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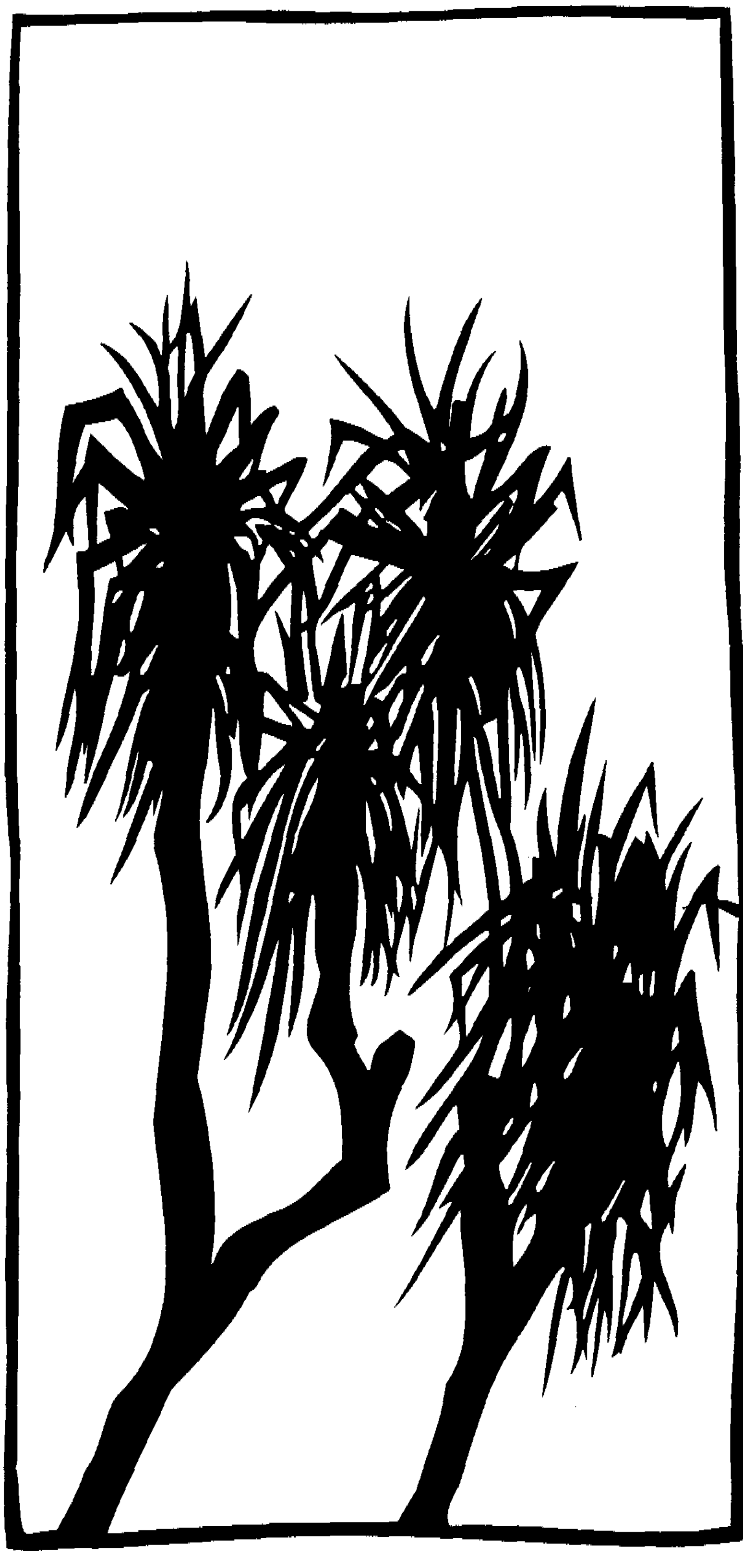

The potential of rapid assessment techniques as early warning indicators of wetland degradation

RA van Dam
C Camilleri
CM Finlayson

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supervising scientist

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RA van Dam, C Camilleri & CM Finlayson

Wetland Protection and Management

Environmental Research Institute of the Supervising Scientist (***eriss***)

Locked Bag 2, Jabiru, NT, 0886

Summary

In recent years, the need to develop assessment techniques that provide advanced warning of significant wetland stress or degradation has been recognised. This paper aimed to identify rapid, yet realistic and reliable methods for the early detection of pollutant impacts on wetland ecosystems, particularly those in the wet-dry tropics of northern Australia. In doing so, it describes the ideal attributes of early warning indicators, and their subsequent selection for wetland research. It then evaluates the potential of existing methods of assessment as early warning indicators of wetland degradation due to pollutant impacts. Particular attention is paid to rapid assessment techniques, covering a range of trophic levels and levels of biological organisation.

Due to a number of favourable characteristics, phytoplankton were considered to potentially be the most promising indicators of wetland degradation, and the scope of application of toxicity assessment and monitoring methods warrants further investigation. Rapid toxicity bioassays using invertebrates and vertebrates were also considered to be an essential part of an early detection program for wetlands, while biomarkers represented a promising tool for achieving true 'early warning' of potential pollutant impacts. Given further refinement and development, rapid methods of monitoring aquatic community assemblages were also considered potentially useful tools for the early detection of wetland degradation. Finally, to gain effective use from an early warning system for wetlands, its incorporation into an ecological risk assessment framework was recommended.

Contents

1 Introduction

Wetlands are under ever-increasing pressure from human activities. Many wetlands have already been degraded or lost through agricultural, urban and industrial development of coastal areas and inland waterways (see Dugan 1990, 1992, Finlayson et al 1992, Finlayson & Moser 1992). Recent assessments on the status, management, and future research needs of wetlands both in Australia and elsewhere recognised the need to develop assessment techniques that would provide advanced warning of significant wetland stress or degradation (Bunn et al 1997, Finlayson 1996, Finlayson et al 1997). As such, the Environmental Research Institute of the Supervising Scientist (***eriss***), situated in the Alligator Rivers Region (ARR), in the wet-dry tropics of northern Australia, is in the process of evaluating the potential of early warning indicators for predicting and assessing the extent of wetland degradation. The goal is to identify rapid, yet realistic and reliable methods of detecting potential adverse effects on wetland ecosystems, and to incorporate such measures into an ecological risk assessment framework being developed to assist wetland policy makers and managers to make considered decisions for the protection and management of wetlands.

We present an overview on wetland characteristics and major causes of wetland degradation, with specific attention to pollutant impacts, followed by a discussion on the ideal characteristics of early warning indicators and their selection. Examples of different types of early warning indicators currently in use, and their potential for predicting and assessing wetland degradation are then considered. Finally, the incorporation of early warning techniques as part of an ecological risk assessment framework is discussed. Examples from the wet-dry tropics of northern Australia are used to illustrate the applicability of such a framework for effective wetland management.

