Managed Aquifer Recharge - Risks to Groundwater Dependent Ecosystems - A Review

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Water for a Healthy Country Flagship Report to Land & Water Australia

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The work contained in this report was supported by Land & Water Australia and components were contributed by partner organisations as follows;

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Appendix 4: Deborah Reed, RPS Consultants
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Groundwater dependent wetland in the lower South East of South Australia. Photo courtesy of Peter Cook, CSIRO Land and Water

A new species of stygofauna (a melitid amphipod) in groundwater from the vicinity of Port Lincoln, South Australia. Photo courtesy of Remko Leijs, Flinders University of South Australia
EXECUTIVE SUMMARY

Objectives:

This report was produced as the product of a consultancy project for Land & Water Australia entitled ‘Managed Aquifer Recharge – Risks to Groundwater Dependent Ecosystems – A Review’ commissioned in October 2008 and completed in December 2008. The report was commissioned at the request of the Australian Government Department of the Environment, Water, Heritage and the Arts to inform and support the development of Guidelines for Management of Aquifer Recharge under the National Water Quality Management Strategy Phase Two of the Australian Guidelines for Water Recycling.

The report was commissioned due to the sparcity of information on this specific topic, as revealed in a public consultation draft of these guidelines in May 2008. The study scope included evaluating from wider literature the potential impacts of managed aquifer recharge on all types of groundwater-dependent ecosystems, identifying relevant methods for measuring impacts, and determining, where possible, acceptance criteria for environmental risk assessment and methods to manage risks. The terms of reference included completion of a report identifying impacts of managed aquifer recharge operations on groundwater dependent ecosystems where impacts have been recorded. Recognising that this information was scarce, the terms of reference also included synthesis of relevant information to make recommendations on effective approaches to measure and manage such impacts for adoption into the Draft Guidelines for Managed Aquifer Recharge.

Publication of this review provides a reference document in support of section 5.11 of the Guidelines (Hazard Identification and Preventive measures - Aquifer and groundwater dependent ecosystems).

Methods:

The work was addressed as a series of studies each focussed on a different group of groundwater-dependent ecosystem receptors. These are on aquifer microorganisms; stygofauna; wetland, riparian and terrestrial phreatophytic vegetation; and fauna and flora of connected wetlands, streams, lakes and marine environments. This work is recorded in Appendices 3 to 6. A literature review of impacts of managed aquifer recharge on groundwater dependent ecosystems was also undertaken (Appendix 1). Results of investigations of potential impacts on various ecosystem receptors of two proposed managed aquifer recharge projects using reclaimed water in coastal aquifers (Appendices 7 and 8) were undertaken. A case study where managed aquifer recharge was used soley to protect a mound spring in the south-west margin of the Great Artesian Basin (Appendix 8) was reported. A literature review and laboratory experimental design of the fate of simizine in aquifers was also completed. Findings of these studies were then synthesised in a format that allowed measurement methods, potential impacts of changes in groundwater level and quality, and potential assessment criteria to be identified relevant to the stages of risk assessment in evolving projects, consistent with the Guidelines for Managed Aquifer Recharge, (Sections 2, 3 and Appendix 9). Remaining gaps in knowledge were identified.

Outcomes:

The recommendations arising from the consultancy report (Appendix 9) have been adopted in their entirety by the Working Group developing the Guidelines for Managed Aquifer Recharge (at Section 5.11). The consultancy report was reviewed and revised to form this current report which is referenced in the Guidelines as a source of more detailed information in support of this aspect of the Guidelines.

Managed Aquifer Recharge operations may affect groundwater-dependent ecossyems via changes in water table elevation or piezometric head at springs and as a result of water
quality changes. Groundwater dependent ecosystem receptors in the sequence from most to least sensitive to groundwater level changes are aquatic fauna, algae, wetland vegetation, riparian vegetation, terrestrial vegetation, larger stygofauna and smaller stygofauna species. Declines in groundwater levels near groundwater dependent water bodies in depositional environments can also result in formation of acid-sulphate soils with consequences to water quality and aquatic and soil life. Falling groundwater levels increase the energy required by plants to extract groundwater. If the rate of decline of water table exceeds the rate at which a plant can extend its roots then the plant suffers water stress, and without other sources of water could die. Rising water tables can result in anoxia within the root zone and stress plants. If the rate of fall or rise of watertable exceeds that to which groundwater fauna and flora can adapt then ecosystem health is impacted.

Water quality changes due to recharge of water that contains greater concentrations of water quality hazards than native groundwater can also have adverse impacts on ecosystems. For some of the hazards that could potentially be present in recharge waters it is likely that the water quality objectives to meet environmental values of the aquifer and/or of recovered water applications will be a tighter constraint than ecosystem constraints. Where ecosystem protection is an identified environmental value of an aquifer the corresponding water quality objectives need to be met at the boundary of the attenuation zone. A well designed system will have an attenuation zone that does not extend to any groundwater-connected surface water features or phreatophtic vegetation.

Groundwater microorganisms have been shown to readily adapt to new biogeochemical conditions arising from introduction of water of different quality to a native groundwater. Water. However sustaining microbial function requires that excessive nutrient concentrations are prevented from reaching the saturated zone or spreading in the aquifer.

Stygofauna similarly have been shown to increase in number when exposed to small concentrations of nutrients, but do not survive in higher concentrations when groundwater is polluted. At some sites, on restoration of nutrient concentrations to antecedent conditions stygofauna populations have returned. Stygofauna responses to other water quality hazards including temperature change are unknown.

For terrestrial, riparian and wetland phreatophtic vegetation, effects of water quality changes beyond the temporary attenuation zone due to MAR are required to be acceptable where groundwater-dependent ecosystems are an environmental value of the aquifer. Australian Guidelines for Water Recycling Phase One (2006) provides indicators of risk to plant health of irrigation with water of various nutrient and salt concentrations which may be used to ascribe water quality objectives at the margin of the attenuation zone. If these are more stringent than water quality objectives for other environmental values of native groundwater and uses of recovered water, these may dictate the size of the attenuation zone or the level of pre-treatment before recharge.

Aquatic fauna and plants are a highly diverse group of ecosystem receptors. They have in common that they require maintenance of a minimum groundwater level for their survival. Their responses to water quality changes can vary from highly sensitive to resilient and ecotoxicoctological tools are used to identify indicator species and evaluate the effects of potential hazards that may emanate from MAR projects. A wide range of tests include acute toxicity, lifecycle tests, sub-lethal tests, microbial tests, genotoxicity and mutagenicity, bioaccumulation, toxicity identification evaluation, endocrine disruption and in situ toxicity studies. These are applied to selected reference species which may contain biomarkers to assist in measuring levels of stress.

A number of information gaps were identified in this review:
• Mobility and rates of inactivation in aquifers of micro-organisms that are pathogenic to fauna or flora (e.g. Phytophera)
• While a number of studies on stygofauna have assisted in establishing methods for their evaluation the power of stygofauna sampling to detect population change is weak due to the large proportion of wells with no detections. Further methods will be needed.
• Plant response functions to the magnitude and rate of groundwater level decline have been defined in well-studied areas on the Swan Coastal Plain, but are yet to be made more widely applicable.

These gaps, if addressed by a research program consisting of linked and targeted research projects, would provide data to assist in the application of the Guidelines. Such data would give a more confident basis for regulators and proponents of managed aquifer recharge projects to design, assess and manage projects to ensure environmental impacts are appropriately addressed.
ACKNOWLEDGEMENTS

Specific acknowledgements of third party contributions to this report are given in the relevant appendices.

The authors are grateful for reviews by Sebastien Lamontagne and Simon Toze of CSIRO Land and Water and Jim Donaldson of Land & Water Australia, whose comments have been taken into account in this revised published form of the review. We also acknowledge the valuable help of Teresa Oppy of Land & Water Australia in managing the consultancy and review process.

As part of the development of the Australian Guidelines for Water Recycling Phase Two within the National Water Quality Management Strategy, members of the Managed Aquifer Recharge Guidelines Working Group also provided comment on the draft and approved modifications to section 5.11 of the draft guidelines, derived from this review.
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1. INTRODUCTION

Managed aquifer recharge (MAR) is the intentional recharge to aquifers for subsequent recovery or environmental benefit (Dillon et al, 2008). The Australian Guidelines for Managed Aquifer Recharge provide guidance on protection of public health and the environment in relation to these operations. While the impacts of managed aquifer recharge operations on groundwater dependent ecosystems were discussed in the public consultation draft guidelines (EPHC, 2008), the factual information upon which guidance could be based was sparse. This report is intended to address that gap. The guidelines do not address the issue of impacts on ecosystems of diverting water from streams and lakes as a source of water for recharge as each Australian jurisdiction has water resources allocation policies in place to address entitlements and allocations to divert water that take account of ecosystem impacts.

The terms of reference for this study were:

a) to complete a report identifying whether impacts on GDEs have been recorded either where aquifers containing GDEs are being utilised by MAR schemes or where GDEs occur adjacent to managed aquifer recharge activities;

b) to make recommendations on the effectiveness of the approach of the MAR Guidelines in protecting GDEs and managing the risk of MAR schemes to GDEs.

Further, using case studies, the project is to include, but is not limited to identifying:

- the impacts,
- the cause of impacts,
- current management arrangements,
- ways the impacts could be mitigated/managed,
- other risks, and
- possible lessons for the guidelines.

The authors acknowledged that there is sparse information in Australia (and internationally) with which to specifically address the brief. Hence the approach adopted was to undertake a systematic review of each of the potentially affected ecosystem receptors, the ways in which they are affected and measurement and monitoring methods available that would present possibilities for developing acceptance criteria and where possible to identify preventive measures.

The project drew from existing dispersed research projects, some unrelated to MAR, undertaken by experts in particular receptor organisms or in methodologies for assessing ecosystem impacts, and drew these together to focus on assessment of impacts of MAR on ecosystems. These studies were compiled as a set of parallel reviews of literature and experience by those relevant scientists, and are recorded in the eight appendices to this report that are briefly described below.

1. review international data (only two studies were identified, both in USA, that were not already covered in appendices 3, 4, 7 or 8)
2. undertake a literature review and initiate collection of primary data on the largest known environmental concern that has been identified in Australia at MAR sites using stormwater, namely the fate of herbicides in groundwater
3. harness baseline monitoring from a major ARC project on stygofauna to undertake correlations of abundance and diversity with existing water quality data and in unconfined
aquifers with water level fluctuations, and if possible acquire data of direct relevance to this project in Mt Gambier

4. review the spatial and temporal variations in **aquifer microbiological communities** in the vicinity of two Australian MAR project trials that use reclaimed water (Bolivar ASR (Adelaide) and Floreat Infiltration Galleries (Perth)) and the understanding of the evolution of communities and the consequences for sustainability of microbiological function

5. harness information and data from an NWC project ‘Ecosystem Case Studies: Sustainable levels of groundwater and surface water extraction’, and at sites where ecosystem health (**wetland, riparian and phreatophytic vegetation**) is correlated with indicators of groundwater elevation, to evaluate potential impacts of MAR. (eg Swan coastal plain)

6. review a wide range of **ecotoxicological monitoring methods**, including those in use at the Parafield ASR /ASTR site for their suitability for assessing impacts of MAR on **fauna and algae** in groundwater-dependent ecosystems

7. reports studies undertaken at a proposed reclaimed water ASR project at Willunga Basin where the a number of groundwater ecosystems require protection, the most difficult of which to address was impacts on **coastal reefs**.

8. summarises two further case studies. One gives the basis for a decision not to proceed with a proposed reclaimed water infiltration project on Mosman Peninsula in Perth due to inadequate protection of the Swan River estuary and marine habitat from nutrient-rich discharges. The final case briefly describes reinjection of water at Olympic Dam to protect a Great Artesian Basin mound spring ecosystem.

Case studies permeate Appendices 1, 4, 5, 7, and 8 allowing evaluation of actual impacts, at least of some organisms, or evaluation of some methods used to assess impacts of MAR operations.

This report syntheses from the above the extent of knowledge and knowledge gaps in this field, taking a structured approach to defining impacted ecosystems by combining hydrogeological and ecological perspectives, and where possible to identify appropriate methodologies to assess impacts of MAR projects on groundwater-dependent ecosystems and preventive-measures. Where these extend or deviate from information provided in the current public consultation draft guidelines this is documented to facilitate guideline revision. Where gaps remain these will are identified and prioritised.

**2. SYNTHESIS OF THE REVIEWS AND EXPERIENCES**

Table 1 illustrates the scope of this project to address the range of environmental receptors likely to be affected by MAR operations using available information.
Table 1 The hazards and ecosystem receptors and monitoring methods addressed within this report with a view to providing scientific support to MAR Guidelines. Bracketed numbers refer to the appendix containing the relevant information.

<table>
<thead>
<tr>
<th>Hazards</th>
<th>Affected receptor and associated monitoring methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Excessive changes in water tables or pressures</td>
<td>• Aquifer microbial community (4)</td>
</tr>
<tr>
<td>• Water quality changes (including pesticides (2))</td>
<td>• Aquifer stygofauna (3)</td>
</tr>
<tr>
<td></td>
<td>• Organisms in connected surface water ecosystems (6)</td>
</tr>
<tr>
<td></td>
<td>• Vegetation in groundwater dependent ecosystems (5)</td>
</tr>
<tr>
<td></td>
<td>• Marine organisms (7,8)</td>
</tr>
</tbody>
</table>

This synthesis follows firstly the responses of various ecosystem receptors to hydraulic hazards and subsequently to water quality changes.

2.1 Hydraulic hazards affecting groundwater dependent ecosystems

MAR operations will generally have several effects on water table levels in unconfined aquifers and on piezometric pressures in confined aquifers. (EPHC (2008) defines confined and unconfined aquifers and gives examples of the types of MAR projects relevant to each.) MAR hydraulic effects can be categorised as follows:

• hydraulic heads decrease below the levels that would otherwise have occurred
• hydraulic heads increase above levels that would otherwise have occurred
• The rate of increase and/or decrease in hydraulic heads exceeds that which would otherwise have occurred

Direct hydraulic effects are considered elsewhere in the draft guidelines (EPHC, 2008, in Section 5.8 Pressure, flow rates, volumes and groundwater levels). This report addresses the consequences of these only in relation to ecosystems. It is important to differentiate between MAR operations in confined and unconfined aquifers because the potential for impact on groundwater dependent ecosystems depends strongly on aquifer confinement (Table 2). In unconfined systems MAR operations hydraulic impacts are likely to be localised because over a recharge and recovery cycle the transmission of water pressures in the aquifer depends on physical movement of water within the aquifer. However, the impact on water table is direct, and as seen below, can be consequential for a range of ecosystem receptors.

In a confined system, pressures can transmit over large distances due the low elasticity of porous media and of water, even though the physical movement of recharged water will only be in close proximity to the recharge well. The impact on ecosystems other than the aquifer itself are constrained to leakages between the confined and overlying aquifers and to discharges via springs, such as mound springs or marine springs. The regional leakage effects will in general be negligible for ecosystems because seasonal recharge and recovery cycles average out the upward hydraulic gradients through low permeability confining or semi-confining layers so that
annual and long-term upward fluxes are not distinguishable from those which would have occurred in the absence of MAR. Hence the major hydraulic impacts of MAR in confined or semi-confined aquifers is constrained to specific springs which allow relatively simple measurement of hydraulic head or flow rate as the sole required indicator of impact. For submarine springs, measurement is not so simple, and water quality effects may also need to be taken into account (as per Appendix 7).

Table 2  Ecosystem receptors for unconfined and confined aquifers

<table>
<thead>
<tr>
<th>Ecosystem receptor</th>
<th>Unconfined aquifers</th>
<th>Confined aquifers</th>
</tr>
</thead>
<tbody>
<tr>
<td>(4) Aquifer microbial community</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>(3) Aquifer stygofauna</td>
<td>yes</td>
<td>possible</td>
</tr>
<tr>
<td>(6) Organisms in connected surface water ecosystems</td>
<td>yes</td>
<td>springs</td>
</tr>
<tr>
<td>(5) Vegetation in groundwater dependent ecosystems</td>
<td>yes</td>
<td>springs</td>
</tr>
<tr>
<td>(7,8) Marine organisms</td>
<td>yes</td>
<td>submarine springs</td>
</tr>
</tbody>
</table>

In unconfined systems MAR projects can affect the presence of water above ground, for example in groundwater-dependent lakes, streams and wetlands. In these systems MAR projects may influence the elevation of the water table and the extent to which this varies during the course of time, both seasonally and over the longer term. If the water table is above ground, this is reflected by the presence of water and by the depth of water in the groundwater-dependent wetland, stream or lake. Hence aquatic species that depend on free water could potentially be extinguished if the water level drops below a maintenance level. In this case the primary indicator of ecosystem health will be the elevation of the water level and the extent to which MAR increases or decreases this level in relation to the ground surface.

Phreatophytic vegetation is also affected by changes in water table depth. Wetland, riparian and terrestrial plants are affected in that sequence by a declining water table. Appendix 4 shows that as the watertable drops that phreatophytic vegetation extends its roots downwards to the maximum rooting depth of that species within the physical and chemical constraints of the soil profile. If the water table drops below the depth from which roots can access the capillary fringe above the water table, then plant health will suffer and ultimately die if the low water table condition is sustained. Some terrestrial phreatophytic plants, such as banksia and some eucalypys are known to draw groundwater when water table depth exceeds 10m. The proportion of plant water use met by groundwater tends to decrease where water tables are deep as soil water capacity in the unsaturated zone is larger and was evidently adequate to meet the needs of the maturing plant until it could opportunistically tap groundwater. For these plants, the energy required to tap groundwater from deep roots is less than that of drawing soil water from a depleted unsaturated zone storage capacity, hence a decline in water table depth, even below 10m, can still result in a decline in terrestrial vegetation health.

Declines in hydraulic head also have the potential to influence the geochemistry of wetlands and lakes, and pools in streams. The more general effect of decrease in hydraulic head in organic-rich soils will be to change the system from anoxic to oxic. This will promote a change from anaerobic to aerobic microbial communities and enable plants roots to colonise deeper into soils.
and sediments. In these depositional environments where organic material accumulates, a transition from anaerobic conditions to exposure to air allows oxidation of organic material and attendant changes in pH. Where soils are present that have potential for formation of acid-sulphate soils, if realised will result in low pH and elevated metal concentrations in the environment. If this occurs on the perimeter of water bodies the consequences for aquatic fauna can be just as significant as if the volume of free water had disappeared.

The rate of fall of water table has also been found to be important for the health of phreatophytic vegetation (Appendix 4). A water table that declines at a rate that exceeds the capability for root growth to pursue it will leave the plant stranded and dependent on other sources of water such as rainfall and residual soil moisture to remain healthy. That is plant health can suffer, even though the plant has a capability to potentially extend its root system to tap the watertable, if it has insufficient time to do so its health may suffer.

For rising groundwater levels, aerated root zones may become anoxic and waterlogging may result in plant death and succession to a new plant community. For example riparian zone plants are succeeded by wetland species. The ability of plants to adapt and shifts in plant community distribution may also depend on the rate at which the water table rises. If the amplitude of annual water table fluctuations increases substantially it would be expected that those species that are adapted to a wider range of conditions will prevail, less adaptable species will die and new species may colonise. If native groundwater is saline then elevating water tables, even seasonally, as a result of MAR operations would have the effect of transporting salt into the root zone with adverse consequences for the health of salt intolerant species.

Stygofauna are considered to be relatively mobile in aquifers in relation to the rate of rise and fall of water table. However larger taxa (>3mm), such as amphipods, may potentially become stranded if the water table drops quickly, and their survival beyond two days out of water is unlikely (Appendix 3). In karstic aquifers in stable geological regions, karst features are typically abundant in the proximity of current or former watertables due to reactions between acidic recharge and carbonate minerals over geological time. This implies that at any location in these aquifers there may be a relatively well defined limiting depth to macroporosity. Hence rapid lowering of watertables below such limiting depths could lead to stranding of aquifer fauna in excess of the size of inter-granular pore apertures. Stygofauna, as with indigenous micro-organisms in aquifers are considered to be unaffected by pressure variations that could result from MAR operations.

For groundwater micro-organisms, motility is higher than for stygofauna and their size smaller so they are expected to adapt readily to elevation changes in watertable by chemotaxis (movement along a chemical gradient). Marine organisms are unlikely to be adversely affected by hydraulic effects of MAR operations, as groundwater discharge rates to the marine environment are unlikely to be highly affected by MAR projects and head variations are likely to be highly damped in groundwater discharge zones. Thus, the salinity regime in coastal areas is unlikely to be affected significantly by MAR projects. However MAR projects can be useful to prevent seawater intrusion inland, which is a widespread problem along the Australian coastline. While most marine organisms can tolerate short-term exposure to freshwater, many will also be adversely affected on the medium and longer term.
Table 3. Summary of constraints in relation to lower and upper hydraulic heads and rates of change of hydraulic head for various ecosystem receptors

<table>
<thead>
<tr>
<th>Ecosystem receptor</th>
<th>Threshold level below which water table decline causes harm</th>
<th>Threshold level above which water table increase causes harm</th>
<th>Threshold rate of change of level beyond which harm is caused</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquatic organisms (fauna and algae)</td>
<td>Higher of (a) Level to prevent acid-sulphate formation and (b) pool maintenance level</td>
<td>No head constraint</td>
<td>No rate of change constraint</td>
</tr>
<tr>
<td>Wetland vegetation</td>
<td>Capillary fringe lower than maximum rooting depth</td>
<td>No head constraint</td>
<td>No rate of change constraint</td>
</tr>
<tr>
<td>Riparian and terrestrial phreatophytic vegetation</td>
<td>Capillary fringe lower than maximum rooting depth</td>
<td>Level at which anoxia causes harm</td>
<td>Root growth constraint</td>
</tr>
<tr>
<td>Stygofauna</td>
<td>Base elevation of macroporosity in karst aquifers</td>
<td>No head constraint</td>
<td>Migration constraint of largest species</td>
</tr>
<tr>
<td>Microbiota</td>
<td>No head constraint</td>
<td>No head constraint</td>
<td>No rate of change constraint</td>
</tr>
<tr>
<td>Marine organisms</td>
<td>No head constraint</td>
<td>No head constraint</td>
<td>No rate of change constraint</td>
</tr>
</tbody>
</table>

2.2 Water quality hazards affecting groundwater dependent ecosystems

Changes in water quality within the aquifer and possibly in groundwater discharging to connected groundwater-dependent ecosystems may occur as a result of recharging a source of water to an aquifer which has a different chemical composition or physical attributes to the native groundwater. The effects of such water quality changes on those ecosystems are considered here.

The draft MAR Guidelines (EPHC 2008) focus on the following water quality hazards:

- Pathogens
- Inorganic chemicals
- Salinity and sodicity
- Nutrients
- Organic chemicals
- Turbidity and particulates
- Radio-nuclides
To these we add temperature, as this has an influence over biological processes in the aquifer in the vicinity of the recharge facility. However mixing with other groundwater, the thermal mass of aquifer material and the dominance of atmospheric heat sources and sinks suggests MAR projects are unlikely to result in temperature changes in connected ecosystems.

Changes to the aquifer redox environment are clearly the main potential water quality impact of MAR schemes. The redox environment can be modified either by adding significant amounts of reductants (particulate organic carbon, dissolved organic carbon, \(\text{NH}_4^+\), etc) or oxidants (\(\text{O}_2\), \(\text{NO}_3^-\), etc) into aquifers. Adding reductants increases the risk to generate anoxic conditions in the subsurface, while adding oxidants to "reduced" (oxygen-free) aquifers can increase the weathering rates of some minerals (pyrite, etc) with potential consequences for water quality (increased release of heavy metals, etc). These issues are specifically addressed in sections 5.2 and 5.4 of the Draft Guidelines for Managed Aquifer Recharge.

Table 4 summarises the likelihood of ecosystem responses as a result of MAR operations. For some receptors, such as aquifer microorganisms and stygofauna very little is known of their sensitivity to these water quality parameters. For others parameters, such as radionuclides, in order to comply with other requirements of the guidelines for human health and environmental protection, it is most unlikely that water would be admitted to the aquifer that would adversely impact on any ecosystem receptors. This also applies to nutrients where the receptor is phreatophytic vegetation. For turbidity and particulates, additional operational requirements to manage clogging would also ensure that environmental receptors are unaffected. For parameters such as pathogens that may cause diseases in plants and animals, it is likely that inactivation will occur in aquifers analogous to the inactivation of bacteria, protozoa and viruses that are pathogenic to humans. If this hypothesis is correct then it is unlikely that water discharging to connected ecosystems or taken up by phreatophytic vegetation would contain any such pathogens if they were present in the source water.

Nutrients are a likely issue for nearly all ecosystem receptors. Appendix 4 shows their central role in establishing spatial and temporal geochemical changes in aquifers and corresponding biodiversity of microbial communities. This is particularly so in the immediate vicinity of the location where recharged water is introduced into the aquifer. With moderate nutrient concentrations and cyclic recharge and recovery, functional groups of microorganisms were found to be stable. Where bioavailable nutrient concentrations were higher and appeared to exceed the biological assimilation capability of the aquifer, the redox-reduced zone in the aquifer was found to expand. For sites with no cycling of groundwater movement, this suggests that nutrient assimilation processes are unstable and may be unsustainable.

Appendix 3 reveals that small increases in nutrients appear to increase population size of stygofauna. However, at higher nutrient concentrations, such as near sewage polluted sites, eutrophic conditions markedly reduce biodiversity of stygofauna and reduce population numbers. No stygofauna were found in highly polluted aquifers, but at one site where groundwater pollution had abated stygofaunal populations were again found. Hence for both microorganisms and stygofauna there is a capacity to tolerate and even benefit from nutrients, but the bounds on sustainable nutrient loadings in relation to requirements for other health and environmental protection values (as per EPHC Section 5.4 Nutrients: nitrogen, phosphorus and organic carbon, which also addresses).

For organisms in connected surface waters and marine waters the Australian Guidelines for Fresh and Marine Waters (NWQMS 2004) indicate receiving water quality objectives for nutrients. For vegetation, the response to marginally more additional nutrients is likely to be positive. Limitations imposed on the quality of MAR source water by environmental values of
the aquifer and intended uses of recovered water are likely to be tighter than the requirements to restrict nutrients for phreatic vegetation uptake. The Phase 1 Australian Guidelines for Water Recycling (NWQMS, 2006) provides guidance on maximum nutrient concentrations for irrigation of various species of Australian and introduced plants and trees, which may be used to test this assumption, noting that some native species are intolerant of nutrient loads that are considered beneficial for some agricultural crops. Similarly, salinity and sodicity tolerance for vegetation is addressed in NWQMS (2006). It is unlikely that this would be an issue for phreatophytic vegetation except where groundwater is saline and the water table rises inducing ‘dryland salinisation’ analogous to that which occurs when recharge rates increase as a result of land clearing. Recovery of water for productive use should result in a groundwater equilibrium that avoids this scenario.

There are a large number of organic chemicals that can potentially be present in natural catchment waters, urban stormwater, reclaimed water and treated water for drinking. These include pesticides, hydrocarbons, polycyclic aromatic hydrocarbons, disinfection by-products and emerging chemicals of concern such as endocrine disrupting chemicals, personal care products, pharmaceuticals and flame retardants. There are also a large number of organisms requiring protection, and neither their dose-response to each of the plethora of chemicals, nor the effects of interactions between these substances nor the compounding effects of other environmental stresses such as water or salinity stress will be well known in the near future. Hence the use of reference organisms for ecotoxicological studies provides a powerful tool for defining the effects of changes in water quality on these species (Appendix 6) as applied at MAR sites (Appendix 1).

For aquifer microbiota there is ample evidence of adaptation to assimilate at least some of the trace organic substances that may be present in anthropologically impacted source waters. Further discussion is provided in Dillon and Toze (eds) (2005) and in Appendix 4 (last section). Degradation has been shown to be enhanced in many cases by the presence of co-metabolites (nutrients), and especially in warmer aquifers (20 degrees C). Some substances are known to degrade under aerobic conditions (eg nonylphenol) and some under anaerobic conditions (eg chloroform). Hence it is possible that at least some trace organic substances can discharge to connected wetlands, streams or lakes if they are in close proximity to the recharge facility, and especially if cyclic recharge and recovery is not in annual balance. Aquifer attenuation studies for significant trace organics, such as the herbicides atrazine, simazine and diuron (described in Appendix 2) will indicate the likelihood that these substances would reach a connected surface water body or the marine environment. This may lead to determining set-back distances for MAR projects from such groundwater-impacted ecosystems or designing MAR operational plans to inhibit discharge to such ecosystems of water recharged by the MAR project. The ecotoxicological tools described in Appendix 6 then provide a way of determining the potential impacts on aquatic organisms (including marine organisms) if water from the MAR operation was to discharge to such potentially affected ecosystems.

Again it is likely that any MAR operation meeting the requirements for public health and environmental values for the intended uses of recovered water and of the ambient groundwater, would find that the trace organics concentrations available to phreatophytic vegetation would be within their tolerance. This would certainly be the case if ecosystem support is required to be met at the edge of the assigned MAR attenuation zone excluded all groundwater-dependent ecosystems.
### Table 4  Probable ecosystem receptor response to various potential water quality hazards

<table>
<thead>
<tr>
<th>Ecosystem receptor</th>
<th>Pathogens</th>
<th>Inorganic chemicals</th>
<th>Salinity and sodicity</th>
<th>Nutrients</th>
<th>Organic chemicals</th>
<th>Turbidity and particulates **</th>
<th>Radio-nuclides ***</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>(4) Aquifer microbial community</td>
<td>unknown</td>
<td>likely*</td>
<td>unlikely</td>
<td>likely</td>
<td>unknown</td>
<td>unlikely</td>
<td>unlikely</td>
<td>likely</td>
</tr>
<tr>
<td>(3) Aquifer stygofauna</td>
<td>unknown</td>
<td>unknown</td>
<td>likely</td>
<td>likely</td>
<td>unknown</td>
<td>unlikely</td>
<td>unlikely</td>
<td>likely</td>
</tr>
<tr>
<td>(6) Organisms in connected surface water ecosystems</td>
<td>unlikely</td>
<td>Unknown # - use ecotox methods</td>
<td>unlikely</td>
<td>likely</td>
<td>unknown - use ecotox methods</td>
<td>unlikely</td>
<td>unlikely</td>
<td>unlikely</td>
</tr>
<tr>
<td>(5) Vegetation in groundwater dependent ecosystems</td>
<td>unlikely</td>
<td>unlikely</td>
<td>unknown</td>
<td>unlikely</td>
<td>unknown - depends on attenuation</td>
<td>unlikely</td>
<td>unlikely</td>
<td>unlikely</td>
</tr>
<tr>
<td>(7,8) Marine organisms</td>
<td>unlikely</td>
<td>unlikely</td>
<td>unknown</td>
<td>likely</td>
<td>unknown - use ecotox methods</td>
<td>unlikely</td>
<td>unlikely</td>
<td>unlikely</td>
</tr>
<tr>
<td>Section of MAR GLs addressing: Acceptance criteria, Preventive measures, Monitoring (validation, verification and operational)</td>
<td>5.1</td>
<td>5.2</td>
<td>5.3</td>
<td>5.4</td>
<td>5.5</td>
<td>5.5</td>
<td>5.7</td>
<td>-</td>
</tr>
</tbody>
</table>

# may also be caused by falling watertables resulting in acid-sulphate soils leading to metal mobilisation
* if inorganic chemicals modify redox status of aquifer

### 3. SUGGESTED CHANGES TO DRAFT GUIDELINES

Some measures that are protective of hydraulic impacts on wetlands are addressed in section 5.8 of that document. However, as a result of this review it is suggested that additional measures relating to the maximum rate of change of groundwater level to allow for adaptation by vegetation be inserted in the acceptance criteria and preventive measures for pre-commissioning residual risk assessment in Table 5.11 of the Draft MAR Guidelines (EPHC 2008).

As indicated in Table 4, preventive measures for water quality impacts on groundwater dependent ecosystems are addressed within other preceding sections of the draft MAR Guidelines (EPHC 2008). Reference to the role of ecotoxicological tools is however missing in Table 5.17 of EPHC (2008) and it is suggested that ecotoxicological assessment be inserted under validation monitoring during pre-commissioning residual risk assessment for cases where
groundwater-dependent ecosystems are within close proximity of the attenuation zone surrounding the MAR site.

Appendix 9 provides text to replace section 5.11 in EPHC (2008). This includes reference to the current reviewed and revised report, which provides the scientific support for this section of the Guidelines.

4. INFORMATION GAPS

The review identified several significant information gaps:

- Mobility and rates of inactivation in aquifers of micro-organisms that are pathogenic to fauna or flora (e.g., Phytophthora)
- While a number of studies on stygofauna have assisted in establishing methods for their evaluation, the power of stygofauna sampling to detect population change is weak due to the large proportion of wells with no detections. Further techniques will be needed and it is suggested that ecotoxicological methods could be considered. In addition, quantitative evaluation of water quality parameters, e.g., nutrients and redox status of groundwater, and aquifer porosity structure, associated with presence of stygofauna are warranted.
- Plant response functions to the magnitude and rate of groundwater level decline have been defined in well-studied areas on the Swan Coastal Plain, but are yet to be made more widely applicable. This will require evaluation of groundwater dependent vegetation where groundwater levels are changing in response to pumping and/or climatic change.
- An evaluation of simazine fate in anoxic aquifers is currently underway. Responses of aquifer microbial communities to pesticides, disinfectants, and other chemicals present in reclaimed water that is used as source water for managed aquifer recharge need to be better understood at new managed aquifer recharge sites, as data is sparse for reliable predictions pre-commissioning of sustainable water treatment processes in a variety of aquifers. Again, ecotoxicological methods could be of value in building a data library to allow improved prediction.

