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212

Use of field-effects information to derive a surface water guideline value for magnesium in Magela Creek, NT Australia

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Australian Government
Department of the Environment and Energy
Supervising Scientist

The Department acknowledges the traditional owners of country throughout Australia and their continuing connection to land, sea and community. We pay our respects to them and their cultures and to their elders both past and present.

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Contents

List of Figures

List of Tables

Executive summary

1. A key component of closure and rehabilitation planning for the Ranger Uranium Mine in Australia's Northern Territory is the development of closure criteria, targets against which the long term success and sustainability of rehabilitation will be measured. For freshwater ecosystem protection, magnesium associated with magnesium sulfate ($MgSO_4$), a product of the leaching of the large volumes of waste-rock that will be involved in landform reconstruction, has been identified as the chemical constituent placing most constraints on Ranger rehabilitation. Laboratory toxicity testing has shown that Mg toxicity may occur at concentrations close to background due to the very soft freshwaters of Magela Creek receiving waters, though amelioration is provided by Ca. A guideline value protective of 99% of species, at a constant Mg:Ca ratio of <9:1 (providing Ca amelioration), was determined at 2.5 mg L^{-1} Mg.
2. International best practice in guideline derivations promotes multiple lines of evidence to validate any one approach, invoking the need for comparative field-effects information for Mg. For this, responses of macroinvertebrate communities in shallow waterbodies, across a spatial and temporal gradient of exposure to Ranger mine waters dominated by $MgSO_4$, was used to infer effects and determine protective thresholds.
3. Macroinvertebrate community data from littoral macrophyte (aquatic plant) habitat, together with associated environmental data, were collected from intermittent sampling conducted over a 37-year period (1979, 1995, 1996, 2006, 2011 and 2013), in up to four mine water-'exposed' and eight reference billabongs.
4. When all macroinvertebrate data were combined, multivariate analyses showed strong separation of waterbodies by year of sampling, with mine-water contamination accounting for much less variation in multivariate space. The lesser mine-related separation of waterbodies was partly associated with only moderate-strength gradients in contamination with magnesium amongst the minesite waterbodies studied over the 37-year period (cf much higher concentration ranges used to characterise the response-concentration relationships amongst laboratory toxicity test species). Accordingly and with combined 'most-years' data, year of sampling, as a proxy for unknown drivers of interannual shifts in community composition, dominated environment-community relationships (BIOENV analysis).
5. Changes in community composition or structure in minesite waterbodies with increasing Mg contamination over time, were evident using (i) number of taxa and multivariate similarity calculated between minesite waterbodies and reference billabongs for each year of sampling, and (ii) pairwise similarity calculated for all biological data, pooled according to categories of increasing Electrical Conductivity/Mg. For (i), statistically significant declines in taxa number and multivariate similarity were observed with increasing Mg in the four exposed waterbodies, relative to reference waterbodies. For (ii), community composition was less similar as the compared (pairwise) contaminant categories became greater.
6. Before the data could be used to determine threshold concentrations providing ecosystem protection, a weight of evidence (WOE) assessment was used to eliminate (or apportion the contribution of) alternative explanations for the observed biological changes. Five broad classes of potential confounding were evaluated in this study:
    i) Appropriateness of the reference billabongs used (i.e. appropriate experimental design);

ii) Interannual patterns in macroinvertebrate community composition that may have coincidentally corresponded with putative mine water associated biological change;
iii) Variations in sample processing protocol, including inclusion or exclusion of sediment-dwelling macroinvertebrates;
iv) Differences in habitat viz measures of aquatic plants;
v) Water and sediment Contaminants of Potential Concern other than Mg (such as: other major ions, metals including U, ammonia, nitrate and turbidity, low pH and associated release of metals from binding surfaces (including from sediments)); and
vi) Other stressors associated with catchment fire regimes and foraging activities of feral animals (pigs) in the littoral portions of waterbodies. While these would influence habitat and water quality directly (and thereby, would be addressed in iv) and v) above), potential effects were examined specifically.

Consistency of taxa responses to those observed in similar salt studies was a further diagnostic line of evidence invoked to determine whether it supported the cause-effect relationship.