1.1 Wetland definition and characteristics in the Australian wet-dry tropics

Wetlands are exceptionally difficult to define because they form an intermediate zone, along the margins of, and hence interact significantly with, distinct terrestrial and aquatic ecosystems (Bunn et al 1997). As a result, various, and often quite different definitions for wetlands have previously been proposed (Cowardin et al 1979, Davis 1994, Paijmans et al 1985). In identifying wetland regions in the wet-dry tropics of Australia, Finlayson (1995) adopted the definition provided by the Ramsar Convention for Internationally Important Wetlands (Davis 1994). It defines wetlands as:

> *"...areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six metres".*

The deliberate broadness of the definition suits application for the wet-dry tropics of Australia due to the large diversity of wetland habitats and ecosystems present. For the purposes of ***eriss***' Wetland Protection and Management research program, Finlayson (1995) identified a range of wetland habitats within the ARR, as follows: intermittently flowing escarpment streams, waterfalls and plunge pools, intermittently flowing lowland streams, permanent billabongs within stream channels, seasonally inundated floodplains, estuaries and tidal reaches of streams, coastal mangrove swamps and salt marshes. The major features of these habitats have been described (Finlayson et al 1988, 1997, Finlayson & von Oertzen 1993, Storrs & Finlayson 1997), although it is noted that the classification of wetland types does not follow those commonly used in Australia (see ANCA 1996, Paijmans et al 1985, Bunn et al 1997) due to inconsistencies in these schemes and the uneven information base. Additionally, Storrs and Finlayson (1997) have pointed out that many wetlands in the region are physically and ecologically linked and can not be easily separated on the simple habitat basis used in the classification schemes.

In addition to the significant spatial variations associated with wetland habitats, many also exhibit large temporal variations. For example, many freshwater wetland systems are subjected to episodic or seasonal flooding and/or drying (Paijmans et al 1985, Pascoe 1993), while estuarine/marine areas with extremely high tidal influences exhibit temporal variations on a different scale. This is certainly true of wetlands in the wet-dry tropics, with annual rainfalls of up to 2,000 mm occurring almost exclusively over a 3 month period from December to February, resulting in an extreme annual cycle of flooding and drying in freshwater wetlands (McQuade et al 1996, Finlayson et al 1990), while tides of up to 7 metres influence estuaries and tidal reaches of rivers up to 70 km inland (Finlayson & Woodroffe 1996). Given this spatial and temporal complexity, it is imperative that a holistic approach is adopted when assessing potential impacts on wetlands (Storrs & Finlayson 1997). This not only requires an understanding of the nature and processes occurring within wetland habitats, but also the interactions that occur between habitats and their catchments.

1.2 Major causes of wetland degradation

The reasons for wetland degradation and loss have been the subject of much scrutiny in recent years. On a broad scale, wetland degradation and loss have been attributed to the fact that the aquatic environment has long been treated as a 'free good', available to be exploited for social, cultural and economic gain (Gardiner 1994). More specifically, Bunn et al (1997) identified four major causes of wetland degradation in Australia:

i) altered water regime,

ii) habitat modification,

iii) pollutants,

iv) exotic species.

In Australia, it is considered that the majority of wetland degradation and loss has been primarily caused by habitat modification, such as the clearing, draining or filling of wetland areas, and altered water regimes (Bunn et al 1997). These have generally been associated with the expansion of primary industries such as agriculture, horticulture, and mining, as well as urban expansion. However, along with changes in habitat and water regime, industrial and urban development also bring with them the threat of pollutant impacts on wetlands. It is these impacts, or primarily the methods for their assessment and early detection, that are the focus of the remainder of this discussion.

1.3 Pollutant impacts on wetlands

The major types of pollutants impacting on wetlands can be more or less categorised according to the human activity from which they originate, and are summarised in table 1. Mineral extraction and processing (mining) operations produce wastewaters with high levels of inorganic compounds such as heavy metals (eg Zn, Cu, Pb), arsenic and cyanide, and are a major source of aquatic pollution in the wet-dry tropics of northern Australia. Major pollutants arising from agricultural activities include excessive nutrient inputs through run-off of fertilisers, and point and diffuse inputs of a large array of pesticides (Bunn et al 1997). In addition, oil-related industries (eg oil exploration, transport, refining) are associated with contamination of waterways with petroleum hydrocarbons, heavy metals (eg in drilling muds) and crude oils and oil products in general. Areas of intensive shipping and/or boating (eg harbours, marinas) contaminate waterways with hydrocarbons and antifoulants, while urban development results in the pollution of wetlands through land and road run-off, and sewage effluent, all of which may contain many potentially hazardous substances.

Table 1 Summary of human activities and major pollutants impacting on wetlands, and the type of pollutant source

Anthropogenic activity	Major pollutants	Source
Mineral extraction and processing	heavy metals, arsenic, cyanide	point
Agriculture	nutrients, insecticides, herbicides	diffuse/point
Oil exploration/transport/refining	hydrocarbons, heavy metals, crude oils, oil products	diffuse/point
Pulp and paper inustry	chlorinated compounds	point
Boating/shipping	hydrocarbons, antifoulants	diffuse
Urbanisation and associated acitivities	land and road run-off, sewage effluent	point/diffuse

Pollutants can enter aquatic environments via point sources, or non-point (diffuse) sources into one or more wetland habitats. Regardless of the source, a pollutant in a wetland system will be transported through a range of different habitats, all of which possess various distinct characteristics which will be affected to varying extents by the pollutant, but which will also help determine the fate of the pollutant. Adverse effects on wetland habitats could be caused either directly by the pollutant, or indirectly, via pollutant-induced alterations to the processes linking the habitats (eg changes in nutrient cycling, loss of migratory species). Furthermore, delayed effects may occur as pollutants deposited in sediments are re-mobilised and transported downstream during flood events (Pascoe 1993), or alternatively, as they accumulate in sediments due to conditions of low flows.

In aiming to protect wetland ecosystems from pollutant impacts, it is desirable that effects are detected and acted upon before significant environmental impacts occur. Both Finlayson (1996) and Bunn et al (1997) emphasised the need to develop assessment techniques that would provide advanced warning of significant wetland stress or degradation. The following discussion represents the initial phase in evaluating the feasibility and potential of using early warning indicators, preferably those which provide rapid yet realistic results, to detect the onset of larger scale wetland degradation.

