6

Monitoring ecological change in wetlands

QUANTITATIVE ASSESSMENT OF MANGROVE INVERTEBRATE FAUNA

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ABSTRACT

Assessment of mangroves habitats in northern Australia is nearly always in response to development proposals in or near the coastal zone which may have an impact on coastal biota and habitats.

Thus mangrove surveys are usually undertaken over short periods of time, and in the more remote areas of Australia are typically performed by one or two specialist biologists working as consultants. The resultant species list of flora and fauna almost always then reflects the specialisation of the biologists who collected the data.

Regulatory agencies have the unenviable task of trying to determine the type, probability and scale of any impacts on mangrove habitats from a proposed development. The agencies are also charged with the responsibility of determining how acceptable any impact may be. This can only be done by assigning some 'value' to the mangrove resources under potential impact. Assignment of 'value' can only be made by reference to other mangrove habitats.

As there is no standard method of assessment how is it possible to compare the data for different stands of mangroves and arrive at a 'value'?

Recent research in Darwin, the Gulf of Carpentaria and the Kimberley is presented which shows that one metre x one metre quadrat sampling of the mangrove invertebrate fauna of the forest floor is a useful technique for comparative assessment of mangrove faunas on both regional and local scales.

Keywords: mangroves, invertebrates, quantitative sampling, Darwin, Gulf of Carpentaria, Kimberley.

1 Introduction

Mangroves are a conspicuous and dominant feature of the intertidal zone along most of the northern Australian coastline. Mangrove habitats are best developed in sheltered embayments or estuaries where fine sediments with a high organic content are trapped and where some freshwater influence occurs annually.

The mangrove flora of northern Australia has now been relatively well documented and few new species of flora are to be expected, but the same cannot be said of mangrove fauna, where much work remains to be done. There have been substantial advances in our knowledge of the mangrove fauna of tropical Australia in recent times and although much of this new information has yet to find its way into publication, it is now possible to identify many of the species associated with mangroves by reference to collections held in museums.

There are obvious and consistent pantropical trends in mangrove floras which show a decline in productivity, diversity and structural complexity with increasing latitude (Saenger & Snedaker 1993). On a local scale, gradients in productivity, diversity and structural complexity are often found across the intertidal zone. This is due to the combined and interrelated influences of highly seasonal rainfall, high pan evaporation rates, high soil salinities and frequency and duration of tidal flooding.

We might expect that the fauna associated with mangroves exhibit these same regional and local trends and it is widely assumed that within Australia there is an increase in diversity (and biomass) of mangrove fauna with decreasing latitude (Hutchings & Saenger 1987). The data upon which this view is based are almost entirely qualitative and there has been little uniformity in the methodologies used to collect data on mangrove fauna.

Usually, mangrove surveys are undertaken over short periods of time, and in the more remote areas of Australia are typically performed by one or two specialist biologists. The resultant species list of flora and fauna almost always then reflects the specialisation of the biologists who collected the data. A crustacean taxonomist will produce a comprehensive list of crabs, but will often fail to even see any of the birds exhaustively documented by an ornithologist in the same mangrove.

It is impossible to compile a comprehensive list of all the fauna associated with a mangrove habitat in a short period of time. Sampling of large mobile elements of the fauna is both difficult and expensive. The degree of difficulty and expense increases as the size and structural complexity of the mangrove increases, particularly as many of the best mangrove stands in northern Australia are in very remote areas.

Are there techniques for sampling mangrove fauna which are fast, accurate, and relatively inexpensive?

Recent research in Darwin and the Gulf of Carpentaria has shown that 1 m x 1 m quadrat sampling of the mangrove invertebrate fauna of the forest floor is a useful technique for comparative assessment of mangrove faunas on both regional and local scales.

Why use the mangrove invertebrate fauna of the forest floor? There are three major reasons for selecting this component of the fauna for quantitative assessment.

- There are usually just tens of species and hundreds of individuals in each 1 m x 1 m quadrat. Therefore identifications and counts of abundance are completed quickly and cheaply and the materials needed to collect, fix and preserve the organisms are not bulky or expensive.
- The fauna of the forest floor is sedentary. After settlement as a larva, or arrival as a juvenile most of the species do not appear to move very far. Therefore the sampling effort required to produce accurate measures of diversity, abundance and biomass is much less than that usually required for larger and more mobile fauna such as birds and pelagic fishes.
- Most of the sedentary mangrove fauna of the forest floor are herbivores or detritivores feeding directly on the fallen leaves and other litter from the mangrove trees above them or feeding on the decomposing organic matter rotting on the forest floor. The majority of resident carnivores on the forest floor are feeding on the resident herbivores and detrivores. Therefore it is a reasonable expectation that the estimates for diversity, abundance and biomass of a mangrove fauna are indirect estimates of the primary productivity at that site, although it is important to recognise that tidal export of organic material from mangroves in the form of litter and detritus can remove significant proportions of the primary productivity from a site.

In this paper the results of studies on the mangrove fauna of Darwin (Hanley 1993a), the McArthur and Roper Rivers in the Gulf of Carpentaria (Hanley 1993b, Hanley & Banks 1995), and the southern Kimberleys (Hanley 1995a,b) are compared for evidence of regional variations in faunal diversity (species richness).

2 Methods

Mangroves at four different locations Darwin, McArthur River, Roper River, and King Sound were surveyed to determine the species richness of the mangrove invertebrate fauna of the forest floor.

At each location four creeks were selected, or in the case of the King Sound samples, small island embayments supporting mangroves. At each of the four creeks or islands, two sites were selected in what is known as tidal creek bank vegetation - or its regional equivalent. Unfortunately, due to time and budget constraints it was not possible to sample at two sites on each of the four creeks surveyed in the McArthur River region.

For a full description of habitats sampled, the rationale behind site selection, the quadrat method used and the collection preservation, identification and sorting of fauna see (Hanley & Couriel 1992, Hanley 1993a,b, 1995a,b, Hanley & Banks 1995).

Quadrats were $1m^2$ and were placed randomly within suitable habitat. Five replicates were sampled at each site and the data compiled and analysed using Statgraphics Plus on a 486 DX2-66 IBM pc clone.

All material collected has been accessioned into the permanent collections of the Museum and Art Gallery of the Northern Territory. Comprehensive species lists of the fauna collected at each location are available in Hanley (1993a,b, 1995b).

3 Results

A comparison of the results for species richness per square metre in the mangroves of Darwin, the Gulf of Carpentaria (McArthur, Roper), and the southern Kimberley (King Sound) is provided in table 1.

All of the data in table 1 were compiled using the same quadrat sampling methodology. Although the structural diversity and other floral characteristics vary considerably between the 4 study sites, all of the sites were located at about the same height on the shore and are considered to represent examples of optimum mangrove development - for each particular region.

The estimates of species richness of mangrove fauna per square metre range from a low of 6.45 for mangroves at the mouth of the McArthur River to a high of 12.20 for mangroves in Darwin Harbour.

When the means for the regions and creeks/islands within each region are subjected to an analysis of variance (table 2) the results indicate highly significant differences in the mean species richness between locations and between creeks/islands.

Table 1 Mean number of species per quadrat estimated for 4 locations and 16 creeks or island embayments (data compiled from Hanley 1993a,b, Hanley & Banks 1995)

Level	Quadrats	Mean	Std. Err	95% confidence limits	
Grand Mean	100	8.59	0.39	7.82	9.36
A: Location					
Darwin	40	12.20	0.56	11.09	13.30
McArthur	20	6.45	0.85	4.76	8.13
Roper	40	7.42	0.56	6.32	8.53
King Sound	40	7.23	0.56	6.12	8.33
B: Creek/Island					
Sadgroves	10	14.8	1.12	12.60	17.00
Reichardt	10	12.3	1.12	10.10	14.50
Bleesers	10	12.2	1.12	10.00	14.40
Hudson	10	9.5	1.12	7.30	11.70
Rosie	5	4.3	1.60	1.12	7.48
Pine	5	6.7	1.60	3.51	9.88
Home	5	9.10	1.60	5.91	12.28
Mule	5	5.7	1.60	2.51	8.88
Nayarnpi	10	10.6	1.12	8.40	12.80
Last	10	5.9	1.12	3.70	8.10
Towns	10	7.1	1.12	4.90	9.30
Roper	10	6.1	1.12	3.90	8.30
Sunday	10	4.4	1.12	2.20	6.60
Tallon	10	7.9	1.12	5.70	10.10
Whirlpool	10	6.7	1.12	4.50	8.90
Talbot	10	9.9		7.70	12.10

Table 2 Analysis of variance for mean species richness at 4 different locations in north Australia

Source of variation	Sum of squares	Degrees of freedom	Mean square	F-ratio	Significance level
Main effects					
A:Location	695.53	3	231.84	19.088	0.0000
B:Creeks/Islands	523.58	12	43.63	3.592	0.0002
C:Sites	177.50	12	14.79	1.218	0.2797
Residual	1360.40	112	12.15		
Total (corrected)	2757.00	139			

A multiple range test of the differences between means for each of the four locations shows that the number of species per square metre is significantly higher in Darwin mangroves when compared to the other three locations. The estimates of species richness for King Sound mangroves are very similar to those reported for both the Roper River and McArthur River regions of the Gulf of Carpentaria.