7. Precepts of (eco)epidemiology were used to assess confounders and evaluate the final lines of evidence for inferring Mg-related change. This weight of evidence assessment concluded that changes in lentic macroinvertebrate communities in Ranger mine site waterbodies over time could predominately be attributed to Mg increase. However, there remained the possibility that K and/or HCO_3 ions, known in the literature to be more toxic than Mg, interacted with Mg to affect toxicity and the associated indirect ecological interactions observed in the field. This aspect requires further investigation.
8. Hazardous Concentrations for 1% of taxa (HC1) (or 99% taxa protection) for community structure and taxa number response measures were calculated for Georgetown Billabong, i.e. 5.6 and 3.9 mg L^{-1} magnesium respectively. The HC1 value for similarity amongst classes of site contamination (from response measure (ii) in 5 above) was 4.1 mg L^{-1} Mg. These values were consistent with (i) the laboratory guideline value of 2.5 mg L^{-1} Mg, and (ii) results from a mesocosm study reporting EC1 values of 1.5 and 2.3 mg/L magnesium affecting phytoplankton algal biomass and zooplankton community structure, respectively.
9. For the exposed waterbodies, there were indications of greater tolerance to Mg with increasing separation and isolation of the waterbody from the main Magela Creek channel. A possible explanation is the longer prior exposure to Mg leading to either population acclimation, species replacement and/or adaption.
10. The Mg:Ca ratio in the exposed waterbody demonstrating least tolerance was ~3.5:1, indicating that the Ca amelioration that might have been expected based upon laboratory predictions (i.e. Mg:Ca <9:1) was not evident. (A similar lack of Ca amelioration was also evident in the mesocosm study referred to 8 above.) The derived laboratory Mg:Ca ratios protective of organisms may not apply under the longer-term Mg exposures observed in the field, there may be ecological interactions associated with the exposure to Mg in the field that override any Ca amelioration, and/or other (unknown) antagonistic ionic interactions may be interfering with the protective role of Ca.

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1 Introduction

1.1 Rehabilitation of Ranger uranium mine

The Supervising Scientist Branch (SSB), within the Australian Government's Environment and Energy Department, is responsible for the protection of the Alligator Rivers Region (ARR) environment from the effects of uranium mining. The ARR is located in Australia's wet-dry tropics, to the east of Darwin, and includes World Heritage and Ramsar listed Kakadu National Park (KNP). The Ranger uranium mine is surrounded by KNP (Figure 1), this high conservation location imposing stringent requirements on both mine operations and closure.