2 Early warning indicators

2.1 Definition and attributes of early warning indicators

An early warning indicator can be described as a measurable biological, physical or chemical response in relation to a particular stress, prior to significant adverse affects occurring on the system of interest. Importantly, it need not be directed exclusively at the biological level. That is, subtle changes in water quality, or physical parameters, such as erosion or saline intrusion can act as early warning indicators of more widespread environmental degradation. In this respect, an early warning indicator can be very much scale dependent. The underlying concept of early warning indicators is that effects can be detected, which are in effect, precursors to, or indicate the onset of actual environmental impacts. Such 'early warning' then provides an opportunity to implement management decisions to prevent serious environmental harm occurring.

Ideal attributes of early warning indicators have previously been discussed (Cairns & van der Schalie 1980, Cairns et al 1993, McCormick & Cairns 1994, Cairns et al 1994), and are summarised in a modified form, below.

To have potential as an early warning indicator, a particular response should be:

- *anticipatory*: should occur at levels of organisation, either biological or physical, that provide an indication of degradation, or some form of adverse effect, before serious environmental harm has occurred,
- *sensitive*: in detecting potential significant impacts prior to them occurring, an early warning indicator should be sensitive to low levels, or early stages of the stressor,
- *correlated to actual environmental effects*: knowledge that continued exposure to the stressor, and hence continued manifestation of the response, would eventually lead to significant environmental effects is important,
- *timely and cost-effective*: should provide information quickly enough to initiate effective management action prior to significant environmental impacts occurring, and be inexpensive to measure while providing the maximum amount of information per unit effort,
- *regionally relevant*: should be relevant to the ecosystem being assessed,
- *socially relevant*: should be of obvious value to, and observable by stakeholders, or predictive of a measure that is,
- *easy to measure and interpret*: should be able to be measured using a standard procedure with known reliability and low measurement error, while it should be capable of clearly distinguishing that a response is caused by some anthropogenic source, not by natural factors as part of the natural background (ie high signal : noise ratio),
- *diagnostic*: should be specific to a stressor, or specific group of stressors, to increase the confidence that an effect is in fact due to the stressor, or to assist in identification of the stressor,
- *broadly applicable*: alternatively, an early warning indicator should predict potential impacts from a broad range of stressors,
- *nondestructive*: measurement of the indicator should be nondestructive to the ecosystem being assessed.

The importance of the above attributes cannot be over-emphasised, since any assessment of actual or potential environmental degradation will only be as effective as the indicators chosen to assess it (Cairns et al 1993). However, an early warning indicator possessing all of the above attributes cannot exist. For example, an easily measured biochemical biomarker will provide a fast assessment of a potential impact, but without long term baseline data, the significance of the information with respect to background variation will be unclear. Conversely, a long term monitoring program will provide excellent baseline data from which small perturbations will be obvious, but may be neither time- nor cost-efficient. Subsequently, decisions will be required as to which attributes are more important for a particular purpose, and appropriate indicators chosen based on those attributes.

2.2 Selection of early warning indicators for wetlands

Economic and ecological considerations will always limit the number of indicators which can be assessed. As such, they must be selected in order to maximise relevant information and minimise redundant information (Cairns et al 1993). Therefore, prior information on the type of chemical stressor entering, or potentially entering a wetland system will be of great use when selecting indicators for their assessment. In listing a wetland site as internationally important under the Ramsar Convention, a contracting party must describe the ecological character of the wetland (Finlayson 1996). Part of this process requires the provision of information on human-induced factors that have affected or could significantly affect the benefits and values of international importance (Finlayson 1996). Such information on pollutant inputs could be utilised to aid in determining which of the biological components of an ecosystem will be affected. Decisions can then be made regarding the selection of the most suitable early warning indicators, to form an adequate 'suite' of indicators, as part of an early warning system (EWS).

In assessing wetland degradation, an indicator for one habitat may not be relevant for another habitat. Universal indicators, relevant across all habitats would be ideal for wetland research as they would standardise pollutant-related responses over spatial scales. However, this is not likely to be feasible, as, for example, a particular indicator species (eg crustacean, fish or aquatic macrophyte) may only be represented in certain habitats. In order to most comprehensively assess pollutant impacts on wetlands, indicators of ecosystem health are required that take into account, or cover a range of habitats. The term *landscape ecotoxicology* has recently been used to describe the process of examining the potential adverse effects of chemicals on biological systems over large spatial scales (Cairns & Niederlehner 1996). It focuses on assessing endpoints appropriate to the spatial and temporal scales across which a pollutant is dispersed. This is the type of approach that needs to be adopted when dealing with impacts on wetland ecosystems, given their complexity and inter-relatedness.

Another factor to consider when selecting appropriate indicators, or endpoints for assessing wetland degradation, is that of time. One of the attributes of an early warning indicator is that it be time-efficient. This is of considerable priority when assessing wetland degradation from both an environmental and management point of view. That is, environmental managers require time from the point of detection of effects, to consult with stakeholders, and if desired, implement preventative or remedial action. An effect that takes too long to detect may result in more significant environmental degradation occurring before action can be taken. Similarly, the managerial process that ensures the information is updated in a timely fashion, to enable necessary consultation and remedial actions, is essential (Finlayson 1996).

3 Examples of early warning indicators

Of the four major causes of wetland degradation described earlier, *pollution* has received by far the most attention regarding its environmental impacts and their prediction. As a result, the vast majority of biological and chemical early warning techniques and methodologies have been developed to assess the impacts of pollutants on aquatic ecosystems. Of these, some have potential application for assessing the impacts of other anthropogenically-related disturbances, such as changes in water regime, physical/habitat modification, and the introduction of exotic species. Some currently used early warning indicators are described below, and their potential for predicting and assessing pollutant impacts on wetlands are discussed.

3.1 Biological indicators

As Cairns et al (1993) noted, only biological material can indicate effects of chemical stressors in an ecosystem. Much research has focused on low levels of biological organisation, such as at the sub-organismal level (eg enzyme activities, haematological parameters), or simply on organisms from low trophic levels (eg bacteria, primary producers), with the underlying assumption being that any adverse effects at low levels of organisation will eventually be transferred to higher biological levels and ultimately manifested as visible environmental impacts at the ecosystem level. As such, an adequate monitoring program for detecting environmental effects will require the use of measures at several levels of biological organisation (Cairns et al 1993, Zakharov & Clarke 1993). It should also be noted that although the emphasis is on field assessment, laboratory-based bioassays currently remain the basis for predicting the hazard of recent or impending pollutant threats to the environment (Cairns et al 1993), and therefore are essential tools for early warning systems. Subsequently, in evaluating potential early warning indicators of wetland degradation, both the use of toxicity bioassays, and monitoring (the long term monitoring of biological and/or physico-chemical parameters) were considered.

