition phase (usually April) when major flow has subsided. Three 'control' sites are located upstream of mining activity, while seven potentially 'to-be-disturbed' sites are located downstream. Data from each site consist of counts of numbers of individuals of each species in each sample. Patterns within multivariate data can be explored using ordination techniques to display the relationships between samples in multi dimensional space based on their compositional similarity. Dissimilarity (or similarity) indices quantify the compositional relationships between pairs of samples, and have been suggested as a measure of difference for use in the BACIP design (Faith et al 1991). An important research issue has concerned the choice of dissimilarity index for monitoring in the ARR, ie one sensitive to changes in community composition arising from a disturbance and likely to provide greater statistical power in an analysis to detect an impact. Faith et al (1991) found from a study of benthic macroinvertebrate communities in a creek polluted by earlier uranium mining, that alternative dissimilarity indices varied by an order of magnitude in their statistical power. The BACIP requirement for independent replicates may place constraints on the frequency of sampling. Seasonal effects may limit the frequency of sampling such that several years of baseline pre-disturbance information is required to achieve the required statistical power in tests for effects; the ARRRI is actively pursuing this important research issue.

Recommendations arising from the ARRRI experience

Environmental controls

The widespread use of the organochlorin pesticide endosulfan in cotton growing districts of northern NSW and southern Queensland poses a hazard to aquatic ecosystems in these regions. Endosulfan, in its application, has been shown to be toxic to fish and other aquatic life (Bacher et al 1992). Chemicals applied to crops may enter aquatic ecosystems in surface water runoff from agricultural land and as an accidental consequence of application by aerial spraying. The toxicological hazard posed by endosulfan to the aquatic environment occurs as a sequence of recurrent 'pulse' disturbances following intermittent, large rainfall events and subsequent uncontrolled dispersion of contaminated runoff waters to streams and associated lagoon habitats. Management should aim to prevent uncontrolled dispersion of these chemicals (though total containment of excess contaminated water may not be a practical alternative). Regulators may require the formulation of site specific engineering solutions to reduce the load of contaminants entering aquatic systems. Potential solutions may include treatment of irrigated crop runoff water, or partial containment of runoff waters followed by controlled release at times when the toxicological hazard posed by these chemicals is assessed to be negligible. A program of biological monitoring in receiving waters can provide evidence of the extent to which management goals for water quality have been achieved.

Biological monitoring

Choice of organisms

The selection of organisms for monitoring should follow an objective assessment of the potential of the biota for monitoring. Choice of organisms will depend on the objectives of the program, the requirements of particular techniques, and consideration of the effects of the particular environmental hazard. As noted above, benthic macroinvertebrate and fish communities were nominated as being most suitable for monitoring in the ARR (Humphrey et al 1990). However, the apparent virtues of these groups may not apply elsewhere. For instance, Lake (1986) advised against the use of fish communities on the basis that fish communities in Australia have been modified by the presence of introduced species, and also because species richness is low. These attributes of fish communities are certainly true for the inland rivers of northern NSW and southern Queensland and thus, study of community structure *per se* may not be appropriate here.

Fish have been identified from laboratory toxicity studies to be sensitive to the pesticide endosulfan, relative to invertebrate organisms which have been tested (Chapman & Sunderam

1992) – hence a disproportionate level of resources might be devoted to assessing the effects of pollutant inputs on fish populations (as opposed to fish community structure, see above). Other taxa may have attributes which recommend them to particular techniques, eg ease of husbandry, low handling mortality and toxicological sensitivity. It is essential that additional research is conducted to satisfy the husbandry requirements of, and to provide sufficient background biological information upon, sensitive species and/or life stages.

Monitoring studies of benthic macroinvertebrate communities in the ARR aim for detailed taxonomic identification of samples to the level of species (Humphrey & Dostine 1993). This requirement may not be necessary elsewhere, particularly if the emphasis is not on the detection of subtle changes. The taxonomic level required to provide sufficient statistical power at reasonable cost needs to be considered.

Choice of methods

Solutions to monitoring requirements in the cotton growing districts of northern NSW and southern Queensland may differ substantially from those developed for the ARR. Programs for the latter focus on the avoidance of detrimental change in particular streams, rather than, say, the amelioration of impacts over a broad geographical area. Nevertheless, some of the monitoring approaches adopted by ARRRI may in part be valid for particular applications. There appear to be three classes of study necessary for effective monitoring of the effects of pesticide contamination upon aquatic ecosystems: (i) definition of the extent of the problem, (ii) evaluation of the success of control measures to diminish the toxicological hazard at the source, and (iii) description of the response of broad-scale systems (eg within and amongst stream catchments) to variation in the levels of such contamination. These are discussed in turn below.