These gaps, if addressed by a research program consisting of targeted research projects, would provide data to assist in the application of the Guidelines. Such data would give a more confident basis for regulators and proponents of managed aquifer recharge projects to design, assess, and manage projects to ensure environmental impacts are appropriately addressed.

5. REFERENCES


APPENDIX 1
LITERATURE REVIEW ON ECOTOXICITY STUDIES IN RELATION TO MANAGED AQUIFER RECHARGE
Anu Kumar, CSIRO Land and Water

Contents
A1.1 Introduction
A1.2 Ecotoxicological effects of recovered ASR water in Florida
A1.3 Fish monitoring study in Orange County, California
A1.4 Use of bioanalytical tools for on-line monitoring
A1.5 References

A1.1 Introduction

Current groundwater-quality risk-assessment methods rely upon the detection of specific chemical compounds in environmental samples. The presence of such chemicals at a specified receptor point and at concentrations above set, intervention/trigger limits is then considered to be likely to cause adverse effects in the environment. Intervention/trigger limits are derived from toxicity tests which measure the impact of a single chemical on test organisms under laboratory conditions, thus providing a link between chemical measurements and possible biological impacts. However, problems exist with these methods. Environmental samples, especially groundwater, are a complex mixture of ionic species, and the impact of mixtures of chemicals on the biosphere is difficult to quantify due to interactions between species in solution.

In a mixture of chemicals, the interaction of ionic species might cause additive, antagonistic or synergistic toxic effects (Berenbaum, 1989; Rand 1995; Penttinen et al., 1998 and these are difficult to quantify using analytical methods. Analytically, it is difficult to measure the concentrations of all the compounds in a groundwater sample which might adversely affect the environment. Even if measured, variations in the physico-chemical characteristics of groundwater mean that concentrations of chemicals are not necessarily easy to relate to effects on the environment.

Bioassays are relatively inexpensive and simple to use, and appear to be useful in quality monitoring, remediation monitoring and contamination-screening applications. The performance of bioassays in groundwater quality assessment may be complicated by matrix effects which are not normally encountered in laboratory or surface-water applications, and some additional chemical analyses might be needed for validation. The advantage of bioassays over conventional chemical methods in groundwater applications is that only one or two tests are required, and the removal of toxicity can be used as the remediation target. Bioassays applied to groundwater assessment provide an appropriate environmentally relevant end-point, rather than chemical intervention levels.

Two case studies of ecosystem effects in relation to managed aquifer recharge have been identified, both in USA. One addresses the ecotoxicological effects of recovered ASR water on receiving aquatic ecosystems as part of the Everglades restoration plan, in Florida (Johnson, 2005). The other covers fish biomonitoring data and associated characterisation of water quality of Santa Ana River water, which at base flow is primarily tertiary treated.
effluent and is used as the source of water for ASTR for potable recovery at Orange County California (Woodside and Wehner 2007).

A1.2 Ecotoxicological effects of recovered ASR water in Florida

The ecotoxicological effects of various mixtures of surface water and native groundwater collected from the Caloosahatchee Aquifer Storage and Recovery (ASR) Pilot Project area was evaluated by Johnson (2005). In this study, the aquatic toxicity tests and bioconcentration studies were conducted based on U.S. Environmental Protection Agency (EPA) methods and Florida Department of Environmental Protection (FDEP) guidance. A set of assays were selected and evaluated during Phase 1 of the Ecotoxicology Project. The methods were applied during both the "dry" and "wet" seasons, representing conditions during periods of low precipitation and high precipitation, respectively. Chronic toxicity studies using sensitive standard fish, frog, and invertebrate species were conducted; bioconcentration studies using fish and freshwater mussels were also evaluated.

The aquatic species used in the range-finding tests were the fathead minnow, *Pimephales promelas*, two species of daphnid, *Ceriodaphnia dubia* and *Daphnia magna*, and the South African clawed frog, *Xenopus laevis*. The following adverse effects were evaluated in these toxicity tests:

- Survival (*P. promelas, C. dubia, D. magna*, and *X. laevis*),
- Malformations (*P. promelas* and *X. laevis*),
- Reproduction (*C. dubia* and *D. magna*), and
- Growth (*X. laevis*).

Surface water (background and treated), groundwater, and mixtures of these waters were tested; a laboratory control water was also included. The 7-day chronic fathead minnow test evaluates effects on fish embryos including morphological deformities during embryological and larval development as well as survival. The 7- day and 21-day daphnid chronic tests evaluate the survival of these sensitive freshwater invertebrates as well as effects on reproductive ability. During the 7-day *Ceriodaphnia* test, young (>24 hours old at test initiation) will mature and typically produce 3 broods of young; this life cycle allows the evaluation of reproductive capacity and survival over a short test period. The advantage of the *Daphnia* life cycle test (although longer) is that this species is more tolerant of high ion concentrations in the water (as compared to *Ceriodaphnia*) and can be used under a broader range of environmental conditions. FETAX tests are initiated using frog embryos, and during this short exposure period (96 hours) effects on embryological development as well as embryo survival are evaluated.

The exposure solutions (treatments) for the tests were:

- Laboratory control water; moderately hard reconstituted freshwater (MHR*);
- Background surface water, 100-percent unaltered (BSW*);
- Treated surface water, 100-percent (TSW*) – treatment previously discussed;
- 20-percent groundwater diluted with treated surface water;
- 50-percent groundwater diluted with treated surface water;
- 80-percent groundwater diluted with treated surface water; and
- Groundwater, 100-percent unaltered (GW*).

Only one of the four bioassay tests conducted demonstrated an effect in the test endpoints evaluated. The 7-day chronic bioassay using *C. dubia* demonstrated a statistically significant reduction in reproduction in the background surface water (BSW) sample when compared to the laboratory control (MHR), the “treatment” of the surface water through the bench-scale ASR pilot removed the toxicity observed in the background surface water. The groundwater also had an effect on *C. dubia*. This 7-day chronic test demonstrated a reduction in
reproduction in the 80- and 100-percent groundwater samples compared to the control; this indicates that the groundwater (full strength and diluted to 80 percent) affected the reproductive ability of C. dubia. The groundwater, once diluted by 50 percent using “treated” surface water, did not show any effects. The remaining range-finding tests (P. promelas, D. magna, and FETAX) did not show any response to the treatments. The primary treatment that affected the species tested under these short chronic assays was the full-strength groundwater. However, since there is no scenario in the ASR program where 100-percent groundwater is discharged from an ASR well, this observation of toxicity is benign and not unexpected.

The bioconcentration studies were conducted as static-renewal (continuous flow) exposures using peristaltic pumps distributing fresh test solutions from head tanks to exposure vessels containing fish and mussels. The objective of the bioconcentration tests was to evaluate the potential uptake of metals and radium (mussels only) from the treatment solutions during the 28-day exposure period. Trace metals and radium (226/228) did not bioconcentrate in fish or mussel tissues during the two 28-day bioconcentration studies conducted during the “dry” and “wet” seasons.

Based on this investigation Johnson (2005) suggested that the bioconcentration studies could be also conducted using in situ exposures. This approach would not require the transport of large volumes of water to a laboratory and the control water for these studies should be the background surface water used for the ASR. Johnson (2005) recommended that both of the species used in the development of the bioconcentration protocols were acceptable and can be used in future onsite bioconcentration studies.

A1.3 Fish monitoring study in Orange County, California

In an average year, the Orange County Water District (OCWD) diverts 246 million m$^3$ per year of Santa Ana River (SAR) water for recharge into the Orange County Groundwater Basin. OCWD’s recharge facilities include 26 facilities that cover over 6.1 km$^2$. SAR base flow, which is comprised primarily of tertiary-treated effluent, contains approximately 4 to 6 mg/L of dissolved organic carbon (DOC). SAR storm flow contains up to 25 mg/L of DOC. Woodside and Wehner (2002) conducted extensive water quality studies to verify the safety of recharging the basin with SAR flows. These studies include chemical characterisation and biological assessment.

Fish biomonitoring was evaluated in a feasibility study to determine if it could provide a supplement to chemical monitoring. Such investigation can provide additional assurance that unidentified or uncharacterised chemicals with potential toxicity are not present at biologically effective concentrations in the water. Fish histopathology and vitellogenin induction was used as end points in this study.

Numerous studies have been carried out in fish to detect the effects of endocrine disrupting chemicals (EDCs) both in the field and in laboratory settings, and gonad histology and serum VTG levels are widely applied as the core endpoints for disturbances of the pituitary-gonadal axis. Also in the new OECD test guidelines under development for fish, these two parameters are proposed as candidate endpoints for screening and testing of potential endocrine active compounds and are currently subject to validation by the OECD and associated scientific groups. Fish histopathology helps in identifying target organs of toxicity and mechanism of action. There is an enhanced sensitivity of histological monitoring compared to classical ecotoxicological testing, since effects on the histological level will be visible at lower dosage, compared to toxicological endpoints such as mortality or behavioural changes.
In oviparous vertebrates, vitellogenin (VTG) is one of the most studied estrogen responsive genes. Vitellogenin, a phosphoprotein and complex precursor egg yolk protein, is synthesised in the liver in response to E2 binding to hepatic estrogen receptors. Vitellogenin undergoes transformation in the liver where it is lipidated, phosphorylated and glycosylated before being secreted into the bloodstream and taken up by the developing oocytes via receptor-mediated endocytosis (Tong et al. 2004; Barucca et al. 2006). Vitellogenin is typically associated with mature females, however male and juvenile fish also possess the hepatic estrogen receptor and are able to produce vitellogenin in response to E2 or other estrogenic compounds (Barucca et al. 2006). Therefore, the assessment of the presence of and level of VTG protein in male or juvenile fish has been widely used as a biomarker for analyses of estrogenic effect in the environment (Tyler and Routledge 1998; McArdle et al. 2000; Kidd et al., 2007)).

During fish monitoring studies, Woodside and Wehner (2002) selected three exposure groups: a negative control, positive control, and the test water. The test water was from a shallow well adjacent to the SAR. Three rounds of analyses with fish were conducted in 2004 and 2005. In the first two rounds, Japanese Medaka fish were analysed for tissue pathology, vitellogenin induction, reproduction, and gross morphology. In the third round, fish were analysed for vitellogenin induction, reproduction, limited tissue pathology, and gross morphology. In the first two rounds, no statistically significant differences in gross morphological endpoints, gender ratios, tissue pathology, or reproduction were observed between the test water (shallow groundwater adjacent to the SAR) and the control water. In the third round, no statistically significant differences were observed in reproduction, tissue pathology (limited to evaluation of gonads and ovaries), or vitellogenin induction between the test water and the control water. Integrated biological and chemical assessment provided evidence that the SAR was a suitable source of recharge water for the groundwater basin.

A1.4 Use of bioanalytical tools for on-line monitoring

The use of biological tests alone will typically not provide specific information about the actual toxic compounds causing the effects. Bioanalytical tools such as enzyme sensors detecting specific groups of substances could be helpful (Fennouh et al., 1997). In the view of remediation evaluation taking account of decontamination and detoxification the combination of chemical analysis and biological (i.e. ecotoxicological) tests might increase the explanatory power of both evaluation techniques (Brack et al., 1999). Remediation effort might take months to years. It would be rather laborious to regularly analyse biological effects to supervise toxicity reduction efficiency of the used remediation technique. On line analyses by automated systems could circumvent this problem. Van der Schalie et al. (2001) studied the effect of groundwater remediation in a real time manner. They used non-invasive real time monitoring of four physiological parameters derived from the ventilatory pattern of adult blue gills (Lepomis macrochirus). This research was based on the knowledge that coughing rate of fish is a sensitive tool to detect sub-lethal toxicities (Carlson and Drummond, 1977).

Küster and coworkers (2004) developed on line biomonitor using luminescent bacteria, Vibrio fischeri as test organisms to control the toxicity reduction efficiency of several different groundwater remediation techniques. The biomonitor was used for a period of over 1 year and was able to detect even minor differences and changes in detoxification efficiency of remediation techniques tested in parallel. The bacteria proved to be highly sensitive to the contaminated groundwater and the biomonitor showed a long standing time despite the highly corrosive groundwater present in Bitterfeld, Germany. The bacterial biomonitor was demonstrated to be a valuable tool for remediation success evaluation. Dose response relationships were generated for the six quantitatively dominant groundwater contaminants.
(2-chlorotoluene, 1,2- and 1,4-dichlorobenzene, monochlorobenzene, ethylenbenzene and benzene). The concentrations of individual volatile organic chemicals (VOCs) could not explain the observed effects in the bacteria. An expected, mixture toxicity was calculated for the six components using the concept of concentration addition. The calculated EC50 for the mixture was still one order of magnitude lower than the observed EC50 of the actual groundwater. The results pointed out that chemical analysis of the six most quantitative substances alone was not able to explain the effects observed with the bacteria. Thus chemical analysis alone may not be an adequate tool for remediation success evaluation in terms of toxicity reduction.

A1.5 References


APPENDIX 2

PESTICIDE ATTENUATION IN AQUIFERS DURING MANAGED AQUIFER RECHARGE: A LITERATURE REVIEW

Rai Kookana, Ali Shareef, Tasha Waller and Peter Dillon, CSIRO Land and Water

Contents
A2.1 Background
A2.2 Atrazine
A2.3 Simazine
A2.4 Diuron
A2.5 Methodology
A2.6 References

A2.1 Background

Urban stormwater is a resource that is increasingly being harnessed for managed aquifer recharge in Australia and the opportunity for recovering water for use in drinking water supplies is being explored. Due to significant pesticide usage in urban areas (roadside kerbs, parks, sports fields, home lawns, and industrial areas) persistent and mobile pesticides are commonly detected in stormwater. These include urea and triazine herbicides (namely, diuron, atrazine and simazine) and organophosphate insecticides such as diazinon and chlorpyrifos. Presence in stormwater of pesticides, requires adequate attenuation during pre-treatment or in the aquifer, to facilitate potential potable reuse of recovered water..

There is a large body of literature on environmental fate and attenuation of pesticides engineered treatment systems, in soils and in water bodies. However, most studies have focussed on aerobic biodegradation of pesticides in unsaturated zone of soils, and a smaller number of studies have been undertaken on pesticide attenuation in ground waters, especially on atrazine, which is a well known groundwater contaminant. Since the groundwater contamination due to agricultural use of pesticides is often restricted to unconfined aquifers (mostly aerobic) most studies have focussed on aerobic biodegradation of pesticides.

The technique of ASTR relies on recharging deep aquifers which may be aerobic or anaerobic in nature. The conditions in such aquifers are often quite different from unconfined aquifers that may get contaminated with pesticides. Indeed, there is little information available on pesticide attenuation under anoxic conditions. For example, a literature search revealed that there is no report on anoxic degradation of simazine and only one study on diuron. However, several studies have examined atrazine behaviour under anoxic conditions. There is no study on anaerobic biodegradation of pesticides in Australian aquifers.
A brief review of literature for each of the three compounds that are identified as potential concern in ASTR, namely atrazine, simazine and diuron, is provided below.

A2.2 Atrazine

Atrazine is among one of the most widely used herbicides and is commonly detected in surface and groundwaters, due to its relative low sorption affinity to soil particles and refractory nature, that is, high environmental persistence (an average half-life of 60 days in USDA database, Wauchope et al. 1992). For example, according to a review by Funari et al. (1995), some 32 herbicides, 19 insecticides and 2 fungicides had been detected to that time in ground waters from various parts of the world. Atrazine had the largest number of datasets (41) where it was detected in groundwaters. This constituted 38% of the total tested groundwater samples. More recently, the National Water Quality Survey in USA reported pesticides in surfacewater and groundwater from monitoring of 178 streams and >2700 wells across USA over a decade (Gilliom and others, 2006). This study has reported widespread occurrence of atrazine in surface water as well as groundwater across USA. Surface water in 57% of the stream had pesticides above benchmark values and atrazine was among the most frequently detected herbicides in surface and groundwaters. In Australia also, atrazine and its degradation products have been detected at low concentrations in groundwater and surface waters (Stadter et. al. 1992, Kookana et. al. 1998; AATSE 2002).

A2.2.1 Atrazine degradation in aerobic soils and water

Most studies in the literature cover aerobic soils and groundwaters as contamination with herbicides often occurs in shallow aquifers that are aerobic. The studies on anaerobic conditions are relatively sparse. As reviewed by Seyboyd et al. (2001) half-lives of atrazine in aerobic soil have been reported to range from 14 days to 1 year depending on the study conditions, however reports on anaerobic soils and waters are conflicting in nature. While some studies report faster degradation under anaerobic conditions (e.g. Kruger et al. 1993), others (e.g. DeLaune et al. 1997) have noted the opposite. The differing study conditions make it difficult to make direct comparisons. Very few studies have been conducted on the fate of pesticides under aerobic or anaerobic aquifers conditions or even simulated experimental conditions.

Atrazine has numerous metabolites and their pathways have been well described (Fig. 1) in the literature (Scribner et al. 2000). However, the two metabolites formed upon dealkylation of atrazine that are most commonly found in surface and groundwaters are desethylatrazine (DEA) and desisopropylatrazine (DIA). In fact, DEA has been reported to be the most mobile among the atrazine metabolites (Kruger et al. 1996).

A2.2.2 Wetlands sediments

Atrazine has been reported to degrade under strongly reducing conditions found in wetland soils. Seybold et al. (2001) studied atrazine and metolachlor degradation in a wetland soil and water microcosms, with a redox potential reaching -200 mv in 14 days. In anaerobic soil a half life of 38 days was noted for atrazine and 62 days for metolachlor. The key metabolites formed for atrazine were hydroxy atrazine and desethylatrazine. They observed that atrazine degraded in a shallow submerged anaerobic sediment water column and noted that 98% of residue had disappeared over a 2 year period. Chung et al. 1996 also noted limited atrazine degradation (<20% over 38 weeks of incubation) in wetland sediment under anaerobic conditions (-50 to -150 mV). They used much higher concentrations (10 mg/L) than expected in groundwaters.
A2.2.3 Anaerobic degradation by bacterial inoculate: column studies

A few column studies carried out under anaerobic conditions using atrazine degraders have shown co-metabolic biodegradation of atrazine. Crawford et al. (2000) studied biodegradation of atrazine under different redox conditions by creating vertical separation of oxic, anoxic and reduced zones (400 to -400 mv) with the help of N2 sparging in a column study. Biodegradation was measured using atrazine mineralising bacterium inoculation (M91-3) in the presence and absence of electron donor glucose and electron acceptors oxygen and nitrate. They noted that the inoculant was capable of catabolising atrazine under
denitrifying conditions. Anaerobic biodegradation of atrazine can be accelerated by glucose in the presence of nitrate but not in the absence of nitrate. This was consistent with Ghosh and Philip (2004), who studied atrazine degradation under an anaerobic environment employing mixed microbial consortia (a sludge from an anaerobic digester of a sewage treatment plant) and noted that atrazine degradation was a cometabolic process and addition of C and N increased the rate of degradation by the consortium. However, in this study the high C content of the wastewater reduced the rate of degradation. In another study (Stucki et al. 1995) of atrazine mineralisation under carbon limiting conditions, it was observed that while under aerobic conditions atrazine was completely removed by the atrazine degrading enrichment culture, whereas under oxygen deficient conditions atrazine was incompletely removed. Supply of 66 mg/L nitrate resulted in removal of 99.9% of atrazine from the effluent under denitrifying conditions.

A2.2.4 Degradation under conditions simulating anaerobic aquifers conditions

A Danish study compared effects of aerobic and anaerobic conditions on degradation of 14 herbicides in aquifers (Albrechtsen et al. 2001). The study conditions ranged from batch, column to field injection experiments. At a field site samples were collected from plough layer to sandy aquifer at a depth of 7.7 m below and radiolabelled atrazine was added to it. Atrazine was reported to be stable and was not found to be mineralised at any depth. However, in aquifer samples from four different aquifers (2.4 to 8.3 m bgs) atrazine was completely transformed or degraded over a 200 day period under sulphate reducing conditions but did not degrade under denitrifying, Mn and Fe reducing conditions. In another experiment reported by Albrechtsen et al. (2001), atrazine, 2,4,5-T, DNOC and tritiated water were released simultaneously in an anaerobic aquifer and monitored multilevel samplers at horizontal distances 0.2 to 1.5 m down gradient from the source for up to 240 days. No indication of degradation in the anaerobic aquifer was found for atrazine and 2,4,5-T. However, DNOC showed rapid initial degradation followed by a slower phase. The study concluded that redox conditions and pH strongly affected the degradation potential of pesticides (depending on their chemistry). This study and others have highlighted the relatively lower degradation of most pesticides especially under anaerobic conditions as a serious limitation to attenuation capacity of aquifers.

A2.3 Simazine

Both atrazine and simazine belong to the same family of s-triazines and both are common contaminants in surface and groundwater. The main difference among the two compounds, according to the USDA database of Wauchope et al. (1992), is that simazine has a much smaller aqueous solubility (6.2 mg/L) as compared with atrazine (33 mg/L) but similar persistence (average half life of 60 days). This is one of the reasons why the literature review by Funari et al. (1995) reported smaller data sets (18 versus 44) on simazine contamination of groundwaters. In the case of simazine a much smaller positive frequency of only 2%, as compared to 38% for atrazine, was noted by them. In the recent monitoring study in USA (Gilliom and others 2006) simazine was among the five most commonly detected herbicides used in non-agricultural areas.

A2.3.1 Aerobic degradation

Many studies on simazine degradation in aerobic soils and sediments have been carried out and there are similarities in the breakdown of the two herbicides (e.g. review by Erickson and Lee, 1989). Indeed, four s-triazine herbicides atrazine, cyanazine, simazine and propazine all degrade in soil in similar fashion and form at least one of two common dealkylated metabolites, DIA and or DEA, as shown in Figure 2 (Scribner et al. 2000).
A2.3.2 Anaerobic degradation

We could not find any study in published literature on degradation of simazine under anaerobic conditions. We are not sure if the degradation behaviour of simazine is comparable to atrazine under anaerobic conditions.

![Common metabolites of triazine herbicides](image)

Figure 2.2. Common metabolites of triazine herbicides (taken from Scribner et al. 2000)

A2.4 Diuron

Diuron belongs to phenylurea subclass of herbicides and is commonly used in both rural and urban environments for a range of weed control situations. Diuron is much more persistent than atrazine (average half-life in USDA database is 90 days, Wauchope et al. 1992); however its sorption is 2-4 times higher than atrazine (Oliver et al. 2003). Diuron has been commonly detected in surface waters and groundwaters (Kookana et al. 1998; AATSE, 2002, Gilliom and others, 2006). In the major monitoring study in USA (Gilliom et al. 2006) diuron was among the five most commonly detected herbicides in non-agricultural areas.

A2.4.1 Aerobic biodegradation

Diuron is mainly degraded through biodegradation as its hydrolysis and photolysis has been found to be generally very slow (reviewed by Giacomazzi and Cochet 2004). While isolates have been identified capable of using diuron as a sole source of carbon, mixed microbial cultures of bacteria and fungi in aquatic environments have been found to be more efficient in diuron degradation (Ellis and Camper, 1982). Partial degradation of diuron can lead to accumulation of 3,4-dichloroaniline (3,4-DCA), because the daughter product is relatively more stable than its parent but can slowly degrade under both aerobic and anaerobic
conditions (as reviewed by Giacomazzi and Cochet 2004). The metabolite (3,4-DCA) is considered to be highly toxic to some organisms (especially Crustaceans) and is classified as a secondary poisonous substance (Giacomazzi and Cochet 2004).

A2.4.2 Anaerobic biodegradation

Most studies on diuron degradation have been carried out under aerobic conditions (Giacomazzi and Cochet 2004). The literature review found only one study so far that demonstrated the anaerobic pathway of diuron degradation. Attaway et al. (1981) studied the biodegradation of diuron by sediments under anaerobic conditions in seven different media inoculated and enriched with culture. This sole study showed that the herbicide was degraded within 25 days but the degradation pathways under anaerobic conditions (reductive dechlorination) were different from those under aerobic conditions (sequential N-demethylation). The study reported accumulation of 3-(3-chlorophenyl)-1-1-dimethylurea. Anaerobic biodegradation of another urea herbicide (isoproturon) was found to be comparatively much slower than its aerobic biodegradation (Issa and Wood, 2005).

![Degradation pathways for diuron](image)

The above literature review show that the redox state of the aquifer is likely to have a major bearing on pesticide degradation and therefore the rate of degradation can vary substantially among different aquifer conditions. Therefore, it is paramount that the attenuation of commonly detected pesticides in stormwater in Australia is studied under the locally relevant conditions. The objectives of the planned study therefore are (i) to address
the knowledge gap on degradation of pesticides, especially under anaerobic conditions, and (ii) to establish the attenuation rates of pesticides under the specific conditions relevant to the local aquifer (e.g., temperature and presence of cometabolites) of interest for a more accurate assessment of risks under MAR.

A2.5 Methodology

The methodology used in this study will be essentially similar to our previous published studies on attenuation of a range of organic compounds under aerobic and anaerobic conditions simulating the redox state of the aquifers being used for ASTR (Ying et al. 2004; 2007). The redox state, temperature and biogeochemical conditions of aquifers would be simulated under laboratory microcosm studies utilising the core material and groundwater collected from the aquifer. The sediments spiked with selected pesticides (three herbicides, namely atrazine, simazine and diuron were chosen based on local monitoring data on stormwater, Page et al. 2008) would be incubated under aerobic and anaerobic conditions under both sterile and non-sterile conditions. The disappearance of pesticide residues of parent compounds with time and production of key metabolites and breakdown products, relevant for the incubation conditions, would be monitored using appropriate chromatographic analytical techniques.

A2.5.1 Aquifer sediment and associated groundwaters

Aquifer sediments would be collected from aerobic and anaerobic aquifers at existing sites earmarked for ASTR project. In total three conditions are to be represented: aerobic, nitrate-reducing and sulphate-reducing conditions through these aquifer samples. Fresh aquifer samples are to be collected to preserve their aerobic and anaerobic state according to the protocol described by Vanderzalm (pers communication). Associated ground water samples are collected freshly are to be utilised for spiking with herbicide mixtures and for use in degradation experiment.

A2.5.2 Biodegradation experiments

Biodegradation of three herbicides in selected aquifer materials will be undertaken under aerobic and anoxic conditions (nitrate-reducing and sulfate-reducing conditions). The protocols to be followed have been published by Ying et al. 2004; 2007. These protocols would be slightly modified for this project.

A2.5.3 Aerobic treatment

The method is adopted from Ying et al. 2007. Aquifer material (5 g) is weighed into 20mL scintillation vials. Half of the vials containing aquifer material are sterilised by autoclaving at 120 ± 1°C under 300 kPa chamber pressure for 30min for three times within 3 days, and used as the sterile controls. One millilitre of the groundwater sample spiked with the three herbicides is then added into each vial in a laminar flow cabinet (the filter-sterilised groundwater and effluent solutions are to be added to the vial containing the sterilised sand). All vials are then incubated in an incubator at 20± 1°C until collected for sampling.

Anaerobic treatments

The anoxic microcosms will be constructed using Hungate tubes that would be flushed with nitrogen gas and sterilised by autoclaving. All activities to construct the anoxic microcosms will be undertaken in an anaerobic chamber. The following protocol will be used. Aquifer material (5 g) is weighed into each Hungate anaerobic culture tube (16 x 125mm) and sealed with an oxygen impervious butyl rubber bung. Once weighed, half of the sealed
tubes are sterilised by autoclaving as described above for the aerobic treatment and used as sterile controls. These tubes are placed in the anaerobic chamber after sterilisation. One millilitre of the prepared groundwater (sterile or non-sterile as described above for the aerobic microcosms) is added into each tube using a 1mL syringe. All the anoxic microcosms are incubated in the same incubator as in the aerobic study. Sufficient tubes are to be set up for each sample type (i.e. aerobic sediment/groundwater, aerobic sterile sediment/groundwater, anoxic sediment/groundwater, anoxic sterile sediment/groundwater) to allow three replicate tubes of each sample type to be collected for destructive sampling on each sampling occasion. Tubes of each sample type will be taken weekly until 84 days (0, 1, 3, 7, days and then weekly at 7x2, ..., 7x12) into glass. All samplings will be performed in triplicate, and duplicate sterile controls are to be monitored at the same time. On collection, 100 mL of liquid is removed from each tube for microbial analysis and then all collected tubes are to be stored frozen at 20\(^\circ\)C until extraction and analysis in batches.

**Microbiological analysis**

Microbial numbers will be monitored on each sampling occasion using the most probable number technique. A 100 \(\mu\)L aliquot will be collected from each scintillation vial prior to freezing of the vials. Six 10 \(\mu\)L replicates of the collected sample are then used to inoculate 90 \(\mu\)L of nutrient broth (Oxoid) in the first row of wells in a microtitre tray. These inoculated samples are then diluted in ten-fold increments to in the following wells of the microtitre plates. Inoculated microtitre plates are then incubated at 20\(^\circ\)C for 72 hours. Growth is to be confirmed by visual examination of the microtitre tray for evidence of microbial growth in each of the wells, and where necessary confirmation of growth through microscopic examination. The number of culturable microorganisms in each of the original samples is then calculated by the using the most probable number tables.

The total number of samples for various treatments to be collected at different times during the experiments is presented in Table 1.

**A2.5.3 Sorption Experiment**

Sorption coefficient of a pesticide in aquifer determines its rate of movement from the point of injection of groundwater to the point of subsequent recovery. Thus the residence time of a pesticide in aquifer (and by implication the opportunity for attenuation) is dependent on its sorption to aquifer sediments. Studies would be carried out on aquifer sediments for selected pesticides to obtain locally relevant sorption coefficients of pesticides.

The data on sorption would be utilised to obtain the retardation factors for pesticide movement and to estimate transit times in ASTR. This coupled with the rates of degradation would provide a better estimate of attenuation potential of aerobic and anaerobic aquifers used for ASTR and in turn provide a more accurate risk assessment than that based on limited overseas data.

Sorption of each of the three compounds to the aquifer sediments would be tested using a batch equilibration method as previously described by Ying et al. (2007). According to the method protocol, one gram of aquifer sediment is to be weighed into each test tube, and then 10 mL of 0.01M CaCl\(_2\) solution containing 1 mg/L of each compound to be added into each tube. All the tubes are then placed on a mechanical shaker and are shaken for 3 hours. After equilibration, the tubes are centrifuged at 3000 rpm for 15 min. One mL of supernatant is taken out from each tube and analysed by high performance liquid chromatography (HPLC) (described in detail below). The sorption tests will be conducted in 4 replicates at 20\(^\circ\)C.
Sorption coefficient for each compound, \( K_d \) (cm\(^3\)/g), will be determined along with the associated retardation factor, \( R \), representing the ratio of travel time of sorbed species to that of water using the following equations:

\[
K_d = \frac{S}{C}
\]

\[R = 1 + \frac{K_d \rho}{n_e}\]

where \( S \) is the sorbed phase concentration (mg/kg), \( C \) is the aqueous concentration (mg/L), \( \rho \) is the dry bulk density of the porous media [g/cm\(^3\)], and \( n_e \) is the porosity of the aquifer.

### A2.5.4 Residue analysis

Residual concentration of the pesticides left in aquifer sediments samples collected at different times will be determined by high performance liquid chromatography (HPLC) with ultraviolet detection. Extraction of a sample of slurries of the aquifer sediments will be performed by ultrasonication for 10 min each with 20 + 10 + 10 mL of a solvent mixture containing acetone and methanol (1:1 v/v). After centrifugation at 800 \( g \) combined supernatant solutions were combined and concentrated to dryness under a gentle stream of nitrogen, followed by reconstitution in 1.0 mL of methanol. An aliquot of this extract will be injected directly analysed by HPLC.

<table>
<thead>
<tr>
<th>Dates</th>
<th>Time</th>
<th>Sterile Controls</th>
<th>Biotic Test Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Aerobic Sulfate</td>
<td>Anoxic Sulfate</td>
</tr>
<tr>
<td>13-Jan</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>14-Jan</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>16-Jan</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>20-Jan</td>
<td>7</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>27-Jan</td>
<td>2 wks</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3-Feb</td>
<td>3 wks</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>10-Feb</td>
<td>4 wks</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>24-Feb</td>
<td>6 wks</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>10-Mar</td>
<td>8 wks</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>7-Apr</td>
<td>12 wks</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

Results of this study will be published in the scientific literature on completion.
A2.6 References

Funari et al. (1995).
Page, D et al. (2008).
Stadter, et al. (1992)
APPENDIX 3
POTENTIAL EFFECTS OF MANAGED AQUIFER RECHARGE ON STYGOFADNA COMMUNITIES

Remko Leijs, Flinders University of SA and South Australian Museum

Contents
A3.1 Evaluation of literature for evidence of water quality and water level changes on stygofauna communities
A3.2 Review of stygofauna monitoring methods
A3.3 Recommendation for monitoring impact of MAR on stygofauna
A3.4 The biodiversity of the Mount Gambier stormwater recharge sites: a pilot study.
A3.5 Glossary
A3.6 References

A3.1 Evaluation of literature for evidence of water quality and water level changes on stygofauna communities.

Tomlinson & Boulton (2008) present an excellent review of all aspects of subsurface groundwater dependent ecosystems, which includes characteristics of groundwater environments, implications for fauna, biodiversity, ecological processes and ecosystem services.

Pristine (‘unpolluted’) aquifers are best characterized by an oligotrophic environment where chemical energy and carbon sources are scarce and where the living conditions (e.g. temperature, pH, water chemistry and flow velocity) are nearly constant (Goldscheider et al. 2006). The most important environmental factors affecting stygofauna diversity and abundance in aquifers are the amount of dissolved oxygen (DO) and the concentration of dissolved organic carbon (DOC) (Danielopol et al. 1994, Ward et al. 1998). In pristine aquifers the amount of dissolved oxygen varies with the degree of confinement, while DOC is generally lower than in surface waters. The restricted inputs of energy in aquifer systems lead to a reduced productivity, which is reflected in decreased metabolic rates (Danielopol et al. 1994), adaptations to suboxia (DO less than 3mg/L) (Malard & Hervant 1999, Datry et al. 2003), to longer life cycles and lower fecundity (Dole-Olivier et al 2000), and much lower population densities of stygofauna species compared to surface species.