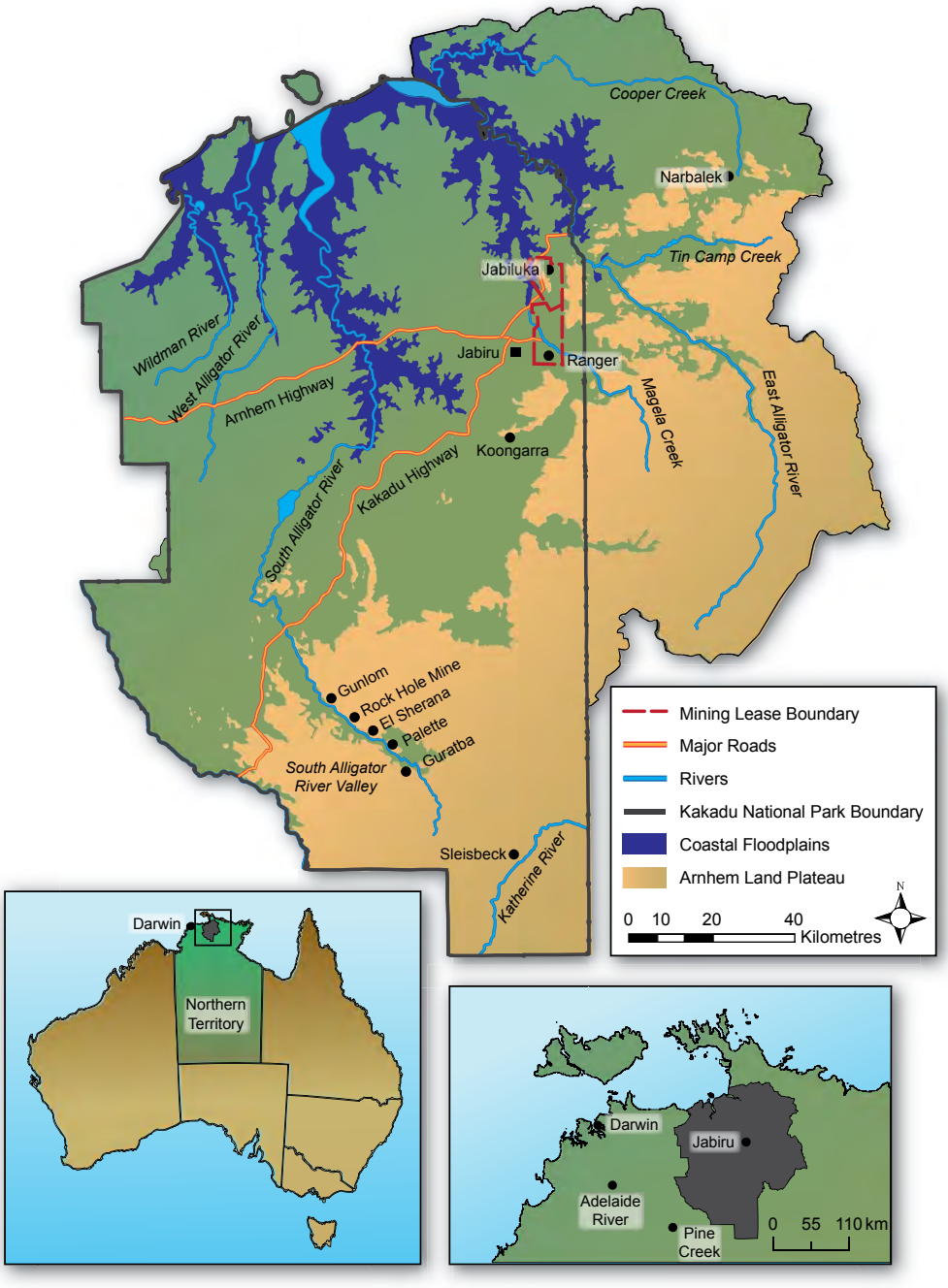


Figure 1 Alligator Rivers Region

The Ranger Mine is bound by a number of Commonwealth environmental requirements (ERs) during operation, closure and rehabilitation (Australian Government 1999) (http://www.environment.gov.au/science/supervising-scientist/supervision/closure-rehabilitation-ranger). They mandate that the activities of Energy Resources of Australia Ltd (ERA) on the Ranger Project Area (RPA) must not impact upon the values, attributes and ecosystem health of Kakadu National Park, nor the health of the regional community.

With respect to rehabilitation at Ranger and ecosystem health outside of the Ranger Project Area, the ERs state that:

1 Environmental protection

1.1 The company must ensure that operations at Ranger are undertaken in such a way as to be consistent with the following primary environmental objectives:

(a) maintain the attributes for which Kakadu National Park was inscribed on the World Heritage list

(b) maintain the ecosystem health of the wetlands listed under the Ramsar Convention on Wetlands (i.e. the wetlands within Stages I and II of Kakadu National Park)

(d) maintain the natural biological diversity of aquatic and terrestrial ecosystems of the Alligator Rivers Region, including ecological processes.

1.2 In particular, the company must ensure that operations at Ranger do not result in:

(a) damage to the attributes for which Kakadu National Park was inscribed on the World Heritage list

(b) damage to the ecosystem health of the wetlands listed under the Ramsar Convention on Wetlands (i.e. the wetlands within Stages I and II of Kakadu National Park)

(d) change to biodiversity, or impairment of ecosystem health, outside of the Ranger Project Area. Such change is to be different and detrimental from that expected from natural biophysical or biological processes operating in the Alligator Rivers Region.

3 Water quality

3.1 The company must not allow either surface or ground waters arising or discharged from the Ranger Project Area during its operation, or during or following rehabilitation, to compromise the achievement of the primary environmental objectives.

Mining and processing activities at Ranger must cease by 2021 and all decommission works must be completed by 2026. ERA, using its own and other (including SSB) expertise, must develop closure criteria for the mine, which are then subject to consideration and approval by the relevant authorities.

1.2 Approaches to deriving water/sediment guideline values

1.2.1 Lines of evidence and their evaluation

A key component of rehabilitation and closure planning for the Ranger uranium mine is the development of closure criteria. These measurable and quantifiable benchmarks are the targets against which the long term success and sustainability of rehabilitation will be measured. They will serve as the basis for issuing of a close-out certificate (ERA 2014a)

As benchmarks or targets, water quality closure criteria are no different from generic water quality guideline values (GVs) in derivation and application. (Further and in principle, derived closure criteria can also be used to inform and adjust water quality objectives already in place for Ranger's current operations should the need be identified.) The Australian and New Zealand Water Quality Guidelines (ANZECC & ARMCANZ 2000) described a hierarchy of approaches towards deriving water quality GVs, with biological

effects data the preferred basis. However, if these effects data are not available, GVs based upon local reference data are recommended.