3.1.1 Bacteria

Toxicity bioassays

Bacterial bioassays have been developed due to their simplicity, speed, cost-effectiveness, and the fact that bacteria are abundant, grow rapidly, and represent a low trophic level, and thus may provide sensitive early warning data of environmental impacts at higher trophic levels (Reteuna et al 1989, Schofield & Davies 1996). However, apart from the well-known Microtox® test, they have not been widely used for toxicity assessment (Reteuna et al 1989). Some bacterial bioassays are discussed, below.

Microtox® assesses the short-term effects (eg 15 min) of pollutants on light production by the luminescent marine bacterium, *Vibrio fischeri*, in order to estimate acute toxicity (Belkin et al 1996). It is a highly standardised laboratory assay, purchased in kit form, and highly useful for comparing pollutant toxicity over space and time. However, its sensitivity to toxicants compared to other aquatic organisms varies greatly, and hence its use as an early warning indicator may be limited. Nevertheless, it can be used, in conjunction with other bioassays, as a screening test of water quality, particularly over time and space. In addition, the sensitivity of Microtox® can be significantly increased if incubation times are extended from 5 - 15 min to 30 - 60 min (C. Blaise, pers. comm.).

While other bacterial bioassays exist (see Reteuna et al 1989, Belkin et al 1996), including modified *V. fischeri* assays (Thomulka et al 1992), it is unlikely at this stage that they could be considered superior to the Microtox® test. However, a bacterial bioassay that could be utilised *in situ*, might be valuable as a tool for assessing wetland degradation. For example, a Glucose U-^{14}C mineralisation assay, which evaluates the inhibitory effects of toxicants on the rate of labelled CO_2 released by *Escherichia coli*, or potentially other bacteria, in the presence of U-^{14}C, has been proposed as being a useful *in situ* bioassay using field organisms (Reteuna et al 1989).

Dutka et al (1989) evaluated the toxicity of Fraser River water (British Columbia, Canada) using a suite of toxicity tests, including several bacterial bioassays, and found that the standard toxicity test organism, *Daphnia magna* was a more sensitive procedure for indicating toxicity than the bacterial assays. While the theoretical basis for using bacterial bioassays to detect the onset of wetland degradation is credible, practical application appears to be lacking. A more in-depth investigation is required to ascertain whether such tools could be applied across the broad spatial and temporal scales required when assessing wetland degradation. In addition, not a great deal is known regarding key microbial processes, particularly in unimpacted systems (Schofield & Davies 1996).

Biological monitoring

Extensive research has been carried out on the abundance of bacterial organisms in the Great Lakes, and how they are affected by contaminants (Munawar & Weisse 1989, Munawar et al 1994). However, the studies emphasised that bacterial assessments should be incorporated into an holistic assessment of the 'microbial loop', the components of which include bacteria, pico- and nanoplankton and phytoplankton. The microbial loop is considered to play a key role in the pelagic food web dynamics of aquatic ecosystems (Munawar et al 1994), and is evaluated in more detail when phytoplankton are discussed.

3.1.2 Phytoplankton

Due to their nutritional requirements, their position at the base of aquatic food webs (dominant primary producer), and their ability to respond rapidly and predicably to a broad spectrum of toxicants (McCormick & Cairns 1994, Stauber 1995), phytoplankton represent perhaps the most promising early warning indicators of wetland degradation due to pollutants. In addition, their sensitivity to changes in nutrient levels make them ideal indicators for assessing eutrophication. McCormick and Cairns (1994) provide a detailed discussion of the importance of algae in, and their contribution to the aquatic food webs, as well as a detailed evaluation of algae as indicators of environmental change.

Toxicity bioassays

Phytoplankton toxicity bioassays are rapid, inexpensive and sensitive. While they are generally carried out in the laboratory using laboratory-cultured algae, bioassays have been developed using natural phytoplankton assemblages, and field, or *in situ* exposures (Munawar et al 1989, Loez et al 1995), in order to better predict effects in the natural environment.

Laboratory algal bioassays using laboratory-cultured species usually assess the effects of toxicants, or natural waters on functional endpoints, most commonly population growth rate, or cell division rate, over 3 to 4 days (72 - 96 h). Other functional endpoints often assessed, include ^{14}C uptake, respiration, fluorescence, ATP and enzyme activity (eg esterases), some of which require much shorter incubation times (eg 2 h), however, growth rate has generally been shown to be the more sensitive parameter (Stauber 1995). Laboratory bioassays are useful in providing information on physiological limits for individual species, but results are difficult to extrapolate to environmental effects. More environmentally realistic laboratory bioassays can be carried out by using natural assemblages of algae, and incorporating key environmental factors. Munawar and Legner (1993) and Munawar et al (1994) describe a sensitive laboratory bioassay using natural assemblages, known as an Algal Fractionation Bioassay (AFB). AFB assesses the effects of toxicants on various parameters (eg ^{14}C uptake, biomass) on various size fractions of algae, by isolating a diverse natural assemblage with a wide variety of algal sizes, and physiological and environmental requirements (Munawar et al 1994). Three size categories are identified and assessed; picoplankton (0.2 - 2 μm), nanoplankton (2 - 20 μm), and microplankton (20 - 200 μm) (Munawar & Legner

1993). This procedure was found to be rapid and sensitive, and could be considered for assessing the health of wetland ecosystems.

The use of laboratory algal toxicity bioassays to assess wetland degradation in the wet-dry tropics of northern Australia is currently in its developmental phase at ***eriss***. Growth inhibition bioassays (72 h) for both a freshwater green alga (*Chlorella* sp.), and a marine diatom (*Nitzschia closterium*) are currently being developed (table 2). *Chlorella* sp. is known to be represented in the freshwaters of northern Australia, while tropical strains of *N. closterium* have previously been identified in Australian waters and used for toxicity bioassays (Florence et al 1994).

Field, or *in situ* bioassays assess the effects of the actual aquatic environment of interest on local, natural phytoplankton assemblages. These can be carried out either in on-site, or mobile laboratories, or in the actual aquatic environment. The latter can be achieved using flow-through bottles, or other types of enclosures, bags, microcosms/mesocosms, and cages (Munawar et al 1989). These techniques are based on the diffusion of toxicants and nutrients through membrane filters into chambers containing phytoplankton (Munawar & Legner 1993). However, due to variability in environmental parameters, in-stream algal productivity measurements are often confounded by relatively high variances (McCormick & Cairns 1994). While on-site laboratory facilities help to minimise such variability, they cannot simulate other necessary environmental conditions, such as light variability.