Both DO and DOC concentrations can be altered by managed aquifer recharge (MAR) and can also vary spatially and temporally within the scale of a MAR operation. It is therefore expected that changes in the water chemistry e.g. by managed aquifer recharge (MAR) will affect the abundance and community structure of stygofauna.

Information on the effects of water quality and water level changes on stygofauna is rather scarce, especially for Australia. The reason for this is that until recently stygofauna in
Australia was only known from caves of eastern Australia (Thurgate et al. 2001). Only in the last decade more systematic surveys have revealed rich stygofauna biodiversity in a range of aquifer types and locations within Australia (Humphreys 2008) including calcrite aquifers in the Pilbara (Eberhard et al. 2005) and the Yilgarn (Humphreys 2001, Watts & Humphreys 2006) regions of Western Australia (WA); limestone and alluvial aquifers in the Kimberley region, WA (Wilson & Keable 1999, Cho et al. 2005); some alluvial aquifers in New South Wales (NSW) and Queensland (Qld) (Hancock & Steward 2004, Hancock & Boulton 2008) and only very recently in South Australia (SA) from limestone aquifers on Eyre Peninsula and the south-east of SA, limestone and fractured rock aquifers in the Flinders Ranges, and fractured rock and alluvial aquifers in the Mount Lofty Ranges (Leys et al., unpublished). The relatively recent discoveries of significant stygofauna biodiversity may be the reason why stygofauna has not been considered earlier as an indicator of ecosystem health in Australia (Tomlinson et al. 2007).

A3.1.1 Effects of water level fluctuations

Information on the effect of water level fluctuation on stygofauna is restricted to a small number of studies from Australia. Hancock (2008, pers. com.) monitored groundwater in the Hunter River Valley, NSW, over three consecutive years of which the first two years were at the end of a six year drought. In the third year the drought broke and significant recharge occurred resulting in local increases in groundwater levels by up to three metres with a subsequent increase in DOC and nitrate levels but no significant increase in DO (Hancock 2008, pers. com.). In response to the recharge event, there was an increase in total abundance of all taxonomic groups (stygobitic dytiscid water beetles, amphipoda, syncarida, as well as stygobitic and epigean copepoda). However, an increase in relative abundance was only apparent for the copepoda, possibly caused by a higher contribution of epigean copepods (Hancock 2008, pers. com.).

Recharge events in the calcrite aquifers of the Yilgarn, WA seem to trigger reproduction in beetles and amphipods (Alford & Cooper pers com.).

Tomlinson (pers. comm.) used experimental microcosms to study how copepoda and amphipoda responded to falling water levels. There is evidence that small bodied fauna (< 1 mm) such as copepods are able to follow declining water levels while larger taxa (>3 mm) such as amphipods may become stranded (Tomlinson et al. 2007). Experiments to study resistance to desiccation of copepods and amphipods showed that 80% of the copepods survived low saturation for 48 hours, but that amphipod survival was poor (Tomlinson, pers. comm.)

A3.1.2 Effects of water quality variations

Although dissolved oxygen (DO) is considered an important environmental parameter in aquifers (Danielopol et al. 1994, Ward et al. 1998), it seems that it is not a limiting resource for most animals in groundwater because the faunal distribution in many studies does not match the oxygen gradients (Malard and Hervant 1999). Hahn (2006) found that oxygen concentrations of 0.5-1 mg/L constitute a critical limit for subsurface fauna. However, Hancock & Boulton (2008) collected stygofauna in four aquifers in eastern Australia with DO levels ranging from 0.23–6.63 mg/L without finding a correlation between DO and taxon richness. A similar range of DO was found in observation wells containing stygofauna in South Australia (0.31-9.26 mg/L) (Leijs & Mitchell, unpublished). Close to 50% of the localities containing stygofauna had suboxic DO levels (<3 mg/L), and 34% of the localities had levels less than 1 mg/L. Although sample sizes are small, most taxonomic groups were
found at the above mentioned range of DO concentrations, however amphipoda were found less often (18%) in suboxic sites than Anaspidacea (75%) or Ostracoda (80%).

Information on the impact of salinity on stygofauna communities is scarce. The author has observed that some taxa are surprisingly resilient (some bathynellids (obligate groundwater animals) in Yilcarn area are found at almost seawater salinity levels. Response to salinity will be species specific and can not be generalised. Effects of groundwater temperature variations are yet to be evaluated.

The impact of organic loadings on stygofauna communities is more evident. Table 3.1 summarizes the most relevant literature on the effects of organic pollution on stygofauna diversity and abundance in a range of aquifer types. From these data it is possible to draw some general conclusions:
- when the subterranean habitat contains stygobitic as well as epigean fauna, epigean fauna tend to displace stygobitic taxa at elevated levels of pollution (Culver et al. 1992, Hallam et al 2008, Sket 1977).
- high levels of organic pollution appear unsuitable for stygobitic as well as epigean fauna (Wood et al. 2002).

Also apparent from the literature in Table 1 is that different taxonomic groups may be more resilient than others. In two of the American studies there is evidence that isopods are more resilient to organic pollution than amphipods (Graening & Brown 2003, Simon & Buikema 1997).

Figure 3.1 A new species of melitid amphipod from the Port Lincoln area (photo: Remko Leijs)
Table 3.1. Review of scientific papers documenting the impact of organic pollution on stygofauna communities (modified from Wood et al. 2008 with new references added)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Location</th>
<th>Aquifer type</th>
<th>Type of pollution</th>
<th>Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culver et al. 1992</td>
<td>Thompson Cedar Cave, Virginia, USA</td>
<td>Cave waters</td>
<td>Sawdust and bark from sawmill operation                                            Elimination of stygobitic amphipod and isopod populations. An increase in the abundance of epigean taxa (Oligochaeta and Chironomidae larvae). Limited recovery after 3 years of the event.</td>
<td></td>
</tr>
<tr>
<td>Fenwick et al 2008</td>
<td>Canterbury Plain, New Zealand</td>
<td>Alluvial</td>
<td>Treated wastewater (moderate) contamination                                         Impact monitored over a gradient showed that stygofauna abundance increased close to contamination source.</td>
<td></td>
</tr>
<tr>
<td>Graening &amp; Brown 2003</td>
<td>Cave Springs Cave, Arkansas, USA</td>
<td>Cave waters</td>
<td>Septic leachate, sewage sludge and cow manure suspected                              Elimination of stygobitic amphipods although stygobitic isopods flourished.</td>
<td></td>
</tr>
<tr>
<td>Hallam et al. 2008</td>
<td>Marrakesh, Morocco</td>
<td>Fractured rock</td>
<td>Leachate from landfill                                                               Well at landfill with elevated nutrient and organic matter levels only harboured epigean fauna, while a less contaminated well had increased abundance of stygobitic species compared to relatively unpolluted wells in the vicinity.</td>
<td></td>
</tr>
<tr>
<td>Holsinger 1966</td>
<td>Banners Corner Cave, Virginia, USA</td>
<td>Cave waters</td>
<td>Septic leachate, sewage                                                              In crease in the abundance of stygobitic isopod and Planaridae populations, as well as an increase in abundance of epigean fauna.</td>
<td></td>
</tr>
<tr>
<td>Masciopinto et al. 2006</td>
<td>Nardo Caves, Salento peninsula, Italy</td>
<td>Limestone and fractured rock</td>
<td>Treated waste water injection                                                        Decline of stygofauna with increased levels of dissolved organic carbon.</td>
<td></td>
</tr>
<tr>
<td>Panno et al. 2006</td>
<td>Illinois’ sinkhole plane, Illinois, USA</td>
<td>Cave waters</td>
<td>Septic leachate, sewage                                                              Elimination of a stygobitic amphipod from one polluted system and recovery from an adjacent previously polluted system. Absence of stygobitic isopods from highly polluted pool, but common occurrence in moderately and slightly polluted waters. Exclusion of stygobitic amphipods from any polluted waters.</td>
<td></td>
</tr>
<tr>
<td>Simon &amp; Buikema 1997</td>
<td>Banners Corner Cave, Virginia, USA</td>
<td>Cave waters</td>
<td>Septic leachate, sewage</td>
<td></td>
</tr>
<tr>
<td>Sket 1977</td>
<td>Podpeška jama, Dinaric Karst, Slovenia</td>
<td>Cave waters</td>
<td>Organic enrichment                                                                   Increase in abundance of stygofauna in the absence of epigean competitors.</td>
<td></td>
</tr>
</tbody>
</table>

(...continues...)
<table>
<thead>
<tr>
<th>Reference</th>
<th>Location</th>
<th>Aquifer type</th>
<th>Type of pollution</th>
<th>Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sket 1977</td>
<td>Jama v Šahnu, Dinaric Karst, Slovenia</td>
<td>Cave waters</td>
<td>Organic enrichment</td>
<td>Stygofauna completely displaced by epigean fauna.</td>
</tr>
<tr>
<td></td>
<td>Postonjna-Planina cave system, Dinaric Karst, Slovenia</td>
<td>Cave waters</td>
<td>Organic enrichment</td>
<td>Increase of epigean fauna further within the cave, and corresponding decline of stygobitic taxa.</td>
</tr>
<tr>
<td>Wood et al. 2002</td>
<td>Peak Cavern, Derbyshire, UK</td>
<td>Cave waters</td>
<td>Paper pulp and peat</td>
<td>Initial exclusion of all taxa and limited recovery of epigean taxa 9 months after detection of pollutant. Recovery of epigean invertebrate community 12 month after pollution events. Recolonizations from unaffected tributaries.</td>
</tr>
<tr>
<td>Wood et al. 2008</td>
<td>Peak-Speedwell Cavern, Derbyshire, UK</td>
<td>Cave waters</td>
<td>Paper pulp and peat</td>
<td>Invertebrate assemblages were more abundant and diverse at sites artificially recharged with storm water compared to reference sites recharged by rainfall infiltration, due to an increase in epigean taxa.</td>
</tr>
<tr>
<td>Datry et al. 2005</td>
<td>Stormwater infiltration Basin, University Claude Bernard, Lyon, France</td>
<td>Alluvial</td>
<td>Stormwater recharge</td>
<td>Pristine, heavy metal polluted and organic polluted rivers were compared. Stygobitic fauna was more affected by organic pollution than by heavy metals.</td>
</tr>
<tr>
<td>Moldovan et al. 2008</td>
<td>Aries River, Romania</td>
<td>Hyporheic</td>
<td>Heavy metals, and severe organic pollution</td>
<td>Pristine, heavy metal polluted and organic polluted rivers were compared. Stygobitic fauna was more affected by organic pollution than by heavy metals.</td>
</tr>
</tbody>
</table>
A3.2 Review of stygofauna monitoring methods

There are several methods available for sampling stygofauna from bores, each with their own advantages and disadvantages with respect to costs of equipment, handling time, effort, and efficiency of recovery of fauna. Regularly used methods are:

(1) Weighted plankton nets (Cvetkov 1968) to filter the water column in a bore.
(2) Various types of pumps to extract and filter standing water from the bore or from the aquifer (after purging the standing water) (Hancock & Steward 2004). Pumps can be used in conjunction with packers to make it possible to pump only from discrete depths (Hahn & Matzke 2005) if the bores are appropriately constructed.
(3) Baited traps or unbaited colonisation traps (Bork et al. 2007).

The various sampling methods have been reviewed by a number of researchers (Hancock & Steward 2004, Hahn & Matzke 2005, Alford et al. 2008, Eberhard et al. in press) which conclude the most effective sampling method is using a weighted plankton net. The disadvantages of this technique are that sampling is restricted to the standing water in the bore and any stratification is disrupted. To sample fauna from the standing bore water as well as the aquifer water it is best to use a combination of plankton net and pumping methods.

Figure 3.2 Weighted plankton net used to sample stygofauna in a well (photo: Remko Leijs)

However, Hahn & Matzke (2005) used pumping techniques to investigate if the taxonomic composition in the standing water from the bottom of bores would be representative for the
aquifer. They found no difference in water chemistry, apart from electrical conductivity, which was higher in the bore, and correlated with a higher amount of detritus in the bore, possibly by increased microbial activity decomposing detritus. There were no differences in taxonomic composition between the standing water in the bore and water from the aquifer after purging the bore, but the abundance of fauna was higher at bottom of the bore than in the water column of the borehole. Fauna composition and abundance was highly variable across bore sites in the same aquifer.

Bork et al. (2008) tested unbaited stygofauna traps in an attempt to standardise the method for sampling for stygofauna biodiversity and abundance. Water chemistry and fauna were collected by pumping from the traps that were fixed at different depths. They found that physio-chemical data and fauna composition in the traps did not differ from that of aquifer, but that fauna abundance was five times higher in the traps than in the aquifer. Alford et al. 2008 compared the efficiency (in fauna collected and time requirements) of plankton nets, pumping and discrete interval sampling, and concluded that net sampling is the most efficient method.

Several studies report that generally the abundance of stygofauna is low in aquifers especially for the larger species (>3mm: such as amphipoda, isopoda, syncarida). Similarly, repeatability of sampling is low. Regularly, fauna are found during the first sampling of a bore and then (almost) never again.

Hancock & Boulton (2008) reported that per region (aquifer) 20-26% of the taxa were recorded only from a single bore. Repeated sampling increased the spatial distribution of some taxa and slightly decreased the percentage of taxa found in a single bore.

Alford et al. 2008 studied the effect of repeated sampling on the percentage of fauna encountered, using taxon accumulation curves. This study was done at a single aquifer with a large number of observation bores (every 100 m in a large grid) A selection of bores were used in clusters of bores that were repeatedly sampled. Some bores in the clusters were randomly chosen to be sampled and others every time. The sampling scheme was statistically sound. They showed that with the most efficient sampling method (weighted plankton net) at least ten samples are needed to recover the majority of the taxa.

Leys & Mitchell, unpublished, found that in South Australian aquifers which are known to accommodate stygofauna, on average less than 10% of the sampled observation bores contained fauna. These low rates of encountering stygofauna may be due to the construction of the bore which is not suitable for fauna to migrate into the standing water of the bore because of the use of filters or slots that are too narrow (in most cases observation bores were constructed to monitor water level and chemistry and not with an aim to monitor groundwater fauna), the open interval of the bore may not intersect fauna bearing passages in the surrounding aquifer matrix, or bores are polluted by organic material from the surface, especially if inappropriately capped.

Although repeated sampling of the same bores may be necessary to obtain a reliable biodiversity estimate, it may also have an effect on the abundance of some slow reproducing taxa, especially when sampling frequency is too high. Some indication of such a diluting effect could be concluded from stygofauna monitoring of the Exmouth, Cape Range, WA water supply area. Monitoring occurred over a period of 9 years using 21 bores that were sampled up to 4 times a year (Goater et al. 2008). The general trend in composition and abundance of the fauna was a decrease in the larger fauna (e.g. amphipods) and an increase in copepods, which may have been a response to the repeated sampling, considering that generation time of amphipods is much larger than that of copepods.

Summarizing, considering sampling effort, costs of equipment and detecting biodiversity and abundance, the weighted plankton net appears to be the best method. Because stygofauna usually occurs in low abundance and variation in taxon richness and abundance between and within bores over time is large, it is desirable to use at least 15-20 bores for monitoring.
A3.3 Recommendation for monitoring impact of MAR on stygofauna

Based on the above literature review, the impact of MAR on stygofauna can best be monitored using a number of monitoring bores in a configuration that allows for measuring the spatial extent of the impact of the recharge.

The objective is to place the monitoring bores around MAR sites in such a way that bores not influenced by MAR such as those up-gradient of the groundwater flow of the recharge site can be used as base line data. Arrays of bores away from the recharge site should give information on the spatial extent of the impact. Ideally the observation bores furthest down-gradient from the recharge site should measure a minimal affect when compared to baseline bore locations, assuming that bioremediation processes will take place.

To minimize the effect of the stochasticity of encountering groundwater taxa it is important that at each distance in the groundwater flow away from the recharge point, there are a number of bores that can act as replicate sites. The best configuration of the monitoring bores will depend on the hydrogeology of the (proposed) MAR site. Piezometry and groundwater modelling should give insight in the extent and direction of the recharge plume and in mixing with the native groundwater.

The requirements of bores for use of monitoring groundwater fauna are as follows. Bore casing, if needed, should be PVC, diameter at least 50 mm, slotted > 3mm, without filters, open intervals in bores should be as large as possible, properly capped, holes in caps should be avoided (not even a little ‘airhole’ because ants will utilize this to get water and have frequently been found drowned in large numbers and thereby changing the water quality in the bore). Larger holes in the cap or uncapped bores attract honeybees (especially in the dryer parts of Australia), but also trap birds and reptiles. Steel casing should be avoided, because corrosion can increase the soluble metal concentrations and significantly fewer fauna have been found in steel cased bores (Leys & Mitchell unpublished).

Preferably, sampling should take place before and after major recharge events. If sampling is needed more than four times a year, and effects of sampling itself on abundance of certain taxa (e.g. slow reproducing amphipods) is suspected, one can opt to sample randomly among duplicate bores. Sampling prior to commencement of a MAR operation will provide background data.

It is important to properly sort, count, preserve and identify the collected stygofauna. Most of the Australian groundwater fauna are still undescribed, and the chance of finding new species is large. Collected fauna therefore should be preserved in absolute ethanol to enable future morphological descriptions and DNA analyses. Samples should be properly labelled (including date of collection, latitude and longitude data, collectors id. etc.), stored in appropriate vials, and ultimately samples should be stored in the collections of a state museum.

A3.4 The biodiversity of the Mount Gambier stormwater recharge sites: a pilot study

Groundwater fauna was sampled in a number of stormwater recharge wells in Mount Gambier, SA. The city of Mount Gambier is built on karstic limestone and stormwater historically drains directly into the limestone aquifer. A pilot study was performed in December 2008 to assess the effect of storm water recharge on the occurrence of groundwater fauna. Because baseline data on groundwater fauna diversity is not available for these recharge wells we compare stormwater recharge wells from high and low level risk catchments, and with biodiversity data from the lower South East of South Australia. Risk levels are based on a pollution risk assessment by Vanderzalm et al. (2007), which involve risks of chemical pollution by industry, fuel/chemical storage, traffic and traffic accidents.
Stormwater recharge wells in the city of Mount Gambier were sampled using the weighted plankton net method. Table 3.2 gives an overview of the fauna collected in seven high risk catchments and five low risk catchments. Although this pilot data set is small, it is possible to make some useful observations:
- None of the sampled bores contained stygofauna species that are known from the wider area around Mount Gambier, such as the mountain shrimp *Koonunga* sp. and the blind amphipod *Uronyctus* sp. *Koonunga* is known from the Englebrechts Cave which is within 650 m from the sampled bores 119 and 158.
- Most of the bores sampled in the high risk catchments contained fauna in reasonable abundance (10-100 specimens), but these fauna mostly consisted of species that are normally found in surface waters (epigean fauna).
- From bores sampled in the low risk catchments, only one (082) contained stygofauna species (Hydrobiid snails (Gastropoda), blind flatworms (Turbellaria) and at least 8 species of mites (Hydracarina)).

The bores in the low risk catchments (155,96,84,357) contained only low numbers of epigean taxa or no fauna at all. The latter bores contained much more black sediment compared to the bores in the high risk catchments, and it was assumed that the load of dissolved organic carbon (DOC), although not measured, was probably too high to sustain fauna. The two bores that did not contain fauna have also low DO levels (resp. 0.65 and 0.41 mg/L).

### Table 3.2. Groundwater fauna sampling of some Mount Gambier stormwater recharge wells. Risk level is based on a preliminary pollution risk assessment by VanderZalm et al. 2007. Abundance is indicated as follows: x – 1-10 specimens; xx –11-100 specimens.

<table>
<thead>
<tr>
<th>Council recharge bore</th>
<th>Catchment</th>
<th>Risk level</th>
<th>Longitude</th>
<th>Latitude</th>
<th>Sample date</th>
<th>DO mg/L</th>
<th>Fauna</th>
<th>Copepoda</th>
<th>Gastropoda</th>
<th>Oligochaeta</th>
<th>Turbellaria</th>
<th>Hydracarina</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>119</td>
<td>423</td>
<td>high</td>
<td>140.77296</td>
<td>-37.82564</td>
<td>01/12/2008</td>
<td>0.38</td>
<td>surface?</td>
<td>xx</td>
<td>x</td>
<td>tree roots*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>220</td>
<td>319</td>
<td>high</td>
<td>140.76817</td>
<td>-37.82331</td>
<td>01/12/2008</td>
<td>3.22</td>
<td>surface?</td>
<td>xx</td>
<td>xx</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>212</td>
<td>293</td>
<td>high</td>
<td>140.74882</td>
<td>-37.82193</td>
<td>01/12/2008</td>
<td>2.56</td>
<td>surface?</td>
<td>xx</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>291</td>
<td>280</td>
<td>high</td>
<td>140.75506</td>
<td>-37.81462</td>
<td>01/12/2008</td>
<td>5.87</td>
<td>surface?</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>158</td>
<td>311</td>
<td>high</td>
<td>140.76538</td>
<td>-37.81789</td>
<td>01/12/2008</td>
<td>4.55</td>
<td>surface?</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>081</td>
<td>72</td>
<td>high</td>
<td>140.78220</td>
<td>-37.83037</td>
<td>01/12/2008</td>
<td>12.35</td>
<td>surface?</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>092</td>
<td>72</td>
<td>high</td>
<td>140.78520</td>
<td>-37.83148</td>
<td>01/12/2008</td>
<td>0.30</td>
<td>surface?</td>
<td>x</td>
<td>Salinity**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>082</td>
<td>?</td>
<td>low</td>
<td>140.78380</td>
<td>-37.83315</td>
<td>01/12/2008</td>
<td>8.52</td>
<td>stygo</td>
<td>x</td>
<td>x</td>
<td>xx</td>
<td>xx1</td>
<td>&gt;8 species</td>
<td></td>
</tr>
<tr>
<td>Tumut Drive Reserve</td>
<td>155</td>
<td>low</td>
<td>140.80922</td>
<td>-37.83290</td>
<td>03/12/2008</td>
<td>4.29</td>
<td>surface?</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardinia St</td>
<td>96</td>
<td>low</td>
<td>140.79315</td>
<td>-37.83353</td>
<td>03/12/2008</td>
<td>0.65</td>
<td>No fauna</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anthony St corner</td>
<td>84</td>
<td>low</td>
<td>140.78916</td>
<td>-37.82952</td>
<td>03/12/2008</td>
<td>0.41</td>
<td>No fauna</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kalimna Crs corner</td>
<td>357</td>
<td>low</td>
<td>140.76257</td>
<td>-37.82986</td>
<td>03/12/2008</td>
<td>1.27</td>
<td>surface?</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Tree roots often form a favourable habitat for stygofauna
** Salinity in that bore was high: 2,730 ppm all the others were between 70 and 430 ppm.

Overall, the sampling results show a similar trend as concluded from the review in paragraph 3.1: It is possible that surface fauna have outcompeted the majority of the stygofauna taxa probably due to elevated organic carbon levels in the bores from the high risk catchments. The pollution levels as reported by Vanderzalm et al. (2007) in these catchments are moderate, and do not seem to affect the fauna. Unfortunately, most of the bores sampled in the low risk catchments appear to have higher organic carbon loads than in the high risk catchments and this could have been the reason for the low abundance of fauna. The elevated organic carbon load in the low risk...
catchments, which are mainly situated in the outskirts of the city, may be due to increased amount of soil and leaf litter in the runoff, compared to the high risk catchments, which are mainly in the centre of the city. In future monitoring of Mount Gambier stormwater recharge wells it is advisable to measure dissolved and particulate organic carbon (DOC and POC) because these parameters are likely the ones that affect the fauna most.

A3.5 Glossary

**Amphipoda** Crustacean order
**Epigean fauna** Surface fauna, sometimes used as a synonym for stygophile when found in groundwater.
**Isopoda** Crustacean order
**Stygobite** Aquatic animal that completes its entire life cycle in groundwater. Often stygobitic species are eyeless and do not have pigment.
**Stygofauna** Aquatic animals found in groundwater; used as a synonym for stygobite.
**Stygophile** Animals that spend part of their life cycle in groundwater.

A3.6 References


Masciopinto C., Semeraro F., La Mantia R, Ingusio S. and Rossi E. 2006. Stygofauna abundance and distribution in the fissures and caves of the Nardó (Southern Italy) fractured aquifer subject to reclaimed water injections. Geomicrobiology Journal, 23(5): 267-278.


APPENDIX 4
EFFECTS ON AQUIFER MICROBIAL COMMUNITIES OF MANAGED AQUIFER RECHARGE

Deborah A. Reed, RPS Environment

A4.1. Brief Literature Review
   A4.1.1 Microbiological response to groundwater contamination
   A4.1.2 Microbiological response to managed aquifer recharge
A4.2 Methods Used in Two Australian Field Studies
   A4.2.1 Molecular microbiological approach
   A4.2.2 Floreat Infiltration Gallery site
   A4.2.3 Bolivar Aquifer Storage and Recovery site
   A4.2.4 Summary of contrasts in study site characteristics
A4.3 Results
   A4.3.1 Floreat Infiltration Gallery site results
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A4.5 References

A4.1. Brief Literature Review

A4.1.1 Microbiological response to groundwater contamination

Environments undergoing changes in the concentrations and availability of nutrients and contaminants such as fertilisation of soils, have been shown to result in highly dynamic microbial communities (Torsvik et al., 1998). In contrast, pristine groundwater represents a relatively stable oligotrophic (low nutrient status) environment compared with terrestrial and surface water bodies (Gilbert, 1994). However, within oligotrophic aquifers, changes in aquifer microbial diversity patterns may result from localised variation in niches such as sediment mineralogy and organic content (Griebler et al., 2002), hydraulic conductivity, sediment grain size (Griebler and Lueders, 2009) and geochemical variation (Takai et al. 2003). Despite groundwater systems generally containing lower microbial biomass compared with soils, it is currently unclear whether this translates into a reduced, similar or higher microbial diversity (Griebler and Lueders, 2009; Balkwill and Ghiorse, 1985; Bone and Balkwill, 1988).

Within contaminated aquifers, anoxic contaminant plumes with distinct redox components are formed where different microbial groups are capable of using locally available electron donors and acceptors (Christensen et al. 2000a). These discrete redox zones are dominated by the different physiologic microbial processes (e.g. Stumm and Morgan, 1981; Lovely, 1991; Lovely and Goodwin, 1988; Chapelle, 2001). Bacteria isolated from such groundwater plumes can play an important role in the degradation of contaminants such as crude oil (Bekins et al. 2001), landfill leachate (Roling et al., 2001), benzene (Koizumi et al. 2002), toluene (Winderl et al. 2007), chlorinated solvent (Doijka et al. 1998), organically rich waste (Kinner et al. 2002), wastewater and trace organics such as pesticides (Pickup et al. 2001). Contaminant/nutrient attenuation is influenced by abiotic and biotic processes, and it is the ability of groundwater microbes to degrade contaminants in aquifers that dictates the success of bioremediation strategies (Griebler and Lueders, 2009). Natural attenuation via indigenous groundwater microbial communities is considered to be the only sustainable component of natural attenuation (Christensen et al. 2000b; Roling and van Verseveld, 2002).
The ability of bacteria to adapt to contaminated groundwater was shown by Herrick et al. (1997) where horizontal transfer of the naphthalene dioxygenase (nahAc) gene occurred at a site contaminated by coal-tar waste. Van der Meer et al. (1998) showed that a new chlorobenzene degradation gene evolved, originating from former non-degradative genes within bacteria indigenous to the aquifer. Genes encoding contaminant degradation such as naphthalene dioxygenase genes have also been shown to be actively transcribed in groundwater where naphthalene degradation was occurring (Wilson et al. 1999) with quantitative estimates of expressed genes involved in trichloroethene (TCE) degradation (Johnson et al. 2005). mRNA analyses have also been used to examine the expression of genes involved in chlorobenzene degradation (Alfreider et al. 2003). Such genetic mechanisms may allow microbial populations to adapt rapidly to changing environmental conditions (Griebler and Lueders, 2009).

Despite the numerous studies of contaminant impacts on groundwater microbial populations at point-source pollution sites (not managed aquifer recharge sites), predictions of these anthropogenic affects on microbial diversity and subsurface biogeochemical cycles are currently not well understood (Griebler and Lueders, 2009). Organic pollutants introduced into oligotrophic aquifers have both shifted or increased microbial diversity (Cho and Kim, 2000; Baker et al. 2001; Rolling et al. 2001; Ferris et al. 2004; Johnson et al. 2004) and conversely resulted in the selection of species within distinct contamination zones (Ferris et al. 2004). Interestingly, wastewater infiltration from farming of intensive stock documented a loss of indigenous microbial diversity through anthropogenic perturbation (Cho and Kim, 2001). Further work is required to fully understand the role of aquifer microbial biodiversity as drivers of these biogeochemical processes and for the resistance and resilience of aquifers against anthropogenic perturbations (Griebler and Lueders, 2009).

A4.1.2 Microbiological response to managed aquifer recharge

Managed aquifer recharge (MAR) source water may include treated effluent or captured stormwater which may contain a number of contaminants and nutrients depending on the level of pre-treatment (wetlands, reservoirs, reverse osmosis, chlorination, ultra violet radiation etc) undertaken before aquifer recharge. The nutrient/contaminant concentration within treated wastewater such as elevated concentrations of nutrients and suspended solids is different compared with oligotrophic groundwater (Dillon et al. 2003). The recharge of treated effluent during MAR thus changes the available nutrients, particulate organic/inorganic matter and physiochemical parameters within an aquifer (Vanderzalm et al. 2006) as a consequence of redox, nutrient and contaminant differences between source water and the receiving groundwater environment.

Changes in available resources within an aquifer can determine the structure and function of microbial communities (Beeman and Suflita, 1987). Consequently recharge of treated effluent may change microbial community dynamics in response to variations in the availability and concentration of electron acceptors and donors during MAR. Competition in response to the change in resource type/concentration has the potential to change the structure and function of aquifer microbial communities. Competitive exclusion and selection are also potential ecological interactions that may result from the expression of contaminant degrading enzymes (Wilson et al. 1999) during MAR.

Groundwater studies that have combined molecular techniques to describe microbial community structure have demonstrated that an interdisciplinary approach (geochemistry, molecular microbial ecology and multivariate statistics) is valuable to comprehensively characterise aquifer biogeochemistry and the changing processes which occur in time and space (Fahy et al. 2005; Haack and Bekins, 2000; Haack et al. 2004). Biogeochemical studies characterising groundwater microbial processes in conjunction with aquifer geochemistry and contaminant removal during MAR are required in order to understand, and predict the ability of aquifers to contribute to the treatment of wastewater for recycling purposes. Studies which fully characterise the microbiology and natural attenuation processes during wastewater recycling within an aquifer undergoing MAR
will inform risk assessments (human health and environmental) (Draft Australian Guidelines for Managed Aquifer Recharge, EPHC/NHMRC/NRMMC 2008). They will also provide information to help manage the environment and associated groundwater dependent ecosystems. Characterising aquifer microbiology during MAR can additionally aid management solutions for sustainable MAR operations such as controlling recharge clogging (Pavelic et al. 2007) and the enhancement of contaminant biodegradation.

A4.2 Methods Used in Two Australian Field Studies

A4.2.1 Molecular microbiological approach

Studies were undertaken at two sites where treated sewage effluent was the source water for managed aquifer recharge, to explore the changes in microbial communities within the aquifer. The two sites were the CSIRO Floreat Infiltration Galleries near the Subiaco Sewage Treatment Plant in Western Australia (Bekele et al. 2008) and the Bolivar reclaimed water aquifer storage and recovery site near the Bolivar Sewage Water Reclamation Plant in South Australia (Dillon et al. 2003).

The aims of the studies were to record the changes in space and time of the overall aquifer microbial community structure in response to MAR operations. Due to the enormous diversity of the indigenous aquifer microbial community it was deemed that a taxonomic study to identify changes in species and their abundance was infeasible. Instead, a more promising approach was to address microbial ecology at a molecular level using multivariate statistical analysis to assess differences in genetic signatures (DNA derived from PCR analyses) of microbial communities from water samples taken from selected piezometers over time, and correlate these with observed changes in water quality, particularly relating to nutrient and redox status. As detailed in Griebler and Lueders, 2009 ‘although details of single organisms matter and are of great interest, ecologists would profit most from uncovering underlying patterns, rules and laws’ (Lawton, 1999). Non-impacted background populations and recharge water were used to define the end-members and to assess the relative impact on the overall community structure at each time and location of groundwater sampling. The details of the methodology for sampling, DNA extractions, PCR analyses, Denaturing gradient gel electrophoresis (DGGE), GelCompare II analysis of DNA patterns, multidimensional scaling plots, principal coordinate analysis and permutational multivariate analysis of variance to determine overall community patterns and significant differences in population structure in space and time are detailed in Reed (2007). Functional classes of bacteria such as sulphate reducing and nitrate-reducing bacteria were also evaluated, using methods described by Reed (2007).

Bacteria were considered in three groups corresponding to their functions representative of three redox states, fermentative, sulphate-reducing and nitrate-reducing. These bacterial groups were cultured from groundwater samples and analysed via DNA fingerprinting profiles of the total culture composition (community structure) for a given sample. For the Floreat site non-culture analysis was also undertaken where DNA was extracted directly from the groundwater sample.