4 Discussion

A definite latitudinal gradient exists in Australia, with respect to mangrove floristic characteristics, such that diversity and productivity both increase with decreasing latitude (Duke 1992, Hutchings & Saenger 1987, Saenger & Snedaker 1993).

The species richness data for mangrove fauna from the King Sound region shows no significant difference from the results collected from that region of the Gulf of Carpentaria which lies at about the same latitude. Both the Kimberley and Gulf regions appear to have significantly lower species richness per unit area when compared to the mangrove invertebrate data recorded from the Darwin region. This suggests that the fauna associated with mangroves may also show a latitudinal gradient in species richness (ie diversity) in which species richness increases with decreasing latitude.

Estimations of the predicted primary productivity of mangroves in the three regions of Darwin, King Sound and the lower Gulf, derived from equations provided by Snedaker and Saenger (1993) suggest that the primary productivity of mangroves in the Kimberley and Gulf region should be about 0.6 of the primary productivity of Darwin mangroves. It is important to recognise that these comparisons apply to mangroves occupying similar positions on the shore in each of the three regions, as there are substantial gradients in productivity across the intertidal zone in each region.

This figure of 0.6 is interesting when compared to the ratios between the estimates of species richness of mangrove fauna which have been calculated for each of these three regions. The ratio between the estimated mean species richness for the Kimberley and the Gulf regions is very close to 1, as is the ratio for predicted primary productivity between the two regions.

With a mean species richness per square metre of 7.23 in the southern Kimberley and one of 12.20 in Darwin the ratio is 0.59. When the estimated mean species richness for the Roper river is compared with that of Darwin the ratio is 0.61. The ratio for mean species richness per square metre on the McArthur River when compared with Darwin is 0.53.

This similarity in ratios between estimates of primary productivity and species richness suggests that estimates of species richness may serve as useful approximations of the underlying primary and presumably secondary, productivity of particular stands of mangroves.

While the data show that there are substantial regional differences in the mean species richness of mangrove invertebrates, it is also true that substantial local differences are also evident. Therefore it appears that the technique might be useful in assessing the relative productivity of a mangrove on both local and regional scales.

However, more data is required across a greater range of latitude and sampling is planned for the Exmouth region (22° S) later this year. Given that rainfall plays a large part in determining mangrove primary productivity, sampling of the mangrove invertebrate species richness along the north-east Australian coast is also required.

Current research in Darwin aims to determine the relationship between primary productivity (leaf litter fall) and secondary productivity (species richness, abundance, biomass) in tidal creek bank mangrove forests. This project is expected to run five years and will provide the opportunity to construct and test predictive models of primary productivity based upon mangrove invertebrate diversity, abundance and biomass data.

The Darwin study is sited on Darwin Harbour, which as a ria system, is one of the two major geomorphological settings which typically provide a broad range of mangrove habitat types in northern Australia.

The second major geomorphological setting which supports a broad range of mangrove habitat types in northern Australia are the large macrotidal rivers such as the South Alligator, the Daly, the Victoria etc.

The infrastructure available at ***eriss***, and its proximity to several large macrotidal rivers provides an opportunity to extend the type of study currently underway in the Darwin region to encompass this second major geomorphological setting.

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SOME CONSIDERATIONS AND REQUIREMENTS FOR MONITORING AND ASSESSMENT PROGRAMS FOR FRESHWATER ECOSYSTEMS OF NORTHERN AUSTRALIA

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ABSTRACT

The development of a biological monitoring program to assess possible impact upon aquatic ecosystems arising from mining in the ARR has been underway at the ***eriss*** *since 1987. The same key principles that have dictated the development of this program must also be considered in programs developed for other management issues in the wetlands of tropical northern Australia. These general principles are discussed in relation to the findings and philosophies derived from the present* ***eriss*** *(mining impact) program.*

Attributes required of biological indicators will differ depending upon the role of the indicator. For monitoring of change (= early detection), rapid responses, robust in time and space, and sensitive to the impact are important. For assessment of impact (= ecological significance of any observed change), selection of populations and communities of organisms that reflect effects at the ecosystem level is paramount. Apart from appropriate choice of indicators, three other factors are required, or must be developed, to underpin a successful monitoring and assessment program: (i) research (knowledge and understanding of ecosystem structure, process and function), and for planned developments (ii) environmental management objectives that are in concordance with the principles of Ecologically Sustainable Development, and (iii) the need to define a level of 'acceptable' impact. Finally, long-term (decadal and longer) monitoring has a key role to play in the management of ecosystems; the various roles and virtues of long-term monitoring, particularly that conducted in protected reference sites, are espoused.

Keywords: biological monitoring, impact assessment, ESD, long-term monitoring.

1 Introduction

The ***eriss*** has been involved with development of a biological monitoring program to assess possible impact upon aquatic ecosystems arising from mining in the Alligator Rivers Region since 1987. Full descriptions of this monitoring program have been presented in Humphrey et al (1990), Humphrey and Dostine (1994), Dostine et al (1993), Humphrey et al (in press), Humphrey (1994), Humphrey et al (1995) and Faith et al (1995). This paper focuses on how the same important principles that have led to development of this program must also be considered in programs developed for other management issues in the wetlands of tropical northern Australia. These general principles will be presented by way of findings and philosophies arising from the present (mining impact) program. Important issues to be raised in the paper include (i) selection of indicators; (ii) other factors required or that must be considered, particularly in assessing the significance of impact; and (iii) how long-term (decadal and longer) monitoring has a key role to play in the management of ecosystems.

By way of defining terms, *environmental monitoring,* an essential tool for the management and protection of the wetlands of tropical northern Australia, is the regular assessment of the environment to see how effective management is in keeping impacts within acceptable limits. Monitoring and assessment are concomitant where *assessment* is the feedback of monitoring results to management, and interpretation of monitoring results to enable managers to decide on appropriate action.

Monitoring and assessment are discussed here with reference to the health or 'integrity' of ecosystems - as a consequence, for example, of threats such as habitat loss/degradation, spread, check and effects of exotic species, chemical contaminants or climate change. (The line of investigation for monitoring and assessment of a resource or a species of high conservation value will generally be more straightforward and direct than that required for ecosystem health.) Dealing essentially with the monitoring of changes/impact upon the biota of ecosystems, it follows that monitoring will employ, foremost, biological methods to assess change or disturbance. It is acknowledged, however, that measurement of other environmental variables (eg water chemistry) is also required for comprehensive monitoring.

2 Selection of indicators for monitoring and assessment programs

Not every facet of an ecosystem can be measured in a monitoring and assessment program (nor is it necessary to consider this), and hence 'indicators' must be selected for this purpose. Indicators serve two primary purposes: firstly, to detect change at a very early stage before substantial and significant impact has occurred (Humphrey & Dostine 1994, Cairns et al 1993), secondly to address the inevitable 'so-what?' questions if changes in an early warning response are detected, ie what is the significance to ecosystems of the observed change (Humphrey et al 1995)?

For *early detection*, the indicator should meet at least three important criteria. Firstly, it should be shown to be sensitive to the impact. This can be determined either by precedence elsewhere for similar types of impact (eg through a literature search), by hazard or risk assessment using, for example, toxicity testing data, or using a 'top-down' approach (Cairns et al 1993) in which ecosystems disturbed by similar types of impact are surveyed and note taken of elements of the biota missing as a consequence of the disturbance. Secondly, the indicator should respond rapidly, and for this, sublethal responses as possible precursors of change at higher levels of organisation may be appropriate. Thirdly, the indicator should demonstrate some degree of constancy in time and space, ie the measured response does not introduce such excessive variation to an appropriate design and statistical test for impact that the test has little power to detect an impact, even when one has occurred.

In the ***eriss*** program, 'creekside' systems are used to measure the responses of early detection indicators to creek waters that might contain mine wastes. Containers holding test animals under a creekside shelter are exposed to water pumped up from the creek. One of these systems is located upstream of the Ranger mine site and draws control waters while another is located several kilometres downstream of the mine and in the event of water release would draw mixed, receiving waters. One of the creekside tests is based on a particularly sensitive, sublethal response of the freshwater snail, *Amerianna cumingii*, namely egg production. This response is measured in the numbers of eggs produced by replicate pairs of snails over a four-day period. Hence, the test fulfils the requirements of 'early detection' and sensitivity to the waste waters of concern (Humphrey et al 1995).