Table 2 Summary of toxicity bioassays in use by the Environmental Research Institute of the Supervising Scientist (***eriss***), or currently under development, for ecotoxicological purposes in the wet-dry tropics of northern Australia

Organism	Test duration/endpoint	wetland habitats represented
Marine alga (diatom)* (*Nitzschia closterium*)	72 h, population growth	estuaries, coastal mangrove swamps**
Green alga (*Chlorella* sp.)	72 h, population growth	lowland streams - floodplains
Duckweed (*Lemna aequinoctialis*)	4 - 7 days, plant growth	permanent billabongs, floodplains
Green hydra (*Hydra viridissima*)	96 h, population growth	permanent billabongs, floodplains
Cladoceran (*Moinodaphnia macleayi*)	3 brood (~ 6 days), reproduction 24 h, feeding inhibition*	permanent billabongs
Chironomid* (*Chironomus crassiforceps*)	5 days, larval growth	permanent billabongs, floodplains**
Purple-spotted gudgeon (*Mogurnda mogurnda*)	96 h, larval survival	escarpment streams - floodplains
Black-striped rainbowfish (*Melanotaenia nigrans*)	96 h, larval survival	escarpment streams - lowland streams
In situ:		
Freshwater gastropod (*Amerianna cumingii*)	96 h, reproduction, juvenile survival	permanent billabongs, floodplains
Black-striped rainbowfish (*M. nigrans*)	96 h, larval survival	escarpment streams - floodplains

* currently under development

** presence in habitat unconfirmed, but likely.

Structural indicators have also been utilised as indicators in algal bioassays. Such indicators include species composition shifts, size assemblage shifts, picoplankton distributions and the disappearance of endemic species. In particular, picoplankton and nanoplankton have been found to be particularly sensitive to a wide range of contaminants, and may have even greater potential as early warning indicators of environmental stress (Sprules & Munawar 1986, Munawar et al 1989, Munawar & Legner 1993, Munawar et al 1994). Microscopy and flow cytometry are two techniques whereby such parameters are measured (Munawar et al 1989).

Biological monitoring

Both functional and structural measures of algal health have been utilised for monitoring purposes. McCormick and Cairns (1994) suggested that algal taxonomic analyses to the species level were desirable in order to maximise information. However, in recognising the resources involved to perform identification at the species level, indices based on genera were considered more feasible. In addition, counts of approximately 500 cells were considered an acceptable means of characterising assemblage structure (McCormick & Cairns 1994). McCormick and Cairns (1994) also suggested that taxonomic indicators be based on the assemblage of diatoms, as they are a dominant and ecologically important group in the phytoplankton of most aquatic ecosystems, while their field collection, processing and identification methods are widely utilised and accepted, and toxicity data are readily available in the literature. In addition, diatoms are known to respond rapidly to changes in water quality and are not highly habitat-dependent (Schofield & Davies 1996). In Australia, the Monitoring River Health Initiative (MRHI) is supporting research into the development of rapid biological monitoring techniques (see *Rapid biological assessment*, below) based on diatoms. Although aimed primarily at rivers, such standardised methods could well be applied to assessments on wetland habitats.

An alternative method of monitoring algal assemblages has been that adopted by Munawar and co-workers for the Great Lakes (Munawar & Weise 1989, Munawar et al 1994). Their assessment of the 'microbial loop', incorporating bacteria, picoplankton, nanoplankton, microplankton as well as the larger phytoplankton appears to be a legitimate and sensitive technique for predicting impacts, at least for lake environments. In particular, the apparent sensitivity of autotrophic picoplankton may deserve further attention. Standardised methods have been developed, and could be evaluated with respect to assessing the health of wetland ecosystems.

3.1.3 Macrophytes

Toxicity bioassays

Aquatic macrophytes have rarely been utilised as early warning indicators of environmental impacts (Sortkjaer 1984, Lewis 1995), however, specific toxicity testing protocols do exist for some genera, and warrant consideration. The importance of aquatic macrophytes is highlighted by their roles in oxygen production, nutrient cycling, control of water quality, sediment stabilisation, and importantly, as a habitat for aquatic and terrestrial life (Sortkjaer 1984, Lewis 1995). In addition, the use of herbicides in many agricultural and land management practices may make them relevant tools for assessing impacts, although phytoplankton may represent a more sensitive indicator for such compounds.

Probably the most frequently used macrophytes for bioassay purposes are the floating duckweeds (*Lemna* spp.) (eg Allison & Holdway 1988, Jenner & Jansen-Mommen 1989, Lockhart et al 1989, Smith & Kwan 1989). Common endpoints assessed include frond chlorosis and necrosis, plant and frond numbers, root length, and dry biomass (Allison &

Holdway 1988, ASTM 1992, Lewis 1995). *Lemna aequinoctialis* is a local duckweed species found in billabong and floodplain habitats throughout the wet-dry tropics of northern Australia. A toxicity bioassay using *L. aequinoctialis* was developed at ***eriss*** in the late 1980s, however the assay was conducted in natural water, low in essential nutrients, and hence test duration was 14 days, to enable sufficient growth (Allison & Holdway 1988). In developing tests which provide rapid results, such a test duration is impractical. Subsequently, a new *L. aequinoctialis* toxicity bioassay is currently being developed by ***eriss***, aiming to utilise a synthetic medium specifically designed to simulate local waters, with the addition of nitrate and phosphate to further stimulate plant growth. Optimal test duration will be determined in terms of response sensitivity and time-efficiency, and ideally, will not exceed 4 days (see table 2).

Biological monitoring

Some research has been carried out regarding the use of aquatic macrophytes as biomonitors of polluted environments (Lewis 1995). Haslam (1982) proposed a method involving the assessment of species diversity, vegetation cover, trophic status, pollution tolerance and physical damage of macrophytes in potentially impacted aquatic ecosystems compared to the vegetation expected in similar non-impacted, or 'reference' sites. While the method was apparently rapid once established, it did not appear to be applicable to a broad range of habitat or stream types. It also appeared to rely heavily on assessment of cover, which is not a particularly sensitive measure of pollutant impacts (Haslam 1982). Sortkjaer (1984) reviewed some previous attempts of macrophyte monitoring including the use of artificial macrophyte communities, but concentrated mostly on the work of Haslam (1982). A benefit of monitoring aquatic macrophyte communities, including riparian vegetation, is that the impacts of stressors other than pollutants can also be assessed. However, this could also be seen as a disadvantage if only pollutant impacts are of interest.

Monitoring of natural macrophyte communities is not likely to represent an ideal method of providing early warning of pollutant impacts on aquatic ecosystems. Problems exist regarding interpretation of species absences, the role of epiphytes, natural spatial and temporal variation, and species responses to water and sediment quality (Schofield & Davies 1996). However, in some wetland regions, such as in the ARR, where the vegetation type/structure has been extremely well characterised, small alterations due to pollutants may be detectable, although it is also likely that they will be masked by the effects of physical disturbances.