A4.2.2 Floreat Infiltration Gallery site

At the Floreat Infiltration Galleries approx 50kL/day was infiltrated via buried trenches containing a polyethylene frame to maintain an even distribution of the water along the trench. A photograph during the construction phase is shown in Figure 4.1. The profile consists of 8m of Spearwood sand overlying Tamala Limestone with the water table within the limestone at a depth of about 10m. Piezometers were installed to record the movement of recharging water within the aquifer as shown in Figure 2. A recovery well at a distance of 50m recovered water on a continual basis at 5 times the combined infiltration rate of infiltration from the two galleries placed 4m apart. The ambient groundwater is aerobic.
**Figure 4.1** Installation of Infiltration Galleries at CSIRO site, Floreat Park, Western Australia, January 2005. Reclaimed water from Subiaco sewage treatment plant is infiltrated in two galleries. Atlantis™ Leach Drain Gallery is shown during construction.

**Figure 4.2** Cross-section of MAR site (modified from Bekele et al. 2005) showing distance and depth of observation bores relative to the infiltration gallery

The infiltration cycle and sampling days used in the aquifer bacterial population and water quality study are detailed in Table 4.1.
Table 1  Infiltration cycle at the Floreat Infiltration MAR Site.

<table>
<thead>
<tr>
<th>Sampling Event (day number)</th>
<th>Calendar Date</th>
<th>Infiltration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29/09/05</td>
<td>Commencement of infiltration</td>
</tr>
<tr>
<td>81</td>
<td>06/02/06</td>
<td>Continuous infiltration</td>
</tr>
<tr>
<td>110</td>
<td>07/03/06</td>
<td>Continuous infiltration</td>
</tr>
<tr>
<td>123</td>
<td>20/03/06</td>
<td>Continuous infiltration</td>
</tr>
<tr>
<td>137</td>
<td>03/04/06</td>
<td>Continuous infiltration</td>
</tr>
</tbody>
</table>

A4.2.3 Bolivar Aquifer Storage and Recovery site

The Bolivar aquifer storage and recovery site uses ‘A-Class’ reclaimed water from the Bolivar to Virginia pipeline which supplies irrigation water to an intensive horticultural area. This is injected into an aquifer at a depth of 100 to 160m below ground via the ASR well. This is surrounded by piezometers and observation wells to monitor changes in pressure and quality of water in the aquifer. The well is designed to recharge and recover almost 200ML/yr. Wells and piezometers were used to evaluate changes in microbial communities in space and time using the same methods as for the Floreat infiltration gallery site. A photograph of the well head and a schematic diagram of the site showing the locations of piezometers and wells used in the microbiological community study are shown in Figs 4.3 and 4.4.

Figure 4.3 Photograph of the well head of the Bolivar reclaimed water aquifer storage and recovery site. Reclaimed water from the dissolved air flotation and filtration plant downstream of Bolivar sewage treatment plant is pumped into the Bolivar to Virginia pipeline for horticultural water supplies and for aquifer storage and recovery.

The ASR cycles and sampling days used in the aquifer bacterial population and water quality study are detailed in Table 4.2.
Table 4.2 ASR cycle at the Bolivar ASR Site.

<table>
<thead>
<tr>
<th>Sampling Event (day number)</th>
<th>Calendar Date</th>
<th>ASR Cycle 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>05/03/02</td>
<td>Start of injection</td>
</tr>
<tr>
<td>30</td>
<td>03/04/02</td>
<td>Injection</td>
</tr>
<tr>
<td>113</td>
<td>25/06/02</td>
<td>End of injection – beginning of storage phase</td>
</tr>
<tr>
<td>143</td>
<td>25/07/02</td>
<td>Start of recovery phase</td>
</tr>
<tr>
<td>218</td>
<td>08/10/02</td>
<td>End of recovery phase</td>
</tr>
</tbody>
</table>

Figure 4.4 Simplified vertical section along a southwest to north east transect through the ASR well showing the location of four sampling stations monitored (ASR well (18777, and wells at 4m (19450), 50m (19181) and 300m (background well, 19035) (not to scale).

1.1. A4.2.4 Summary of contrasts in study site characteristics

1.2.
Table 4.3 summarises and contrasts features of both sites.
Table 4.3: Features of Floreat and Bolivar sites hydrogeology and managed aquifer recharge techniques

<table>
<thead>
<tr>
<th>Floreat: (Infiltration Galleries)</th>
<th>Bolivar: (Aquifer Storage and Recovery)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shallow (10-30m)</td>
<td>Deep (100-160m)</td>
</tr>
<tr>
<td>Sand overlaying limestone</td>
<td>Limestone</td>
</tr>
<tr>
<td>Aerobic</td>
<td>Anoxic (Nitrate reducing)</td>
</tr>
<tr>
<td>Unconfined</td>
<td>Confined</td>
</tr>
<tr>
<td>Infiltration to water table using infiltration galleries</td>
<td>Injection into confined aquifer</td>
</tr>
<tr>
<td>Extraction well at distance from infiltration site</td>
<td>Extraction occurs from injection well</td>
</tr>
<tr>
<td>Extract 5x infiltration rate</td>
<td>Injection and extraction volumes are similar</td>
</tr>
<tr>
<td>Many observation bores at varying distances and depths to capture plum</td>
<td>Selected observation wells and piezometers were analysed along groundwater flow path from ASR well</td>
</tr>
<tr>
<td>Continuous infiltration</td>
<td>Injection phase, storage phase and recovery phase</td>
</tr>
<tr>
<td>Source water from Subiaco Water Reclamation Plant following discharge from Subiaco Waste Water Treatment Plant</td>
<td>Source water from Bolivar Water Reclamation Plant following discharge from Bolivar Sewage Treatment Plant</td>
</tr>
</tbody>
</table>

Although water quality varied with time at each site, Table 4.4 is a snapshot of the water quality contrasts between recharged and recovered water and ambient groundwater at the two sites. In general although the Bolivar recharge water in ASR cycle 2 was more aerated on recharge than the Floreat water, it contained a greater organic load and oxygen demand that consumed oxygen readily within the aquifer.

Table 4.4 Water quality differences between Perth and Adelaide Sites

<table>
<thead>
<tr>
<th>MAR Site</th>
<th>Water Type</th>
<th>Eh mV</th>
<th>EC uS/cm</th>
<th>DO mg/L</th>
<th>Cl mg/L</th>
<th>DOC mg/L</th>
<th>Fe mg/L</th>
<th>NO₃-N mg/L</th>
<th>S0₄²⁻ mg/L</th>
<th>TDS mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Floreat</td>
<td>Wastewater¹</td>
<td>437</td>
<td>1440</td>
<td>3</td>
<td>279</td>
<td>11</td>
<td>0</td>
<td>2.5</td>
<td>64</td>
<td>757</td>
</tr>
<tr>
<td></td>
<td>Rec Water²</td>
<td>170</td>
<td>1160</td>
<td>4</td>
<td>188</td>
<td>5</td>
<td>3</td>
<td>0.7</td>
<td>60</td>
<td>681</td>
</tr>
<tr>
<td></td>
<td>Groundwater³</td>
<td>348</td>
<td>1020</td>
<td>2</td>
<td>161</td>
<td>3</td>
<td>0</td>
<td>0.05</td>
<td>73</td>
<td>650</td>
</tr>
<tr>
<td>Bolivar</td>
<td>Wastewater¹</td>
<td>853</td>
<td>1975</td>
<td>6.0</td>
<td>413</td>
<td>19.5</td>
<td>0.08</td>
<td>2.5</td>
<td>178</td>
<td>1166</td>
</tr>
<tr>
<td>(cycle 2)</td>
<td>Rec Water²</td>
<td>70</td>
<td>2550</td>
<td>0</td>
<td>575</td>
<td>12</td>
<td>0.7</td>
<td>0.01</td>
<td>220</td>
<td>1481</td>
</tr>
<tr>
<td></td>
<td>Groundwater³</td>
<td>61</td>
<td>3507</td>
<td>0.1</td>
<td>931</td>
<td>0.7</td>
<td>1</td>
<td>0.0</td>
<td>271</td>
<td>2023</td>
</tr>
</tbody>
</table>

¹ Waster water used for recharge: tertiary treated for ASR and secondary treated for infiltration galleries; ² Recovered Water (at end of recovery); ³ ambient groundwater collected from background bores outside the influence of the MAR schemes. ⁴ mV SHE (std hydrogen electrode)
A.4.3 Results

A.4.3.1 Floreat Infiltration Gallery site

Table 4.5 shows the nutrient and major ion concentrations, redox status and percentage gene similarity between recharge water and native groundwater (and one or more intermediate piezometers) (at the end of the recharge period or an identified date when results are likely to be significantly different).

Water quality was relatively similar at 15m to that observed in ambient groundwater except for iron and temperature. Organic carbon was higher in ambient water but as discussed below organic carbon naturally varied within the superficial aquifer. Local variation in aquifer conditions, such as grain mineralogy, temperature and hydraulic conditions, can substantially change groundwater microbiology (Griebler and Lueders, 2009). These results support literature observations because despite similarity in water quality between background and 15m samples, microbial populations were only 28% similar. The elevated concentrations of iron at 15m undoubtedly resulted in an increase in iron oxidising bacteria, where an elevation in temperature may have also been unfavourable for some aquifer microbial communities. The background bore was located 177m upgradient from the MAR site and there were natural variations in the background communities over time.

The recharge and ambient groundwater gene similarity is only 6% and therefore the microbial populations of source water and background water are very dissimilar. The recharge water contains a significantly larger number of microbes (total heterotrophic count) than any groundwater samples. Microbial numbers reduce significantly during infiltration to groundwater in unison with substantial improvements in water quality. Despite the significant reduction in number of groundwater microbes from 2.5m to 15m these two piezometers had 20-30% genetic similarity to the recharge water and 13% and 28%, respectively, similarity to ambient groundwater. The microbial principal coordinate similarity analysis (PCO) as detailed in Reed, 2007 suggested that over the whole time series analysed (day 1 to 137), bacterial populations at 2.5m are more similar to source water than at 15m. These results provide some evidence that impacts on groundwater populations decrease with distance from the infiltration location.

It has been shown that bacterial numbers and diversity can vary according to depth within the aquifer (Balkwill and Ghiorse, 1985). The depth of each sampling location varied from near the watertable to up to 14m below water table and therefore variation in bacterial community structures may have also naturally differed according to depth, where differences in local aquifer conditions may affect the bacterial biodiversity present (Kolbel-Boelke and Nehrkorn, 1992). The Perth MAR site was a shallow superficial aquifer compared with the deep confined aquifer in Adelaide. The Perth aquifer thus had greater potential to interact with the surface environment. Organic carbon also varied substantially in the background bore over time, ranging from below Limit of Detection (LOD of 1mg/L) to 8mg/L. The background bore was 177m hydraulically upgradient from the MAR site and demonstrates local temporal variability in water quality as a consequence of recharge to the shallow unconfined aquifer in a semi-urbanised environment.
Table 4.5 Concentrations of nutrients, redox status and most significant PCA and PCO variables at day 123 of study (20/03/2006) during continuous infiltration of secondary treated effluent.

<table>
<thead>
<tr>
<th>Water quality parameter</th>
<th>Recharge water</th>
<th>Piezometer BH1 (2.5m)</th>
<th>Piezometer BH11 (15m)</th>
<th>Native groundwater at Piezometer (-177m)</th>
<th>Extracted Water BH17 (50m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOC (mg/L)</td>
<td>14.00</td>
<td>5.00</td>
<td>0.00</td>
<td>5.00</td>
<td>8.00</td>
</tr>
<tr>
<td>DOC</td>
<td>14.00</td>
<td>7.00</td>
<td>0.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>NO3-N (mg/L)</td>
<td>1.80</td>
<td>1.70</td>
<td>0.00</td>
<td>0.08</td>
<td>0.55</td>
</tr>
<tr>
<td>NH4-N (mg/L)</td>
<td>0.03</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.27</td>
</tr>
<tr>
<td>TKN (mg/L)</td>
<td>1.50</td>
<td>1.50</td>
<td>0.09</td>
<td>0.09</td>
<td>0.68</td>
</tr>
<tr>
<td>Soluble Phosphate (mg/L)</td>
<td>7.20</td>
<td>0.00</td>
<td>0.00</td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>SO4 (mg/L)</td>
<td>68.50</td>
<td>84.20</td>
<td>78.10</td>
<td>78.10</td>
<td>66.30</td>
</tr>
<tr>
<td>Fe (Total)</td>
<td>0.08</td>
<td>0.01</td>
<td>2.90</td>
<td>0.40</td>
<td>3.60</td>
</tr>
<tr>
<td>ORP (mV)</td>
<td>381</td>
<td>404</td>
<td>341</td>
<td>ND</td>
<td>294</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>1.43</td>
<td>9.12</td>
<td>5.11</td>
<td>2.13</td>
<td>2.13</td>
</tr>
<tr>
<td>Total heterotrophic count (cells/mL)*</td>
<td>43,000</td>
<td>9,300</td>
<td>230</td>
<td>930</td>
<td>3.00</td>
</tr>
<tr>
<td>pH</td>
<td>7.15</td>
<td>7.74</td>
<td>6.39</td>
<td>7.57</td>
<td>7.57</td>
</tr>
<tr>
<td>Temp (degrees C)</td>
<td>27.04</td>
<td>26.23</td>
<td>32.21</td>
<td>23.36</td>
<td>23.36</td>
</tr>
<tr>
<td>EC (uS/cm)</td>
<td>129</td>
<td>125</td>
<td>101</td>
<td>106</td>
<td>106</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>135</td>
<td>220</td>
<td>255</td>
<td>240</td>
<td>240</td>
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<tr>
<td>Chloride</td>
<td>286</td>
<td>254</td>
<td>183</td>
<td>158</td>
<td>158</td>
</tr>
<tr>
<td>Gene % likeness ** to recharge water – non culture</td>
<td>100%</td>
<td>20%</td>
<td>29%</td>
<td>5%</td>
<td>31%</td>
</tr>
<tr>
<td>Gene % likeness ** to native groundwater – non culture</td>
<td>6%</td>
<td>13%</td>
<td>28%</td>
<td>100%</td>
<td>29%</td>
</tr>
</tbody>
</table>

*Not included in PCA analysis. The heterotrophic count = bacterial total abundance requiring oxygen.
** Analysed binary matrix data of presence/absence of genes

Reed (2007) used multidimensional scaling plots, principal coordinate analysis and permutational multivariate analysis of variance to determine overall community patterns and significant differences in population structure in space and time. Such techniques may be used for highly dynamic and sparse populations in ecological studies to observe overall trends that a single comparison for a one-off time period is unable to determine. These techniques take account of the natural biological variation which occurs continuously to detect trends that exceed the natural variation. Although the groundwater populations were diverse, the background, infiltration gallery and extraction well communities changed less over time, than for communities present between the infiltration gallery and extraction well (Reed, 2007). Therefore biodiversity was largely unaffected but the various populations within the whole community structure changed more frequently, where it is suggested this resulted from variation in localised aquifer conditions.
i.e. hydrology (Haack et al., 2004), temperature, nutrient types as a result of MAR. The generation time of bacteria where subsurface studies have showed doubling times of between 1 and 320 days (Phelps et al., 1994) allows for these rapid changes, competition and acclimation to changing conditions, which does not change the overall bacterial biodiversity present. That is small differences in aquifer conditions do not lead to the extinction of bacterial types, but to greater competition and thus a more dynamic community.

A.4.3.2 Bolivar Aquifer Storage and Recovery site results

At the Bolivar aquifer storage and recovery (ASR) site water was injected and recovered using the same well. This resulted in reversals of flow directions in the aquifer. Hence data for water quality in Table 4.6 are reported at each site once during injection and once during recovery, to demonstrate the temporal changes that can occur. Statistical analysis was undertaken on DNA fingerprints from samples from 4 wells after bacterial groups were cultured under three different redox states. This allowed genetic likeness to end-members (recharge water and native groundwater) to be assessed.

The results for the sulphate-reducing bacteria suggest that water quality changes that occurred during the injection phase at the injection and 4m well resulted in a low percentage of genes similar to ambient groundwater. Sulphate concentrations were much lower in the recharge water and hence the injection and 4m well, compared with ambient groundwater. It is suggested sulphate-reducing populations would have been very stressed during the injection phase due to the high concentrations of oxygen and therefore a stable sulphate reducing community is not expected during these unfavourable conditions. Indigenous sulphate reducing bacteria are likely to have gone into survival mode by becoming dormant and surviving within micro-climates such as anoxic sub-environments within sediment particles and biofilms (Pedersen and Eskendahl, 1990; Bouwer and McCarty, 1984).

At the end of the recovery phase the genes in samples from ASR and 4m wells became more similar to those isolated from ambient groundwater. Sulphate, dissolved oxygen and redox became more similar between the injection, 4m and ambient groundwater, which resulted in more similar sulphate reducing bacterial communities. It is interesting to note how the sulphate reducing community structure recovered from the unfavourable conditions during the injection phase which did not appear to result in the extinction of ambient sulphate reducing bacterial community structures. Indigenous bacterial diversity can be resilient where bacteria may become dormant (Roszak and Colewel, 1987) during unfavourable conditions (a survival strategy) and may also survive within micro-climates such as within anoxic pockets within sediment particles and within biofilms (Bouwer and McCarty, 1984). Once favourable conditions return such as was seen during the recovery phase i.e. high concentrations of sulphate, organic carbon and low dissolved oxygen and redox conditions these sulphate reducing communities that are present in ambient groundwater were able to re-establish themselves at the injection and 4m well.
### Table 4.6 Concentrations of nutrients, redox status and most significant PCA variables

<table>
<thead>
<tr>
<th>Water quality parameter</th>
<th>ASR well 18777 (0 m)</th>
<th>Observation well 19450 (4m)</th>
<th>Piezometer 19181 (50m)</th>
<th>Native groundwater at Obs well 19035 (300m)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASR Cycle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Injectant Day 30</td>
<td>Recovered Day 218</td>
<td>Injection Day 30</td>
<td>Recovery Day 218*</td>
</tr>
<tr>
<td></td>
<td>DOC (mg/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOC (mg/L)</td>
<td>24</td>
<td>5.5</td>
<td>18.3</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>SO4 (mg/L)</td>
<td>194</td>
<td>273</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NH4-N (mg/L)</td>
<td>0.44</td>
<td>1.96</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NO3-N (mg/L)</td>
<td>0.582</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NO2-N (mg/L)</td>
<td>0.023</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P (soluble) (mg/L)</td>
<td>1.41</td>
<td>0.216</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P (Total) (mg/L)</td>
<td>1.65</td>
<td>0.222</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TKN (mg/L)</td>
<td>3.19</td>
<td>2.97</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fe (Total)</td>
<td>0.056</td>
<td>0.634</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Eh (mV)</td>
<td>865</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DO (mg/L)</td>
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<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EC (uS/cm)</td>
<td>1943</td>
<td>3200</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>6.7</td>
<td>7315</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Temp (degrees C)</td>
<td>20.5</td>
<td>21.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TOC (mg/L)</td>
<td>25.5</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cl</td>
<td>430</td>
<td>849</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gene % likeness to recharge water (NO3 reducers)</td>
<td>100</td>
<td>100</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Gene % likeness to native groundwater (NO3 reducers)</td>
<td>33</td>
<td>18</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>Gene % likeness to recharge water (SO4 reducers)</td>
<td>100</td>
<td>100</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Gene % likeness to native groundwater (SO4 reducers)</td>
<td>18</td>
<td>55</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Gene % likeness to recharge water (fermentative)</td>
<td>100</td>
<td>100</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>Gene % likeness to native groundwater (fermentative)</td>
<td>36</td>
<td>n.d.</td>
<td>27</td>
</tr>
</tbody>
</table>

* Water chemistry analyses for 4m well assumed similar to ASR well at end of recovery based on previous analyses not reported.

* Water chemistry analyses for native groundwater reported for sampling on day 83 (26/5/02) consistent with earlier and later analyses.

Sulphate-reducing and nitrate-reducing bacterial gene similarity undertaken for sampling events 30 and 218. Nitrate reducing bacterial gene similarity undertaken on sampling events 44 and 218 as the 30 day sampling event culture was dropped and the contents lost.

n.d. = not determined

The results suggest that aquifer microbiology changes in unison with water quality parameters (notably redox status) during aquifer storage and recovery within an initially anoxic confined aquifer. These results, along with those of nitrate-reducers and fermentative bacteria are more fully described by multi-dimensional scaling plots, principal component analysis, principal coordinate analysis and permutational multivariate analysis of variance in Reed (2007) and Reed et al. (2008).
The results indicate little difference in the similarity of 4m and 50m nitrate-reducing populations to recharge water between the injection and recovery phases. However, the background nitrate reducing populations became less similar during the recovery phase for the injection and 4m well. The change in nitrate concentration between the injection and recovery phases was greater at the ASR and 4m wells than at the 50m well. The results suggest that bacteria that reduce nitrate during the injection phase were similar to those present in ambient groundwater and therefore injection did not select a different population of nitrate-reducers at the recharge and 4m well.

**A4.4 Conclusions and their relevance to Guidelines for Managed Aquifer Recharge**

Studies of contaminated aquifers have shown that members of the indigenous groundwater microbial populations in impact zones are able to adapt and become stable (Ferris *et al.*, 2004). A stable population indicates a well-adapted microbial community that is able to take advantage of the new resources and environmental conditions (Lin *et al.*, 2005). Essentially these microbes are able to out-compete and exclude other bacterial species because they have the genetic ability to quickly adapt. Genetic ability includes the expression of contaminant degrading enzymes being able to take advantage of the introduced substances not available prior to recharge (Griebler and Lueders, 2009).

Bacteria can have a doubling time of as little as five hours under ideal conditions in the presence of available nutrients and therefore, communities may change rapidly. Consequently, groundwater bacterial populations can be very dynamic, changing to localised perturbations in aquifer conditions (Kolbel-Boelke *et al*. 1988; Kolbel-Boelke and Nehrkorn, 1992). Multi-cellular organisms take longer to evolve and thus longer to adapt to changes in environmental conditions compared with bacteria. Thus the indigenous groundwater bacteria, if their genetic make-up allows, can quickly evolve and express contaminant-degrading enzymes. This ability to quickly out-compete other indigenous and introduced microbes that do not have this ability has been shown to be a major factor in the influence of native groundwater bacteria on the removal of introduced microorganisms such as enteric pathogens from groundwater (Gordon and Toze 2003, Lin *et al.*, 2005, Toze *et al*. 2004, Yates *et al*. 1990). Unlike the groundwater microorganisms, introduced microorganisms such as pathogens do not have the capacity to rapidly react and adapt to environmental changes. Research has shown that groundwater microorganisms are able to remove introduced metabolically bacteria such as *E. coli* and *Salmonella* and more dormant or inert pathogens such as protozoan (oo)cysts and viruses (Gordon and Toze 2003).

However, once the favourable conditions decline as a result of the biodegradation of metabolised species during MAR, the opportunistic indigenous bacterial species may decline back to background levels. The bacterial community structure at the studied MAR sites was able to quickly revert back to that observed in non-impacted groundwater. As a consequence of spatial heterogeneity (Kolbel-Boelke *et al*. 1988; Kolbel-Boelke and Nehrkorn, 1992), natural variability is expected within an aquifer that is unaffected by managed aquifer recharge, and therefore it is unrealistic to expect that detected populations would be identical to those in sampled ambient groundwater. The reversion of aquifer microbial communities as water quality improves to population structures similar to those in background groundwater suggests that MAR at the studied sites has less impact on aquifer biodiversity than on community structure. Competition and bacterial community composition was reversible, corresponding to the changes in aquifer water quality as a result of aquifer treatment of wastewater recharge.

The key messages for Guidelines for Managed Aquifer Recharge from this research are:

- Changes in water quality and aquifer microbial community structure are linked;
- Indigenous microbes have enormous natural variation as they can evolve and change very quickly and have highly advanced adaptive and protective strategies;
• Bacterial community composition was only significantly affected in the zone of greatest water quality impact in the vicinity of the source of recharge;
• On return to ambient groundwater quality, bacterial community composition reverted back to that present before MAR;
• The ability of indigenous groundwater bacteria to respond to nutrient enrichment and a decline in nutrient enrichment from natural attenuation is a result of their ability to evolve quickly due to short generation times (single cellular organisms versus multi-cellular organisms).

On a general note if the aquifer is overwhelmed for sustained periods by nutrients or organic chemicals such as at point-source pollution sites, then some bacteria may disappear. However once the aquifer is restored indigenous bacteria that have been able to go into defence mechanisms, eg by forming spores (Lappin-Scott and Costerton 1990) are highly likely to return to normal levels. Bacterial spores survive in deserts and other extreme environments (Rothschild and Mancinelli, 2001), and so indigenous bacterial species are highly resilient. In managed aquifer recharge, unlike pollution sites, nutrient loadings may be controlled to ensure that the aquifer returns to ambient conditions.

These research results and the literature suggest that indigenous groundwater bacteria are highly adaptive and survive very adverse conditions, proliferate on return of favourable conditions and would therefore appear to very resilient to the impacts of MAR.

A4.5 References


APPENDIX 5
EFFECTS ON GROUNDWATER DEPENDENT VEGETATION
OF GROUNDWATER LEVEL CHANGES INDUCED BY
MANAGED AQUIFER RECHARGE

Stephen Parsons, SKM Consultants, Armadale, Vic

Contents

Introduction
A5.1 Ecosystems and Groundwater Use
A5.2 Ecosystem Response to Changes in Groundwater Levels
A5.3 Methods to Assess Impacts of MAR operations on Potential GDEs
A5.4 Preventative Methods to Mitigate or Eliminate Adverse Impacts on Potential GDEs
A5.5 References

Introduction

This Appendix examines the potential impact of MAR schemes on riparian and terrestrial
vegetation GDEs and on wetland GDEs. The Appendix is limited in scope to considering
quantity impacts (ie, level and flux). The review of literature did not identify any direct
examples of MAR scheme impacts on GDEs, however the impact of the recovery cycle of
MAR schemes can be inferred from studies of groundwater extraction, since the processes
occurring during declining groundwater levels are the same. There are two important
differences however - one is the impact of rising water levels from the recharge cycle of MAR
schemes and the second is the greater seasonal variability in watertables associated with
MAR schemes in unconfined aquifers. While the literature available on these two issues is
limited, that which is available is presented in this Appendix.

This section is primarily concerned with unconfined aquifers. The impact of confined aquifer
MAR schemes on GDEs will be generally be substantially lower than for unconfined aquifers.
This is particularly the case for MAR schemes that, over the long run, inject approximately the
same volumes as are extracted (provided extraction occurs in relatively close proximity to the
injection). MAR schemes in confined aquifers concerned with injection for regional
groundwater benefits, and without local recovery, could over the long run impact on GDEs in
connected watertable (ie, unconfined) aquifers. However, because i) this is a relatively small
sub-set of MAR schemes, ii) the timeframes of impact will generally be long and iii) the nature
of the local impact in the overlying aquifer will be a slowly rising watertable which is less
problematic than a MAR scheme in an unconfined aquifer, this section is focussed on GDE
impacts in unconfined aquifers.

This Appendix is structured around the following sections:
- Section A5.1. Ecosystems and Groundwater Use - Summarises key literature describing
ecosystems (wetlands, riparian and phreatophytic vegetation) use of groundwater.
- Section A5.2. Ecosystem Response to Changes in Groundwater Levels - Summarises
  key literature describing changes in health of ecosystems with changes in depth to
groundwater.
**Section A5.3. Methods to Assess Impacts of MAR operations on Potential GDEs**

Presents recommendations on methods that could be used to assess impacts of MAR operations on GDEs (wetlands, riparian and phreatophytic vegetation).

**Section A5.4. Preventative methods to mitigate or eliminate adverse impacts on potential GDEs**

Recommends possible measures that could be used to reduce impacts of MAR operations on affected ecosystems.

Ecosystems, communities and species are obligately groundwater-dependent if every occurrence relies 1) on groundwater to provide all or part of the water supply, pressure, chemistry, or temperature requirements seasonally, intermittently (e.g. during drought) or persistently or 2) on a shallow watertable during any time of the year. Species or ecosystems are obligately groundwater-dependent if they are restricted to locations of groundwater discharge. In contrast, biota are facultatively groundwater-dependent if groundwater maintains their habitat conditions - such as late season flow - in some locations but not others. As a result, some ecosystems are groundwater-dependent by virtue of their type but most are groundwater-dependent by virtue of their location in the landscape.

**A5.1 Ecosystems and Groundwater Use**

**A5.1.1 Terrestrial Vegetation**

Within Australia there have been very few studies that have identified groundwater use by terrestrial vegetation. Studies of vegetation water use on the Gnangara Mound of the Swan Coastal Plain remain the most detailed, long-term examination of groundwater dependency by terrestrial vegetation within Australia. Recently though, there has been some studies that describe spatial and temporal patterns of groundwater use by terrestrial and riparian vegetation (Thorburn et al. 1993, Mensforth et al. 1994, Zencich et al. 2002, O’Grady et al. 2005a,b,c, Froend and Bertuch, 2007, Bertuch et al., 2007).

Studies on *Banksia* communities of the Swan Coastal Plain provide valuable insights into the functioning of groundwater dependent ecosystems. The Swan Coastal Plain has a highly seasonal Mediterranean climate. Summers are hot, with high evaporation rates and very little rainfall. In contrast, winters are cool and wet and evaporation rates are low. The soils are characterised by deep, strongly leached oligotrophic sands with very little water-holding capacity (Groom et al. 2001a,b). For plant communities to survive, plants must either be well adapted to drought or have access to deep soil water reserves or groundwater.

Dodd and Bell (1993a,b) were among the first to recognise that *Banksia* communities on the Swan Coastal Plain were probably reliant on groundwater. They demonstrated that predawn leaf water potential remained high (close to zero) throughout the year despite estimated annual *Banksia* water use exceeding rainfall and changes in soil water storage, although they were not able to definitively quantify groundwater use. Improved technology and increased use of isotopic tracers have enabled the partitioning of groundwater and soil water uptake (see for example Burgess et al. 2000, Zencich et al. 2002).

By measuring flow in lateral and taproots directly, Burgess et al. (2000) clearly demonstrated that *B. prionotes* became increasingly reliant on deeper water sources during summer as the shallow soils dried. Zencich et al. (2002) used a combination of isotopic and heat pulse techniques to study the seasonality of water sources in *Banksia ilicifolia* and *Banksia attenuata*. They found that both species were, to some extent, phreatophytic, but groundwater use varied as a function of species, season and position in the landscape.

*Banksia ilicifolia* trees in damlands (with average depth to watertable of 2.5m) were highly reliant on groundwater during summer with 100% of their water use requirements being derived from the shallow groundwater. (In the Zencich study referred to in the following paragraphs, damlands, lower slope, upper slope and dune crest sites contained average depths to groundwater of 2.5m, 4m, 9m and 30m respectively). With the onset of autumn
rains, there was a rapid switch from groundwater use to use of near surface soil moisture. In contrast, *B. attenuata* trees were less reliant on groundwater and more reliant on stored soil water although there was an increase in groundwater use during the summer. The amount of groundwater used by this species was a function of position in the landscape. *Banksia attenuata* trees on dune crests did not show evidence of groundwater use even late in summer. However, trees on lower slopes and upper slopes were able to access some groundwater.

Differences in the dependence on groundwater were again reflected in the seasonal leaf potential water relations of the two species. Dampland-associated *B. ilicifolia* maintained high leaf water potentials (> -0.5 MPa) in both summer and winter. By comparison leaf water potential of *B. ilicifolia* trees on the lower slopes in all seasons were lower than *B. ilicifolia* trees in the damplands and water use by dampland *B. ilicifolia* was higher and less seasonal than water use by *B. ilicifolia* trees on the lower slopes.

The study of Zencich et al. (2002) highlighted the temporal and spatial variability in groundwater use strategies. Groom (2004) reviewed these strategies for plant species of the Swan Coastal Plain. Drought tolerance was characterised by tolerance to significantly lower leaf water potentials and lower transpiration. These species were usually shallow-rooted species. Shallow-rooted species that were unable to develop low leaf water potentials were restricted to low-lying positions in the landscape or where groundwater was shallow. In contrast, deeper-rooted species were able to maintain higher leaf water potentials and water use throughout summer as they accessed groundwater at depths of up to 9 m or deep soil water reserves where groundwater was deeper than the maximum rooting depths.

Spatial and temporal dependency on groundwater has been highlighted in a number of studies. On the Chowilla floodplain in South Australia, a number of studies has highlighted the importance of groundwater for maintaining ecosystem function (Bacon et al. 1993, Thorburn et al. 1993, Mensforth et al. 1994). On the Chowilla floodplain, *Eucalyptus camaldulensis* and *E. largiflorens* trees may use small amounts of saline groundwater to sustain trees between large flooding events. These large flooding events leach accumulated salts from the soil profile and replenish the soil water reserves. The observed decline in health of ecosystems on the Chowilla floodplain is related to the changes in the natural flow regimes, resulting from locks along the river and a reduction in the frequency of these flood events.

In the Pioneer Valley in North Queensland, O’Grady et al. (2005a) demonstrated that species within the *Corymbia/Melaleuca* woodlands also exhibited a range of strategies to cope with the extended dry season. *Corymbia clarksoniana* was almost exclusively using groundwater and deep soil water by the end of the dry season. *Corymbia* trees maintained high leaf water potentials and water use. In contrast, conspecifics such as *M. virdiflora* and *E. platyphylla* used only a small proportion of groundwater and, tended to have lower pre-dawn leaf water potentials and reduced daily tree water use. *Lophostemon suaveolens* used no groundwater at all, and had the lowest water use and pre-dawn leaf water potential.