Biological effects data may be sought from a spectrum of test exposure conditions, from simple single-species systems with experimental control over the contaminant of interest, to more realistic and complex field settings, studying assemblages in semi- or natural field conditions. This spectrum is depicted in (Figure 2): single species laboratory tests, microcosms (i.e. small experimental units, including multi-species tests and/or contaminant mixtures), mesocosms (larger experimental units with natural or added communities, located outdoors (semi-field) or indoors) and exposures in natural systems across which there is typically a gradient in contaminant concentrations. The last-mentioned, field exposures in natural systems, provide the greatest environmental realism but often lack experimental control, including pre-disturbance data. Effects in field exposures are typically inferred from contaminant-response correlations and other suitable and supportive lines of evidence.

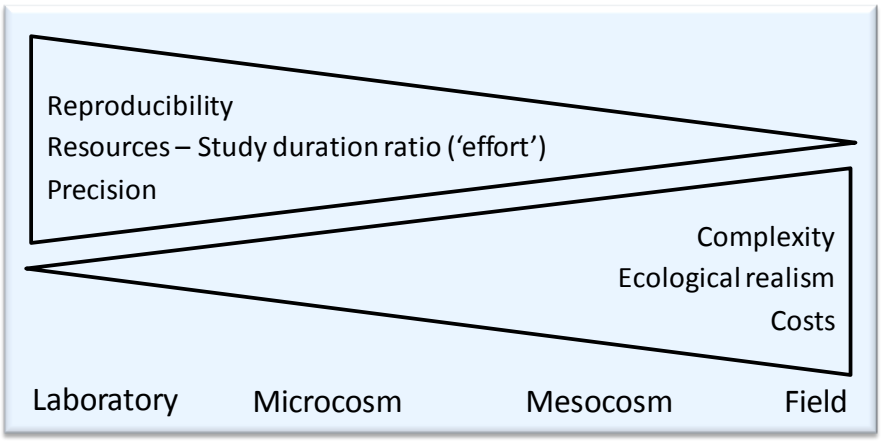


Figure 2 Spectrum of laboratory and field methods for assessing environmental risk. Adapted from Schäffer et al (2008).

GV derivations using field-effects or laboratory toxicity testing information have inherent advantages and disadvantages (eg Cormier et al 2008, van Dam et al 2014b, Chariton et al 2016, Buchwalter et al 2017). Laboratory toxicity testing aims to establish concentration-response relationships under controlled exposures and durations. The ability to isolate the toxicant of interest is a distinct advantage in GV derivations. However, without enhancing the complexity of the experimental design and test equipment, standard toxicity testing is limited in both the number of species that can be practically assessed and in mimicking natural and variable environmental conditions. Nor is such standard testing useful for stressors that are indirect (eg nutrients), are without a toxic chemical mode of action (eg deposited sediment), or that bioaccumulate over extended exposure periods. Further, some important and sensitive species cannot be readily cultured and/or tested under laboratory conditions (eg many aquatic insects), while such testing cannot account for ecological interactions (see Cormier et al 2008, European Commission 2011). Field effects incorporate environmental realism but may suffer from confounding by stressors or environmental variation unrelated to the contaminant of concern; results and interpretation, therefore, may be based upon correlation with weak inference. Mesocosms can control for these confounding covariates and can also incorporate ecological complexity (Perceval et al. 2011, Buchwalter et al 2017); however, they can also introduce other limitations not described further in this report (see Perceval et al. 2011, Chariton et al 2016).

International agencies responsible for developing methods for GV derivations (e.g. US EPA, European Commission, Australia and New Zealand, Canadian Council of Ministers of the Environment) are mixed in their recommendations of approach. The European Commission (European Commission 2011) and Canadian Council of Ministers of the Environment (Canadian Council of Ministers of the Environment 2007) note that field observational studies may be too confounded to be useful in guideline derivations though the derived data are important in evaluating and validating the final GV. Within biological effects information, ANZECC & ARMCANZ (2000) ranked field-effects as preferred over laboratory toxicity testing information for GV derivations.

However, most agencies acknowledge the limitations of historical reliance upon laboratory toxicity testing. Consequently, there has been a shift in the favoured approach towards the consideration of multiple lines of evidence (LOE) of stressor effects and thresholds, using laboratory effects, mechanistic models and field or semi-field data, to help define and derive GVs (see also discussion in Buchwalter et al 2017). Neither the European Commission (European Commission 2011) nor the US EPA (Cormier et al 2008) regards any one LOE of evidence as necessarily superior to others in defining a guideline. Cormier et al (2008) highlighted consistency of result amongst LOEs or conservatism as possible criteria to consider, but emphasised the need for weight of evidence (WOE) evaluation of all the LOEs to confirm causal associations and thereby engender confidence in the final GV selection.