3.1.4 Invertebrates and vertebrates

Invertebrates and vertebrates have been used extensively to assess and monitor the effects of pollutants on aquatic ecosystems. They are discussed together here, as many of the methods, including the use of toxicity bioassays, biochemical markers, and biological monitoring are applicable and similar for both groups.

Toxicity bioassays

Representative vertebrate and invertebrate organisms have long been used for toxicity testing purposes to investigate the effects of pollutants on natural systems. Toxicity bioassays using ecologically relevant endpoints, such as reproduction and population growth, have been used to provide a means of quantifying early warning stress or effects on ecosystems. The enormous range of toxicity bioassays that have been developed are not discussed here, other than noting their potential applicability for assessing impacts on many of the world's wetland habitats.

A range of vertebrate and invertebrate toxicity bioassays have been, or are currently being developed for the wet-dry tropics of Australia, specifically the ARR. They form part of a 'battery' of tests including those already described for phytoplankton and macrophytes (table 2). The overall objective is to develop and utilise bioassays that provide rapid yet sensitive indications of potential impacts on wetland ecosystems. Towards this goal, the use of short term chronic/sub-chronic toxicity bioassays such as the *Hydra viridissima* 96 h population growth bioassay (Markich & Camilleri, in press), the *Moinodaphnia macleayi* 3 brood/6 day reproduction bioassay (Hyne et al 1996), and the *Chironomus crassiforceps* 5 day larval growth bioassay, allow for the detection of effects of low levels of pollutants in both the water and sediment compartments, on sensitive, sub-lethal parameters (table 2). In keeping with the need for rapid bioassays, a short term (≤24 h) cladoceran (*Moinodaphnia macleayi*) bioassay based on feeding inhibition is also being developed, with the ultimate aim of replacing the 3 brood/6 day reproduction bioassay. Allen et al (1995) have demonstrated that toxic effects in another cladoceran, *Daphnia magna*, can be related to an inhibition of feeding. The relationship is well defined, and feeding inhibition has been found to be a reliable and sensitive indicator of ecologically relevant effects such as reproductive and growth impairment (D Baird, pers. comm.).

The aquatic species used in the toxicity bioassays outlined in table 2 represent both a wide range of trophic levels and wetland habitats. Both these attributes are likely to be minimum requirements if impacts on wetlands are going to be adequately predicted, assessed and monitored. However, it may be that some of the many species used for toxicity assessment purposes throughout the world will be represented in a particular wetland of interest, in which case the development of site-specific protocols, and the associated time and costs, may no be required.

Biological monitoring

Biological early warning systems (BEWS)

Biological early warning systems (BEWS) are field-based systems that utilise 'housed' aquatic organisms for continuous toxicity monitoring. Although they could be considered as toxicity testing, their continuous nature is more closely related to the concept of monitoring. Organisms are kept in the actual aquatic environment, or in on-site laboratories receiving flow-through water from the environment of interest, and monitored continuously over large time periods for behavioural and/or physiological responses that indicate the onset of stress (de Zwart et al 1995) Freshwater mussels (eg *Dreissena polymorpha*) have been used as BEWS with great success in Europe, particularly in attempts to monitor pollutant inputs and effects in the Rhine River (Kramer et al 1989, Borcherding & Volpers 1994; de Zwart et al 1995). The method is based on the fact that mussels have been shown to close their shells (valves), which are usually open for respiration and feeding, when exposed to a stressor (Kramer et al 1989). Valve movement detection via electromagnetic induction devices has been utilised to assess the effects of both natural phenomena and pollutants on the aquatic environment, with considerable success. The use of fish and cladocerans as BEWS is also common, with responses such as avoidance, rheotaxis, and ventilatory behaviour being assessed for the former, and swimming behaviour/activity for the latter (Hendriks & Stouten 1993, Balk et al 1994, Hendriks 1994). Again, the majority of the responses are measured electronically, often with the aid of pressure and infra-red sensors.

Although adequate sensitivity appears yet to be fully demonstrated, further advancements in BEWS technology are likely to improve this. Improvements are essential, as the measured response must be sufficiently characterised so as to minimise the occurrence of false alarms.

Nevertheless, BEWS have been demonstrated to be reliable, easy to handle, and both cost- and time-effective (Borcherding & Volpers 1994, de Zwart et al 1995, Sluyts et al 1996). A further advantage is that BEWS can be used to detect biological impacts on aquatic ecosystems due to water quality changes unrelated to pollution, and hence may have potential as early warning indicators of other types of human-induced impacts on wetland ecosystems.

Rapid biological assessment (RBA)

Ultimately, effective long term monitoring may provide the best means of the early detection of pollutant impacts on aquatic ecosystems, including wetlands. Indeed, the monitoring of macroinvertebrate communities has long been established as a tool for assessing the status of aquatic ecosystems and monitoring changes associated with anthropogenic stress (Resh et al 1995). However, traditional monitoring techniques have been relatively costly, and extremely time consuming. The need for fast results has facilitated the development of rapid biological assessment (RBA) techniques that evaluate aquatic macroinvertebrate assemblages at reduced costs relative to those associated with the more rigorous assessments (Resh et al 1995). RBA techniques are only briefly discussed here, and the reader is directed to papers by Chessman (1995), Resh et al (1995), Wright (1995) and Schofield and Davies (1996) for more detailed discussions.

The underlying concept of RBA is to minimise the amount of information required, or minimise the effort required to obtain the information, yet still retain the capacity to detect relevant changes in community structure. Means by which costs and effort are reduced include defining key habitats, identifying organisms to family level only, and reducing the number of organisms processed, by setting a standard count time (eg 1 h) or maximum number of counts per habitat (eg 100) (Chessman 1995, Resh et al 1995, Wright 1995). An emphasis is also placed on producing data that is easily interpretable to water managers, and this has generally been achieved by reducing the information to a metric or biotic index (Growns et al 1995; Resh et al 1995).

One of the most comprehensively studied RBA programs is the River Invertebrate Prediction and Classification Scheme (RIVPACS) in the United Kingdom. This approach is based on comparing monitored river sites against reference unimpacted, or least impacted sites (Wright 1995, Schofield & Davies 1996). Although developed for assessing macroinvertebrate communities, the RIVPACS structure could well be applied to other faunal groups. In Australia, the MRHI is supporting research on the assessment and development of similar techniques for fish, diatoms, phytoplankton and possibly bacteria, macrophytes, and community metabolism (Schofield & Davies 1996). In addition, efforts are being directed towards the adaptation of RBA techniques to estuarine conditions, including tidal reaches of lowland rivers, for which the current methods are not applicable (NRPMP/MRHI 1994). These methods will attempt to take into account some of the specific issues relating to waterbodies in Australia, including the wetlands of the wet-dry tropics, such as flow variability and high spatial and temporal variation. With the added benefit of large areas of essentially unimpacted aquatic ecosystems for reference purposes, the use of RBA for the early detection of pollutant impacts on wetlands of the wet-dry tropics warrants continued consideration.