**A5.1.2 Riparian Vegetation**

Riparian tree species are thought to be more sensitive to drought than other species due to their proximity to reliable water sources, either in-stream water or shallow groundwater (Horton et al. 2001a, Rood et al. 2003). This simplifying assumption however does not reflect the diversity of physiological responses within riparian communities and there has been very little research in Australia on the water use requirements of riparian vegetation, even though plants in riparian communities can potentially access a range of water sources (Dawson and Ehleringer 1991, Thorburn et al. 1994, Drake and Franks 2003, O’Grady et al. 2005 b,c). Leaf-scale measurements of water relations have provided valuable insights into a plant’s ability to respond to environmental stresses.
Horton et al. (2001a) demonstrated that stomatal conductance and net photosynthesis were higher in riparian cottonwoods, salt cedar and willow than terrestrial representatives of these species and that this was principally due to increased water availability with the riparian zone. Furthermore, near streams in semiarid Nevada, Horton et al. (2001b) found that rates of leaf gas exchange and leaf water potential were significantly related to depth to groundwater. During dry years, when river flows were reduced and groundwater was lowered, riparian trees exhibited increased water stress and canopy loss. Smith et al. (1991) examined the water relations of trees in diverted and undiverted reaches of the Sierra Nevada and found that plants at sites with low flows developed significantly lower mid-day leaf water potential and that the extent of mid-day depression in leaf water potential was larger in juvenile plants, presumably because of the limited development of the juvenile root system. Further, there was an inverse relationship between leaf-to-air vapour pressure deficit and maximum stomatal conductance that was not observed on the un-diverted streams, suggesting that trees on undiverted reaches had more favourable leaf water relations.

Chen et al. (2007, 2008) reported on the provision of eight intermittent water deliveries in Xinjiang (Western China), over the period 2000 to 2006. Water was provided to the lower reaches of the Tarim River to improve the riparian ecological conditions. Previously, the stream flow had been dried out for more than 30 years. Data collected during these water deliveries included nine groundwater-monitoring cross-sections with a total of 40 monitoring wells and 30 vegetation plots. The study measured the rise in watertable during the water deliveries, with a rise of 9.87 m below ground level (bgl) before water delivery to 7.74 m bgl after the first water delivery, 3.79 m bgl after the second, and 3.16 m bgl after the third. The watertable responded 450m laterally from the river after the first water delivery and up to 1,050m after the fourth delivery. The composition, type, distribution, and growth of riparian vegetation were closely related to watertable changes, and the range of vegetation responses developed gradually as the water deliveries led to a rising watertable. Chen et al. (2007, 2008) concluded that natural vegetation survival in the area is dependent on connection with the watertable, and that the water deliveries significantly restored natural surface ecosystems in the lower reaches of the Tarim River.

In Australia, E. camaldulensis trees located away from the edges of river channels had lower pre-dawn leaf water potentials than trees located closer to the river and pre-dawn leaf water potential was positively correlated with maximum stomatal conductance (i.e., rate at which leaves can lose water) (Mensforth et al. 1994). In northern Australia, Corymbia bella and Melaleuca argentea trees along the Daly River in the Northern Territory maintained high pre-dawn leaf water potentials during the dry season (O’Grady et al. 2005c). In contrast, pre-dawn leaf water potential of eucalypt trees in open-forests of the surrounding savannas declined during the dry season (Duff et al. 1997, O’Grady et al. 1999, Eamus et al. 2000). Lamontagne et al. (2005) compared the isotopic signatures of groundwater and xylem water to partition groundwater use within the riparian vegetation along the Daly. They demonstrated that trees at positions lower than 5 m above the river level were principally using groundwater, while trees at higher positions within the landscape were principally using soil water. Within the riparian rainforests along streams in Northern Queensland, tree species exhibited a variety of seasonal patterns of pre-dawn leaf water potentials and water use strategies reflecting access to differing sources of water (Drake and Franks 2003).

### A5.1.3 Wetlands

Wetlands comprise areas of temporary or permanently inundated land where the presence of water influences the biological inhabitants and their ecological processes (Boulton and Brock 1999). The biodiversity of wetlands can be high, with many species of permanent and migratory residents. The biological activity of wetlands is largely determined by the hydrological regime, so can fluctuate from periods of intensive aquatic activity when the wetland is full to periods of low biological activity when empty. The persistence of the wetland biological community varies with the coping mechanisms adopted by the constituent species.
Some animal species, such as many microinvertebrates, are able to survive dormant as eggs in the sediments (Jenkins and Boulton 2003). Others, such as birds, are able to migrate from one wetland to another.

Permanent wetlands contain animal and plant species that are often not well adapted to the drying out of the wetland. Where groundwater contributes to the wetland, it can increase the duration of the period of inundation. If the presence of groundwater is essential to the biota and their ecological processes, then the wetland can be considered groundwater dependent. Because the nature of groundwater dependence varies (i.e., entirely, highly, proportional, opportunistic or not dependent; see earlier), it is often difficult to determine groundwater dependence in wetlands.

A number of studies have been conducted across Australia to assess the degree of groundwater dependence of wetlands. The following list is not exhaustive:

- South West Victoria (Barton, et al. 2007) - Groundwater and surface water chemistry was used to distinguish between the relative importance of these two water sources for lakes.
- South west Wimmera (North-west Victoria), SKM (2006) - Used depth to watertable mapping and high resolution digital terrain modelling (along with limited groundwater-surface water chemistry analysis), to assess extent of groundwater dependence of wetlands.
- Burnett catchment (central Queensland coast), SKM (2005) - Groundwater dependence of wetlands was estimated based on depth to watertable maps, degree and duration of wetland inundation and soil maps.
- Alstonville Plateau (Green et al. 2007) - The groundwater dependence of wetlands was determined by combining a hydrogeological assessment of the aquifer with detailed mapping of springs and seepages and associated floral and faunal dependencies.
- Millicent Coast Basin, (SE South Australia) - Fass and Cook (2006) undertook a reconnaissance survey of the groundwater dependence of 37 wetlands for the south east of South Australia. Radon activity and chloride concentrations were used to calculate a simple, steady state, mass balance model. The ratios of (1) groundwater inflow rate to surface water inflow rate and (2) groundwater inflow rate to wetland volume were used to estimate groundwater dependence.

Again, as with terrestrial vegetation, the area within Australia where the greatest research into wetlands and groundwater dependency has occurred, is on the Gnangara Mound. The Gnangara Mound supports some 400 wetlands, which most often occur in depressions between the dominant dune systems of the coastal plain (Yesertener, 2002). Most of these have at least some degree of groundwater dependence.

### A5.2 Ecosystem Response to Changes in Groundwater Levels

#### A5.2.1 Overview and Types of Response to Disturbance

Scheffer et al. (2001) proposed three ecological response functions for describing changes in ecosystem function as a result of disturbance. In the first instance, ecosystem function may decrease in a smooth continuous manner (a). Other ecosystems may be quite inert over a range of conditions until a critical threshold is reached where they quickly attain another stable state (b). The third response is where the response curve is “folded backwards” (c) such that for given environmental conditions an ecosystem can have two (or more) stable states.
Environmental condition (X-axis), in the current context, would principally be related to depth to groundwater and subsequent changes in related hydrological processes such as rate of groundwater water recharge and soil water storage in the unsaturated zone. Ecological condition (Y-axis) can apply to many aspects of ecosystem function and depends largely on the variables being measured (e.g., resilience, species diversity, condition, abundance (Carpenter et al. 2001, Hart et al. 2003, Froend and Zencich 2002)). Many responses are nonlinear and calibrating these responses requires knowledge of the thresholds that induce changes in ecosystem function. However defining the critical thresholds where ecosystem function is disrupted is extremely difficult, as process-based understanding of ecosystem function is limited (Cramer and Hobbs 2002, Hart et al. 2003). Froend and Zencich (2002) review the types of responses that might be expected at each of the different levels of organisation (individual, population or community) within an ecosystem and this provides a useful framework for assessing responses of terrestrial vegetation to declining groundwater availability.

**A5.2.2 Terrestrial and Riparian Vegetation**

This section is divided into the following sub-sections:

- A description of ecological responses due to declining and rising watertables, including conceptual models developed by Naumburg et al. (2005)
- A case study from the Gnangara Mound on the Swan Coastal Plain
- A summary of risk management tools for acceptable levels of groundwater decline in the Gnangara Mound, and their applicability to other areas.
Responses to Declining / Rising Watertable

Declining Watertables

Declining watertables decrease the accessibility of a permanent water source and can therefore result in water stress. There are some possible advantages of declining watertables for vegetation: (i) creates aerated soil profiles at field capacity that become available for new root exploitation (Martin and Chambers 2001), (ii) up to a limit, deeper watertables increase the soil volume available for storage of precipitation and hydraulically lifted water, which can significantly increase plant water use and growth (Jackson et al. 2000) and (iii) high watertables may interact with saline soil layers to reduce vegetation health. If groundwater is high, this may limit the rooting zone to the saline soil layers or introduce saline water to the rooting zone, thus reducing growth (Sorenson et al. 1991, Rengasamy et al. 2003). While a declining watertable can benefit vegetation, the literature contains relatively few examples of positive responses (Naumburg et al. 2005). Therefore in the remainder of this section the focus is on the detrimental effect of declining watertables.

The section is based on a summary of the literature review conducted by Naumburg et al. (2005). The section is divided into direct effects, mitigating factors and confounding factors.

- **Direct effects of water stress.** Declining watertables can decrease plant-available soil water in areas where groundwater is within the rooting zone (Scott et al. 1999). Water deficits stress growing vegetation and lead to numerous physiological changes. As transpiration occurs at the leaf surface, water is pulled up from the soil into roots and through xylem conduits in plants. With a decrease in available soil water, xylem tension increases and leaf water potential becomes more negative (Tyree and Ewers 1991). However, plants can tolerate decreasing water potentials only up to a tissue- and species-specific threshold, beyond which xylem cavitation occurs (Sperry et al. 1998). Consequently, the amount of water transported to leaves decreases, which causes stomatal closure, a reduction in photosynthesis, and, if enough xylem cavitates, branch and crown mortality (Leffler et al. 2000, Sperry and Hacke 2002, Sperry et al. 2002, Cooper et al. 2003). Naumburg et al. (2005) lists xeric (drought tolerant) phreatophytes in the Great Basin (a large, arid region of the western United States) can tolerate water potentials ranging from -4 to -9 MPa, suggesting variable degrees of water stress tolerance in these species. In contrast, less drought-tolerant species, such as riparian trees and shrubs, cannot tolerate water potentials this low. As a consequence of high vulnerability to xylem cavitation, riparian trees are much more likely to experience branch or crown mortality when cut off from groundwater (Segelquist et al. 1993, Pezeshki et al. 1998, Lines 1999, Scott et al. 2000, Shafroth et al. 2000, Horton et al. 2001). On the other hand, phreatophytic shrubs can survive significant watertable drawdowns, but may lose some branches and leaf area (Groeneveld et al. 1994). Thus, large differences in their ability to withstand water stress exist among species utilising groundwater. Knowledge of species-specific differences in their ability to withstand low water potentials therefore becomes key to understanding the degree of vegetation damage under water stress (Naumburg et al. 2005).

- **Mitigating factors:**
  - **Redistribution of roots** - Plant roots can remain in contact with a declining watertable if the rate of decline does not exceed potential root growth rate. Moist, aerated soil layers left behind by falling watertables can facilitate root proliferation with depth. Thus, the rate of watertable declines may be just as important to vegetation health as the absolute change in watertable (Naumburg et al. 2005). Naumburg et al. (2005) postulates that root growth rates for arid shrubs and grasses (3-15 mm/day) may also be possible for xeric phreatophytes but qualifies this by concluding that research on these species is limited and ‘their tolerances to differing rates and amount of groundwater declines remain unclear’.
Alternate sources of water - Vegetation can adjust to declining watertables by utilising water from other sources. In general, root water extraction rates will be highest where the combination of hydraulic conductivity, soil water potential, and root density leads to the lowest amount of energy expenditure (Adiku et al. 2000). Therefore, plants use shallow soil moisture preferentially if it is abundant and switch to groundwater or deeper soil layers as shallow layers become dry. However, differences between species limit the capacity of vegetation to rapidly switch to shallower soil water (if it is there), indicating that each species will be uniquely affected by declines in groundwater levels even in the presence of summer / dry season rainfall (Naumburg et al. 2005).

Hydraulic lift - Hydraulic lift is the process whereby soil water moves upward through the profile via plant root systems, after transpiration has ceased (typically at night), due to heterogeneous soil water potential within the rooting zone. This process could, to some degree, mitigate the effects of decreasing groundwater levels as long as roots maintain contact with the saturated zone (Naumburg et al. 2005).

Water transport capacity and biomass production limitation - With an increasing path length to use water from a deepening watertable, plants can maintain transpiration rates by decreasing leaf water potentials, in order to compensate for the increased resistance. However, this mechanism is limited in its effectiveness due to the species specific thresholds for leaf water potential causing xylem cavitation (as described above). Secondly, because deep roots have a longer transport path, increasing the uptake and transport capacity in deep layers requires a greater biomass investment than in shallow layers. As a consequence, the increased biomass cost of deep roots may limit their proliferation. Naumburg et al. (2005) notes that the importance of this factor is difficult to evaluate because of the almost complete lack of data on deep root distributions (>2 m) in all phreatophytic plants under either constant or fluctuating watertables.

Confounding factors: Shafroth et al. (2000) lists a range of other factors that can impact on the severity of water stress experienced by plants under a declining watertable, including rainfall amounts, soil type and hydraulic conductivity, depth, rate, and duration and timing of watertable decline, and the historical stability of the watertable. To this list Naumburg et al. (2005) adds the effect of pore size (which effects the rate of draining of the soil profile after watertable decline and the height of the capillary fringe), the effect of plant dormancy (whereby a fall in watertable coinciding with a dormant stage is less harmful) and the confounding effect of the interaction of grazing under a falling watertable scenario.

Rising Watertables

Rising watertables can saturate a plants rooting zone, and the resulting anoxia places stress on growing vegetation because of lacking oxygen required for aerobic respiration. In response to anoxia, plant roots switch to anaerobic metabolism. This may initially cause an accumulation of toxic end products and cell damage (Drew 1997). Roots that cannot tolerate extended periods of anoxia usually die. As a consequence of decreased root functioning and death, water transport capacity decreases. Therefore, whole-plant responses may resemble symptoms of drought stress, including the closure of stomata and a decrease in photosynthetic activity (Cronk and Fennessy 2001).

Frequent fluctuations of watertables cause repeated occurrences of anoxia, which create an obstacle for deep root formation (Martin and Chambers 2002). Summarising available literature, Naumburg et al. (2005) concluded that phreatophytes have limited tolerances to prolonged flooding of their roots, which result in limited rooting depths and, potentially, death of entire communities.
Based on their literature review, Naumburg et al. (2005) developed conceptual models for phreatophytic vegetation response to decreasing and increasing watertables. These models are presented in and Figures 5.2 and 5.3 below. The models are prefaced with the conclusion that 'it is difficult to draw definitive conclusions on how fluctuating watertables in water-limited environments affect vegetation. This is largely due to both positive and negative effects involved in both rising and dropping watertables and species differences in stress tolerance’ (Naumburg et al. 2005).

For the falling watertable scenario (Figure 5.2) Naumburg et al. (2005) note that the outcome can range from no observable change to the loss of entire communities, depending on the interaction of biological and physical properties. For example, even if the vegetation cannot produce new roots at sufficiently high rates to maintain contact with the watertable (first decision in flowchart), as long as precipitation is above average or soils have a high water-holding capacity, the effects of a dropping watertable will be delayed or possibly avoided. Conversely, if the vegetation is exposed to additional stresses such as low precipitation, herbivory, or disease, then the consequences will be more severe. At the second decision point, the relative change in depth to groundwater will determine how well root systems can transport water from a greater depth. If the groundwater depth exceeds the maximum rooting depth of a species, groundwater is lost as a direct water source. Groundwater may still be available via hydraulic lift of deeper-rooted species. However, even if a species can still tap into groundwater, the transport limitations may reduce water availability and cause a reduction in aboveground biomass (Naumburg et al. 2005).

For the rising watertable scenario, a critical issue is whether root systems can tolerate and/or adapt to anoxic conditions. Naumburg et al. (2005) stresses the difficulty in evaluating this impact on xeric phreatophytes because of very limited data on their root system responses to flooding. These species may tolerate anoxia longer than flood-intolerant species (Ganskopp 1986), but ultimately their roots die as well (Groeneveld 1990). After the initial root loss, plants may recover if they either have a sufficiently large rooting volume available above the anoxic zone, or if they are able to grow new modified roots that can tolerate anoxic conditions (Groeneveld and Crowley 1988). If too much of the root zone is flooded and the plant loses a significant portion of roots, death may follow. Some plants, while unable to endure extended flooding, may tolerate anoxia for a short period. Naumburg et al. (2005) conclude that the ultimate effect of increasing watertables on vegetation is strongly dependent on the degree of root flooding, length of flooding, and species-specific ability to tolerate anoxic conditions.

The two models developed by Naumburg et al. (2005) describe changes in opposing directions of watertable movement, but both are relevant to operation of a MAR scheme. The common factors effecting the extent of vegetation stress include the relative change in the depth to groundwater and the associated changes in functional root biomass and water uptake capacity. Importantly, Naumburg et al. (2005) conclude that ‘if plants are exposed to frequently rising and falling groundwater, the stress to vegetation may be compounded over that expressed in either conceptual model because of the constant need to readjust root systems to new water levels’. This is an important conclusion in terms of considering MAR impacts on GDEs. Their final note regarding the conceptual models emphasises the need for site specific ecological models to assess the impact of changes in groundwater level, due to the influence of frequency, timing, duration, species-specific traits, and other factors such as grazing and disease, which all potentially affect how severely root systems and water uptake capacity are compromised.
Figure 5.2  Simplified conceptual model of the effects of a declining watertable on a community that was in equilibrium with and using groundwater as a significant source of water. DTW refers to the depth to groundwater, δDTW to the rate of increase in depth to groundwater, RGP to the potential root growth rate, max Droot to the maximum potential rooting depth, and dashed arrow to potential long-term routes. (Naumburg et al. 2005)

Figure 5.3  Simplified conceptual model of the effects of a rising watertable on a community that was in equilibrium with and using groundwater as a significant source of water. DTW refers to the depth to groundwater (Naumburg et al. 2005).
A Case Study: Banksia Woodland on the Gnangara Mound

The unconfined sand aquifers of the Swan Coastal Plain (Gnangara Mound aquifer) in Western Australia support extensive open woodlands dominated by *Banksia* and other phreatophytes. Since the mid-1970's these ecosystems have been subject to declining annual rainfall and watertables. In the summer of 1990/91, a *Banksia* woodland on the Swan Coastal Plain, was subjected to a combination of historical watertable decline due to changing rainfall patterns, and significant groundwater drawdown as a consequence of nearby abstraction. The ecohydrological state of the vegetation at that time was typical of shallow depths to groundwater (<3m); dominated by obligate phreatophytes dependent on access to the watertable throughout the dry summer months. However, increased rates of drawdown in the summer of 1990/91 and poor aquifer recovery during the previous winter, resulted in over 80% mortality of the phreatophytic overstorey species (Froend and Bertuch, 2007). The impacted *Banksia* woodland recovered, but facultative phreatophyte species now dominate the overstorey, suggesting that the ecohydrological state of the site has shifted to one in which the dependence on groundwater access is reduced relative to the pre-impact state (Froend and Bertuch, 2007).

A field experiment was performed over two consecutive summers at the impact sites (and control sites), where the recovered vegetation was subjected to further (rapid) drawdown and its physiological water stress and water source partitioning compared to vegetation at reference sites. The results from both of these sources of data indicated that the phreatophytes at the impacted site were not drought stressed, but were instead meeting their water requirements from deeper portions of the vadose zone. Error! Reference source not found. Figure 5.4 (after Froend and Bertuch, 2007) presents groundwater levels prior to, during and after the sudden change in vegetation health, including levels during the drawdown trials.

![Figure 5.4](image)

*Figure 5.4* Hydrograph of the observation bore at P50 (P50 Obs) and the reference site (Pveg 3). Ranges in groundwater levels are noted for periods before drawdown impacts, immediately after and during the recent drawdown trial (Froend & Bertuch, 2007).
The outcomes of this experiment in terms of conceptual understanding of ecological and hydrological processes are summarised in Figures 5.5 and 5.6 (after Froend and Bertuch, 2007). The key aspect for this study is that while significant ecological decline occurred in the short term, a new ecohydrological state was established, and thus this case followed the scenario 'C' in the Scheffer et al. (2001) model presented in Figure 5.1. This is a further factor for consideration when looking at impacts of extraction (including extraction from MAR schemes) on terrestrial or riparian vegetation. Whether or not affected stakeholders would consider the transitory stage acceptable (which in this case study involved a high mortality rate in the over-storey species) is however a issue for consideration.

**Figure 5.5** Conceptual representation of the observed ecohydrological states at P50 (Froend and Bertuch, 2007)

**Figure 5.6** Conceptual representation of the observed ecohydrological states at P50 and the outcomes of an induced, repeated rapid drawdown event (Froend and Bertuch, 2007).
As described in the previous case study, overlying much of the Gnangara Mound is phreatophytic Banksia woodland that is susceptible to prolonged separation from the unconfined aquifer over the hot, dry Mediterranean summer (Groom et al. 2000a, 2000b; Groom et al. 2001; Zencich et al. 2002; Froend and Drake 2006). The consequences of excessive summer drawdown on phreatophytic Banksia woodland have been recorded several times over the last 22 years. The first was during the summer of 1985 in Whiteman Park adjacent to the Wanneroo and Mirrabooka bore fields. Then during the summer of 1990/91, following two dry winters and a period of extreme hot weather conditions, extensive tree decline was observed near the Pinjar bore field (refer to previous case study). Excessive drawdown during the summer has been shown to cause decline in Banksia woodland species and promote a change in the woodland towards more a xerophytic species composition (Groom et al. 2000a, 2000b; Groom et al. 2001).

The groundwater use of Banksia woodland plants has been found to vary interspecifically, temporally and with topography/depth to watertable; variability which is significant to water resource management, since it potentially determines how hydrological changes will impact upon native vegetation (Froend and Bertuch, 2007). Zencich et al. (2002) determined that dependency on groundwater as a summer water source decreased with increasing depth to watertable; Banksia that had developed in habitat typified by a shallow (<3 m) watertable displayed a far greater dependency on groundwater than individuals of the same species that developed in habitats of moderate (3-6m) and deep (6-10m) depths to the watertable. Froend and Bertuch (2007) suggest that this argument could be extended to vulnerability to groundwater drawdown, i.e. stands of Banksia that are more dependent on groundwater as a summer water source will be more vulnerable to drawdown. Banksia that grow at greater depths to groundwater were shown to meet a larger proportion of their water requirements from the vadose zone, particularly at depth, and developed a deeper rhizosphere to exploit soil moisture beyond the influence of summer evaporation and competition from shallower rooting species (Froend and Bertuch, 2007).

In addition to the total groundwater decline, phreatophytes are also susceptible to the rate, as well as season, of drawdown. Bertuch et al. (2007) cite the following references in support of this proposition: Mahoney and Rood 1992; Stromberg et al. 1992; Tyree et al. 1994; Scott et al. 1999; Groom et al. 2000a; Scott et al. 2000; Shafroth et al. 2000; Horton et al. 2001; Eamus et al. 2006]. This is presumed to be because the rate of watertable decline is faster than the rate of fine root elongation and/or because lowering the watertable is occurring during a time of limited capacity for root growth (Sorenson et al. 1991). Low magnitude and rates of change in groundwater levels as opposed to rapid drawdown, may allow intra- and inter-generational adaptation and persistence of phreatophytes (Scott et al. 1999; Shafroth et al. 2000).

Based on work in Froend et al. (2004) - a study of ecological water requirements on Gnangara and Jandakot Mounds - the Water Corporation (2008) presents risk of impact models for phreatophytic vegetation for three categories of groundwater depth (0 to 3m, 3 to 6m and 6 to 10m). These figures are replicated in Figures 5.7, 5.8 and 5.9. These guidelines will be used by the Water Corporation in order to manage the timing of abstraction and the magnitude and rate of drawdown, to in turn manage risks to phreatophytic Banksia woodland from groundwater decline.

Eamus et al. (2006b), presents a case study on the Banksia woodland on the Gnangara Mound, which includes threshold plant response curves for groundwater declines for the same three groundwater categories described above, using a very similar model, and also developed by Froend. The curves and recommended declines are not replicated here, as the information is essentially replicated in Figures 5.7 to 5.9. However, the attached cautionary notes are repeated below, and are equally valid for the risk models in Figures 5.7 to 5.9. They
describe the problems inherent in development of threshold plant response curves or risk of impact models (Eamus et al. 2006b):

- Different life stages of a single species have different root depths and hence what is appropriate for a mature tree is unlikely to be appropriate for a sapling. The risk plots essentially relate to mature trees
- Using current or recent hydrograph data to define acceptable limits for fluctuations in groundwater depth can be difficult without knowing whether these data represent average or below average years for recharge
- The potential for new root growth probably declines with tree age and thus the rate of increase of groundwater depth and life stage probably determines the degree to which an adaptive response in root growth can be given
- The approach does not take into account the impact of soil texture and hence soil water holding capacity (refer to discussion above for the effect of this influence)

![Figure 5.7](image1)  
**Figure 5.7** Risk of impact for phreatophytic vegetation in the 0 to 3m depth to groundwater range (Water Corporation, 2008).

![Figure 5.8](image2)  
**Figure 5.8** Risk of impact for phreatophytic vegetation in the 3 to 6m depth to groundwater range (Water Corporation, 2008).
These curves are obviously only applicable to *Banksia* species, and even more specifically to *Banksia* within the Gnangara Mound environment. Nevertheless, the principles behind the curves are valid when considering drawdown impacts on phreatophytic vegetation. Most importantly, that the acceptable magnitude and rate of decline is proportional to the existing depth to groundwater. Given the dearth of Australian studies developing ecological response curves for phreatophytic vegetation outside of Gnangara Mound, GDE studies and management approaches outside of Western Australia have used (in a modified form) the response curves developed by Froend and presented in Eaumus *et al.* (2006), eg SKM (2005) for a GDE assessment in the Burnett catchment (Queensland central coast). Given differences in soil type, vegetation and climate between the two regions, adoption of the Gnangara Mound results was considered a conservative management approach for the Burnett area.

In terms of assessing potential MAR impacts on phreatophytic vegetation across Australia, given the lack of suitable data outside of studies in Perth, assessors could use the type of data presented in Figures 5.7 to 5.9, and adopt conservative assumptions in applying those models to their area. (Or alternately conduct expensive and long term groundwater level - vegetation health studies in the proposed MAR area).

The further issue not quantitatively addressed in current literature is the acceptable rate of groundwater rise under injection. The work of Naumburg *et al.* (2005) summarised above, suggests a reasonable approach, in the absence of other data, is that acceptable levels of groundwater rise should be similar to the acceptable levels of decline. However, obviously significantly further work is required in this relatively poorly researched area.

In general, it should be relatively easier for MAR schemes to comply with the types of guidelines presented in Figures 5.7 to 5.9 compared to extraction borefields, because there is no net withdrawal from the aquifer. That is, compliance with the x-axis criteria, 'magnitude of decline', should be relatively more easily achieved. However the y-axis criteria, rate of decline, will be a tighter constraint, as annual water table fluctuations will be larger at MAR operations with seasonal cycles of recharge and recovery than in 'normal' extraction borefields. However, if the rate of decline is measured from the average groundwater level, and not from the injection high groundwater level, achieving this nominal annual target for MAR schemes would be comparable to achieving the target for normal groundwater extraction. The particular MAR operational requirements will be influential here (eg,
wastewater fed MAR schemes with a relatively steady injection will be different to stormwater MAR schemes with a strong seasonal influence).

A5.2.3 Wetlands

Wetlands are typically more susceptible to watertable declines than phreatophytic vegetation, due to the dominance of shallow rooted vegetation in wetlands. Vegetation surrounding wetlands has adapted to seasonal fluctuations in surface and/or groundwater levels. However, wetland species are highly susceptible to non-seasonal changes in water regimes as each species is adapted to a specific water level range, any change in which can ultimately affect its distribution (Wheeler 1999). Long-term persistent hydrological change can cause a shift in community composition and structure as species better adapted to the new conditions become established (Froend et al. 1993). Lowering watertables can result in the loss of species intolerant of drying and their gradual replacement by more drought-tolerant terrestrial species with broader ecohydrological ranges. However, wetlands are generally less susceptible to rising watertables due to greater tolerance of permanent or periodic inundation.

This section focuses on defining an acceptable wetland vegetation response to watertable change. An acceptable change for the aquatic biota of a groundwater dependent wetland is obviously dependent on maintaining the period and depth of inundation to levels as close as practical to natural as possible. Studies from the Gnangara Mound are again the most advanced in Australia in terms of attempting to define impacts of declining groundwater levels on wetland health. Based on a wetland vegetation monitoring program established in the mid-1990s, Loomes and Froend (2007) considered responses at wetlands close and distant from groundwater extraction bores to determine if variations are due to differing watertable decline or other factors.

Their results indicated that to-date, many wetlands display a proportional response to drawdown, under which a progressive change in vegetation condition has corresponded with a progressive groundwater level decline. They conclude that their study, along with other recent work, suggest that the magnitude (m) and rate (m/yr) of water level change are the most relevant (factors) to potential vegetation impact (Froend et al. 2004; Froend Loomes and Bertuch 2006). Based on observed trends from the 11 years of data, Loomes and Froend (2007) propose hydrological criteria to predict ecological responses using a sliding scale to determine the potential risk of impact to wetland vegetation from drawdown of differing rates and magnitudes. Their resultant risk matrix is presented in Figure 5.10.

These categories can only be applied with confidence to wetlands on the Gnangara Mound, but the principles behind their derivation are applied to all groundwater dependent wetlands. As per terrestrial vegetation, given the lack of Australian studies linking wetland ecological condition to groundwater level, this study can serve as a useful starting point for considering acceptable impacts at other sites, giving due consideration to differences in vegetation species, climate and soil type.

In terms of the opposite issue of potential groundwater rises adjacent to/in a wetland, this review has not identified sufficient research to make any meaningful conclusion. The principle suggested for phreatophytic vegetation - that acceptable levels of groundwater rise are likely to be similar to acceptable levels of decline (ie, reverse of Figure 5.10) - is not unreasonable, and it is conceivable that for wetlands, greater levels of rise may be tolerated compared to corresponding acceptable levels of decline.
A broad approach for assessing impacts of MAR operations on GDEs is outlined below:

1) Desktop studies to identify presence of GDEs - Desktop studies to identify the presence of potential GDEs in the MAR area should be undertaken as a ‘first-pass’ assessment. This is likely to involve compiling and overlaying available ecological data sets with hydrogeological maps/data (in particular depth to watertable maps) as well as other supplementary information such as soils maps and possibly aerial photography or satellite imagery. This assessment can be used to determine locations where groundwater is likely to be in reach of the roots of at least some components of the ecosystem. If, for example, this exercise demonstrates that the depth to watertable beneath the only vegetation in the vicinity of the MAR scheme is 30m below surface, it is unlikely the vegetation is groundwater dependent, and hence subsequent steps are probably not warranted.

Table 1 in Eamus et al. (2006a) provides a list of questions that will assist in the process of identifying GDEs. Further, Land and Water Australia (2007a,b,c) commissioned development of a framework for assessment of environmental water requirements of GDEs. Report 1 (LWA, 2007a) in this framework is a compilation of methods (tools) available for identifying GDEs and quantifying their interaction with groundwater (refer to the web-link below). One of the tools in that report applicable to desktop studies of GDEs is ‘mapping’ tools.


2) Assessment of degree of likely impacts - Groundwater modelling (analytical or numerical) can be used to calculate the response of the watertable to a given injection / extraction regime, from which inferences can be drawn about likely GDE responses. If, for example, the modelling indicates that groundwater fluctuation at the nearest GDE is likely to be very small (eg, less than 0.1m) then it is unlikely that GDE impacts will be
significant, and many of the remaining steps may be unnecessary (provided there is a high degree of confidence in the model).

3) **Detailed studies to confirm presence of GDEs** - If the preceding two steps indicate risks to GDEs are potentially significant, field assessment to either confirm groundwater dependence or identify the degree of groundwater dependence may be warranted. (The actual need for this will be based on the likely degree of risk and GDE value). Field techniques for this purpose are discussed at the end of this section.

4) **Establish management targets** - Management targets to limit impacts on GDEs should be set. The type of risk management frameworks developed by Froend *et al.* and described in previous sections of this Appendix are likely to be helpful here, but significant caution should be applied in attempting to use these in a different setting. Ecological expert input will be required here, either in adapting frameworks such developed by Froend *et al.*, or in developing new management targets. Given the paucity of data describing the response of ecosystem function to changes in groundwater regime, there is likely to be significant uncertainty in these management targets, which heightens the importance of baseline and operational monitoring (refer below).

5) **Baseline monitoring** - Establishing a suitable baseline data set before implementation of the MAR scheme is a key part of the monitoring process, in order to establish a reference point against which future deviation can be assessed. Further, because other factors beyond the change in groundwater level can impact on GDE health, ideally the baseline monitoring will also include a control site (or sites) in order to evaluate the effect of other factors on ecosystem response.

6) **Operational monitoring** - Having established the presence of a GDE and established a management target (for example maintaining groundwater depth within a certain range during the growing season), it is important to have a vegetation response that can be measured routinely and which will indicate that ecosystem function is being maintained. The response of management to a trigger being breached will vary depending on the nature of the breach and the management objectives. For example, the management targets may need to be adjusted if monitoring indicates that the environmental condition of a GDE has declined to a level greater than is acceptable, or that a GDE appears to be more resilient than predicted (Sinclair Knight Merz, 2001). Resilience, however, is particularly hard to detect with short term monitoring because of the time-lag between change in groundwater regime and vegetation response. Eamus *et al.* (2006a) lists five criteria to decide which characteristics should be measured, being those which: i) have a defined relationship with groundwater levels, ii) characterise risk to the environment, iii) are cost-effective and practical, iv) have early warning capability and v) consider the ‘lag’ effect effects between changed groundwater levels and environmental condition and/or health.