Recent work in developing water quality criteria in the USA is focusing on improved ways to use field data for this purpose, mainly through development of methods to disentangle effects of confounding stressors (Suter & Cormier 2013). The outcome is greatly enhanced inference and evidence of cause and effect of stressors, particularly for those stressors and organisms which are difficult to study under laboratory conditions (see above) (Cormier & Suter 2013a, Coffey et al 2014, Chariton et al 2016). Consistent with recent trends internationally, the revised Australian and New Zealand Water Quality Guidelines (ANZG 2018) includes a more formal promotion of the use of multiple LOEs and subsequent WOE assessment for defining and deriving GVs. While in principle, field-effects derivations are preferred over those derived from laboratory toxicity testing, acceptable quality and strength of evidence are key determining factors that must be demonstrated before such a priori precedence can be assumed.

1.2.2 Methods to derive water quality guidelines using field data

Laboratory approaches to GV derivation typically record the responses of several taxa to a single chemical stressor, with the results combined into a species-sensitivity distribution (SSD) to derive a GV protective of a (high) proportion of species. SSDs have been used to derive GV for uranium (van Dam et al 2017), magnesium (van Dam et al 2010), manganese (Harford et al 2015b) and ammonia (Mooney et al in press) using organisms local to Magela Creek.

As described above, there has been much research published in recent years on methods to derive water quality guidelines using field data. Amongst field approaches, two common and established methods include:

1. Concentration-response relationships

1. The abundances or relative proportions of sensitive taxonomic groups (eg Figure 3A) or community summaries such as taxa number (eg Figure 3B) are plotted against corresponding ambient (or cumulative antecedent) contaminant concentrations. This entails comparing the response to, or expressing it as a proportion of, the same

response measured at uncontaminated reference sites. A 'hazardous' concentration (HC) at which an abrupt step change is observed (Figure 3A) or at which a small percentage of the response is adversely affected (eg. 5 or 10 percent) (Figure 3B) may then be determined.

2. Species-sensitivity distributions (SSDs) based on exposure-response relationships of assemblages of organisms

2. Species-sensitivity distributions (SSDs) describe the relationship between a contaminant and the varying sensitivity of different taxa to the contaminant across a concentration gradient. The sensitivity of each taxon may be represented by (e.g.) the highest concentration at which the taxon is recorded in the field, or a concentration above which only (say) 5% of the observations of the taxon occurs (ie in this case, 95% or XC95 where XC = Extirpation Concentration). SSDs are usually displayed as a plot showing the fraction of affected taxa on the y-axis and the contaminant concentration on the x-axis. The fraction of affected taxa is calculated by ordering the taxa from highest to lowest sensitivity and calculating for each taxon the cumulative frequency across the contaminant range. The resulting plotted distribution (of proportion of taxa affected vs contaminant concentration) is modelled using a weighted cumulative distribution function. From this modelled relationship, a conservative (e.g. 5th percentile of the distribution of taxa), is then determined (in this case HC5). See example in Figure 4.

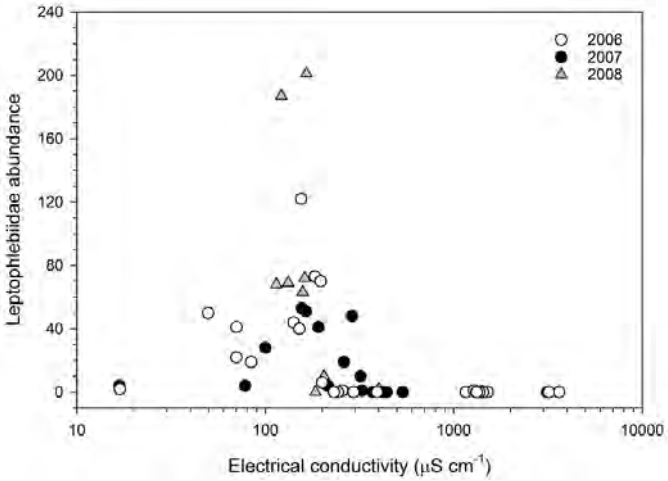
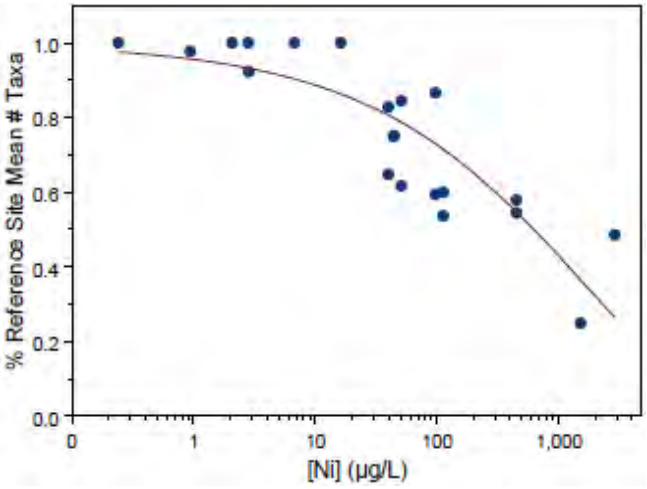