Humphrey et al (1995) presented egg production data of snails over a time series of tests conducted over three Wet seasons. The tests were conducted outside of periods of release of mine waters with data for both upstream control and downstream 'to-be-disturbed' sites. Briefly, the principle of a statistical test for impact is to compare upstream and downstream data over a time series, before and after impact. This requires that in the baseline, pre-impact phase of data collection, there is constancy of the responses between the two sites, ie the data from the two sites track one another. Humphrey et al (1995 figure 2) demonstrated such constancy in the snail egg production data. The response, therefore, is robust in time and space and would contribute high statistical power in any test for impact - fulfilling the third important criterion for selection of 'early detection' indicators. Not all species studied in creekside systems, however, 'behave' as well as *A. cumingii* in such constancy of response between sites (Humphrey et al 1990, 1995).

For *assessment of the ecological significance of any observed change*, the indicator should be shown to be a good surrogate for changes occurring at the ecosystem level. Studies of communities of organisms can meet this need, providing the sensitivity of the particular group to the impact of concern is no less than for other assemblages (Humphrey et al 1995, Faith et al 1995). 'Keystone' taxa, important in structuring ecosystems, that might be directly or indirectly affected by the disturbance might also be useful surrogates. Alternatively, an 'early detection' indicator could be used if linkages to higher-level responses could be made; however, such linkages may be difficult to establish (see Humphrey et al 1995). In the ***eriss*** mining impact program, macroinvertebrate and fish communities are used as 'assessment' indicators, these groups having relatively high species richness and known to contain elements sensitive to metal contamination. Data on these groups should offer the best means to assess the significance of mining-related effects on ecosystems as a whole (Bishop et al 1995, Faith et al 1995, Humphrey et al 1995).

3 Assessment of impact

3.1 Research: knowledge and understanding of ecosystem structure, process and function

Apart from the appropriate choice of indicators, other factors are required, or must be developed, to underpin a successful monitoring and assessment program. The first requirement is research that would increase knowledge and understanding of ecosystem structure, process and function. The reader is referred to the paper by Pidgeon and Humphrey (this volume) who discuss the role of research in biological monitoring programs. An important factor raised by Pidgeon and Humphrey (this volume), namely, that a sound research base can help in developing and assessing different management models, is expanded upon below.

3.2 Environmental management objectives and principles of Ecologically Sustainable Development

The need for environmental management objectives that take into account principles of Ecologically Sustainable Development is essential wherever there are planned developments. For any environmental protection regime, the monitoring and assessment program that is developed must be underpinned by criteria against which the adequacy of the protection measures will be judged. (See also McBride, this volume.) Of interest here is the way in which an environmental management objective can be formulated in an ESD framework, on the basis of assessing the degree of ecological significance (or harm) of impacts.

In the Supervising Scientist's current revision of environmental management objectives for mining at Ranger, two ESD principles that are directly relevant to ecosystem protection have been incorporated, namely:

1) The precautionary principle
This requires firstly, that biological criteria are included in any control regime, and secondly, that management intervention takes place in response to 'triggers' from 'early detection' indicators (to prevent irreversible environmental damage).

2) The conservation and maintenance of biological diversity
This requires firstly, use of assessment indicators, ie community-based monitoring; secondly, adequate baseline data in an appropriate statistical design framework to guarantee that an impact no greater than a prescribed amount would go undetected; and thirdly, as a logical consequence of this, some definition of a limit of acceptable change.

3.3 Determining a level of 'acceptable' impact

Can a level of 'acceptable' impact be defined, or re-expressing this: is it possible to quantify and define a level of impact that is 'ecologically sustainable'? We believe a resolution to this issue may be possible when experimental designs are expanded to incorporate data from a broad geographical range (ie for streams, information from adjacent streams and/or beyond). Thus, the observed change must be viewed in the perspective of undisturbed (or reference) sites and also if available, sites disturbed by a similar type of impact from elsewhere. (See Humphrey (1994) and Humphrey et al (1995) for a more complete account of this topic.) Examples of such broad-scale designs include:

- Predictive modelling, eg the basis of the national Monitoring River Health Initiative being conducted throughout Australia (using stream macroinvertebrate communities). This approach has been successfully applied in the UK (eg Wright 1995).
- Index of Biotic Integrity applied in some parts of the USA (Karr 1993).
- Disturbance gradients derived from multivariate ordination and applied to particular types of disturbance in the marine environment. In particular, Warwick and Clarke (1993) in an analysis of production data from marine benthic invertebrates, derived a gradient of disturbance due to oil pollution over broadly-distributed sites from the north-east Atlantic shelf. In a multivariate ordination the sites ranked clearly from 'undisturbed' to 'very disturbed'. Thus for a particular site, the question could be posed: 'where does the disturbance of interest lie when superimposed on the derived disturbance gradient, eg is the impact 'trivial' (lying towards or amongst the space of undisturbed sites) or 'severe' (lying amongst those sites clearly polluted by similar types of disturbance)?' We need to determine whether a similar approach could be applied in freshwaters to specific types of impact.
- The ***eriss*** has recently expanded its (Magela Ck) monitoring using macroinvertebrate and fish communities to include data from several control streams. Data from a range of undisturbed sites in adjacent streams can subsequently provide a 'yardstick' by which to characterise natural variation in community structure.

Some of these broad geographical approaches have the potential to address the issue of 'ecological sustainability'. It might be possible, on the basis of natural variability of undisturbed sites in similar types of streams (in concert with data from disturbed sites), to define boundary limits of 'acceptable change' then use this to prescribe an appropriate change of the assessment indicator for use as environmental criteria in planning and in experimental design.

4 The role and value of long-term monitoring programs

The final part of this paper considers the need for long-term - 10 years and longer - monitoring programs. There have been moves recently in Australia to develop a national framework for the long-term monitoring of biodiversity to fulfil international agreements (Redhead et al 1994, Marine Environment Conference 1995). One idea that is emerging from workshops on the topic is the establishment of long-term reference sites in a number of key areas around Australia, preferably in protected areas, that would establish baseline conditions and enable natural variability to be described. Obviously, coordination would be required between data sets from protected areas and areas of concern (disturbed, to-be-disturbed). Long-term monitoring programs should always be set up initially, to address an issue, ie they shouldn't be collected in the absence of a suitable hypothesis. Refinement, of course, can follow.

Although problems surround the establishment and implementation of long-term monitoring programs (eg assuring interest and continuity beyond the average time of residency of the scientist at a particular institute - see also Cullen (1990) and McComb and Maher (1990)), such programs are, nevertheless, extremely valuable and with careful planning and design, should be instigated to address the important management issues for northern wetlands. Some of their virtues for inland waters have been described by Cullen and Lake (1994) and for the marine environment at the Marine Environment Conference (1995). These and other desirable features of such programs are listed here:

- Provide a baseline (i) to separate natural variation from human-related changes, (ii) to detect and quantify major changes, and responses to manipulation and recovery - especially
  - *slow processes* (succession, population dynamics of long-lived species eg *Melaleuca* affected by salt-water intrusion, riparian vegetation affected by grazing cattle)
  - *extreme events* (severe flooding, drought)
  - *episodic events* (El Nino)
  - *complex phenomena* (interacting factors such as impact of introduced species)
  - *subtle processes* with high variability, evident as trends over long time scales and where controls may be absent
- Observe the performance, sustainability of a resource.
- Provide a serendipitous source of 'inspiration', address issues at short call (answer the unexpected), allow formulation of testable hypotheses about functioning of ecosystem processes and indicators. The classic example here is Gene Liken's work at Hubbard Brook which proved the occurrence of acid rain in North America.
- Fulfil international obligations: early evidence of global change, monitor biodiversity and status of (endangered) populations and communities.

- Test assumptions behind broad-scale models for monitoring (eg temporal constancy important for predictive modelling and repeated-measures designs), see Humphrey et al (1995) and Faith et al (1995).
- Provide the framework in which results from small-scale/short-term studies or surveys can be viewed.
- Lead to improved awareness of ecological time scales.

5 Conclusions

The extensive experience of the ***eriss*** in developing monitoring programs to assess the impact of mining in northern Australia has meant that it is well placed to advise upon, initiate and conduct monitoring programs - alone or in collaborative or cooperative partnerships - for other management issues in the Wet-Dry tropics of Australia. The important factors that must be considered in planning for and implementing such programs have been discussed above. Final comment is reserved for long-term monitoring needs. As discussed above, there is expert opinion in Australia of the need to establish long-term reference sites in a number of key areas around Australia, preferably in protected areas, that would establish baseline conditions. Parts of the ARR would be ideally suited to meet this need for wetlands of the Wet-Dry tropics. Some studies on fish and macroinvertebrates of ARR streams are particularly valuable in this respect as they extend now for longer than five (up to ten) years. One of these studies, benthic invertebrates of the upper South Alligator River is, in fact, contributing important information for the current national Monitoring River Health Initiative (ie testing the assumption of temporal constancy important for predictive modelling).