Eamus *et al.* (2006a) also notes that the ability to make predictions of the impact of a modified water regime on ecosystem components depends on an understanding of the relationship between the ecosystem component (e.g. wetland vegetation, phreatophytic vegetation, macro-invertebrates) and the water regime (Froend and Zencich 2001). Hence consideration needs to be given to understanding and quantifying this relationship at all ecological levels (community, population and individual), as all are linked; an individual species response has implications for population response which in turn influences community composition or structure (Froend and Zencich 2001).

Techniques for operational monitoring are described below.

*Features of vegetation that can be measured to monitor ecosystem health / function*

Steps 3, 5 and 6 (and possibly 4) above require techniques for determining vegetation health or function. Table A5-1 is reproduced from Eamus *et al.* (2006a) which provides a summary of a range of techniques for assessing vegetation function. Eamus *et al.* (2006a) draws
attention to two points regarding the choice of measurements to make: First, there is generally a lag between a decline in groundwater availability and an observable vegetation response. Once the watertable and associated capillary fringe are beyond the root zone, changes in stomatal conductance may occur within a short time (a few days), but changes in leaf area index may require months to be evident and changes in recruitment to the adult population of trees will not be observed for years. Second, field measurements of any variable will be ‘noisy’. Variation among different trees, different species at a site, or among sites, can be very large and consequently replication of measurements at all scales should be designed such as to ensure an adequate signal to noise ratio is achieved to allow detection of the impact of the change in groundwater availability.

Table A5-1 is predominantly concerned with vegetation (and can be applied to phreatophytic vegetation and riparian vegetation). The Land and Water Australia (2007a,b,c) framework reports provide a broader set of tools for assessing GDE function, as well as providing more detail on some of the tools presented in Table A5-1. In particular, the ‘tool-box’ report (Report 1) presents a compilation of methods available for identifying GDEs and quantifying their interaction with groundwater. Tools in that report of relevance here include:

- Tool 3 - Pre-dawn leaf water potential
- Tool 4 - Stable isotope analysis – vegetation
- Tool 5 - Long term observation of systems response to change
- Tool 10 - Plant water use modelling
- Tool 13 - Root depth and morphology

The Toolbox report can be accessed at the following web address: http://www.lwa.gov.au/environmentalwater/Research/A_framework_to_provide_for_the_assessment_of_environmental_water_requirements/indexdl_4563.aspx

When undertaking ecological monitoring, it is also important to measure environmental variables that will influence ecological communities. In addition to groundwater levels, (the primary variable of interest in this Appendix), Eamus et al. (2006a) suggests the following potential variables may influence vegetation health:

1) water quality (nutrient concentrations, salinity, toxicants);
2) soil water-retention capacity and soil stratigraphy (water retention layers above water table);
3) climatic information (rainfall and maximum temperatures during summer/early autumn) can be useful in determining the cause of changes to vegetation;
4) records of past fires (as these may have strong impact on vegetation composition and can compound effects of other environmental factors, such as water regime, on wetland vegetation);
5) depth to the saturated zone of water (usually the capillary fringe above the watertable);
Table A5.1  A range of techniques is available to measure aspects of ecosystem function that might be expected to respond to a change in hydrologic regime (after Eamus et al. 2006a)

(Comparison with control (reference) sites greatly improves the utility of the measurements. These should be close to the experimental sites with the same species and soil types present as at the experimental site).

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Technique</th>
<th>Commentary</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf water potential</td>
<td>Pressure bomb</td>
<td>Simple, cheap, but a nearby reference (control) site is required because it responds to climate (rainfall, temperature, solar radiation, vapour pressure deficit) rapidly. Access to canopy for leaves can be difficult.</td>
<td>Myers et al. (1997)</td>
</tr>
<tr>
<td>Stomatal conductance</td>
<td>Leaf diffusion porometer</td>
<td>Simple, cheap, rapid, but a nearby reference (control) site is required because it responds to climate (rainfall, temperature, solar radiation, vapour pressure deficit) rapidly. Access to canopy for leaves can be difficult.</td>
<td>Thomas et al. (2000)</td>
</tr>
<tr>
<td>Transpiration—tree or canopy scale</td>
<td>Sapflow sensors for trees; eddy covariance or Bowen ratio for canopies</td>
<td>Technically difficult and time-consuming; not cheap. A nearby reference (control) site is required because it responds to climate (rainfall, temperature, solar radiation, vapour pressure deficit) rapidly.</td>
<td>Zeppel et al. (2004)</td>
</tr>
<tr>
<td>Leaf area index; canopy fullness; crown health</td>
<td>Visual assessment; hemispherical photographs; LAI analyser, or remote sensing</td>
<td>Visual assessment easy, rapid and cheap. Leaf area index responds seasonally. Hemispherical photographs technically difficult to analyse. Leaf area index analyser not suitable for all vegetation structures. Remote sensing increasingly available but expensive and not available for all sites and best suited to long-term (2+ years) studies.</td>
<td>O’Grady et al. (2000)</td>
</tr>
<tr>
<td>Growth rate</td>
<td>Band dendrometers</td>
<td>Simple, cheap and can be left in the field for years.</td>
<td>Prior et al. (2004)</td>
</tr>
<tr>
<td>Cover and abundance of indicator plant species</td>
<td>Fixed plots measured over time</td>
<td>Simple and relatively cheap. Provides a measure of community response to shifting water availability gradient. Most often applied where distinctive gradients in groundwater use are evident, e.g. wetland fringes. Requires repeated measures over the long-term (3+ years).</td>
<td>Froend and Looimes (2004)</td>
</tr>
<tr>
<td>Community distribution/zonation change</td>
<td>Visual assessment in fixed plots measured over time</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A5.4 Preventative methods to mitigate or eliminate adverse impacts on potential GDEs

The following actions and strategies may be applicable to mitigate or eliminate adverse impacts of MAR schemes on GDEs (in terms of level and flux):

- Avoid areas of shallow watertable in MAR scheme site selection
- Within the selected MAR scheme location, site injection and extraction bores / infiltration basins in areas of higher elevation in the landscape
- Within the selected MAR scheme location, site injection and extraction bores / infiltration basins away from GDEs
- Restrict volumes injected or extracted so that groundwater levels remain within a specified range, or within a seasonal target range at a reference monitoring well.
- Where GDE impacts are likely to be significant, use horizontal wells or spread out injection / extraction bores as much as possible in order to avoid interference effects and hence magnified local watertable declines/rises, ie, plan the layout of the borefield to minimise drawdown/drawup to the greatest extent possible.
- Monitor the watertable at key sites and allow operational flexibility to react when trigger points are reached (eg, stop injecting / extracting or inject / extract more / less at different locations within the scheme or install and initiate use of standby bores)
- Allow a percentage of injection above planned extraction volumes for environmental benefit
- Start MAR scheme with an injection cycle before the extraction cycle
- Make greater use of / construct a larger buffering storage to allow greater operational flexibility
- Where practical and ecological sensible, use water extracted from the MAR scheme to directly compensate effected GDEs (eg, groundwater extraction from the Gnangara Mound is used to supply water to some high value wetlands effected by groundwater extraction (Loomes and Froend, 2007).

A5. 5 References


Wetland Plants. Water Authority of Western Australia and the Environmental Protection Authority.


Land and Water Australia 2007a, CSIRO Land & Water and Sinclair Knight Merz Pty Ltd. A framework for assessing the environmental water requirements of groundwater dependent ecosystems; Report 1 Assessment toolbox. Prepared for Land & Water Australia by Resource & Environmental Management Pty Ltd.

Land and Water Australia 2007b, CSIRO Land & Water and Sinclair Knight Merz Pty Ltd. A framework for assessing the environmental water requirements of groundwater dependent ecosystems; Report 2 Field Studies. Prepared for Land & Water Australia by Resource & Environmental Management Pty Ltd.

Land and Water Australia 2007c, CSIRO Land & Water and Sinclair Knight Merz Pty Ltd. A framework for assessing the environmental water requirements of groundwater dependent ecosystems; Report 3 implementation. Prepared for Land & Water Australia by Resource & Environmental Management Pty Ltd.


APPENDIX 6
REVIEW OF ECOTOXICOLOGICAL MONITORING METHODS FOR GROUNDWATER DEPENDENT ECOSYSTEMS

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A6.1 Ecotoxicity tests

There are many MAR schemes in Australia comprising of fully operational schemes, those under trial and proposed new sites. Monitoring is essential to ensure that preventive measures are effective. Monitoring to date has included physico-chemical parameters (e.g. conductivity, pH, turbidity) and chemical analyses (e.g pesticides, metals and EDCs) however the toxicity of the waters to aquatic species is generally unknown. Biological assessments that examine the effect of exposure to MAR treated water on aquatic organisms, are rarely utilised. The biological data may greatly augment chemical data by indicating the availability of contaminants in the recovered water and importantly, the potential for negative effects on receptor systems.

Ecotoxicological testing is a procedure that uses living organisms to estimate chemical effects. Organisms that are utilised in these tests need to be; economically or ecologically important, sensitive to contaminants, have easily measurable and identifiable endpoints. In addition, they should be a native species or a species that is comparable to native species, be readily available or easily reared in the laboratory and the organism’s biology is well documented. If feasible, biomonitoring and ecotoxicological investigations could provide additional assurance that unidentified or uncharacterized chemicals with potential toxicity are not present at biologically effective concentrations in the recovered water.

A generally applied approach for risk assessment of such complex mixtures is based on chemical target analysis for example of priority pollutants and modelling of toxicity using individual compound toxicity data. However, this approach is meaningless if key contaminants are not known a priori (Ankley and Mount, 1996). Numerous studies combining chemical and biological approaches for hazard assessment of complex environmental mixtures indicate that priority pollutant concentrations are a poor indicator of toxicity (Jacobs et al., 1993 and Samoiloff 1983). Non-target analysis of complex mixtures allows the detection and identification of a broader range of compounds; however, it is time consuming and not
The results are often difficult to evaluate, since toxicological data of the compounds identified are missing (Reemtsma et al., 1999).

Toxicity assessment using bioassays is an established alternative for hazard assessment of complex environmental mixtures. This approach provides an integrative parameter for the presence of compounds affecting the applied test system. A priori knowledge of key contaminants is not necessary and interactive toxicity among the components is reflected by the results. However, bioassays alone do not provide information on the compounds causing the measured effects and therefore are not a sufficient basis alone for risk reduction measures such as remediation or emission control.

Summarised In this section, are the ecotoxicological methods available and their utility to discriminate impacts of MAR on groundwater dependent ecosystems, primarily on aquatic organisms, and identify methodological gaps.

A6.2 Acute toxicity tests

Standard protocols for toxicity testing have been developed over the years by international bodies such as the Organisation for Economic Co-operation and Development (OECD), the United States Environmental Protection Agency (US EPA) and Environment Canada. Detailed toxicity test conditions and culture methods are provided in the manual developed by USEPA (2002a) for the following principal test organisms:

Freshwater Organisms:
1. Ceriodaphnia dubia (daphnid)
2. Daphnia pulex and D. magna (daphnids)
3. Pimephales promelas (fathead minnow)
4. Oncorhynchus mykiss (rainbow trout) and Salvelinus fontinalis (brook trout)

Estuarine and Marine Organisms:
1. Mysisopsis bahia (mysid)
2. Cyprinodon variegatus (sheepshead minnow)
3. Menidia beryllina (inland silverside), M. menidia (Atlantic silverside), and M. peninsulae (tidewater silverside)

These organisms are easily cultured in the laboratory, are sensitive to a variety of pollutants, and are generally available throughout the year from commercial sources. To be valid, a test must meet certain specified conditions. These vary between different protocols, and test reports should explain which protocol was used and why any variations were introduced. When an aquatic test begins, water quality should be measured at least once each day. Dissolved oxygen (DO) is particularly important and should not fall below 60 per cent saturation to avoid stress to the animals: OECD (1992). Large variations in other parameters, such as temperature and acidity, are not permitted. The concurrent testing of reference toxicants, such as zinc, phenol, potassium chloride and others, provides assurance that test cultures are not deteriorating with time and their sensitivities are not changing, and that overall laboratory performance remains satisfactory.

The objective of acute toxicity tests with effluents and receiving waters is to identify discharges of toxic effluents in acutely toxic amounts. Data are derived from tests designed to determine the adverse effects of effluents and receiving waters on the survival of the test organisms. The recommended effluent toxicity test consists of a control and five or more concentrations of effluent (i.e., multi-effluent-concentration, or definitive tests), in which the endpoint is

1. an estimate of the effluent concentration which is lethal to 50% of the test organisms in the time period prescribed by the test, expressed as the LC50, or
2. the highest effluent concentration at which survival is not significantly different from the control (No-Observed-Effect Concentration, or NOEC).
Receiving water tests may be single concentration or multi-concentration tests. The LC50 is determined by the Graphical, Spearman-Karber, Trimmed Spearman-Karber, or Probit Method. The NOEC is determined by hypothesis testing. Data analyses procedures are well described in the USEPA protocol (2002a). In addition, statistical packages such as Toxstat and Tidepool are widely used for data analyses.

A6.3 Lifecycle tests

Acute toxicity tests last for short periods, usually a few days, in the life of adult animals. Adults are usually stronger and more resistant to toxicants than very young animals. It is obviously better to have toxicity data for the most sensitive stage of an animal's life than for one stage only, and such data can be obtained by testing animals over their whole life cycle.

Life-cycle tests are easiest to conduct with short-lived animals such as small water fleas, which mature so fast that a newly hatched female can produce three broods of young in as little as one week. With these animals, it becomes possible to complete life-cycle toxicity tests cheaply and quickly. For example, a seven day life-cycle test with the American species *Ceriodaphnia dubia* provides a chronic test of reproductive capability that takes only three days longer than an acute 96 hour test with adult fish: Mount and Norberg (1984).

Various Australian species of water flea have been tested. *Moinodaphnia macleayi* has been used as a test species for uranium mining wastes in the Northern Territory; (Van Dam et al., 2002). In Sydney, reliable results were obtained with *Ceriodaphnia cf dubia*, which consistently produced more young in less time than any other species (Julli et al., 1990). This animal has not yet been assigned a definitive name. It resembles the northern hemisphere *Ceriodaphnia dubia* more than any other species but there are some taxonomic differences. It yielded similar toxicity data to imported American animals when tested under similar conditions (Johnston et al., 1990). *Ceriodaphnia cf dubia* is a very attractive test animal because it is an Australian species which can be subjected to chronic life-cycle toxicity tests using established protocols.

A6.4 Sub-lethal tests

Sub-lethal tests are tests in which concentrations of the test chemical are not high enough to kill the test animals. They are not synonymous with chronic tests. Sub-lethal tests examine effects other than death, and they may extend for short or long periods. Chronic tests examine the effects of long-term exposure to chemicals, often over most of an organism's life, and they may measure lethal or sub-lethal effects. A sub-lethal test is often a chronic test and vice versa, but not always (Calow, 1998).

Life-cycle tests which measure reproductive effects in water fleas are one type of sub-lethal test. Another example is the early life-stage (ELS) test for fish. This was developed by the United States EPA on the North American fathead minnow *Pimephales promelas* (Norberg and Mount, 1985) and OECD guidelines are available (OECD, 1992). The Environmental Research Institute of the Supervising Scientist (ERISS) has adapted it to various local tropical fish species to assess mine waters in the Northern Territory (van Dam et al., 2002).

The United States EPA test is a seven day sub-chronic test. It measures the effect of chemicals on the most sensitive larval stages, immediately after hatching, but not on the whole life of the fish (US EPA 1994). Stress from toxicants, at any stage during this period, changes the timing of progression from one stage to the next and may reduce the fish's chance of survival.

The most common endpoints measured in ELS tests are growth of the larvae, abnormalities in body form and changes in the yolk. Significant differences between control and exposed groups are used to identify the "lowest observed effect concentration" (LOEC) or the "no
observed effect concentration" (NOEC). The LOEC is the lowest concentration, of those
tested, at which a significant effect was observed. The NOEC is the next concentration below
this. It is the highest concentration, of those tested, at which no significant effect was
observed. When LOEC and NOEC figures are quoted, it is important to describe the test
conditions and duration.

One primary concern to environmental regulators is the potential toxicity and pathogenicity of
introduced microorganisms towards non-target organisms (Environment Canada 1997). Amphibians are an ideal model species because of their use in standardized toxicity assays,
their susceptibility to pathogenic bacteria, and their potential for exposure (Anver and Pond
1984). As a key component of wetland ecosystems, amphibians may be exposed to
remediated effluents through groundwater discharge to wetlands (Todd 1980). Standardized
toxicity assays, such as FETAX (ASTM 1998), allow for the testing of complex effluents for
both acute toxicity and teratogenicity. FETAX is a 96 hour whole embryo developmental
toxicity test that uses the South African clawed frog, *Xenopus laevis*. It was developed to
rapidly and inexpensively determine the deleterious effects of sublethal levels of contaminants
(ASTM 1998). Initially FETAX was designed to be used as an indicator of potential human
development health hazards. However, it is applicable to aquatic toxicity assessments and
can be used to assess single chemicals or complex mixtures.

A6.5 Test species used in Australia

A common laboratory aquatic test is the 96 hour LC50 test with adult fish. A number of fish
species, such as the rainbow trout and the fathead minnow, are commonly used overseas. In
Australia, native species like Duboulay’s rainbowfish, *Melanotaenia duboulayi* and Murray
rainbowfish, *Melanotaenia fluviatilis* are easy to breed in the laboratory and robust and easy to
handle in toxicity tests. Duboulayi’s rainbowfish and a number of closely related rainbowfish
are native to Australia and provide an Australian counterpart to the well-known test species

Fish are obvious in the public’s perception, but invertebrates are just as important in aquatic
ecosystems. The most common invertebrate tested is *Daphnia magna*, a water flea less than
4 mm long. Water fleas are crustaceans, related to shrimps and crabs. Under normal
conditions they reproduce asexually, and this makes it easy to maintain genetically uniform
populations in the laboratory. Water fleas are sensitive to many toxicants and are, eg,
considered to be essential test animals for many purposes, such as assessment of pesticides
in the United States. Acute tests with *Daphnia magna*, usually conducted over 48 hours under
static conditions, have been written into protocols by the American Society for Testing and
Materials (1980, pp 548-572) and the OECD (1987). *D magna* is not found in Australia, but
similar tests have been developed with local species, primarily *Ceriodaphnia cf dubia* and
*Daphnia carinata*, have become local standard species: Julli, *et al.*, 1990; Rose *et al.*, 1997).

Bioassays using tadpoles of native frogs such as *Crinia signifera*, *Limnodynastes
tasmaniensis* have also been developed and validated to assess acute and teratogenic effects
of contaminants and effluents.

A6.6 Biomarkers: Histopathological, biochemical and physiological changes

Biomarkers at the suborganismal level of organisation (biochemical, physiological and
histological) have also been considered valuable measures of stress. Rapid assessment of
an organism’s health can be made by measuring the degree of dysfunction that a contaminant
has produced. Overt signs of toxicity are usually preceded by changes that can be detected
by the methods of histopathology, ethology, biochemistry and physiology. In histopathological
examination, eg, organs such as the liver are sectioned and examined under the microscope for evidence of disease, damage or deformity. A further, practical advantage of histopathology using small fish is the possibility of embedding the animals in situ which enables a quick overview of various relevant organs, thus facilitating fast and comprehensive screening. Routine techniques can be used and to a large extent the basic general pathological principles apply to fish (Wester and Vos, 1994). There is an enhanced sensitivity of histological monitoring compared to classical ecotoxicological testing, since effects on the histological level will be visible at lower dosage, compared to toxicological endpoints such as mortality or behavioural changes. This not only enhances the discriminative potential of the experimental setting, but will also contribute indisputably to improved animal welfare by helping move towards the refinement of tests.

A recent technological development in fish histopathology is the use of immuno- and enzyme histochemical methods to identify potential biomarkers of early hepatic change at the molecular and cellular levels. These include several types of metabolic changes (enzyme activity and protein/oncogene expression) (Grinwis et al., 2000, 2001; Vincent et al., 1995) to detect early preneoplastic lesions and to analyse progression towards carcinomas (Kohler et al., 1998); another application of immunohistochemistry is the detection of VTG.

In mammals, biochemical and physiological responses have been correlated with whole-animal responses, and toxicologists can understand what effects physiological changes have on the whole animal. In aquatic toxicology relatively little work has been done in this area and it is extremely difficult to relate test results to undesirable environmental changes. Sublethal biochemical changes resulting from exposure of individuals to chemicals are called biomarkers (Hyne and Maher, 2003).

Despite uncertainty about the ecological significance of these tests, they do show some promise as early warning indicators of long-term pollution stress. For example, enzyme changes in the blood and organs of fish have been used as biomarkers for determining the effects of pulp mill effluents. Other effects which have been examined include respiration and oxygen consumption, bone and cuticle development and deformities and regulation of ionic balance. These are only some of the biochemical and physiological responses which can be affected by toxicants (Connell and Miller, 1984, pp 45-46).

Organophosphorus (OP) and carbamate pesticides inhibit the activity of an enzyme in the brain, acetylcholinesterase (AChE), which affects the transmission of nerve signals from the brain to muscles in mammals, birds, reptiles and fish. Inhibition of AChE levels has been used as biomarker for OP exposure in shrimp (Abdullah et al., 1994) and fish (Kumar and Chapman, 2001), providing rapid quantitative predictions of toxic effects in both laboratory and field (Kumar and Chapman, 1998; 2002). Such biomarkers should not be used as a replacement for convectional aquatic toxicity techniques, but be useful to demonstrate links between sub-lethal biochemical changes and adverse effects on natural populations (Hyne and Maher, 2003).

**A6.7 Microbial tests**
The microbial community, including protozoa, algae, bacteria and fungi, comprises the bulk of the biomass in many ecosystems and fulfills crucial roles in energy flow pathways and nutrient regeneration (Cairns et al., 1992). Microorganisms possess the majority of the same biochemical pathways as higher organisms and species interactions between the different taxonomic groups are similar in complexity to those occurring in higher organisms (Stauber and Davies, 2000). Despite their importance in degrading organic compounds, and in recycling metals and nutrients, the development of microbial toxicity tests has lagged behind the use of invertebrates and fish.
Microorganisms are particularly suitable for toxicity testing due to their short generation times (allowing short term chronic exposures), sensitivity and reproducibility. Because microorganisms have a large surface area and their cell membrane is in direct contact with soil or water, they are often more sensitive to contaminants than invertebrates and fish (Walsh and Merrill, 1984).

A typical microbial toxicity test uses a sub-lethal response which can be measured easily. For example, some marine bacteria emit light which can be measured by a light-sensitive cell. Under chemical stress, the amount of light emitted decreases (Tarkpea et al., 1986). This property is used in a number of toxicity test kits (eg Microtox®), which are useful in ranking the relative toxicity of a large number of samples, in rapidly detecting changes in toxicity in complex effluent streams, and in giving early warning of toxicant effects on bacterial cultures such as those used in sewage treatment plants (Dutka et al., 1988). Such tests may not, however, provide a particularly good indication of effects on other species or in ecosystems. Microtox bioassay has been shown to be sensitive both to the presence of reduced compounds and semi-volatile organics in groundwater (Toussaint et al., 1995; Boyd et al., 1997) but is generally considered to be less sensitive than the Daphnia tests (Mitchell and Dunn, 1994).

Another genetically engineered microorganism, P. putida mt-2 KG1206, has been constructed to monitor toluene analogues in groundwater samples collected from petroleum hydrocarbon contaminated sites (Kong et al., 2007). The protocol of this simple bioluminescent assay consisted of mixing one volume of groundwater sample with four volumes of broth culture, followed by bioluminescence measurement, after 30 min.

A6.8 Genotoxicity and mutagenicity
Mutagenicity tests are available as high-throughput systems, which are appropriate for use as biological detectors. While the relevance of mutagenicity testing with respect to human health is widely agreed on, the relevance for aquatic organisms is still under discussion. Most mutagen identification studies were based on the Salmonella/microsome assay as developed by Ames et al. (1975) for the detection of chemically induced mutagenesis using histidine-dependent Salmonella strains and observing the reverting to histidine independence after exposure to mutagens (Mortelmans and Zeiger, 2000). Most studies apply the basic strains TA100 indicating base pair substitution as reversion event and TA98 indicating frameshift mutations.

The addition of the microsomal fraction of rat liver homogenate S9 as an exogenous activation system allows the detection and discrimination of direct and indirect mutagens. The selectivity of mutagen identification has been significantly enhanced by applying additional strains deficient in nitroreductase (TA98NR) and transacetylase (TA98/1,8-DNP6) (Fernandez et al., 1992; DeMarini, 1996) for the discrimination of nitroarene mutagenicity. Appropriate alternatives applied for genotoxicity identification are the umu-test on the basis of the activation of the SOS DNA repair system in Salmonella typhimurium TA1535/pSK1002 (Bobeldijk et al., 2001 and 2002) and the Mutatox assay using a dark mutant strain of Vibrio fischeri (Thomas et al., 2002). Both are promising approaches with respect to high-throughput analysis even if the discriminating power is lower than that of the multi-strain Ames approach.

A6.9 Bioaccumulation Tests
Most potentially toxic chemicals of environmental concern express their toxicity after being absorbed or accumulated by exposed animal or plant life. Accordingly both the bioavailability of a chemical and its capacity to be absorbed or accumulated are important factors in determining risks from exposure. Low concentrations of some chemicals, such as mercury, cadmium or DDT, may have little or no measurable effect in the short term but may cause long-term biological effects because they accumulate in living organisms. These and other
chemicals may enter the environment for many years before their biological impacts are detected. Thus it is better to be able to measure or predict such problems through laboratory or field-based testing. Organic chemicals that bioaccumulate have a number of features in common. They have a low solubility in water, a high solubility in lipids (or body fat) and they degrade slowly (Connell, 1988).

**Bioaccumulation** is a general term for the accumulation of chemicals by living organisms as a result of intake both in the food and also from the organism’s environment (water, soil/sediment or air). Determination of a bioaccumulation factor (BAF) is extremely important in the risk analysis of a chemical. This is dependent on the pathway of chemical accumulation, whether by water, soil/sediment, air or food. **Bioconcentration** describes the accumulation of a chemical in a living organism by absorption from the environment, rather than food intake. The concept is of particular importance for aquatic organisms, where chemicals cross the permeable surface of the gills in large animals or across the body surface in small animals and plants. It does not operate effectively in air-breathing aquatic animals such as insects, waterbirds, seals or whales (Connell, 1988). Bioconcentration is quantified as the bioconcentration factor (BCF), the ratio between the concentration in an organism and the concentration in the environment (eg water) once equilibrium is reached. A high BCF means a high level of bioaccumulation of a given chemical. **Biomagnification** describes the accumulation of chemicals in a living organism from food intake. Biomagnification along a food chain will result in the highest concentrations of a chemical being found at the top of the food chain.

### A6.10 Toxicity identification evaluation (TIE)

The major disadvantage of effluent testing using a suite of aquatic toxicity tests via this procedure is that although toxicity is detected, its cause is not identified. To address this problem, the United States EPA has developed a set of toxicity characterisation procedures called Toxicity Identification Evaluation (TIE). In TIE, various treatments are applied to the effluent: its acidity may be altered, it may be aerated vigorously, or passed through selective absorption columns, or treated with specific chemicals. Toxicity is re-evaluated after each treatment, and any loss of toxicity may be attributed to those chemicals that would have been affected by the treatment used: Norberg-King et al (1991).

The US-EPA presented a protocol for toxicity identification evaluation (TIE) in aqueous samples including the following three phases:

I. Toxicity characterization by assignment of toxicity to general groups of toxicants such as heavy metals, nonpolar organics or ammonia applying methods to render these toxicant groups biologically unavailable (Norberg-King et al., 1991) See Table 6.1.

II. Identification of suspected toxicants (Mount and Anderson, 1989)

III. Confirmation of the suspected cause of toxicity (Mount, 1989)

<table>
<thead>
<tr>
<th><strong>Manipulation</strong></th>
<th><strong>Target chemical group</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>pH adjustment (pH 3 and pH 10 (Microtox®) or 11 (microalgae))</td>
<td>Hydrolysis or precipitation of chemicals</td>
</tr>
<tr>
<td>pH graduation (pH 7, 8 and 9)</td>
<td>pH sensitive compounds (e.g. ammonia)</td>
</tr>
<tr>
<td>C18 solid phase extraction and methanol elution</td>
<td>Non- or moderately-polar organic compounds</td>
</tr>
<tr>
<td>Addition of EDTA</td>
<td>Divalent metal ions (e.g. Cu^{2+}, Zn^{2+})</td>
</tr>
<tr>
<td>Addition of sodium thiosulphate</td>
<td>Oxidative chemicals (e.g. Cl⁻, Br⁻)</td>
</tr>
<tr>
<td>Aeration</td>
<td>Volatile or oxidisable chemicals</td>
</tr>
<tr>
<td>Filtration (± removal of particulates)</td>
<td>Toxicants associated with particulate matter</td>
</tr>
<tr>
<td>Baseline (un-manipulated sample)</td>
<td>None</td>
</tr>
</tbody>
</table>
A6.11 Endocrine disruption related approaches
In recent years endocrine disrupters have became an increasing human and environmental health concern. Since Purdom et al. (1994) reported that numerous sewage treatment work (STW) effluents were estrogenic to fish, increasing attempts have been made to identify compounds which cause estrogenic and androgenic effects in aquatic environments. The predominating estrogens in STW effluents and estuary water were the natural hormones $17\beta$-estradiol and estrone as well as the synthetic hormone $17\alpha$-ethynylestradiol (Harries et al., 1996; Desbrow et al., 1998; Williams et al., 1999). Environmentally relevant concentrations of these compounds were sufficient to account for elevated levels of vitellogenin observed in caged and wild male fish (Harries et al., 1997; Tyler et al., 1998; Jobling et al., 1996; Routledge et al., 1998).

A number of in vitro assays have been developed to screen for chemicals and waters (including WWTP effluents) for estrogenic / anti-estrogenic and androgenic / anti-androgenic activity. These assays discriminate chemicals that are capable of binding to the steroid receptors and, in some cases, transcriptional activation. The most commonly used in vitro assays used for screening estrogenic or androgenic potentials are listed in Table 6.2. These in vitro assays differ in their sensitivities, applications and endpoints, with each assay having various advantages and disadvantages in their use and the information which they provide. A group of commonly used in vitro assays include the reporter gene assays, which include the yeast estrogen screen (YES) and the estrogen responsive chemically activated luciferase reporter gene expression (ER-CALUX) assays. The advantages of the reporter gene assays include the use of eukaryotic cells, can distinguish between agonist and antagonist (in most cases), their cost-effectiveness, ease of use and, due to their extensive validation, can be standardised relatively easily for inter-laboratory comparisons. In vitro assays have been established to screen for androgenic and anti-androgenic activity of chemicals. The yeast androgen screen (YAS) is suitable to investigate agonists as well as antagonists of the androgen receptor (Sohoni and Sumpter, 1998).

### Table 6.2. Examples of in-vitro tests for assessing the effects of EDCs

<table>
<thead>
<tr>
<th>In-vitro assay</th>
<th>Test</th>
<th>Distinguishes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Competitive ligand binding assays</td>
<td>ER$\alpha$, ER$\beta$, AR</td>
<td>Receptor agonists</td>
<td>(Soto et al., 1998)</td>
</tr>
<tr>
<td>Cell proliferation</td>
<td>E-screen</td>
<td>ER receptor agonists and antagonists</td>
<td>(Soto and Sonnenschein, 1995)</td>
</tr>
<tr>
<td>Recombinant receptor/reporter gene assays</td>
<td>YES, YAS, ER-CALUX, AR-CALUX, MVLN cells, HGELN cells</td>
<td>hER, rtER, AR, agonists and antagonists</td>
<td>(Routledge and Sumpter, 1996)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Sohoni and Sumpter, 1998)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Gutendorf and Westendorf, 2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Van den Belt et al., 2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Legler et al., 2002a)</td>
</tr>
<tr>
<td>Protein based assays</td>
<td>Trout hepatocytes</td>
<td>ER, prolactin, SHBG, EROD, VTG</td>
<td>(Toomey et al., 1999)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Laville et al., 2004)</td>
</tr>
</tbody>
</table>

Note: ER-estrogen receptor, AR-androgen receptor, YES-yeast estrogen screen, YAS-yeast androgen screen, ER-CALUX-human T47D breast cancer cells, MVLN cells-MCF-7 breast cancer cell line, HGELN-HeLa cells-human cervical cancer cells, hER-human estrogen receptor, rtER-rainbow trout estrogen receptor, SHBG- sex hormone binding globulin, EROD-7-ethoxyresorufin-O-deethylase, VTG-vitellogenin

Measuring the effect of EDCs in whole organisms such as invertebrates, amphibians, fish and birds has advantages over using cellular in vitro assays (Campbell et al. 2006). For example, assays at the whole organism level can assess population effects, life-cycle, fecundity,
deformities, growth, reproductive deficiencies and offspring development. In the OECD and US EPA, programmes have been established for screening EDCs. The US EPA is currently developing and validating in vitro and in vivo assays to determine the potential for chemicals to cause endocrine disruption in humans or wildlife. To achieve this goal the US EPA is using a two-tiered approach. The first tier involves a screening battery and is intended to identify chemicals affecting the estrogen, androgen or thyroid hormone systems through any of the recognised modes of action. Whereas, tier 2 testing is intended to confirm, characterise, and quantify those effects for estrogen, androgen and thyroid active substances in invertebrates, amphibians, fish, birds and mammals (USEPA 2007).