Figure 3 Concentration-response relationships derived for field data:

A. leptophlebiid mayfly intolerance in mine-receiving waters with EC exceeding 200-300 µS cm^{-1} (van Dam et al 2014b).



B. HC5 (the concentration that would be predicted to reduce taxonomic richness by 5%) for Ni at 20 µg L^{-1} for a mine-receiving water (Hydrobiology 2016).

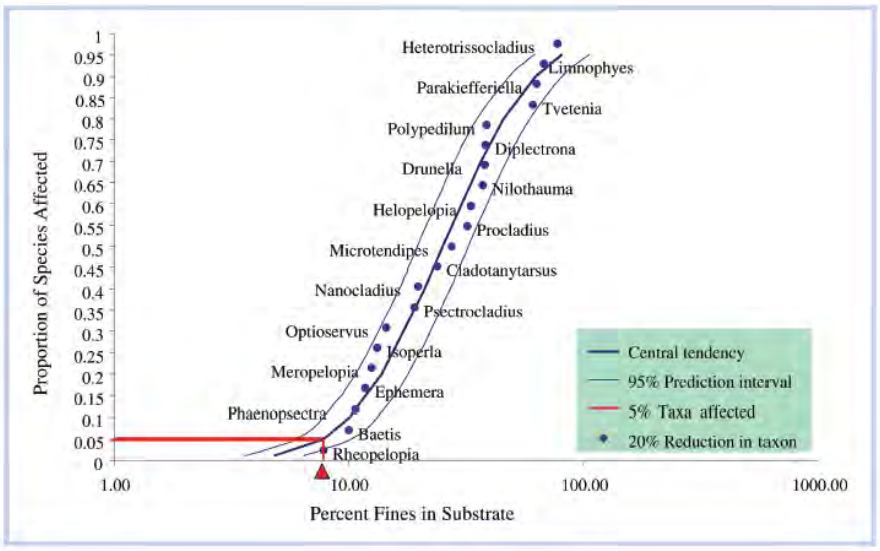


Figure 4 Species sensitivity distribution based on field data for the effects of sedimentation on stream benthos. Each point is the value of percent fines at which a taxon's abundance was reduced by 20%. The 20% reduction was calculated with respect to a maximum abundance estimate taken from 90th-quantile regression plots of the relative abundance of individual species of invertebrates. Abundances of 5% of the species are reduced by at least 20% at 7.8% fines. From Cormier et al (2008).

A less commonly applied variant of approach 1 considers analyses based upon measures of multivariate similarity of taxa compositional data between all possible pairs of classes of site contamination across a gradient (Kefford et al 2010).

Other techniques to improve use of field data for water quality objective setting (i.e. from above, viz disentanglement of effects of confounding stressors) have focused on either use of more sensitive and diagnostic biological indicators (i.e. physiological and genetic biomarkers), or improved statistical modelling approaches. Many of the diagnostic methods have only been developed in recent years and hence were not available when early data used in the present study were being gathered. Chariton et al (2015), for example, described the improvements in diagnostic information inherent in genomic (DNA and RNA) information, stressor-specific biomarkers and toxicodynamic-toxicokinetic models to predict exposure and bioaccumulation, viz expanded understanding of ecology, and thereby improved knowledge of the effects of individual stressors in a system.

Trait-based indicators, using physiological sensitivity or other biological information on biotic assemblages, offer improvements over traditional taxonomic-based monitoring methods. A number of these indicators have been demonstrated to be stressor-specific, thereby allowing the disentangling of effects of multiple stressors. A SPEcies At Risk (SPEAR) method for the detection of the effects of salinisation on freshwater communities of south-eastern Australia was developed by Schäfer et al (2011).