3.1.5 Biomarkers

The use of biochemical biomarkers as early warning indicators of potential environmental effects stems from the fact that changes in the biochemistry of individual organisms often precede effects at the organismal, and therefore, potentially ecosystem level. Two such biomarkers, the mixed function oxidase, or cytochrome *P*-450 system, and metallothioneins, are discussed below. It should be pointed out that many types of biomarkers have been developed, and can be used either for toxicity assessment purposes or as biological monitoring tools. While the two types of biomarkers discussed below are generally used for monitoring purposes, others can also be used as measures of toxicity in laboratory bioassays. For the purposes of the present discussion, biomarkers are discussed separately from toxicity bioassays and biological monitoring.

Mixed function oxidases

The mixed function oxidases (MFOs) are a group of enzymes (isoenzymes) which metabolise lipophilic xenobiotics (poorly-water soluble foreign compounds) (Goksøyr and Förlin, 1992). Exposure to such a compound results in an increase in activity (induction) of MFOs, or *P*-450 isoenzymes. Such induction of *P*-450 is an extremely useful biomarker of chemical exposure, and hence potential adverse effects in the aquatic environment, particularly with respect to persistent organochlorine pesticides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and complex effluents from pulp and paper mills (Haux & Förlin 1988, Holdway et al 1995). Measurement of total *P*-450 or *P*-450 isoenzyme activity can be made at both a molecular and biochemical level, in a diverse array of aquatic and terrestrial organisms, including fish and aquatic invertebrates, and is both time and cost-effective (Payne 1984). An enormous body of literature exists regarding the use of *P*-450s in aquatic toxicology, and has been summarised in a number reviews (eg Payne 1984, Haux & Förlin 1988, Ahokas 1990, Goksøyr & Förlin 1992).

As *P*-450 induction is a normal compensatory response to xenobiotic exposure, there is no guarantee that adverse effects on individuals, let alone populations and ecosystems, will occur. However, although limited in terms of indicating actual toxic effects, evidence of exposure should be sufficient to implement more detailed investigations regarding water quality and potential adverse effects. Due to their specificity, induction of *P*-450 isoenzymes are most useful as biomonitors in cases where the pollutant(s) is known, and known to result in induction. However, the specificity of the MFO system could also be utilised to actually identify the presence of a single chemical or group of chemicals (Haux & Förlin 1988). The utility of the MFO system has been demonstrated for monitoring pulp mill effluents in the northern hemisphere (Haux & Förlin 1988, Kloepper-Sams & Owens 1993), and the techniques could well be applied to assess or monitor potential pollutant effects on wetland ecosystems.

Metallothionein (MT)

Metallothioneins (MTs) are widely occurring metal-binding proteins which act primarily as metal donors for essential biochemical processes (Roesijadi 1992, Livingstone 1993). However, MTs are induced by, and bind excesses of both essential and non-essential metals, and hence are also involved in metal detoxication. MT induction can be readily measured in fish and aquatic invertebrates using either electrochemical or immunoassay techniques (Olafson & Sim 1979, Hogstrand & Haux 1990). MTs are sensitive and specific indicators of metal contamination, particularly cadmium, copper, zinc, mercury and silver (as reviewed by Roesijadi 1992, Livingstone 1993) and provide early warning of potential adverse effects on individuals, which may lead to deleterious effects in population structure and ultimately ecosystems (Roesijadi 1992).

MTs generally suffer the same limitations as MFOs. That is, they tend to be indicators of contaminant exposure rather than toxic effects, while their induction is also a normal compensatory mechanism in response to elevated levels of particular metals, in order to help maintain homeostasis. Animals may well be able to persist for indefinite time periods with elevated levels of MTs, and show no deleterious effects. Nevertheless, as with MFOs, an indication of exposure should be sufficient to implement more detailed investigations, and if early detection is considered a priority, MTs should be considered a viable early warning indicator of potential adverse effects due to metal contamination of wetland ecosystems.

Other biomarkers

There exists many other types of biomarkers, of either a biochemical, physiological, or even immunological nature. Other biochemical biomarkers include enzymes such as serum sorbitol dehydrogenase, esterases (eg acetyl cholinesterase, butyryl cholinesterase), as well as various anti-oxidant enzymes (Kloepper-Sams & Owens 1993, Förlin et al 1995, Holdway et al 1995, Johnston 1995, Walker 1995). Condition indices such as liver and gonad somatic index, and condition factor have also previously been utilised to assess organism health, while haematological parameters such as haemoglobin content, and white and red blood cell counts, have also been found to vary due to contaminant exposure (Everaats et al 1993, Kloepper-Sams & Owens 1993).

The assessment and development of immune responses as biomarkers has also received considerable attention in recent years. The potential of immune cell functions as biomarkers stems from immune cells' ability to rapidly proliferate in response to the introduction of foreign compounds (Holdway et al 1995). The field of aquatic immunotoxicology is only in its infancy, while fish immune systems are not yet particularly well understood. Nevertheless, quite sensitive methods for assessing immune dysfunction in aquatic animals have been developed, including macrophage responses, mitogenic responses, and natural cytotoxic activity (Weeks et al 1992, Barry et al 1995).

Many biomarkers have been demonstrated to give early warning of environmental effects of chemicals prior to serious effects occurring upon individuals or populations (Walker 1995). As such, it is likely that, in conjunction with other ecotoxicological and biological monitoring techniques described in this discussion, appropriately selected biomarkers would help to provide adequate early warning of pollutant-related impacts on wetlands.

3.2 Physico-chemical indicators

Physico-chemical monitoring of a waterbody is known to be insufficient to fully characterise its status, or reliably detect adverse impacts. However, it has been recognised as being a vital component of an integrated assessment utilising both physico-chemical and biological measures for assessing a waterway's condition (Schofield & Davies 1996).

The monitoring of standard physico-chemical parameters such as pH, dissolved oxygen (DO), biochemical oxygen demand (BOD), total organic carbon (TOC), hardness, conductivity, salinity, and nutrients such as nitrogen (N) and phophorus (P) can be of use in several ways. Firstly, it provides a record of the physico-chemical characteristics of the waterbody, which when continued over an extended period, provides a record of the variation in the characteristics over time. Unusual changes in any of the parameters will provide an indication that the characteristics of the water, and therefore potentially the water quality, are changing. Secondly, many physico-chemical parameters have the ability to alter the toxicity of particular pollutants. For example, pH, hardness and TOC are all known to modify the toxicity of a range of heavy metals. Subsequently, knowledge of the behaviour of such parameters is of great importance, particularly in regions where elevated levels of pollutants already exist. The majority of standard physico-chemical water quality parameters are simple, inexpensive and quick to measure, and should be used to complement any ecotoxicological or biological monitoring study.