In Australia, there is limited information on EDCs especially in relation to the changes to reproductive development of native fish species in streams receiving treated sewage effluent. The ecological implications of EDCs have been demonstrated by a seven-year whole lake experiment at the Experimental Lakes Area in north-western Ontario, Canada, published recently by Kidd et al., 2007. This study showed that chronic exposure of fathead minnow (Pimephales promelas) to ng/L concentrations of the potent 17α ethynylestradiol led to near extinction of fish species from the lake. The implication of this finding to Australian environment is not clear.

**A6.12 In situ toxicity studies**

*In situ* toxicity studies in the field can be very powerful in confirming the presence of toxic materials in waterways and giving rapid response to pollution incidents. In general, most chemicals would be expected to be less toxic in the field due to reduced persistence and bioavailability, although some can be more toxic. Several studies have concluded that the acutely toxic effects of many chemicals in the field can be accurately predicted from laboratory studies (Rand,1995 ; US EPA, 1999).

Current approaches to evaluating contaminated sites generally divide the site into separate ground water and sediment/surface water units of concern. The transition zone provides unique habitats and refuges, contaminant attenuation and removal, nutrient and carbon cycling, and food for macrobenthic organisms. Most methods have not estimated organism exposure or effects at the point of exposure. USEPA investigation on ecological effects in the transition zone at contaminated sites has shown how important it is to know where the contaminants are and the role ground water discharge and recharge have on exposure of organisms in the transition zone. In transition zones, organisms range from microbes to fish. Ecologists who have focused on the water column and sediments are now employing tools such as cores and recolonisation devices, as well as in-situ chambers for toxicity tests, to look at effects within the transition zone ecosystem (USEPA report by Duncan et al.).

**A6.13 Early warning systems for groundwater testing**

More advantageous in the view of a fast identification of toxic substances in a complex contaminated environmental sample would be the use of on line biomonitors. In terms of remediation control there could be two ways. A change of toxic effects could be related to either a known substance or a group of substances which have a special mode of action. Recently, several authors have developed systems to accomplish these goals of parallel evaluation and identification. Either the combination of chemical and biological tests as in (Bobeldijk et al., 2001) who combined solid phase extraction, HPLC fractionation and the umu test to detect genotoxic substances in industrial and hospital waste waters or tests using bioanalytical tools like enzyme sensors to detect organophosphates in the presence of different organic solvents (Fennouh et al., 1997) are used.
Biological early warning systems could be more meaningful in terms of toxicity to higher organisms such as fish (Van der Schalie et al., 2001), crayfish (Bloxham et al., 1999), nematodes and gammarides (Gerhardt et al., 2002, Gerhardt et al., 1998). But only biomonitors which use such microorganisms as bacteria (Schwedt et al., 1997; Gu et al., 1999; Gu and Gil, 2001) or microalgae (Twist et al., 1997) can be used for the monitoring of highly toxic groundwater, because the effected/toxicated test organisms can be easily replaced by new untoxified organisms using parallel bioreactors for cultivating the organisms (Guilhermino et al., 1999 or Gu et al., 1999).

Biological early warning systems with higher organisms have to cope with disadvantages making them infeasible as a real time biomonitors of highly toxic samples and/or groundwater with low oxygen concentrations. If the test organisms get killed or severely affected each time they contact the samples, successive samples have to be highly diluted before contact. Preliminary tests with an automated “Daphniabimonitor” (Lechelt et al., 2000) showed that the toxicity of the Bitterfeld/SAFIRA groundwater to *Daphnia magna* was too high to be tested with the original sample. Testing the groundwater with groundwater contaminant sensitive organisms like Daphnia and fish would require another test design. Namely the groundwater would have to be diluted to a non-toxic end before testing. Dilution of the groundwater might, however, mean to strip the highly volatile organics from the sample before testing, so only the groundwater sample with the aquifer medium and non-volatile substances would be tested.

Therefore biomonitors using microorganisms are best to use in these circumstances, where the samples, although highly toxic, still contain the volatile substances during testing. Van der Schalie et al. (2001) stated that "no single sensor system should be expected to be, by itself, an optimal continuous, real time monitoring system . . . it would be better to identify an optimal suite of sensors that complement one another . . . ". This would require development of an on line biotest battery which ideally would combine the above bacterial biomonitor with other on line test systems to ensure a successful detoxification evaluation.

**A6.14 A current ecotoxicological study at an Australian managed aquifer recharge site**

Currently CSIRO is carrying out preliminary investigations of the ecotoxicological effects on aquatic fauna and algae of stormwater, before and after wetland treatment and water after recovery from storage in an aquifer at the Salisbury aquifer storage transfer and recovery (ASTR) project. The main objective of this project is to carry out direct toxicity assessment (DTA) of the storm water at different stages of treatment. Ecotoxicological assessment will provide additional evidence of the quality of water produced by this system and the efficiency of of the main passive treatment processes. Toxicity assessment will be conducted at the site on water and sediment samples collected from different stages of storm water treatment using ecotoxicological approaches as described in Table 6.3. This work is in progress. Ultimately, these ecotoxicological methods and relevant physical, chemical, and biological data and modelling results generated from other water quality and ecological studies may be used in the preparation of a regional-scale probabilistic ecological risk assessment for MAR. It is likely that in due course some of those projects would supply water into surface water systems.

Chronic toxicity studies using sensitive standard alga, bacteria, fish, frog, and invertebrate species are being conducted. Bioconcentration studies using fish and yabbies are also being conducted. It is important to evaluate a set of screening-level aquatic toxicity tests methods to assess the toxicity and/or bioconcentration potential of recovered waters. Details of the methods are given below.
Table 6.3. Ecotoxicological bioassays selected and the endpoints to be measured

<table>
<thead>
<tr>
<th>Test Organism</th>
<th>Endpoint Measured</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strom water bioassays using water samples collected at different treatment stages to check efficiency of storm water treatment</strong> (five stages of treatment representing five different samples)</td>
<td></td>
</tr>
<tr>
<td>1.1 Microtox, <em>Vibrio fischeri</em></td>
<td>Luminescence inhibition in bacteria (5 and 15 minutes)</td>
</tr>
<tr>
<td>1.2 Alga, <em>Chlorella</em> sp</td>
<td>Growth rate after 72 h exposure</td>
</tr>
<tr>
<td>1.3 Duckweed, <em>Spirodela</em> sp</td>
<td>Number of fronds after 7 days exposure</td>
</tr>
<tr>
<td>1.4 Waterflea, <em>Ceriodaphnia dubia</em></td>
<td>Number of neonates in three broods during 8 days exposure</td>
</tr>
<tr>
<td>1.5 Purple spotted gudgeon, <em>Mogurnda adspersa</em></td>
<td>Survival, growth and teratogenicity after 7 days exposures</td>
</tr>
<tr>
<td>1.6 Tadpoles, <em>Lymnodynastes tasmaniensis</em></td>
<td>Embryo-larval survival, growth and teratogenicity after 5 days exposure</td>
</tr>
<tr>
<td><strong>Whole sediment/pore water testing for assessing performance of wetland system</strong> (Two samples representing inlet and outlet sections)</td>
<td></td>
</tr>
<tr>
<td>2.1 Midge, <em>Chironomus tepperi</em></td>
<td>Whole sediment test, Growth, survival and emergence after 7 days exposure</td>
</tr>
<tr>
<td><strong>Biomarkers using in-vitro techniques</strong> (five stages of treatment representing five different samples)</td>
<td></td>
</tr>
<tr>
<td>3.1 Yeast, <em>Saccharomyces cerevisiae</em> specific for endocrine disrupting chemicals</td>
<td>Estrogenicity, Anti-estrogenicity, Androgenicity, Anti-androgenicity based on microplate method</td>
</tr>
<tr>
<td>3.2 Natural hormones and synthetic hormones in solvent extracts</td>
<td>ELISA kit for E2, EE2, E1 and DHT analyses based on microplate method</td>
</tr>
<tr>
<td>3.3 Acetylcholinesterase inhibition specific for organophosphate and carbamate pesticides</td>
<td>Enzyme assay based on microplate reader method</td>
</tr>
<tr>
<td>3.4 Ames test</td>
<td>Genotoxicity</td>
</tr>
<tr>
<td><strong>In-situ approach for assessment of wetland performance</strong> (two samples representing inlet and outlet sections)</td>
<td></td>
</tr>
<tr>
<td>4.1 Midge, <em>Chironomus tepperi</em></td>
<td>Survival and growth over 7 days exposure</td>
</tr>
<tr>
<td>4.2 Water flea, <em>Ceriodaphnia dubia</em></td>
<td>Survival and growth over 48 hours exposure</td>
</tr>
<tr>
<td>4.3 Tadpoles, <em>Lymnodynastes tasmaniensis</em></td>
<td>Survival and growth over 4-6 weeks exposure</td>
</tr>
<tr>
<td>4.4 Yabby, <em>Cherax destructor</em></td>
<td>Bioaccumulation of metals over 4 weeks exposure</td>
</tr>
</tbody>
</table>

The methods are described below.
A6.14.1 Direct toxicity assessment

1.1 Microtox, *Vibrio fischeri* | Luminescence inhibition in bacteria (5 and 15 min)

This acute test is a commercially available bioassay that measures the decrease in luminescence of the marine bacterium *Vibrio fischeri* in response to toxicants. It is a rapid bioassay (5- and 15-minutes exposure) that uses internationally standardised protocols (Azur Environmental 1998). Although this bacterium is an estuarine species, it is particularly sensitive to a wide range of organic compounds and this cost-effective test has a rapid turn-around time.

1.2 Alga, *Chlorella* sp | Growth rate after 72 h exposure

This chronic test measures the inhibition in growth of the freshwater single-celled alga *Pseudokirchneriella subcapitata*, previously known as *Selenastrum capricornutum*. The protocol follows the USEPA (1994) and the OECD guideline (1984) with modifications by Stauber et al (1994). Algal growth (cell yield) is measured after a 72-h exposure and the bioassay is run in standard test medium without the addition of the complexing agent EDTA. The sample is filtered (0.45 µm) prior to testing to remove particulate matter and indigenous micro-organisms that can interfere with the test results.

In summary, this test involves exposing laboratory cultured alga to the test material in 250mL test flasks. The test is usually undertaken on a range of concentrations of a test material, eg 100, 50, 25, 12.5 and 6.3% effluent. The test vessels are inoculated with algal cells in log-growth phase. The test vessels are then incubated in a constant light and temperature environment for 96 hours. At the end of the exposure period, the number of *Selenastrum* cells are counted. Statistical analyses are then applied to the test data to determine for example, the concentration of the test material causing a 50 and 25% inhibition in algal growth. The test data can then be used to estimate concentrations of the test material likely to cause toxicity to micro algae in the environment.

1.3 Duckweed, *Spirodela* sp | Number of fronds after 7 days exposure

The common duckweed *Lemna* sp. is a small flowering aquatic macrophyte occurring in quiescent fresh waters in both tropical and temperate regions. Duckweed is a food for waterfowl and small animals, and provides food, shelter and shade for fish and other aquatic organisms. In addition to being an ecologically important group, duckweeds are a sensitive and useful test organisms for testing turbid and coloured waters. This gives the duckweed an advantage over algal toxicity tests which may require filtering, compromising the sample integrity. The duckweed test is a simple, sensitive and cost-effective.

In summary, this test involves exposing laboratory cultured *Lemna* sp. to the test material, and measuring growth in the number of colonies and fronds produced over a 7 day exposure period. The test is usually undertaken on a range of concentrations of a test material, eg 100, 50, 25, 12.5 and 6.3% effluent. At the beginning of the test, the test vessels are inoculated with 9-12 duckweed colonies, each with 2-4 fronds. The test vessels are then incubated in a constant light and temperature environment for 7-days. Test solutions may be renewed every 2 to 3 days if required. The number of fronds in each test vessel are counted daily. At the end of the exposure period, the number of *Lemna* colonies and fronds are counted. Statistical analyses are then applied to the test data to determine for example, the concentration of the test material causing a 50 and 25% inhibition in duckweed growth. The test data can then be used to estimate concentrations of the test material likely to cause
toxicity to aquatic macrophytes in the environment. This test follows either USEPA Test Method OOPTS 850.4300, ASTM (1998) or OECD Guideline 221, depending on the objectives of the test.

1.4 Waterflea, *Ceriodaphnia dubia* | Number of neonates in three broods during 8 days exposure

*Ceriodaphnia dabvia* is known to be important in aquatic food-webs and highly sensitive to contaminants. This cladoceran has been used in Australia as a bioindicator organism for the ecotoxicological investigations. Acute toxicity test will be initiated with the collected ASR samples for 48 hours. If no toxicity is observed, chronic bioassays will be followed using standard protocols (USEPA, 1996).

The chronic (7-day partial life-cycle) toxicity test using the freshwater crustacean *Ceriodaphnia dubia* is one of the most commonly used chronic tests for the assessment of potential harm posed by contaminants to freshwater aquatic ecosystems. This test is commonly used throughout North America using USEPA protocols and is usually performed along side the 48 hour acute test using the same species. A considerable volume of toxicity data exists for this species, making the acute and chronic *Ceriodaphnia* tests ideal for validating ecological risk assessments. In summary, this test involves exposing laboratory reared juvenile *Ceriodaphnia* (<24 hrs old) to the test material in 20mL test chambers. The test organisms are drawn from cultures demonstrated to be healthy and free of stress. The test is usually undertaken on a range of concentrations of a test material, eg 100, 50, 25, 12.5 and 6.3% effluent. A total of 10 juveniles are exposed to each test concentration plus control treatment until 80% of control organisms produce their 3rd brood of young (about 7-days). The test solutions are renewed and the numbers of young produced for each animal are counted daily. The number of surviving *Ceriodaphnia* are counted in addition to the number of young produced. Statistical analyses are then applied to the test data to determine the concentration of the test material effecting reproduction and survival. The test data can then be used to estimate concentrations of the test material likely to cause acute and chronic toxicity in the environment. The method is based on the *Ceriodaphnia* Survival and Reproduction Test developed by the USEPA (1994).

1.5 Purple spotted gudgeon, *Mogurnda adspersa* | Survival, growth and teratogenicity after 7 days exposures

Many bioassays have been developed for the early life stages of fish. Due to the sensitivity of early life stages (ELS) of fish, a broad spectrum of targets of environmental pollution can be tested. An embryo-larval toxicity test which determines the toxicities of single or complex mixtures (i.e. industrial effluents) to embryo and early larval stages has been developed for an Australian fish species, the purple-spotted gudgeon, *Mogurnda adspersa*, (Hyne 1999). This bioassay can measure following endpoints: percentage hatch of embryos at the termination of test (i.e. hatchability); the delay/inducement of embryo hatching (i.e. hatching time); mortality of embryo/larvae (i.e. mortality); incidence of deformities or abnormal behaviour at test termination (abnormality) and growth (relative to control) at test termination.

1.6 Tadpoles, *Limnodynastes tasmaniensis* | Embryo-larval survival, growth and teratogenicity after 5 days exposure

An embryo-larval toxicity test which determines the toxicities of single or complex mixtures (i.e. industrial effluents) to embryo and early larval stages has been developed for an Australian frog species, the grass-spotted frog, *Limnodynastes tasmaniensis*. This bioassay can measure following endpoints: percentage hatch of embryos at the termination of test (i.e. hatchability); the delay/inducement of embryo hatching (i.e. hatching time); mortality of
embryo/larvae (i.e. mortality); incidence of deformities or abnormal behaviour at test termination (abnormality) and growth (relative to control) at test termination.

**A6.14.2 Whole sediment toxicity assessment**

| 2.1 Midge, *Chironomus tepperi* | Whole sediment test, Growth, survival and emergence after 7 days exposure |

Aquatic sediments provide a habitat for many organisms, however sediments are also a major repository for many of the more persistent chemicals that are introduced into surface waters. Although certain chemicals are highly sorbed to sediment, these compounds may still be bioavailable to the biota. Whole sediment toxicity tests using midges seek to determine whether contaminants in the sediment are toxic.

In summary, these tests involve exposing 2\textsuperscript{nd} instar larvae of midges to the test sediment and assessing survival over a 7-day period. The test is essentially a screening test where midges are exposed to undiluted sediment.

The survival of each sediment sample is statistically compared with the control or reference sediment treatments. The test data can then be used to determine whether the test sediments may have an acutely toxic effect on benthic invertebrates.

**A6.14.3 Biomarkers in vitro toxicity assessment**

| 3.1 Yeast, *Saccharomyces cerevisiae* specific for endocrine disrupting chemicals, Yeast estogenic screen (YES) and Yeast androgen screen (YAS) |

The recombinant yeast estrogen and androgen assay have been previously described by Routledge and Sumpter (1996) and Sohoni and Sumpter (1998). In the yeast estrogenic assay (YES), the human estrogen receptor (hER) is integrated into the main chromosome of the yeast (*Saccharomyces cerevisiae*). The yeast contains expression plasmids carrying the reporter gene $\text{lac-Z}$ (encoding the enzyme $\beta$-galactosidase), which is used to measure the receptors activity. In YES, the hER is expressed in a form that is able to bind with estrogen-responsive sequences. Upon binding an active ligand, the estrogen-occupied receptor interacts with transcription factors and other transcriptional components to modulate gene transcription. The reporter gene $\text{lac-Z}$ is expressed and $\beta$-galactosidase is secreted into the medium, where it metabolises the chromogenic substrate, chlorophenol red-$\beta$-D-galactopyranoside (CPRG), which is normally yellow, into a red product that is measured spectrometrically at an absorbance of 540 nm. Extraction of (xeno) estrogens/androgens will be carried out by solid phase extraction (SPE). All extracts will be evaluated for estrogenic and anti-estrogenic activities.

| 3.2 Natural hormones and synthetic hormones in solvent extracts |

(E1, E2 and EE2) in the extracts will be measured using ELISA kits from Japan EnviroChemicals Ltd (Tokyo, Japan). The quantification limit for each estrogen is 0.05 ng/L. Method established.

| 3.3 Acetylcholinesterase inhibition specific for organophosphate and carbamate pesticides |

*In Vitro* assay using pure AChE enzyme using microplate method. Method established.
3.4 Ames test

Most mutagen identification studies were based on the Salmonella/microsome assay as developed by Ames et al. (1975) for the detection of chemically induced mutagenesis using histidine-dependent Salmonella strains and observing the reverting to histidine independence after exposure to mutagens (Mortelmans and Zeiger, 2000).

A6.14.4 In-situ Toxicity Assessment

4.1 Midge, Chironomus tepperi | Survival and growth over 7 days
Second instar midge larvae will be placed in cages at wetland and monitored for survival, growth and emergence of larvae over seven day in-situ exposure.

4.2 Water flea, Ceriodaphnia dubia | Survival and growth over 48 hours exposure
24 old waterflea neonates will be placed in cages and monitored for survival over 2-day in-situ exposure.

4.3 Tadpoles, Lymnodynastes tasmaniensis | Survival and growth over 4-6 weeks exposure
Frog embryos and early stage tadpoles will be collected from clean areas and placed in the enclosures at all sites. These growing tadpoles will be monitored at regular intervals for mortality, growth, deformities and metamorphosis.

4.4 Yabby, Cherax destructor | Bioaccumulation of metals over 4 weeks exposure
Assessment of the bioavailability of contaminants will be carried out using yabbies, Cherax destructor of approximately 15 cm in length. Prior to use, the yabbies will be maintained in flow through system and will be fed on trout pellets for at least two weeks. Two cages of bird wire and light weld mesh construction will be used for each site. Six yabbies will be placed in each cage horizontally on to the sediment surface. They will be in direct contact with the wetland sediments. They will be placed in the cages for four weeks and collected at intervals of two weeks. After exposure periods, four yabbies will be collected from each cage and placed in coolers and transported to the laboratory. They will be kept frozen at –20°C for further heavy metal analyses.

A6.14.5 Quality assurance and analysis of results

Each bioassay will include a negative control using standard test media. If the water quality parameters (e.g. conductivity, pH, hardness, alkalinity) of the sample are outside the standard criteria for the test species, additional controls will be incorporated in the toxicity test to determine the effect of these water quality parameters alone on the test species. Prior knowledge of the sample’s water quality characteristics is essential to incorporate these controls in the toxicity test.

A positive control, reference toxicant, is included in each test to ensure that the test species are responding to a known toxicant in a reproducible way. The reference toxicant phenol is used for the Microtox® test and copper for the microalgal and water flea bioassays.

Each bioassay will also include water quality monitoring throughout the test as per the standard protocols (e.g. pH, conductivity).

Results will be analysed by the following methods:

- All of the toxicity data will be analysed according to USEPA statistics protocols (USEPA 1994) using ToxCalc 5.0.23 (Tidepool Software). Toxicity information from each bioassay will include:
• Concentration-response curves
• EC/IC50 (the sample concentration to cause a 50% effect)
• LOEC (the lowest sample concentration to cause a significant effect)
• NOEC (the highest concentration of sample to have no effect)

A6.15 Concluding remarks
Bioassays are powerful and in many cases successful tool to identify and confirm previously unknown or unexpected toxicants in the environment. On the other hand, the approach is expensive, time-consuming and may end in “disappointment” with respect to the identification of toxicants. Thus, it is quite important to discuss the criteria for the successful application of bioassays on the basis of the available experience. These criteria include the selection of indicative objects of investigation and methodological requirements. The discussion is based on the assumption that ecotoxicological testing aims at the identification and confirmation of individual toxicants even if there might be other objectives such as the comparison of pollution patterns.

Ecotoxicology is a young science in Australia; it is developing well and has the potential to play a critical role in protecting aquatic ecosystems. Indeed, the greatest problem may not be so much in predicting toxicity, as in failing to understand the ecosystems that are to be protected. Australian aquatic toxicologists can produce criteria for the protection of aquatic ecosystems, but these will have little practical value without a better understanding of the structure and function of the ecosystems themselves. Toxicity assessment can help to protect aquatic ecosystems, but those ecosystems need to be better understood in order to define what is to be protected and why.

For the success and significance of biological assessment, the selection of toxicological endpoints is crucial and pre-determines the identified toxicants. At least two types of bioassays may be considered:
(i) Specific cellular and sub-cellular test systems for the detection of Ah, estrogen or androgen receptor binding compounds and mutagens. High-throughput properties and high discriminating power of such bioanalytical tools suggest that they may be ideal detectors in effect-based testing. However, users should be aware of the limited significance of the results in ecotoxicological hazard assessment.
(ii) In contrast to the tests mentioned above, toxicity testing on the basis of aquatic organisms such as bacteria, algae, invertebrates or fish claim to detect aquatic toxicity rather than one individual specific effect. They typically integrate many specific and nonspecific effects. Since no test system is able to detect all specific effects, the application of a test battery enhances both the chances of toxicant identification and the significance for hazard assessment. The availability of chemical analytical tools is also of great importance in such investigations. For typically hydrophobic chemicals, GC-MS is the standard method. However, positive toxicant identification may require additional tools including UV, IR and NMR spectroscopy. For typically more hydrophilic aquatic contaminants, LC-MS methods are often required.

It is evident that successful MAR projects may impose high methodological demands with respect to both ecotoxicological and chemical tools. Thus, an important criterion for a successful monitoring program is the joint interdisciplinary activity of ecotoxicologists and analytical chemists rather than isolated efforts. Keeping these criteria in mind, an integrated approach of biological and chemical assessment is a promising tool and still the only way towards an appropriate assessment of hazards due to unknown contaminants. More research should be done to enhance knowledge in fast and efficient effect diagnosis in complex environmental matrices in order to allow rational approaches to identification of source water pre-treatment needs and acceptable uses of recovered water.
A6.16 References


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APPENDIX 7

STRATEGIES FOR ASSESSING POTENTIAL IMPACTS OF ALDINGA RECLAIMED WATER ASR SCHEME

Greg Ingleton, SA Water Corporation

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A7.1 Introduction

The SA Water Corporation, in partnership with a non-government third party, are developing an aquifer storage and recovery scheme at Aldinga, located on the Adelaide Plains, approximately 30km south of Adelaide. The purpose of the Aldinga Aquifer Storage and Recovery (ASR) Scheme is to inject and store treated Class B quality effluent from a large nearby wastewater treatment plant (Christies Beach WWTP) in a confined aquifer over winter and spring, in order to supplement the supply to the Willunga Basin irrigators during their irrigation period in summer and autumn. Under current arrangements, 100% of the summer and autumn effluent volume from Christies Beach WWTP is supplied to the local growers, however this volume falls short of current peak irrigation demands and prohibits expansion of the irrigation network in the area. As reclaimed wastewater is not used during winter and spring, it is discharged to the marine environment via the Christies Beach WWTP outfall. The Aldinga ASR scheme is designed to reduce the marine discharges during low irrigation demand periods by enabling the storage of the treated effluent in the Aldinga aquifer.

A7.2 Background

The South Australian Environment Protection Agency (EPA) has granted approval, via and Emergency Authorisation, for a trial cycle of the Aldinga ASR scheme. The trial commenced in March 2009, with injection of up to 2ML/day/bore into two injection bores. A total volume of 100 megalitres (ML) was injected during March and April 2009, with extraction of this water beginning two weeks after the end of the injection cycle, and continue for two months until 100ML of water is recovered. It is acknowledged that although 100ML will be extracted from the aquifer during May and June 2009, less than 90% of the injected water will be recovered. This is due to the mixing of the injected water with the native groundwater along the interface between the native and injected water.

The 100ML trial is designed to gather the required information to allow the EPA, Department of Water, Land and Biodiversity Conservation (DWLBC) and the Adelaide and Mt Lofty Ranges Natural Resource Management Board (AMLR NRMB) to gain confidence in this scheme and grant the required approvals for on-going operation of the scheme. Based on the success of the 100ML trial, an application will be made to the relevant agencies to commence the operational stage of the scheme.

As the reclaimed water has a higher nutrient concentration than the native groundwater, an exemption from the Environment Protection (Water Quality) Policy 2003 is required from the
The reclaimed water is not fit for drinking purposes, but it does meet the Department of Health requirements for irrigation purposes (Class B – restricted use).

The operational stage involves the injection of up to 400 ML of reclaimed water from Christies Beach WWTP into the aquifer over winter and spring, with extraction of the 400 ML to occur during the following irrigation season. The reclaimed water will be filtered at the Aldinga site prior to injection through three bores located along the eastern boundary of the Aldinga property, with these bores being used for the extraction of the water once the irrigation season begins. Based on the success of the trial (100ML) and the first operational stage of the scheme (400ML), SA Water will investigate the expansion of the scheme to accommodate up to 1.2 GL of injectant in the near future, and potentially up to 5GL thereafter.

The aquifer to be used for this scheme is the Port Willunga Formation which is a confined aquifer located between 30 metres and 70 metres below the surface at the injection site. The natural groundwater in the aquifer at this location is brackish, with total dissolved salts (TDS) being around 2500mg/L. The TDS of the reclaimed water is around 730mg/L. The native groundwater flows in a southwesterly direction from the foothills of the Mt Lofty Ranges towards the sea, as shown in Figure 1.

Extracted water will be discharged into a 90 ML lagoon within the adjacent Aldinga WWTP site, where it will be chlorinated (to Class B - restricted use) and directed to the third party mains for distribution to the irrigation network.

A7.3 Case Study

As this is one of the first commercial ASR schemes using treated effluent in South Australia, additional effort has been invested to ensure that the scheme is sustainable and successful. There are a number of issues and obstacles associated with this scheme, mainly due to the injection of treated effluent into a regional aquifer, and the subsequent fate of any unrecovered injectant. The specific ecosystems of concern, and methods of tracking and reducing impacts, are detailed below. These are in an order associated with the proximity to the point of injection, and not necessarily in order of risk.

A7.3.1 Impacts on local Stygofuana

An assessment of the stygofuana was carried out in the aquifer, using suitable bores within a short distance to the point of injection. The results were inconclusive, due to the lack of suitable bores for stygofuana assessment in the area, as most bores were equipped with a submersible pump, or contained iron-casing, both of which impact on the accuracy of stygofuana sampling (Leijs pers comm. 2008). From the limited sampling that was conducted, only a few animals were found in the confined aquifer, and they were the same species as that which dominated the overlying unconfined aquifer. Future assessment will be undertaken in proposed monitoring bores, which are due to be installed during the 400ML operational stage. It is hoped that this future assessment will enable an accurate assessment of stygofuana within and outside of the aquifer storage zone to allow quantification of the impacts of injecting reclaimed water on stygofuana health.
Figure 7.1 Map showing the direction of groundwater movement in the Port Willunga Formation Aquifer. The site for the ASR scheme is highlighted in yellow.

A7.3.2 Impacts on local groundwater users

As humans are part of the ecosystem, the potential impacts on local users has been included in this report. The two potential impacts are from the increased pressure within the aquifer resulting from injecting water into the aquifer, and the potential escaping of injectant from the storage zone (bubble) and subsequent flow toward privately-owned bores. The closest privately-owned bore to the injection site is approximately 200 metres south, south-east of, and slightly upstream of the nearest injection bore. The maximum head build-up associated with the first operational stage of the scheme (400ML) has been estimated to be around 30 metres in the middle of the well field, with the pressure gradient extending horizontally 100 metres upstream and 200 metres downstream of the injection bores. As such, the closest privately owned bore is located outside of the area of influence. The potential increase in
pressure at the privately-owned bore will need to be addressed if future expansion of this scheme (above the 400ML injectant stage) is to occur.

The second issue associated with anthropogenic users is the potential for injected water to escape from the aquifer storage zone and impact on the water quality of a privately-owned bore. As the injectant may contain small concentrations of pathogens and other contaminants, there may be health issues associated with the use of the groundwater at these privately-owned bores. For the 400ML operational stage, modelling has shown that injectant will not affect the nearest privately-owned bore, which is approximately 200 metres south, south-east of, and slightly upstream of the nearest injection bore. Issues may arise if the scheme expands in the future, as there is a potential for privately-owned bores to be within the aquifer storage zone, dependent on the location of future injection bores. The risk of potential impacts on privately-owned bores, for both the 400ML stage and the future expansion of the scheme, will be mitigated and managed through the following processes.

- Chlorination of the treated effluent prior to leaving Christies Beach WWTP. This disinfection will remove a large percentage of the pathogen load leaving the plant, however due to the high chlorine demand of the treated effluent, the chlorine residual will not be persistent and will not be present at the point of injection.

- Monitoring of water quality at the treatment plant and at the injection bores, including in-line real time measurements, regular grab samples, and flow-weighted composite samples, will identify any water quality issues prior to injection. In-line gauges have been equipped with an alarm system and a live telemetric connection to ensure that any out-of-spec readings are acted upon immediately.

- Residence time within the aquifer, which will encourage the die-off of any remaining pathogenic organisms. According to a report by Toze and Hanna (2002), the majority of pathogens are removed during the first 28 days of residence in an aquifer, mainly due to predation by indigenous groundwater microorganisms. The main risk is when extraction of water occurs shortly after injection, as the injection bores will also be used as the extraction bores, therefore recently injected water will have a lower residence time than water injected earlier in the injection cycle. During the operational stage, when 400ML will be injected into the aquifer, the residence time between the completion of the injection cycle and the commencement of the extraction cycle will be at least one month, and possibly up to three months. As the highest concentration of pathogens is at the point of injection, the lowest concentrations will be at the outer fringes of the aquifer storage zone, in the water that was injected at the start of the injection cycle. Fortunately, this is also where privately-owned bores are likely to be located.

- Monitoring at dedicated bores throughout the operation of the project, and in the months following the end of each extraction cycle. Specifically, water quality samples will be taken from both privately-owned bores and observation bores around the ASR site.

- Informing the private bore owners. In the area of the ASR, many landowners currently use treated wastewater for irrigation purposes. These people are already aware of the constraints associated with the use of treated effluent, however if their bore does draw from the aquifer storage zone in the future, additional information and signage etc will be required. Current use of groundwater at the nearest privately-owned bore is for toilet flushing and some minor irrigation around the house, due to the high conductivity of the native groundwater.

- Chlorination of the extracted water prior to distribution to the irrigation mains. This final disinfection will ensure that the risk of pathogen contamination of the end-product is mitigated.

With these strategies in place it is expected that the risk to human health will be minimised. Further consultation is required between SA Water and DWLBC regarding the development of new privately-owned bores in the future, as the location of these bores may need to be assessed in regards to the extent of the aquifer storage zone and the subsequent potential impact on water quality at these bores.
A7.3.3 Impacts on remnant vegetation stands

Three areas of remnant vegetation have been identified within a short distance of the Aldinga ASR. The first site, known as the California Road Wetlands, is 6km north-east of the ASR site, however this is upstream of the injection site. As such, there is little risk of impacts occurring at these wetlands from ASR operations. The wetlands have been identified as being dependant on groundwater flows in the Port Willunga Formation aquifer, therefore if future expansion of the ASR scheme occurs, this area will need to be investigated to ensure no impact occurs. The second site is the Aldinga Scrub, located 2km south-west of the ASR site, which is a remnant coastal native vegetation stand located within the Aldinga Scrub Conservation Park. Groundwater from within the perched Semaphore Sand aquifer sustains vegetation along with permanent and temporary waterholes. The perched aquifer is separated from the Port Willunga Formation by the Hindmarsh Clay aquitard. The third site is the Washpool Lagoon, located 4km south-west of the ASR site. The connectivity of this site to the Port Willunga Formation aquifer has not been investigated at this stage of the scheme, but will be studied in the near future as part of the coastal influence investigations (described below).