Methods for diagnostic analysis and stressor identification are also being rapidly developed, through specific analysis methods (see reviews in Chariton et al (2016) and van Dam et al (2014b)) which may be combined with laboratory, field and/or modelling data in formal weight of evidence (WOE) assessments (eg Cormier et al 2008, Coffey et al 2014). Amongst the gradient and change-detection methods are Bayesian inference, (distance-based) multivariate analysis, boosted regression trees, improvements to field-based SSDs, Threshold Indicator Taxa ANalyis for threshold detection, and integrating toxicity

identification evaluation with multiple regression models (see reviews by Chariton et al (2016) and van Dam et al (2014b)). Some of these methods are expanded upon elsewhere in this report. In addition to using data from multiple sources, the use of a variety of methods to analyse the data further strengthens WOE assessments in order to best determine final water quality GVs (see Cormier et al 2008).

1.3 Contaminants of concern for Ranger

For ecosystem protection, the physical/chemical 'contaminants of potential concern' (CoPCs) for Ranger receiving waters/sediment were originally derived by Brown et al (1985a))[1]. A subsequent hazard and risk assessment was conducted by Frostick et al (2012) who reviewed the original suite of CoPCs. They compared water quality data (2005-06 and 2010-11) from representative water types expected to leave the RPA, against national (ANZECC & ARMCANZ 2000) and locally-derived GVs. The majority of Brown et al's (1985) list of CoPCs was assessed as posing very low risk to aquatic ecosystems at and post-closure. The remaining CoPCs identified as most important and limiting[2] for relevant receiving waters/sediments were magnesium and sulfate (with electrical conductivity (EC, always corrected to 25ºC) a surrogate measure of both), uranium and manganese (Frostick et al 2012).

Recently, ammonia has been identified as an additional CoPC due to (i) commissioning of a brine concentration plant at Ranger, which discharges large volumes of distillate containing residual amounts of ammonia to the environment, and (ii) the potential for seepage of ammonia from the tailings and brines deposited in the rehabilitated mine pits (Turner et al 2016). Mooney et al (in press) derived a guideline value for total ammonia nitrogen of 0.4 mg L^{-1} TAN at pH 6.4 and 32ºC (typical pH and temperature conditions for natural billabong water quality). Exceedances of this value have not occurred in minesite waterbodies antecedent (12 months) to the macroinvertebrate sampling described in this report.

Of the five listed CoPCs, magnesium has been identified as the most likely limiting CoPC for Ranger rehabilitation and decommissioning. In particular, construction of the final landform of the Ranger rehabilitated site will involve large-scale use of waste rock that will generate significant loads of $MgSO_4$ salts to surface runoff and shallow groundwaters, dispersing to adjacent Magela Creek after mine-site closure (ERA 2014b). Predicted concentrations of CoPCs in ground and surface waters are being modelled, and the ecological risks associated with these will need to be determined. This ecological risk assessment will require closure criteria for the CoPCs, including Mg. Derivation of such criteria requires information from as many LOEs as possible for comprehensive evaluation.

Associated with the hazard and risk assessments cited above, as well as additional recent ecological risk assessments identifying knowledge needs for Ranger closure (R. Bartolo, A. Harford, S. Iles and R. van Dam, unpubl. data), two potential contaminant pathways involving sediment quality are the subject of ongoing research. These are the potential for build-up of sulfate and/or uranium in sediments of onsite or offsite waterbodies, and subsequent mobilisation of contaminants through sediment-surface water fluxes. Under

[1] The full suite of CoPCs included: SO_4, NH_3-N, NO_x, total P, phosphate, TSS, pH, alkalinity, turbidity, TOC, DOC, Ca, Mg, Mn, Fe, Cu, Pb, Cd, Zn, Cr, V and U.

[2] Limiting: In the absence of mitigation, potential for offsite biological impact based on concentrations present

(natural) wetting and drying conditions of billabong sediments, sulfate build up in sediments can give rise to acid sulfate soils with potential for oxidation and subsequent acidification, deoxygenation and release of metals. Sediment-related CoPC are considered elsewhere in this report (section 5.1.2).

This report focuses on a significant aspect of field effects information for $MgSO_4$ only, information from which will then contribute to a separate, broader WOE assessment drawing in additional (including laboratory toxicity and mesocosm) information. A number of sources of field biological-effects information relevant to Ranger are available for $MgSO_4$, including results of a multiple-assemblage (phytoplankton, diatoms, zooplankton, macroinvertebrates), semi-field (mesocosm) experiment (McCullough 2006), toxicity monitoring results examining freshwater snail reproduction, and responses of macroinvertebrate communities in onsite, lentic (still-water) waterbodies.