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ECOLOGICAL IMPACT OF CONTAMINANTS ON WETLANDS: TOWARDS A RELEVANT METHOD OF RISK ASSESSMENT

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ABSTRACT

Assessing the potential impact of contaminants on aquatic ecosystems has routinely been achieved by the use of standardised laboratory toxicity tests. Existing toxicity test procedures suffer from significant shortcomings, however, including a lack of generality regarding their predictive power, particularly when dealing with environmental conditions which deviate significantly from those prevailing in tests, and in an inability or unwillingness on the part of those producing such tests to deal with the problem of contaminant bioavailability. Organisms in nature occupy a wide range of environmental conditions and habitat types, and this is embodied in the use of the niche concept by ecologists. Clearly, niche will determine the routes by which organisms are exposed to contaminants, and must be considered when predicting the possible ecological impacts of such chemicals on natural food webs.

Protection of wetland systems from the adverse effects of contaminants presents a unique problem to ecotoxicologists, not least because such systems are often regarded as sinks for waste, including contaminants. Thus while such systems are widely regarded as buffers to retard the wider spread of contaminants, it must not be forgotten that accumulation of materials within these systems could impair their future function. Furthermore, this accumulation has implications for trophic transfer and consequent transport of material out of the systems by mobile species. We need to be more flexible in our approach to dealing with contaminants in wetlands, particularly in a shift away from general measurements of toxicity (animal-in-a-bottle methods) to more site-specific approaches, which deal with impacts on local species, under realistic local exposure scenarios. In particular, the role of sediments and suspended particulate matter as sources of and sinks for contaminants requires further investigation.

Keywords: ecotoxicology, risk assessment, wetlands, contaminants

Ecotoxicology: getting beyond dead bugs in jars

In dealing with the impacts of contaminants on natural ecological systems, ecotoxicologists have only just begun to address the problem of biological diversity. Despite recent efforts to improve and synthesise our understanding of interspecific variation in response to exposure, the database of information currently available for use in chemical risk assessment is still largely biased towards aquatic organisms in general, and one or two species in particular (eg *Daphnia, Oncorhynchus*). While such data at least provide a benchmark in determining chemical hazard, they only provide a starting point in any site-specific risk assessment.

From an ecological standpoint, the current approach to risk assessment advocated in Europe and North America suffers from a bias towards those species (see above) and environments (temperate agro-ecosystems) which is driven by the short-term needs of regulators in those regions, rather than by any strategic, scientifically-based goal. In addition to the problem of interspecific variation in sensitivity under standard laboratory conditions (eg Baird et al 1991, Siriwardena et al 1993, Forbes et al 1995), there is an additional problem regarding the lack of generality in exposure scenarios used in hazard assessment. The test media used tend to be moderate/hard waters with neutral-alkaline pH, low dissolved organic carbon and no particulates, producing data which inadequately assess the potential toxicity of a compound in natural waters, which may be acidic, of low buffering capacity and contain substantial amounts of particulate and dissolved carbon. Together, these shortcomings in the hazard assessment process have traditionally been offset by the use of so-called 'safety factors' when employing hazard data in risk assessment. However, the explosion of interest in the science of ecotoxicology in the last few years has opened up the dubious practice of safety factors, largely advocated by regulators rather than scientists, to closer scrutiny.

In order to advance the practice of ecological risk assessment, there is an urgent need to subject the above areas to detailed systematic scientific enquiry. Moreover, there is also a need to re-assess the whole practice of hazard assessment, which is biased towards assessment of median lethal concentrations, to allow it to be used in a more precise ecological context - with a greater emphasis on nonlethal effects such as growth, reproduction and behaviour.

How can we use toxicity tests to generate ecologically-useful information?

When making an assessment of the risks associated with the use or release of a particular chemical in a specific location, what is clearly needed is information pertinent to the species which occur within the area. In managed conservation areas such as national parks, for example, it may be appropriate, indeed essential to provide information on the toxicity of a chemical (eg a herbicide used in weed management) to local species - carrying out laboratory studies where possible. In addition, it may be necessary to study local conditions more closely, in order to assess the appropriateness of the laboratory data obtained to predict impacts in different microhabitats, where bioavailability of the test compound may differ from the laboratory situation. If the species present within the system are accorded different degrees of conservation value, eg if there is an endemic or endangered species which requires an extra degree of protection, it may be appropriate to develop management models which use ecologically-relevant endpoint data available in tests to predict response to exposure at the population level, integrating toxicity information with other environmental factors. In this way, ecotoxicologists, working closely with ecologists, can design toxicity tests which are ecologically relevant to the prevailing situation. This approach is discussed in more detail below.

Wetlands: pollutant sinks or conservation havens?

Despite their intrinsic conservation importance, wetland areas are increasingly valued in terms of the 'services' they provide for neighbouring ecosystems (Turner & Jones 1991). Indeed, wetlands adjacent to human settlements are often tacitly regarded as sinks for the wastes produced by towns and cities. While this has been a powerful and persuasive argument for their conservation, since it can easily be seen to have direct economic value, it is clearly also a dangerous one, since wetlands effectively become surrogate landfill sites. While it is true that wetlands obviously have a tremendous capacity to accumulate and store chemical wastes, including contaminants, the concept of assimilative capacity of such systems is rarely addressed, except perhaps in terms of their capacity to store nutrients and some trace metals (Finlayson 1994 & Finlayson et al 1986).

What is even more surprising is that the potential fate of accumulated contaminants, and the possibility of transfer out of the system—for example, by migratory birds accumulating material through the diet—has rarely been studied. In the published studies carried out on this subject, the results obtained have ranged from those in which accumulation of material has clearly detrimental effects on the ecosystem and its associated fauna to others in which it is difficult to see any obvious effect beyond that of simple contamination, although in the latter case, this may be due to the relatively short duration of the experimental studies, which generally run for less than one year.

Where purpose-built wetland filters are concerned, despite the upsurge in interest in this topic in recent years, and a considerable amount of published research (eg Cooper & Findlater 1990), the picture which emerges is again that little is known concerning the ultimate fate of contaminants entering such systems: instead, the emphasis has been on how to best construct efficient filters, and on producing black-box models of nutrient flux. In short, there is a clear lack of information regarding the sustainability of the structure and function of wetlands when they receive significant contaminant inputs. In addition, we have failed to consider how to manage these systems after they have reached their assimilative capacity. While short-term conservation gains may be had by advocating wetlands as contaminant sinks, it is doubtful whether such arguments have a place in a serious conservation strategy.

What are the contaminant risks facing the wetlands of Kakadu, and how can they be assessed?

Kakadu National Park is one of the most important wetland conservation sites in Australia, and has international significance, as evidenced by its current World Heritage Site status (Finlayson et al 1988). Despite its splendid isolation, Kakadu still faces numerous threats to the long-term sustainability of its ecosystem, both from the direct impacts of man's activities (mining, tourism) and through indirect consequences (invasive species and their control). Of these, perhaps the greatest emphasis, from an environmental management standpoint, has been given to avoiding the adverse consequences of uranium mining. The immediate management issues arising from uranium mining have now largely been addressed and an environmental monitoring program has been developed by the Environmental Research Institute of the Supervising Scientist (***eriss***), composed of laboratory and field *in situ* tests, together with a comprehensive community-level monitoring program (Humphrey et al 1995 & Bishop et al 1995). However, despite its isolation, Kakadu is not immune from developments around its borders, and other contaminant issues continue to encroach on the park, such as the need to control invasive weed species using chemical spraying. For example the spread of *Salvinia molesta*, an invasive macrophyte species originating in South America, has resulted in the need to investigate possible control by spraying with a surfactant-based mixture (Finlayson et al 1994). Impacts on the local fauna have been studied at ***eriss*** using the same ecotoxicological test methods originally developed for the monitoring of uranium mining impacts (see below) - illustrating the versatility of the methods developed. What is certain is that the threats to the ecological integrity of Kakadu are likely to remain, and increase in the future, particularly regarding the impact of tourism, which will inevitably increase as the 'Top End' continues to develop - contaminant issues related to tourism include release of toxic hydrocarbons (eg PAHs from boat-engine and motor oils), and surfactants. These and other issues necessitate a more flexible, site-specific approach to the assessment of risks from contaminant exposure than that normally used in 'generic' environmental regulation, where information is required to be applied across all environments. The major difference, and indeed the key advantage of the site-specific approach is that the data generated are immediately relevant to the site in question. This approach can be summarised in four key points:

- use local species, where possible/appropriate,
- study local environmental conditions more closely, in particular, paying attention to those factors which might influence contaminant bioavailability (eg pH, suspended solids load),
- use ecologically relevant test endpoints, preferably those which relate to population parameters (eg mortality, reproduction, developmental time),
- use the information derived from the local environment to design tests relevant to the field situation of concern.

How does the *eriss* approach differ from other ecotoxicology research programs?