Temporal and spatial extent of pesticide contamination

Organisms which accumulate organochlorine pesticides in body tissues (eg freshwater mussels (Ryan et al 1972, Storey & Edward 1989) and fish (Bacher et al 1992)) can be used as indicators of the spatial and temporal extent of pollution and hence identify zones and times of particularly high concentrations of bio available pesticide. (Though other techniques may be applicable in determining the spatial extent of pesticide contamination including the incidence of mouthpart deformities in chironomid larvae (see Warwick et al 1987), and skeletal deformities in frogs (see Tyler 1983; 1991), bioaccumulation provides an unambiguous indication). Broad-scale patterns in residue levels can be assessed relative to differences in management practice (thereby indicating where remediation efforts need to be concentrated) and will assist in selection of control sites, or at least allocation of sites to classes of relative impact.

Studies for evaluating the success of control measures

Techniques analogous to the ARRRI creekside monitoring system may be employed to assess the success of various control measures designed to reduce the toxicity of irrigation water draining from crops (eg carbon filters in irrigation channels). The BACIP design can be applied to test for effects. Factors to consider here are the adequacy of controls and the temporal replication required for sufficient power, as discussed above. Another approach to evaluate the success of on-farm control measures designed to limit pesticide dispersion would be to examine long-term change in the responses of captive organisms exposed to potentially contaminated water exported from individual management areas to aquatic systems. The ARRRI creekside procedures include relatively lengthy tests (up to 15 days) to obtain data on sub-lethal end-points for detection of impact. However, data from acute tests, eg mortality of fish larvae held in in-stream containers, may suffice in the waterways of the cotton growing districts of NSW and Queensland for assessment of the effects of pesticides in the water column. The BACIP design is not applicable in this case.

Broad-scale studies

The short-term effects of endosulfan, at least, can be investigated via appropriately designed experimental studies to allow powerful tests of relevant hypotheses. Ideally, this would involve controlled replicated studies at a range of spatial scales: from mesocosm studies (albeit with some caution, see below) to whole system (eg billabong) manipulations as discussed in the final section below. Green (1989) presents a design appropriate for any spatial scale incorporating multiple independent control and 'to-be-disturbed' sites from which samples are collected prior to and after impact (on at least one occasion), and the variable of interest is 'change between before- and after-impact times'. (This represents a 'transposition' of the BACIP design – with independent spatial rather than temporal replication.) Green (1989) outlines details for power analysis for both a univariate design and a multivariate extension. An alternative multivariate approach to this design, of course, would be to apply dissimilarity measures as the descriptor of difference for each site between times. This approach would allow a large number of variables (species) to be used, as for the BACIP design. Power analysis is required to determine the replication needed to detect, with high probability, the prescribed level of impact. Power analysis may indicate that the planned study cannot achieve sufficient power for sensitive statistical tests of null hypotheses (Bernstein & Zalinski 1983).

The above design assumes that both treated and untreated sites can be measured, both before and after treatment. But, it may be that, although treated and untreated sites are available (or even a range of degrees of treatment), there is no 'before' measurement. On this occasion the treatment effect may be implicated if, when the site-samples are ordinated (eg see Faith & Norris 1989), a significant gradient corresponding to the treatment is recovered. (If required, an approximate guide to the number of points (sample sites) required to identify a correlation with a given level of significance can be estimated using conventional statistical tables.) Ter Braak (1989) has popularised this gradient approach to monitoring, and discusses applied examples.

Field experiments and related issues

Field experiments provide the most persuasive evidence of causal links between purported impacts and observations of change in natural systems (Keough & Quinn 1991, Underwood 1991). Further, field experiments allow identification of the biological variables most sensitive to perturbation and hence most useful in subsequent monitoring. Variation of the levels of experimental impact enables predictions to be made of the behaviour of systems at a level of impact beyond that currently observed.

Congruence between field experiments and observed changes in natural systems increases confidence in attributing changes to observed environmental perturbations (Cooper & Barmuta, in press). For example, Clements et al (1988) used stream mesocosm experiments to predict macroinvertebrate community responses to heavy metals in natural streams.

Although mesocosm studies, such as enclosures in lakes or artificial streams, offer ease of replication and manipulation, they may introduce artefacts which limit the extrapolation of results to natural systems. For instance they may incorporate little of the heterogeneity inherent in natural systems, and exclude large mobile organisms and predators, or limit immigration. They may be colonised by a resilient unrepresentative fauna which underestimates the responses of natural communities to toxicants. Other problems include the multiplicity of potential end-points (an increase in Type I error rates is one consequence), temporal phasing differences between replicates and inappropriate test design (Smith & Mercante 1989). Mesocosm experiments are regarded as being useful in delineating direct toxic effects on organisms, but less useful in determining long-term, large-scale effects which may arise through trophic interactions (Cooper & Barmuta, in press). Caution should be exercised if the results are to be applied to the setting of water quality standards; indeed, recognising the above potential problems, the US Environmental

Protection Authority no longer accepts mesocosm data for this purpose (Baird D, pers comm). The alternative is to manipulate natural systems such as, for example, billabongs. Large-scale manipulations are costly and difficult to replicate; there may be ethical constraints limiting the type of manipulation; and poor replication may provide insufficient power to adequately test hypotheses. Nevertheless, suitable approaches to the design and analysis of unreplicated whole-ecosystem experiments have been developed and these are discussed by Carpenter (1990).

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