The measurement of priority, suspected and/or known pollutants in a waterbody will also provide potentially useful information. Chemical monitoring will generally assist in identifying the toxic components in a waterbody, but is unlikely to be able to provide adequate early warning, unless gradual increases at very low levels (ie below the toxic threshold), due to processes such as seepage from contaminated soils or groundwater, can be detected. Nevertheless, the use of speciation and chemical equilibrium models, incorporating chemical measurements and physico-chemical data will certainly assist in the prediction of bioavailable fractions of pollutants and hence potential toxicity, and may provide a form of early warning. Chemical monitoring is relatively simple, and rapid, with standard methods existing for most major organic and inorganic chemicals, but unlike physico-chemical monitoring, is relatively expensive. Unless pollutant inputs are suspected, or known to be entering a wetland system, chemical monitoring may not be a regular requirement.

4 Early warning indicators and ecological risk assessment

The successful implementation of an early warning system (EWS) requires that it fits into an appropriate management framework. That is, a framework that will allow effective use of the EWS, and provide an adequate mechanism of feedback when required. ***eriss*** is currently in the process of developing an ecological risk assessment (ERA) framework for use in wetland environmental management, primarily, but not totally in northern Australia (NLC/***eriss*** 1997). The concept of early warning relates to both major processes of ERA; risk assessment and risk management, as described below.

The risk assessment process attempts to quantify the risks associated with a pollutant input into an aquatic ecosystem. As part of this, *effects assessment* provides the opportunity to assess the effects, or toxicity of a pollutant to regionally relevant aquatic organisms, using the types of toxicity bioassays described above. If the ERA is predictive (ie the pollutant is yet to enter the waterbody of interest), then the detection of a toxic compound provides early warning that, if released into the aquatic environment, it may potentially result in serious harm. However, it essential that the end-points of interest in effects assessment are considered ecologically relevant (Pascoe 1993). For this reason, toxicity bioassays assessing end-points such as reproduction, maturation, growth and death are generally utilised. Field analyses of chemical concentrations, and the use of modelling as described for physico-chemical indicators will provide further information on the likelihood of aquatic organisms being exposed to the pollutant (ie *exposure assessment*), by determining its presence, speciation, partitioning, and degradation. Again, outcomes of this, when compared with those from *effects assessment* will provide early warning of potential adverse effects.

Management decisions arising from an ERA (ie risk management) require ongoing monitoring to assess their effectiveness. The purpose of the monitoring is to ensure that the quality of the waterbody of interest is not altered more than the level chosen during the risk management process (Sortkjaer 1984). Such a monitoring program would include an early warning system with appropriate indicators having been selected according to both information obtained during the ERA, on the pollutant and its potential effects, and the habitat characteristics. If the desired level of protection cannot be maintained under the specified conditions, the early warning system should be capable of detecting effects prior to the occurrence of any serious environmental impacts. The original risk management and reduction decisions are then reassessed, and remedial action implemented accordingly. For such monitoring purposes, the use of biomarkers, which detect subtle effects prior to ecologically significant effects, are likely to be of value. Even toxicity bioassays, particularly *in situ* assessments will be of use, provided sub-lethal effects are observed, and they are known to be sensitive. Rapid biological assessment of community assemblages would also represent an appropriate monitoring tool, again assuming adequate sensitivity.

The use of ecological risk assessment to predict, manage and monitor pollutant impacts on wetlands should be applicable, as long as their spatial and temporal complexity are recognised, and adequately accounted for, in ways described earlier.

5 Conclusions

Wetland degradation and loss has been recognised as being a serious and global issue. As such, recent reports on wetlands identified the need to develop appropriate early warning indicators of wetland degradation. The primary aim of the present paper was to discuss the potential of existing methods of assessment, particularly rapid assessment techniques, as early warning indicators of wetland degradation due to pollutant impacts. Particular emphasis was placed on the assessment of wetlands in the wet-dry tropics of northern Australia.

Various types of biological indicators were discussed, including toxicity bioassays and biological monitoring techniques for bacteria, phytoplankton, macrophytes, and invertebrates and vertebrates. Of the organisms assessed, the most promising indicators of wetland degradation may well be the phytoplankton. This is due to their trophic position as dominant primary producers, their abundance, their predictable and rapid response to a wide range of toxicants, their relationship to changing nutrient levels, and the fact that many rapid, reliable and sensitive techniques have been developed for their assessment. Of the methods, rapid biological assessment of diatom assemblages, and assessments of the microbial loop, incorporating bacteria and phytoplankton may be useful, standard, yet adaptable techniques for assessing the status of different wetland habitats. Toxicity bioassays, using either single species or natural assemblages are also well developed for phytoplankton.

Toxicity bioassays using invertebrates and vertebrates are generally rapid and reliable, and are also an important tool for assessing the potential effects of pollutants on aquatic ecosystems. They are used extensively throughout the world, and have been used with success at ***eriss*** for predicting potential impacts of pollutants on wetlands in the wet-dry tropics of northern Australia. They remain an essential part of any early detection program. The use of biochemical biomarkers in invertebrates and vertebrates are a promising tool for achieving true 'early warning' of potential pollutant impacts, and depending on the nature of the pollutant may have great value for predicting wetland degradation. Their obvious value is as a monitoring tool to assess the effectiveness of risk management and reduction decisions. Other types of monitoring also likely to be important in the early detection of pollutant impacts on wetlands, include rapid methods of monitoring community assemblages, currently being developed for a range of organism types.

As wetlands often comprise a wide range of habitats with broad areas of ill-defined watercourses, and spatial and temporal ephemerality, representatively monitoring them is an extremely difficult challenge. No one indicator will be representative of all the habitat types, although some may have broader distributions than others. By selecting a suite of indicators appropriate to the spatial and temporal scales across which a pollutant is dispersed, an adequate early warning system (EWS) for predicting wetland degradation may be established. This includes selection of indicators based on a knowledge of the inter-relationships between wetland habitats, and the integration of toxicity testing (using several species), and biological and physico-chemical monitoring. In addition, the use of *rapid assessment* early warning indicators provides, in effect, a 'double-edged sword', whereby effects are not only detected early, but also quickly, and therefore further investigations or remedial action are even swifter in their implementation.

Finally, ecological risk assessment should provide a suitable framework to allow the effective use of an early warning system for pollutant impacts on wetlands, from both a predictive and retrospective perspective.

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