Uniquely, the ecotoxicology research group at ***eriss*** has had the scientific advantage of being able to focus its research on a single scientific issue: the impact of uranium mining and its associated activities within the Alligator Rivers Region (including Kakadu National Park). In employing aspects of the approach to risk assessment outlined above, a series of aquatic toxicity tests has been developed using only local species, and has been designed to assess the hazard associated with potential release of runoff water stored on the ERA Ranger uranium mine site into Magela Creek..

Following the controversial decision to allow water release (stored in retention pond 2) to the creek during the 1994–95 Wet season, a monitoring program was initiated. However, it was never actually fully utilised as due to social pressures no water was released from this pond. Prior to these decisions being made, water from the pond was collected and used to run a series of single species check-monitoring toxicity tests. Testing was carried out from December 1994 to March 1995, providing independent verification of results from ERA environmental lab. The results were consistent with tests run in previous years, and the need for such an independent toxicity testing facility was highlighted by a cross-check of laboratory methods and analyses.

Conclusions and future directions

It is clear that environmental managers faced with having to deal with site-specific problems of contamination are finding the information available from generic laboratory tests to be of limited value. There is an urgent need to develop methods of site-specific hazard assessment which are more flexible, and use available data as a starting point to develop more relevant water quality guidelines for their own particular area. The approach developed at ***eriss*** is a good example of how this can be achieved, although it is clear that further work of this nature is necessary to establish methods of dealing with site-specific problems of environmental variability and its effects on the bioavailability of contaminants to local flora and fauna.

Highly-seasonal wetland areas such as Kakadu pose a particular challenge, since the environmental conditions vary greatly in space and time, posing the problem: how do we establish limits for the laboratory test environment? The answers to this and other questions raised here will have relevance for the management of not only the wetlands of Kakadu, but for other wetland areas of the wet-dry tropics.

That such management strategies are urgently required is best illustrated by the fact that the bulk of the population in the world's tropical areas live adjacent to wetland areas (eg the Sunderbans in Bangladesh). These areas are being drained and exploited at an ever-increasing rate, yet are expected to continue to absorb contaminants, due to spiralling economic development and rising populations. Despite this, our understanding of the assimilative capacity of these systems is poor, although widespread impairment of ecosystem function seems almost inevitable. In order to remedy this situation, action is urgently required to study the role of wetlands both as sinks and sources of contaminants, and in particular, what are the critical ecosystem functions which must be maintained in a sustainable management policy.

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BEHAVIOURAL RESPONSES OF THE TROPICAL FRESHWATER BIVALVE *VELESUNIO ANGASI* EXPOSED TO URANIUM

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ABSTRACT

Uranium has been identified as the prime potential contaminant downstream of the Ranger Uranium Mine, in the Magela Creek, Alligator Rivers Region, Northern Territory, Australia, as a consequence of mine waste water releases. The freshwater bivalve Velesunio angasi *is ubiquitous and abundant throughout the Magela Creek catchment. Bivalves are well known to respond to adverse chemical contaminants by isolating their soft tissues from the aquatic medium by valve closure. The sensory acuity and associated repertoire of behavioural response can be employed to assess subtle effects exerted by chemical contaminants, such as uranium, that ultimately can influence the survival of an aquatic organism. Therefore, the valve movement behaviour of* V. angasi *was measured during exposure to varying levels of waterborne uranium, in the context of using the valve movement behaviour of this bivalve species to indicate the biological significance of elevated uranium water levels. The results indicate that several components of the valve movement behaviour of* V. angasi *provide quantifiable and ecologically interpretable sub-lethal endpoints for the rapid and sensitive evaluation of waters containing elevated levels of uranium.*

Keywords: behavioural response, bivalve, *Velesunio angasi*, biosensor, freshwater, uranium, mine waste water, Magela Creek, Ranger, water quality.

1 Introduction

Uranium has been identified as the prime potential contaminant, from a toxicological perspective, downstream of the Ranger Uranium Mine (RUM) in the Magela Creek, Alligator Rivers Region, Northern Territory, Australia, as a consequence of mine waste water releases (Noller 1991). Since mine waste waters entering the Magela Creek would flow into the river systems of Kakadu National Park, which has been included on the World Heritage List (as well as being inscribed to other international agreements, eg International Convention on Wetlands), any releases from the Ranger mine site need to be carefully controlled to avoid environmental detriment. Moreover, since uranium occurs in physico-chemical forms that may be readily transported and redistributed within the Magela Creek (Noller & Currey 1983, Johnston et al 1988), they pose a potential hazard to populations of aquatic organisms exposed to mine waste waters. However, few studies have investigated the biological responses of aquatic organisms, particularly invertebrates, exposed to uranium (see Holdway 1992, Hyne et al 1993).

The freshwater bivalve, *Velesunio angasi* is ubiquitous and abundant throughout the Magela Creek catchment (Humphrey & Simpson 1985), and its filter-feeding habit ensures passage through the body of large volumes of water on a daily basis, containing metals in several physico-chemical forms. However, bivalves are also known to minimise the exposure of their soft tissues to the aquatic environment by valve closure, when exposed to waters containing unsatisfactory levels of chemical contaminants, such as trace metals (eg Doherty et al 1987, Sálanki et al 1991). The sensory acuity (via specialised sensory regions including the osphradium) and associated repertoire of behavioural response can be employed to assess subtle effects exerted by chemical contaminants, such as uranium, that may ultimately influence the survival of an aquatic organism. As hazard assessment (ie concentration-response) tools, behavioural studies reflect sublethal toxicity and often yield a highly sensitive estimate of the lowest observable effect concentration (LOEC) (Little 1990). Valve movement behaviour is one of the more sensitive biological early response measures (BERM) to a variety of aquatic contaminants, in comparison with those used in other aquatic animal phyla (see review by Kramer & Botterweg 1991). Indeed, rapid biological assessment techniques form an important beginning in a hierarchical testing scheme to assess the ecological risk associated with environmental contaminants (Forbes & Forbes 1994).

As part of a biological monitoring program for Magela Creek, Humphrey et al (1990) identified a requirement for employing aquatic organisms, such as *V. angasi*, as rapid early response systems of contaminants present in mine waste waters. Moreover, the regulatory authority of the RUM, the Northern Territory Department of Mines and Energy, has stipulated that biological toxicity testing should be conducted prior to any mine waste water releases, as a supplement to water chemistry data, commencing from the 1993-94 Wet season. Therefore, this study aims to investigate the usefulness and biological significance of the valve movement behaviour of the freshwater bivalve *V. angasi* in evaluating waters containing elevated concentrations of uranium, relative to background, under experimental conditions, that are predicted to occur in the receiving waters of the Magela Creek. This paper provides a summary of the methodology used to measure the valve movement behaviour of *V. angasi* in the laboratory, as well as selected results of the behavioural responses of *V. angasi* to a variety of uranium exposures.

2 Methods and Materials

2.1 Collection, acclimation and maintenance of *V. angasi*

Specimens of *V. angasi*, covering a wide size range, were collected from Mudginberri Billabong (see figure 1 in Brown et al 1994), a minimally-impacted permanent waterbody in the Magela Creek, situated about 12 km downstream of the Ranger mine site, and air-transported to the Sydney laboratory within 24 h of collection. The animals were acclimated to a synthetic Magela Creek water (see Brown et al 1994 for composition and description) in a plexiglass aquarium (without substratum) under flow-through conditions (95% molecular replacement every 8 h) for at least 10 days before experimental use. The pH and temperature of the experimental water were regulated at 6.0 ± 0.1 and 28.0 ± 0.1°C, as described in Brown et al (1994). Photoperiod was kept constant at 12 h light:12 h dark. The selected temperature and pH represent median values derived from main wet season water quality of the Magela Creek (Humphrey & Simpson 1985, ***eriss*** unpublished data). Animals were fed at a constant rate on a diet composed of unicellular green algae and standardised aliquot volumes of an aerated cattle manure suspension. This mode of nutrition was found to be optimum in maintaining a uniform valve movement behaviour (measured in terms of the duration and amplitude of valve gape; see below) and metabolic rate (measured in terms of oxygen consumption) for specimens held under laboratory conditions for up to 10 months, and also for periods of up to 10 days after termination of feeding.

2.2 Measurement of valve movement behaviour

The valve movement behaviour of *V. angasi* was continuously measured using an on-line computer-based data acquisition system, as shown in figure 1. In summary, a fine nylon thread was attached from a clasp on the shell valve of a horizontally positioned individual and connected to a spring-loaded lever-arm of a linear variable displacement transducer (LVDT), so that the nylon thread was immediately vertical to the attachment on the arm of the transducer. The LVDT was adjusted so that the transducer arm was in a downward position when the animal was closed (at rest). Therefore, any opening response by the animal would result in an upward deflection of the transducer arm. The analog DC signal of the LVDT was digitised and acquired by a Maclab, where the acquired data is able to be manipulated using specific Macintosh software (ie Chart 3.6). An acquisition rate of 2 samples/sec was the minimum required to accurately measure the most subtle behavioural patterns. Sálanki & Véró (1969) demonstrated that the valve movement behaviour of immobilised specimens (ie where one shell valve was fixed to a substrate) of the freshwater bivalve *Anodonta cygnea* in the laboratory, did not deviate to any discernible extent from unrestrained activity under environmental conditions.