A7.3.4 Impacts on the Marine Environment

One of the main environmental concerns with this ASR scheme is the potential for injected water to reach the marine environment, which is located approximately 3km west of the ASR injection bores. It is clear from the findings of the Adelaide Coastal Waters Study that nitrogen from wastewater discharges is having a significant impact on seagrass meadows and associated seagrass-dependent biota. As the ASR scheme is part of a strategy to reduce effluent discharges to the marine environment, the loss of injected water from the aquifer to the marine environment is not conducive to the aim of this ASR scheme. There are a number of uncertainties associated with the risk of nutrient enrichment of the marine environment from aquifer discharges, including:

- A lack of suitable monitoring wells along the coast. Modelling results of the discharges from the aquifer to the marine environment have varied significantly, and uncertainty also exists around the influence of seawater intrusion on the direction of groundwater flow at the coastal interface.
- A lack of evidence as to the point of discharge of Port Willunga Formation aquifer water to the marine environment. The point of discharge is important as it may provide information regarding the impact of these discharges, especially if the discharge were to occur within near-shore reefs, as has been suggested.
- The positive impacts of nutrient attenuation during the residence time within the aquifer. Modelling has shown that due to the low transmissivity of the aquifer, it would take at least 30 years for the injectant to reach the coast. The extent of nutrient removal during this period is unknown.

To address the potential impact on the marine environment, the following strategy has been proposed and will be implemented during the first years of the 400ML operation stage of the scheme. The main components of the strategy include:

- Quantifying the volume of water injected versus the volume of water extracted, to ensure that full recovery is obtained. This will be achieved using in-line flow gauges on both the injection and extraction pipelines.
- Measuring water quality at monitoring wells downstream of the injection point, to identify water moving away from the aquifer storage zone. These monitoring wells will have the capacity to be converted to extraction wells if the volume of escaping water is significant. From previous ASR experience and from extensive modelling of this scheme, it is expected that up to 100ML of injected water will remain in the aquifer after the first two cycles. Once the aquifer storage zone is established, it is expected that less than 30ML per year will be unrecoverable, due to the mixing of the injected water with native groundwater at the interface of the two water types. As the modelled
The volume of aquifer ambient discharge to the marine environment is between 700ML/yr and 1800ML/yr (Lamontange et al. 2005) the unrecoverable injectant should be undetectable prior to reaching the marine environment, due to dilution within the aquifer.

- A full assessment of the direction of groundwater movement at the coastal interface, in order to quantify the impacts of seawater intrusion and the volume of discharge from the aquifer to the marine environment.

As the first trial injection cycle was only just completed when writing this report, the accuracy of modelling for injectant water movement and recovery percentages has not been tested. From the homogeneity of the aquifer media recorded when drilling the three injection bores, and the low transmissivity recorded during the bore step testing, it is likely that the modelling results will reflect the actual operational data. Actual data will be compared with modelled data throughout the trial and operation stages of this scheme, to enable model calibration and inform potential changes to operational and monitoring strategies.

A7.4 Discussion

A7.4.1 Risk Assessment and Risk Mitigation

A risk assessment workshop was conducted a few months prior to the proposed injection starting date, to ensure that all risks were identified and addressed. This risk workshop was attended by staff from numerous disciplines within SA Water, as well as members of two other government agencies, consultants and SA Water contractors, which enabled the capturing of risks relevant to the agencies. The "Resolver ballot" method was used during the workshop to allow a fair voting system. The attendees at the workshop came up with 37 risks, covering numerous environmental, social and economic issues. The risks were sorted into five main categories, being

- Legal and ownership
- Potential health risks to users
- Community perception
- Water quality issues
- Environmental issues

The usual practice in risk assessment workshops is to prioritise the resulting list of risks, with the high-risk activities requiring a mitigation strategy to be developed. As the storage of treated effluent in an aquifer is a high-risk activity, and expansion of the scheme is essential to SA Water’s future reuse and sustainability targets, mitigation strategies and adaptive management actions were developed for all identified risks as required by Australian Guidelines for Water Recycling (Phases 1 and 2).

These mitigation strategies and adaptive management actions were assessed and modified by the project stakeholders, and provided the basis for the modelling exercise and the proposed monitoring strategy. One example of the risk, mitigation strategy and adaptive management actions is displayed in Table 7.1.

A7.4.2 Modelling Outcomes

A number of modelling exercises were conducted on the available data from the site and from information gathered during a small trial conducted on a neighbouring property during 2001. Previous modelling data from PHREEQ software (showing the interaction of injected contaminants on aquifer water quality) and monitoring results obtained during this initial trail in 2001 showed no adverse impacts to native groundwater quality. Although a simple numerical model was used to estimate the extent of the aquifer storage zone for the 100 ML trial scheme in 2009, a more detailed modelling exercise was underway at the time of writing this report.
Table 7.1 Risk mitigation strategy, showing three levels of adaptive management triggers and actions

<table>
<thead>
<tr>
<th>Category</th>
<th>Risk</th>
<th>Mitigation Strategy</th>
<th>Adaptive management Strategy Level 1</th>
<th>Adaptive management Strategy Level 2</th>
<th>Adaptive management Strategy Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Quality issues</td>
<td>Sustainability of aquifer as a long term storage (physical and chemical clogging etc)</td>
<td>Additional filtration prior to injection will reduce the possibility of physical clogging by suspended particles. For biological clogging, a suitable backwash program will be developed once transmissivity info and injection efficiency curves are developed during the first few days of operation.</td>
<td>Injection efficiency drops by 30% - <strong>Action</strong> - increase backwash frequency by 30%. Check monitoring results from post-filtration location to ensure low suspended sediment.</td>
<td>All bores showing a 50% reduction in injection efficiency - <strong>Action</strong> - double backwash frequency, check that SS levels are low and that the filtration unit is working to spec. Possibly increase alum dosing rate to allow additional removal of SS and phosphorus (ensure aluminium levels do not exceed EPP guidelines if alum rate is increased)</td>
<td>Major clogging from all bores - <strong>Action</strong> - establish whether it is physical or biological clogging. If physical, install additional filter prior to injection and increase alum dose rate to highest possible concentration (without exceeding aluminium EPP values). If clogging is from biological activity, increase frequency of backwashing, do as above for alum dosing, increase injection pressure for around 30 minutes (exact time to be established during operation) prior to backwash. This will assist with the dislodging of biofilms.</td>
</tr>
</tbody>
</table>

This detailed modelling uses the information gathered from the calliper testing, drillers log and the step test results obtained during bore development in September 2008, plus additional monitoring information from the injection cycle, and will provide information regarding the changes in hydraulic head during injection and extraction, the migration and extent of the injectant plume, and the salinity and volume of recovered water. The results from these modelling exercises will be compared with the actual data throughout the operation of the scheme, and both the modelled information plus the data gathered will be delivered to the EPA, DWLBC and AMLR-NRMB for review on a regular basis. As modelling information is only as accurate as the data used to run the model, and information is limited at the planning stage of an ASR scheme, it was agreed that a trial cycle be undertaken to allow the gathering of data for the on-going verification and calibration of the model.

**A7.4.3 Agency issues**

The main concern associated with this scheme was the fact that there are no ASR schemes using treated effluent of this quality in Australia, and as such, precedents have not been set. The three main issues associated with this scheme were:

- Injected water quality will exceed the *Environment Protection (Water Quality) Policy 2003* for parameters including nitrogen, phosphorus, colour and some heavy metals.

The native aquifer water quality exceeded the *EPP (Water Quality)* concentration (for
drinking water, a default environmental value prior to implementing a process to set environmental values in this area) for salinity, total phosphorus, boron, iron and zinc. As the injected water exceeds the EPP Act concentrations, an exemption from the EPP Act is required from the EPA.

- As the injection bores are located on the SA Water property boundary, the aquifer storage zone extends under privately owned properties. Therefore, the landowners must give consent for this activity to occur, due to the requirement for an exemption from the EPP Act. For the 400ML operational stage of the scheme, only three properties will be located above the aquifer storage zone.
- The fate of the unrecovered injected water, as detailed above.

These three issues were the focal point during inter-agency meetings prior to the commencement of the trial phase of the scheme. Discussions were mainly centred on the mitigation strategies that would be employed, and how these issues would be monitored and addressed if anything unusual were to be identified. It was acknowledged that the removal of nutrients and heavy metals from the effluent prior to injection was a costly and energy intensive exercise, and the excess nutrients and heavy metals would cause minimal environmental harm due to the lack of groundwater-dependent biota around the injection site and the inert nature of these parameters when in low concentrations. It was acknowledged that the movement of injected effluent to the near-by privately-owned bore was not acceptable at this stage, due to the uncertainties associated with the removal of pathogens and other contaminants whilst in the aquifer. Therefore monitoring of water quality at the privately-owned bore, plus within observation wells outside of the modelled extent of the aquifer storage zone, was important to mitigate this risk. This monitoring will also provide information regarding the fate of contaminants in the injected water when in the aquifer.

A7.5 Conclusion

From the information outlined above, including the identified risks, mitigation strategies and current unknowns, it is clear that on-going data acquisition will be an essential component of risk reduction for this ASR scheme. Significant funding and effort will be invested in monitoring during the first few cycles of the scheme to ensure that in the short term all risks are known and mitigated, and to inform operational changes, and in the long term, to gain confidence in this type of scheme and allow the expansion of the scheme in future years. As SA Water continue to explore options for wastewater reuse, the benefits of an efficient ASR scheme appear to be conducive to meeting the reuse objectives. Due to climatic conditions in the Adelaide area, wastewater reuse for irrigation during winter months will not occur. As such, winter storage is required to meet the increasing summer demands for local irrigators. The development of an ASR scheme to allow winter storage, whilst simultaneously reducing the environmental impacts associated with wastewater discharge to the marine environment, is a logical approach to the current water resources issues being experienced in this State. It is hoped that with further information from the operation of this small-scale scheme, large schemes will be developed to further reduce the pressure on limited water resources. As water resource issues are influencing projects and policies in many government departments, it is essential that responsible agencies work together to further develop this type of scheme.

A7.6 References


Leijs, R. Personal communication on-site, August 2008.

APPENDIX 8

ASSESSED ECOSYSTEM IMPACTS OF TWO MANAGED AQUIFER RECHARGE PROJECTS

Peter Dillon, CSIRO Land and Water

A8.1 Mound spring protection
A8.2 Nutrient discharge to an estuary

Acknowledgements: Don Armstrong, Lisdon and Assocs and Keith Berry, BHP Billiton, and Stephen Appleyard, WA Environmental Protection Authority and Blair Palenque and Nick Turner, Water Corporation of WA, are thanked for assistance with information.

These contrasting case studies address positive and negative impacts of managed aquifer recharge on ecosystems. The first describes a groundwater reinjection project to protect a mound spring in the Great Artesian Basin from the effects of groundwater extraction near Olympic Dam in northern South Australia. The second is an overview of a proposed project to infiltrate reclaimed water on the Mosman Peninsula in Perth to support irrigation. The project did not proceed due to concerns about nutrient rich groundwater discharge into the estuary of the Swan River and to a marine fish habitat protection area. These complement the project, described in more detail in appendix 7.

A8.1 Mound spring protection

The water supply for the Olympic Dam Mine, located in northern South Australia, is derived from the southwestern margin of the Great Artesian Basin. The initial wellfield development in 1986 (Borefield A) was sited 100 km north of the mine in an area with a large number of artesian springs, known as Mound Springs (Figure 8.1). The springs are the only permanent surface water features in an otherwise arid region. Habitat isolation has led to the evolution of several unique aquatic ecosystems with high diversity of endemic or relict plants and animals having high conservation value. The springs are also of considerable cultural significance, since prior to European settlement they provided the only water supply in times of drought thus playing an important role in Aboriginal culture.

By 1994 wellfield abstraction of up to 12 ML/d (139L/s) had resulted in declining artesian pressures in the vicinity of certain springs close to the abstraction area of Borefield A (Figure 8.2). At Bopeechee Spring, typical flow rates had declined from 0.5 L/sec to 0.3 L/sec. Marked natural short term variations in flow rate had masked the declining trend, however flow rates dipped to 0.15 L/sec in late 1994 and water demand was projected to temporarily increase to 16ML/d (185L/s). From groundwater modelling and correlations between groundwater pressures and spring flow rates it was projected that the spring could cease to flow by mid-1996.

Due to the cultural and environmental sensitivity and because of concerns regarding the possible impact of changed water chemistry on the spring biota, any engineering scheme designed to maintain flow could not introduce water from elsewhere directly into the spring.

A proposal was developed by Western Mining Corporation to reinject water into the same aquifer near the spring to increase the artesian pressures in the vicinity of the mound spring and maintain spring flow. Analytical and numerical modelling forecast that injection of 2.3L/s (1.3% of abstraction) into an existing observation well located 4000m from the spring would produce a widespread pressure increase of 0.4m, sufficient to offset the additional drawdown until the commissioning of a second wellfield (Borefield B) located 90 km further into the...
Great Artesian Basin. A formal proposal was developed and submitted for internal and governmental comment.

Issues considered and addressed prior to injection included:
- Bore and aquifer hydraulics - Flow tests, pipeline, casing screen and aquifer pressure losses were considered in design of the injection system.
- Bore and aquitard integrity - The double pressure cemented injection bore was constructed to high pressure artesian standards, capable of containing the required pressure.
- Predicted pressure response - The predicted pressure rise constituted only a small portion of total pressure loss at the spring, but the focus at this time was to stabilise the trend of declining pressure to maintain flow.
- Effects on groundwater chemistry - Injected water chemistry was almost identical to that of the receiving water as it came from the same aquifer. Travel time from the injection well to Bopeechee Spring was calculated at about 200 years, under continuous injection.
- Clogging - Chemical similarity of waters indicated that precipitation due to mixing would not be significant. The system was designed to remain sealed and was sterilised on establishment to minimise the risk of bacterial infestation.

Trials were conducted at low flow rate, and subsequently a larger injection pump installed, and an automated backflushing sand media filter installed to address initial clogging problems. Water was pumped 3000m from GAB06 about 6.6km west of the Spring to a monitoring well GAB20, which has a depth of 120m, and together with the spring was located on the east side of the Norwest Fault zone where the aquifer had low transmissivity.

Reinjection commenced in October 1995 at a rate of 2.3L/s. The trend of declining pressure in the Northeast Sub-Basin was rapidly reversed. The reinjection rate was maintained between November 1995 and April 1996. Reinjection was halted briefly in mid-1996 while the system was upgraded to a rate of 5.2L/s. During the period of system down-time, pressure dropped to its lowest recorded level. At the higher injection rate, pressure rapidly recovered to its highest level since 1994. The new more distant wellfield (Borefield B) was operated from September 1996, allowing a reduction in local abstraction and regional recovery of aquifer pressure. The reinjection system was shut down in early 1998.

The flow rate from Bopeechee Spring was maintained by the additional artesian pressure generated by reinjection (Figure 8.3). In first half of 1996, pressure in the Wellfield Sub-Basin declined to the lowest recorded levels as the abstraction rate reached 16 ML/d. Despite this the Spring flow rate steadily increased over this period. Reinjection at a rate of about 4.6L/s apparently had a similar impact on Northeast Sub-Basin aquifer pressure and Spring flow rate as did reduction of the local abstraction rate from 16 ML/d to 6 ML/d. Approximate expenditure of about $300 000 capital costs plus operating costs ensured the conservation of a spring with high conservation value. Remote injection produced no visual impact at the spring site other than the obvious continuation of spring flow.

Since the commencement of operation of Borefield B with the associated limiting of the Borefield A flowrate to a maximum of 6 ML/d, spring flow at previously affected sites has recovered.

References:
Figure 8.1 Map of Borefield A region showing Bopeechee Spring, source well GAB06 and reinjection well GAB20, and the Norwest Fault zone (where aquifer transmissivity is low). Source: K. Berry (1997).
Figure 8.2 Decline in aquifer pressure and spring flow rate in response to increased abstraction 1986 to 1994 from the Olympic Dam Borefield A.  Source: K. Berry (1997)
Figure 8.3  Bopeechee Spring protection by reinjection of water.  (a) pressures in subbasin decline during increase in abstraction 1995-1996.  (b) Springflow increases as a result of reinjection.  (c) rate of reinjection in GAB20 that provided maintained the spring flow.  Source: K. Berry.
A8.2 Nutrient discharge to an estuary

This final example, briefly describes a proposal for managed aquifer recharge at Mosman Peninsula in Perth using reclaimed water from Subiaco Wastewater Treatment Plant that did not proceed, primarily because of concerns for ecosystem protection of the Swan River and a marine fish habitat protection area in the adjacent coastal area of the Indian Ocean. Of importance is the strategic advice developed by the Environment Protection Authority, to balance the need for protecting groundwater from salinisation, the environmental consequences of proceeding with projects, and the need for developing information without exposing the public and the environment to unnecessary risk.

Mosman Peninsula is a 4km long and 2km wide strip of prime residential land that lies between the Indian Ocean and the Swan River and is an established part of the Perth metropolitan area (Figure 8.4). In this area two golf courses, local government and some residential groundwater users were experiencing saltwater intrusion into irrigation wells. This created demand for an alternative reliable source of irrigation water. Since 1995 several schemes have been proposed to use reclaimed water from local sewers or Subiaco sewage treatment plant (Blair and Turner 2004). The area coincides with one of many opportunities that Scatena and Williamson (1999) identified for managed aquifer recharge in the Perth region. In this case recharge of reclaimed water to the superficial aquifer was proposed to push back the saltwater interface and meet irrigation demand without the need for a separate reclaimed water reticulation network.

A preliminary study commenced in 2003 using existing data to model the migration of recharged water accounting for salinity contrasts (and density effects) in the aquifer (Prommer et al. 2004) (Figure 8.5). This allowed evaluation of the likelihood of increased discharge of nitrogen to the Swan estuary and to a coastal fish breeding ground in the adjacent Indian Ocean. These were considered to have the most potential for adverse environmental impacts. The scenario modelled was for recharge of 1.5 GL/year along the axis of the peninsula combined with an additional 375 ML/yr abstraction by groundwater users. This was considered the 'worst case' scenario for nitrogen discharge to sensitive aquatic ecosystems. This scenario assumed there was no additional treatment to reduce nutrient concentrations in sewage effluent, and that there was no denitrification within the aquifer. Hence it was a maximal risk assessment for nitrogen discharge to the estuary and the marine environment.

The study revealed that the discharge of nitrogen was likely to be problematic. Although existing groundwater was nutrient-rich as a result of fertiliser use, including on golf courses and domestic gardens, and the concentration of nitrogen in recharged water would be similar or less, the projected flux of nitrogen discharging to the marine environments after about seven years was considered excessive. Previous eutrophication of the nearby Cockburn Sound, where proliferation of algae in the water and on the leaves of seagrass, had been attributed to excessive nutrient discharges from industry and the catchment as noted by the EPA (2005). Management of the Mosman recharge operations would therefore be required to protect marine environmental values.

There were two options available. The first would be to form a sound understanding of the mechanisms of groundwater flow to the marine environment and to maintain heads so as to prevent saline intrusion but also to prevent excessive groundwater discharge. In the karstic Tamala Limestone, solution features could potentially provide preferential groundwater flow direct to the marine environment. With distributed privately-owned groundwater extraction, it would not be possible to manage groundwater gradients across both shorelines to achieve these tight constraints.

Alternatively the nutrient composition of the recharge water would need to be substantially reduced so that the quality of water discharging to marine environments on both sides of the peninsula did not breach any water quality objectives of the relevant environmental values.
Figure 8.4 Mosman Peninsula between the Indian Ocean and Swan River showing a proposed pipeline from Subiaco Wastewater Treatment Plant at the north of the map. Shaded areas are largely vegetated areas that in whole or part could potentially have been irrigated with recycled water (after Blair and Turner, 2004).

Figure 8.5 Simulated spread of recycled water in the superficial aquifer on Mosman Peninsula under one scenario with four injection wells after 7 years of recharge (after Henning Prommer et al. 2004; Blair and Turner 2004).

The costs of including denitrification in the wastewater treatment were judged by the proponents of the scheme at the time to make the project infeasible. Hence the plans were shelved.
In addition to nitrogen, other hazards were also considered. A study of pathogen survival in this aquifer (Toze et al. 2004) showed that pathogen inactivation would occur readily within the vicinity of the recharge facilities, and would not reach other groundwater users nor the shorelines within the survival times of any pathogens recharged. The question of the potential impact of endocrine disruptors in sewage effluent remained unresolved. It was determined that further research was required to determine methods to accurately measure their concentrations and fluxes, and their potential fate and impacts on ecosystem receptors in the Swan River and in the marine fish habitat protection area in the adjacent Indian Ocean. Another issue remaining to be examined is the potential for heavy metals to accumulate in soils where infiltration galleries are used to recharge treated wastewater. There was a question as to whether in some cases, particularly with wastewater from industrial sources, the metals may become bioavailable, or become mobilised through changed environmental conditions. This was also identified as an area for further research.

The EPA (2005) also considered two environmental benefits of managed aquifer recharge. These include the improvement in water quality, particularly the decrease in salinity, that may be achieved by reversing saline intrusion, or by recharge into brackish aquifers. The second is the restoration of groundwater levels through managed aquifer recharge where groundwater levels have declined. This can have benefits more generally for restoring environmental benefits of caves, and for freeing up allocations of water to allow rivers, wetlands or vegetation to be maintained or restored.

References:


Based on this report it was recommended that section 5.11 of the Draft Managed Aquifer Recharge Guidelines be amended to read as follows. These changes were agreed to in early 2009 by the Working Group for Managed Aquifer Recharge Guidelines and the Joint Steering Committee responsible for the Australian Guidelines for Water Recycling, and were subsequently incorporated into the Guidelines for Managed Aquifer Recharge.

5.11 Aquifer and groundwater-dependent ecosystems

Ecosystem receptors that require protection are; indigenous microorganisms and stygofauna in aquifers, the fauna and flora of wetlands, streams, lakes and springs that depend on groundwater, and riparian and terrestrial phreatophytic vegetation. Managed aquifer recharge may impact on these receptors through excessive changes in groundwater levels, excessive rates of change in groundwater levels or excessive changes in water quality, in particular of the water quality hazards identified in sections 5.1 to 5.7. Such changes may reduce or eliminate habitat or impact directly on the receptor species. Declines in groundwater levels near groundwater-dependent water bodies in depositional environments can also result in formation of acid-sulphate soils with consequences to water quality and aquatic and soil organisms.

All aquifers contain microorganisms, and the microbial populations that can biodegrade unwanted constituents in recharge water must be sustained. Such populations may be native to the soil and aquifer, or introduced in recharge water.

Acclimation of microbial populations may be required for efficient biodegradation of some contaminants. Some nutrients may act as cometabolites of organic contaminants, and attempts to reduce these in recharge waters may increase contaminant persistence. However, overloading with contaminants can result in environmental conditions that no longer support biodegrading organisms. In some cases, the bounds are unknown; but reducing concentrations of contaminants, notably nutrients, before recharge is thought to be desirable for protecting and sustaining the microbial function and environmental health of the aquifer and for uses of recovered water. Advances in microbial ecology methods in conjunction with multi-variate statistics allow changes in microbial function to be detected (eg Reed 2009). However, a simpler indicator of sustainability of microbial function is to detect no persistent (non-cyclic) shift in redox state to anaerobic conditions in the aquifer beyond the recharge facility. Migration of an anaerobic zone away from the recharge facility suggests that the microbial ecosystem function is impaired and that nutrient loadings are excessive. Cyclic fluctuations in redox state surrounding an ASR well, which became anaerobic between injection and recovery cycles, were found to restore ambient microbial function in recovery cycles (Reed, 2009).

The term ‘stygofauna’ encompasses all animals that occur in subsurface waters. Australian stygofauna includes a highly diverse range of microscopic (<1 mm) to large (20–100 mm) aquatic groundwater invertebrates, and several species of blind fish (Humphreys 2006). These fish have adapted to live in total darkness, limited space, and low energy environments that have limited food webs lacking in predators (Gilbert and Deharveng 2002).

Stygofauna have been found in fresh and saline aquifers that have macroporosity (such as caves and fissures), and in pores of alluvial aquifers. Although they are found in all continents...
(except Antarctica), a large proportion of stygofauna species are highly endemic and localised. Their habitats range from aerobic, energetically rich upper layers of an alluvial aquifer in contact with aquatic or terrestrial ecosystems, to deeper fine-grained anaerobic substrates (Danielopol 1976, Eber 1983).

Distinct communities are adapted to each niche in the range. In general, a direct relationship exists between increasing depth and increasing morphological and physiological specialisations, as the groundwater becomes increasingly oligotrophic and lower in oxygen. This continues until only highly specialised organisms can persist in the anoxic conditions (Boulton et al 2003). Biodiversity also decreases with depth. Groundwater animals migrate actively within the interstitial space to find their preferred habitat (Danielopol 1989).

In general, knowledge of stygofauna ecology is limited but growing. A review by Leijs (2009) showed that stygofauna populations and biodiversity were found to increase when exposed to water containing small amounts of nutrients, but that stygofauna were not found in polluted groundwater containing excessive nutrients. Restoration of stygofaunal communities after the passive remediation of sewage-contamination was also evident. Leijs (2009) also reported that larger stygofauna (>3mm) could be stranded if groundwater levels dropped rapidly, and did not survive beyond 2 days above the watertable. If water table drops below the maximum depth of karst features, loss of habitat is likely to adversely impact stygofaunal communities. The responses of hyporheic organisms (ie organisms that live in the interface between groundwater and surface water bodies) to managed aquifer recharge are unknown; however, their location suggests an inherent capacity to deal with variations in flow and quality.

The weighted plankton net is considered the most reliable method for detecting stygofaunal biodiversity and abundance. However because stygofauna abundance is low and very variable between bores and within bores over time, differentiating impacts of managed aquifer recharge is likely to require at least 15-20 bores for monitoring (Leijs, 2009). This suggests that geochemical change be used as a surrogate indicator, as for microbial populations, and that validation monitoring and ecotoxicological studies be undertaken at selected sites where monitoring requirements can be met.

The health of phreatophytic vegetation (wetland, riparian and terrestrial vegetation) is affected by falling groundwater levels that increase the energy required by plants to extract groundwater, and each species has a limit to the extent of its maximum rooting depth. If the rate of decline of water table exceeds the rate at which a plant can extend its roots then the plant suffers water stress, and without other sources of water could die. Thus both magnitude and rate of the decline in water table were found to influence phreatophytic vegetation for a range of phreatophytic species with different rooting depths in a review of Parsons (2009). Rising water tables can result in anoxia within the root zone and stress riparian plants, although wetland inhabitants are evidently more tolerant of this, as water level changes due to surface inflows can be rapid.

For phreatophtic vegetation, effects of water quality changes beyond the temporary attenuation zone due to MAR are required to be acceptable where groundwater-dependent ecosystems are an environmental value of the aquifer. Australian Guidelines for Water Recycling Phase One (2006) provides indicators of risk to plant health of irrigation with water of various nutrient and salt concentrations which may be used to ascribe water quality objectives at the margin of the attenuation zone. If these are more stringent than water quality objectives for other environmental values of native groundwater and uses of recovered water, these may dictate the size of the attenuation zone or the level of pre-treatment before recharge.

A range of measurement methods for vegetation health include; leaf water potential, stomatal conductance, transpiration measurement, leaf area index, growth rate, cover and abundance (Eamus et al 2006). Groundwater level and quality measurements between the managed
aquifer recharge site and the groundwater-dependent vegetation provide an indicator of the need for vegetation-based monitoring to assess impacts. Increasing the separation distance between the location of the managed aquifer recharge site and the groundwater-dependent ecosystem is the simplest approach to mitigating potential impacts of managed aquifer recharge operations.

*Fauna and algae inhabiting groundwater-dependent water bodies*, such as springs, streams, wetlands, and lakes are highly diverse. As with groundwater-dependent vegetation they also require a minimum groundwater level to be maintained for their health and survival. Without water their habitat is lost.

Aquatic fauna and plants are a highly diverse group of ecosystem receptors and their responses to water quality changes can vary from highly sensitive to resilient. Ecotoxicological tools are used to identify indicator species and evaluate the effects of potential hazards that may emanate from managed aquifer recharge projects. Kumar (2009) has reviewed the range of ecotoxicity assessment techniques that have been developed and applied as environmental indicators of impacts of recycled waters on aquatic organisms. These methods include acute toxicity tests, lifecycle tests, sub-lethal tests, microbial tests, genotoxicity and mutagenicity, endocrine disruption, bioaccumulation, toxicity identification evaluation, and *in situ* toxicity studies. These are applied to selected reference species some of which may exhibit biomarkers to assist in measuring levels of stress.

As with other ecosystem receptors, surrogate parameters are commonly used and the Australian Guidelines for Fresh and Marine Waters (ANZECC-ARMCANZ 2000b) specify three sets of water quality criteria to satisfy aquatic ecosystem protection, covering systems with high conservation value, slightly to moderately disturbed systems and highly disturbed systems. Ecotoxicology tools described by Kumar (2009) are a useful supplement where information is lacking for the effects of particular water quality hazards introduced, or aquatic species potentially impacted, by the managed aquifer recharge project.

**Box 5.3 Discharge to marine ecosystems**

For managed aquifer recharge operations that have the potential for marine discharge of recharged waters, although it can be difficult to predict the impacts of such discharges, these can be significant. For example Ingerson (2009), Blair and Turner (2004) and Western Australian Environment Protection Authority (2005) evaluated potential impacts of managed aquifer recharge with reclaimed water on groundwater discharge of nutrients to coastal reefs, fish nurseries and a tidal estuary. At one site, where the aquifer is karstic and very close to the sea, adequate protection could not be demonstrated, so the project did not proceed. At the other, sufficient evidence was available at pre-commissioning risk assessment stage to indicate that trials could be managed adequately and the project is proceeding with groundwater level and quality monitoring to allow more rigorous assessment.

Managed aquifer recharge in some cases may have the sole purpose of sustaining flow or levels in springs (eg Berry and Armstrong 1997) or other groundwater-dependent water bodies.

Biodiversity conservation (including migratory birds) is a major consideration for high conservation value wetlands including Ramsar wetlands (the Ramsar Convention is an international treaty for the conservation and sustainable use of wetlands), and would need to be evaluated when assessing nearby managed aquifer recharge projects. General criteria for assessing the risks and appropriate level of management for groundwater-dependent ecosystem protection are provided in Table 5.17. Management methods to mitigate undesirable effects of managed aquifer recharge on ecosystems are described below.
**Pesticides and antibiotics**

It is necessary to prevent long-lasting pesticides and antibiotic substances being recharged at concentrations that would impair the function of ecosystems within the aquifer (see Section 5.5).

**Volumes and rates of recharge and recovery**

Volumes and rates of recharge and recovery should be constrained, so that effects of managed aquifer recharge remain within thresholds determined to protect targeted communities at indicative monitoring points (e.g., wells or springs). Use of multiple low-rate extraction wells or horizontal collectors on extraction wells can be used to reduce drawdown of groundwater levels. In some situations where thresholds are unknown, selected indicator species and related influencing variables (e.g., meteorological) should be monitored over sufficient time to determine the relative influence of managed aquifer recharge on the ecosystem.

**Siting of managed aquifer recharge projects**

Avoid recharge and recovery in unconfined aquifers in areas with shallow water tables. In such locations ecosystems are likely to be significantly impacted by managed aquifer recharge operations. Increasing the distance between areas of recharge or recovery and groundwater-dependent ecosystems will reduce the amplitude and rate of water level variations affecting the ecosystems. No connected surface water body should lie within the aquifer attenuation zone.
<table>
<thead>
<tr>
<th>Entry-level or simplified assessment</th>
<th>Maximal and precommissioning residual risk assessment</th>
<th>Residual risk assessment (operational)</th>
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<tr>
<td><strong>Acceptance criteria</strong></td>
<td><strong>Acceptance criteria</strong></td>
<td><strong>Residual risk assessment</strong></td>
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<tr>
<td>• Avoids recharge in close proximity to sensitive groundwater-dependent ecosystems</td>
<td>• Modelling shows that hydraulic head variations in groundwater-dependent ecosystems are within historical range, or are closer to historical range than they would be without the project, or that heads do not fall below minimum levels for ecosystem maintenance, and rates of decline are within those determined acceptable for the vegetation present</td>
<td>• Confirmation of achievement of precommissioning residual risk assessment criteria, based on additional field and laboratory measurements and modelling</td>
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<td>• Avoids unconfined aquifer with shallow water table for recharge and recovery of water</td>
<td>• Modelling shows that mass and concentrations of nutrients and contaminants discharged to ecosystems are within acceptable range for indicator species present and receiving waters remain within the water quality criteria for the relevant ecosystem (ANZECC-ARMCANZ 2000b)</td>
<td>• Modelling and data from trials, including groundwater geochemical monitoring, or ecotoxicological studies reveal no adverse effects on aquifer ecosystems, connected water bodies or groundwater-dependent ecosystems</td>
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<td>• Aquifer unlikely to contain stygofauna (ie aquifer is anaerobic or has no macropores)</td>
<td>• Aquifer unlikely to contain stygofauna (ie aquifer is anaerobic or has no macropores)</td>
<td><strong>Preventive measures</strong></td>
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<tr>
<td>• Site selection as above</td>
<td>• Reduce concentrations of long-lasting pesticides and antibiotic substances to benign levels for the species present in the ecosystem, and in any biological treatment systems in use</td>
<td>• As for pre-commissioning residual risk assessment</td>
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<td>• Reduce nutrients, metals and turbidity to acceptable levels, where they impact on groundwater-dependent ecosystems or surface water systems receiving purge water</td>
<td>• Further reduce concentrations of water quality hazards</td>
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<td>• Operate the system to avoid inducing increased hazard concentrations via increased discharge of groundwater into the ecosystem</td>
<td>• For ecosystems with high ecological value, establish criteria (eg groundwater level range, maximum rate of change of groundwater level, concentration range of pertinent constituents) for one or more relevant observation wells, and evaluate monitoring results obtained during the trial</td>
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<td>• Move site to increase separation from groundwater dependent ecosystems. Ensure attenuation zone excludes GDEs</td>
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<td>Validation monitoring</td>
<td>Entry-level or simplified assessment</td>
<td>Maximal and precommissioning residual risk assessment</td>
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<tr>
<td>Operational monitoring</td>
<td>• Estimate or measure annual injection and recovery volumes</td>
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na = not applicable