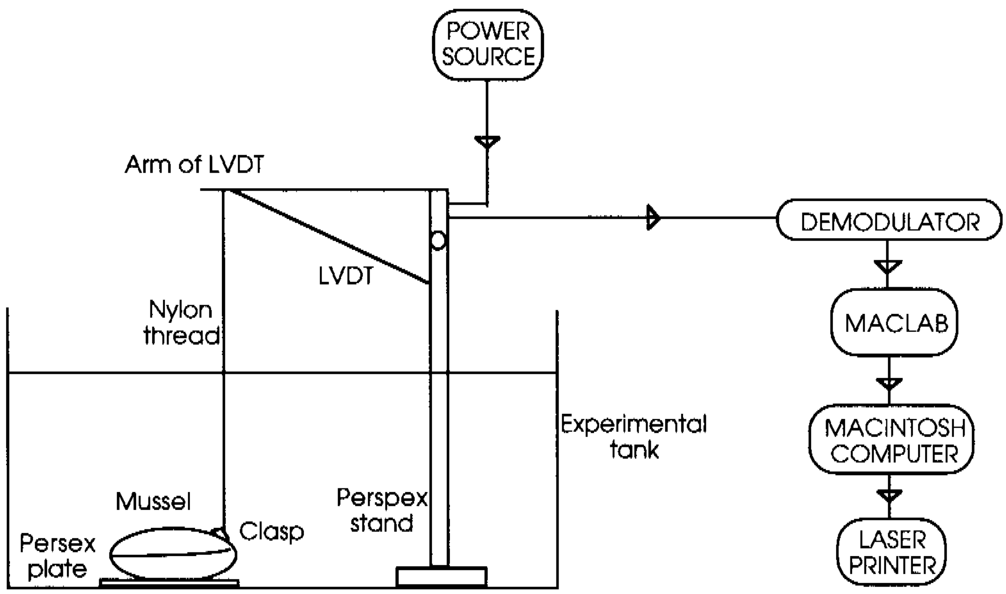

Figure 1 Schematic presentation of the experimental computer-based data acquisition system used to measure and record the valve movement of *V. angasi*. LVDT: linear variable displacement transducer

2.3 Experimental design and evaluation of valve movement behaviour

The experimental design consisted of two consecutive 48 h exposure periods. The initial 48 h period served as a control phase, in which the valve movement behaviour of each individual was measured upon exposure to a background concentration of uranium (as the uranyl ion (UO_2); 0.1 µg/L; see Section 2.4). This was subsequently followed by a 48 h exposure period to a variety of constant UO_2 concentrations (initiated by addition of a UO_2 spike to the experimental aquaria and followed by pre-equilibrated experimental water containing UO_2 at a constant concentration). Several experimental runs, consisting of two consecutive 48 h exposure periods to the background UO_2 concentration, were conducted to adjust for any temporal changes in valve movement behaviour not associated with higher UO_2 exposures. Preliminary studies revealed that 24-36 h was the minimum period necessary to adequately characterise an individual's valve movement behaviour, in the context of displaying several reproducible valve movement signatures or patterns, when exposed to a variety of UO_2 concentrations. Bivalves were neither physically handled, disturbed, nor fed during the consecutive 48 h exposure periods. Individual specimens were exposed to UO_2 at a variety of selected (nominal) constant water concentrations, ranging from 0.1 to 3000 μg/L, that were derived from preliminary range-finding experiments to best characterise the nature of the concentration-response relationship.

To evaluate the valve movement behaviour of individuals, the following characteristics were selected: the mean (i) duration and (ii) amplitude of valve gape per open period, and (iii) the frequency of phasic muscular contractions (hereby referred to as valve adductions) per hour, per open period. A typical valve movement pattern displaying these selected characteristics is shown in figure 2. Owing to the inherent variability between individuals, with respect to their duration and amplitude of valve gape, and their frequency of valve adductions during an open period, established during preliminary studies, all experiments were conducted using individuals as self-controls. An exposure index (EI) was calculated for each individual exposed to a particular UO_2 concentration, by dividing the mean response value for each selected behavioural characteristic (see above) for the second 48 h exposure period (E), by the mean response value of the respective behavioural characteristic for the first (ie control) 48 h period of exposure (C). Therefore, the E.I. = (E)/(C). An EI of 1 would indicate an identical mean value for a particular valve movement characteristic for the two consecutive 48 h exposure periods. For example, an EI of 0.10 for an individual exposed to 2000 μg/L UO_2 would indicate a ten-fold reduction in the mean value of a selected valve movement characteristic, relative to background. For each specimen, the mean value of each selected valve movement characteristic was derived from at least four periods of valve opening during each 48 h exposure period. Individuals displaying less than four periods of valve opening were not included in the results.

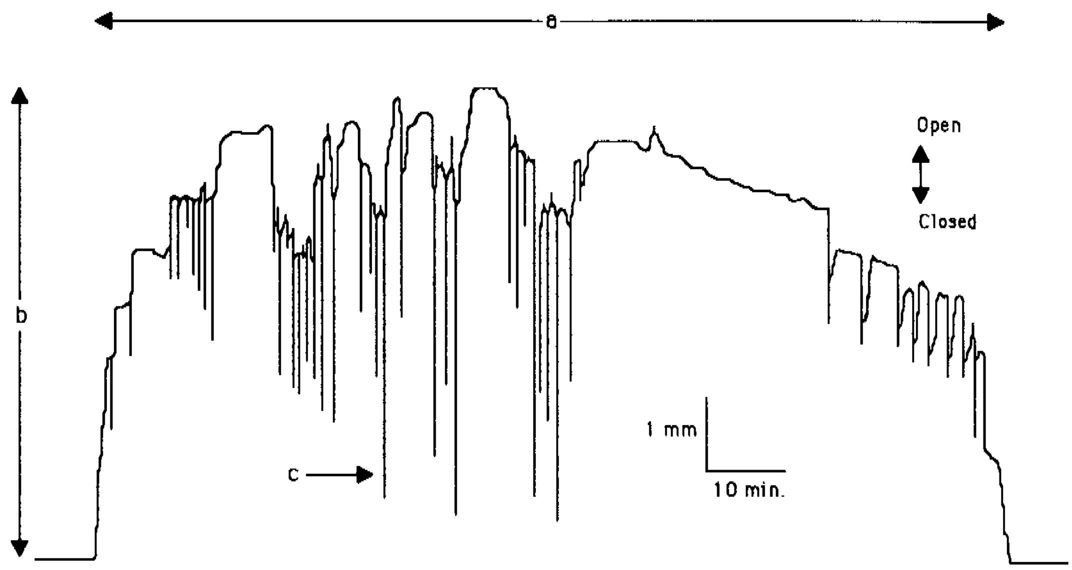

Figure 2 A typical valve movement pattern for the freshwater bivalve *V. angasi*, showing (a) the duration and (b) amplitude of valve gape, and (c) the frequency of phasic muscular contractions (valve adductions) per unit time, per open period

In addition to the continuous exposure of individuals to constant uranium concentrations over two consecutive 48 h periods, they were also tested for their rapidity of valve movement response. This test involved the addition of a uranium spike to the aquaria while an individual, exposed to the background uranium concentration, was at an appropriate point throughout an open period. Spikes were added only after 3–4 open periods had been previously characterised in the same run for an individual. This test was practically feasible due to the highly reproducible and rhythmic patterns of valve movement demonstrated by individuals exposed to the background uranium concentration. This allowed any potentially confounding interpretation of a deleterious behavioural response by an individual to the spiked uranium concentration to be minimised, via a direct comparison to previous open periods to normalise for any extraneous activity.

2.4 Chemical analysis of the experimental waters

A complete description of the analytical techniques used to measure the physico-chemical parameters of the experimental waters are described elsewhere (Brown et al 1994). The results of chemical analyses of the experimental waters showed that the mean concentrations of all ions were within 10%, usually 5%, of their nominal concentrations. Measured, not nominal, concentrations of UO_2 were used to assess the behavioural responses of *V. angasi*. Uranium may occur in the environment in several oxidation states; however, the hexavalent (UO_2^{2+}; uranyl ion) state is most likely to occur in oxidised waters, and hence, has been used to represent uranium in this study.

2.5 Statistical analysis

A four-parameter logistic model (Günther et al 1989) was employed to best fit the sigmoidal relationship between selected behavioural responses and the measured total uranyl exposure concentration. The EC_{50} (ie effective concentration of UO_2 showing a 50% reduction in the response of a particular behavioural characteristic, relative to background) was also derived from this model. The BEC_{10} (10% bounded effect concentration), an alternative measure to the NOEC (no-observed effect concentration) was estimated using the approach outlined by Hoekstra & van Ewijk (1993).

3 Results and Discussion

3.1 Exposure of *V. angasi* to UO_2

Figure 3 shows typical valve movement patterns for specimens of *V. angasi* exposed to elevated UO_2 concentrations, relative to the background. It is evident that at UO_2 concentrations greater than 350 µg/L there is an appreciable decline in both the duration and amplitude of valve gape, and an increase in the frequency of valve adductions, where these responses become more pronounced with increasing UO_2 concentration. A non-linear sigmoidal relationship was shown to exist for all three behavioural characteristics when plotted as a function of the logarithm of UO_2 concentration. These relationships were defined by a decline in the duration (figure 4a) and amplitude (not shown) of valve gape, and an increase in the frequency of valve adductions (figure 4b) with increasing UO_2 concentration. Based on both the BEC_{10} and EC_{50} values for each of these characteristics the relative sensitivity of *V. angasi* exposed to UO_2 for ~48 h can be ranked as follows: frequency of valve adductions > duration of valve gape > amplitude of valve gape. For the most sensitive valve movement characteristic (ie frequency of valve adductions) the BEC_{10} and EC_{50} were 230 µg/L (0.85 µM) and 450 µg/L (1.67 µM), respectively (figure 4b). Comparable results have been shown for cladocerans, hydra and fish from Magela Creek under similar experimental conditions (Holdway 1992, Hyne et al 1993).

The nature and rapidity of the valve movement responses of *V. angasi* to varying spiked concentrations of UO_2 during exposure to the background UO_2 concentration are illustrated in figure 5. It is evident that there is an almost immediate (ie within 30 sec) increase in the frequency of valve adductions after exposure to the spike of 350 µg/L UO_2. Moreover, this trend continues for all exposures to the spikes above 350 µg/L UO_2. However, for all spiked exposures above 350 µg/L UO_2, the time taken for valve closure after the spike is progressively reduced with increasing UO_2 concentration. For example, valve closure takes less than 15 minutes at spiked UO_2 concentrations equal to or greater than 1000 µg/L, but appears to be unaffected at UO_2 spike concentrations less than ~250 µg/L. In fact, a non-linear sigmoidal relationship provides the best fit between the time taken for valve closure after the spike and the logarithm of UO_2 concentration (not shown), a finding consistent with response time to loss of rheotaxis in rainbow trout exposed to cyanide (see van der Schalie 1986). The relatively short time between UO_2 exposure and initial response in *V. angasi* can aid in detection of adverse effects in their incipient stages. The immediate detection of environmental changes is essential for the success of any protective response. Moreover, results obtained for the rapidity of behavioural response, in terms of the time taken for valve closure after the UO_2 spike, closely mirror the behavioural response patterns measured at 48 h, indicating that the former may serve as an early-warning measure of longer exposures. The behavioural responses of *V. angasi* to exposure periods longer than 48 h are currently being investigated.

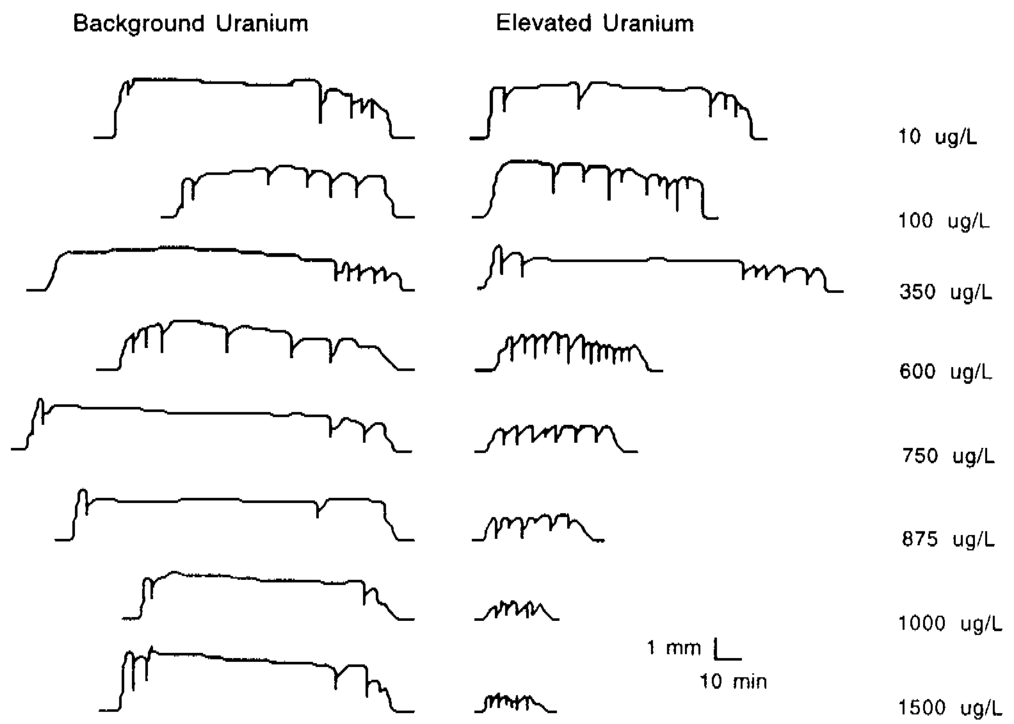

Figure 3 Typical valve movement patterns of *V. angasi* exposed to elevated UO_2 concentrations, relative to background UO_2 (0.1 µg/L) exposures

Valve closure, in response to elevated metal levels in water, effectively reduces metabolic rate functions, such as filtration rate (ie feeding potential) and oxygen consumption (Jorgenson 1990). Such short-term measures can be linked mechanistically and quantitatively to longer-term measures of organism performance, such as life-history traits ie growth and fecundity (Belanger et al 1986, Jorgenson 1990). Therefore, the behavioural responses of bivalves are measurable, adaptive features which contribute to their evolutionary fitness, and hence, can serve as sensitive, quantifiable and ecologically relevant endpoints for the assessment of environmental stress.

Based on the valve movement patterns exhibited by *V. angasi* in response to a variety of UO_2 concentrations, it is of interest to pose the following question. Are aberrations in valve movement patterns manifestations of UO_2 toxicity or simply non-detrimental physiological adaptations? Overt UO_2 toxicity was not apparent; complete valve closure was not evident even at the highest UO_2 concentration (figures 3, 4a & 4b; see below). In light of the results of numerous experiments investigating the valve movement responses of several species of freshwater bivalves to a variety of trace metals in this laboratory, it is believed that such responses are non-detrimental physiological adaptations to metal stress, induced by neurological mechanisms, an assertion supported by previous studies (see Rózsa & Salánki 1991, Salánki 1992). Like other trace metals, such as Cd, Cu and Pb, UO_2 shares a common mode of interference at nerve membrane ion channels (Lin-Shiau & Fu 1980) within the chemosensory sites of the tissue, particularly the osphradium, located on the roof of the exhalent siphon.

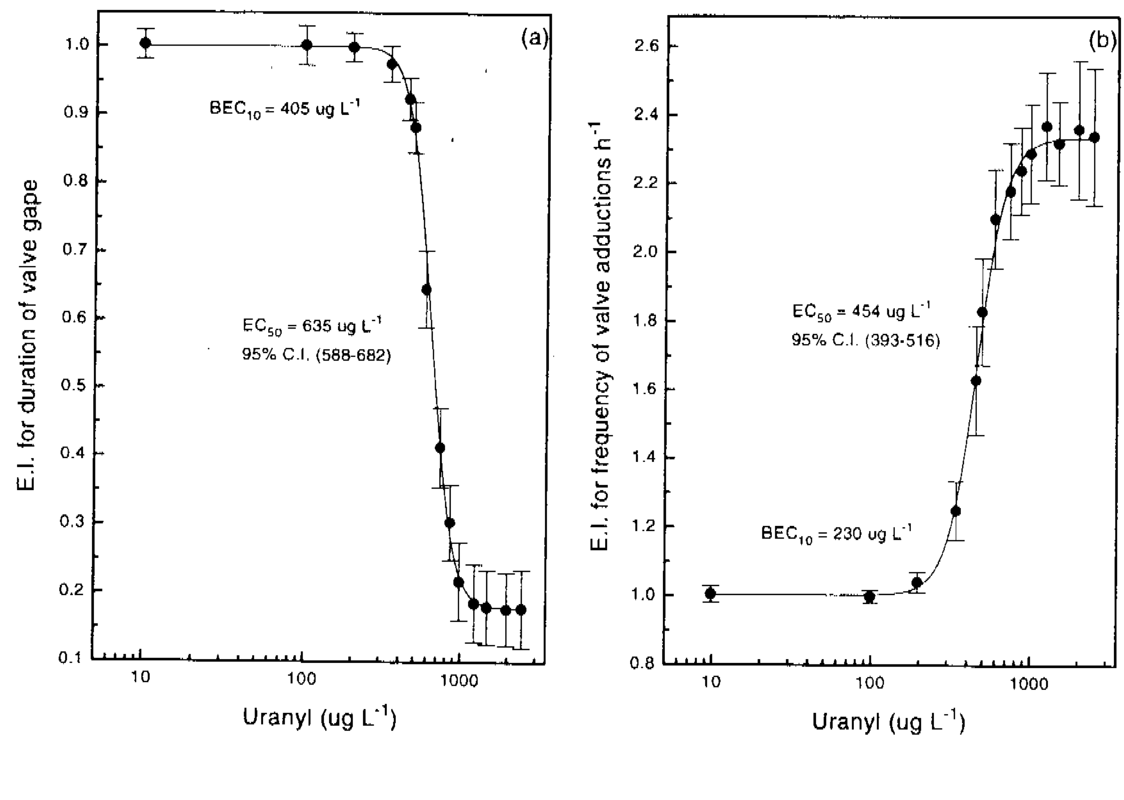

Figure 4 Sigmoidal log-linear concentration-response relationships for *V. angasi* exposed to varying UO_2 concentrations, showing meal EI values for (a) the duration of valve gape and (b) the frequency of valve adductions per hour, per open period.

Chemoreception plays a dominant role in mediating the behaviour of aquatic organisms, because chemoreceptive membranes are directly exposed to the aquatic medium at some stage of the organism's normal behavioural processes. Bivalves have evolved an adaptive, defensive response (ie shell closure) that enables them to tolerate, survive and overcome transient chemical stress in their aquatic environment. This behavioural response results in a temporal, rather than a spatial, escape. The aberrant behavioural responses of *V. angasi* exposed to elevated UO_2 concentrations, relative to background, indicates that the tolerance of their (chemo-)sensory organs and nervous system may have been exceeded, which may further lead to physiological and/or biochemical damage.

It is interesting to note that exposure of *V. angasi* to the highest UO_2 concentration used in this study (ie 3000 µg/L) was not high enough to cause complete valve closure. This is exemplified in Fig. 4a, where E.I. values for the duration of valve gape were not measured below ~0.15, indicating that a specimen of *V. angasi* exposed to 3000 µg/L UO_2 is open at least 15% of the time, relative to the background exposure. Thus, partial isolation may reduce the effects of short-term exposure to UO_2 without incurring the full metabolic disadvantage of complete valve closure. In terms of the methodology, the LVDT proved to be very sensitive to both partial and complete valve movements by *V. angasi*. Its ability to measure with great precision, the duration and amplitude of valve gape, and particularly the frequency and intensity of valve adductions, certainly enhances the ability of an investigator to discern complex behavioural responses, and more importantly, how these may alter in response to varying UO_2 exposures. Parallel studies to this have investigated (i) the effects of bivalve size and age on the behavioural responses of *V. angasi* to varying UO_2 exposures, (ii) the ability of *V. angasi* to recover (ie recovery times) to a 'normal' behavioural repertoire, after exposure to varying UO_2 concentrations.

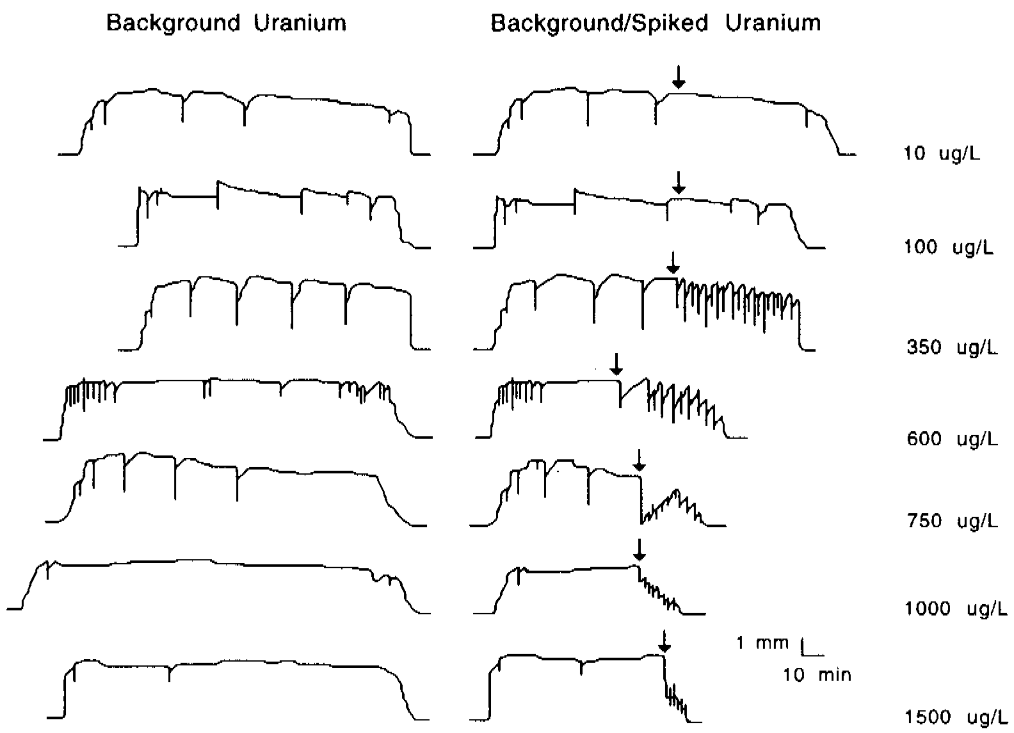

Figure 5 Rapidity and nature of the behavioural responses of *V. angasi* exposed to spiked UO_2 concentrations (indicated by arrows) superimposed on background UO_2 (0.1 µg/L) exposures

3.2 UO_2 speciation and bioavailability

To properly interpret, and hence predict, the behavioural responses of *V. angasi* exposed to UO_2 requires a thorough knowledge of its chemical speciation in solution, since it is well established that trace metal toxicity to aquatic organisms is related to the activity of certain chemical species, rather than the total metal concentration (Brown et al 1994; Markich & Jeffree 1994). Since aquatic organisms absorb only certain physicochemical forms of a metal, it is clear that water quality guidelines for the protection of aquatic life, that are based on total metal concentration, may be inappropriate. Therefore, it is necessary to investigate which form(s) of a metal are bioavailable, and to ascertain whether theoretical and analytical methods of chemical speciation are able to predict and evaluate the bioavailable metal fraction. Experimental studies have been conducted in this laboratory that attempt to interpret the response of *V. angasi* exposed to UO_2, in terms of its chemical speciation and bioavailability (Brown et al 1994, Markich unpublished results). Such studies have established that the behavioural responses of *V. angasi* are closely correlated with inorganic uranyl species, as predicted using the geochemical speciation code HARPHRQ. The addition of dissolved organic carbon, in the form of a model fulvic acid, was shown to ameliorate the behavioural responses of *V. angasi* exposed to varying UO_2 concentrations, by complexation of UO_2 with the model fulvic acid. Furthermore, the behavioural responses of *V. angasi* were shown to vary with pH where, for example, the duration of valve gape became progressively smaller for a given UO_2 concentration with a decline in pH from 6 to 5. Geochemical modelling of the speciation of uranium in solution may assist in predicting its impact on biota exposed to elevated concentrations of UO_2 in natural waters, resulting from releases of uranium mine effluent. Therefore, in the context of the Magela Creek catchment, the incorporation of biological testing, into the legislative framework for deriving acceptable dilutions for mine waste water releases into Magela Creek, will assess current legislation (ie based on chemical and hydrological criteria), and will provide an important means of assessing ecological risk. Moreover, if scientifically defensible water quality guidelines for the protection of freshwater life are to be developed for the loading of metals into the Magela Creek catchment, it is essential that consideration be given to the speciation and complexation capacity of a metal, such as uranium, in these surface waters.

3.3 Comparison of laboratory and field investigations

To identify underlying mechanisms governing biological responses in the field situation, it is necessary to establish, by laboratory experimentation, an appropriate biological response database documenting and describing the relationships between the concentrations of UO_2 and their effects on behavioural processes. One of the early problems identified in assessing the effect of environmental contamination on aquatic organisms was the lack of comparability between results in the laboratory and those in the field. One proposed solution to this dilemma was to the use of realistic water qualities to simulate environmental conditions in laboratory studies, as was done in this study. Several studies, including one from this laboratory, have shown that the use of reconstituted test waters in toxicity studies may provide a high level of realism that facilitates extrapolation of laboratory results to field situations, especially if a local species is employed. This concept is currently being investigated using *V. angasi* exposed to UO_2 in both natural and simulated Magela Creek wet season waters.

4 Conclusion

Overall, the results indicate that several characteristics of the valve movement behaviour of *V. angasi* provide quantifiable and ecologically interpretable sub-lethal endpoints for the rapid and sensitive evaluation of waters containing elevated levels of uranium. Moreover, the use of *V. angasi* to assess water quality, may minimise human value judgement with respect to the level of water quality that is satisfactory for aquatic life.

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