Results and evaluation of the lentic waterbody macroinvertebrate study are provided here, with mesocosm results to be published separately. The present study examines responses of macroinvertebrates to Mg concentrations in all natural and artificial waterbodies on the RPA which have, for various and sustained periods over time, exceeded laboratory-derived GVs (van Dam et al 2010). Periods prior to, and following, GV exceedance have been accompanied by collection of macroinvertebrate assemblage data, thereby providing an opportunity to independently assess the relevance of the laboratory-derived GV. Data and preliminary analyses used in the current report are provided in Jones et al (2008), Humphrey et al (2008b) and Humphrey et al (2012).

1.4 Biological effects information for magnesium sulfate

Globally, the impacts associated with increasing salinisation of freshwater ecosystems, a consequence of rising saline groundwater, alterations, to stream flows, pollution from mines and road de-icing have received considerable attention (eg Cañedo-Argüelles et al 2013, Herbert et al 2015, Cañedo-Argüelles et al 2016). In southern Australia, salinisation of freshwater ecosystems associated with Na^+ and Cl^- dominated groundwater has been well studied (see reviews of Nielsen et al (2003) and Cañedo-Argüelles et al (2016)). Setting water quality objectives for saline mine waters discharged to surface waters is also the subject of recent research focus where, typically, ionic constituents are quite different, for example: Ca^{2+}, Mg^{2+}, HCO_3^- and SO_4^{2-} dominance in coal mining effluents of southern Appalachian streams of USA (eg Cormier et al 2013, U.S.EPA 2016), Mg^{2+} and SO_4^{2-} dominance in at least three different mine sites in northern Australia (WA and NT) (van Dam et al 2010, van Dam et al 2014a, van Dam et al 2014b), and $Na^+ > Mg^{2+}$, $Cl^- > SO_4^{2-}$ dominance in coal mining effluents of the Fitzroy River catchment, central QLD (Mann 2012, Prasad et al 2014).

Increases in salinity of freshwater ecosystems may affect aquatic organisms in two ways: 1) direct toxicity through physiological changes (particularly associated with disruption of ionoregulation (ionic homeostasis), acid-base regulation (pH homeostasis) and potentially membrane integrity, S Cormier, personal communication), and 2) indirect effects associated with changes in community structure, and changes to species interactions and ecological processes (ANZECC & ARMCANZ 2000). Both increases and decreases in salinity (see Harford et al 2013) can have adverse effects.

Work by Mount et al (1997) and more recently Mount et al (2016) and Erickson et al (2017) determined the toxicity of, and interactions between, several major ions K^+, HCO_3^-, Mg^{2+}, Cl^-, SO_4^-, Na^+ and Ca^{2+} to standard test species. Na and Ca demonstrated the lowest

toxicity, while Ca was more significant at reducing the toxicities of other major ions. van Dam et al (2010) observed higher than expected toxicity for the major ions, $MgSO_4$, in very soft freshwaters of Magela Creek attributing the toxicity to just the Mg^{2+} ion. Toxicity was ameliorated by Ca, with a Mg:Ca (mass) ratio of 9:1 affording significant protection from Mg toxicity. A GV protective of 99% of species for Mg at a constant Mg:Ca ratio of <9:1 was determined to be 2.5 mg L^{-1} Mg. (An operational Limit for Magela Creek of 3 mg L^{-1} Mg is based on this site-specific guideline value, and has been in place since 2013 (Sinclair et al 2014)). Clements and Kotalik (2016) examined the responses of aquatic insect communities to exposures of different salts, specifically, $NaHCO_3$, $MgSO_4$, and NaCl, in four mesocosm experiments. They observed greater sensitivity of assemblages (in particular, mayflies (Ephemeroptera)) to $NaHCO_3$ and $MgSO_4$ exposures. Effects were also greater in stream waters naturally low in ionic strength (60–72 µS/cm) compared to responses in stream waters of moderate ionic strength (200–250 µS/cm).

Field mesocosms (tubs) deployed in the Magela Creek channel in 2002 were dosed with $MgSO_4$ and colonised naturally with macroinvertebrate, zooplankton, phytoplankton, and attached diatom communities. Results of this work (McCullough 2006), including recent re-analyses of the data (interim results reported in Supervising Scientist (2018)), demonstrated that phytoplankton (algal biomass, in the form of chlorophyll *a*) and zooplankton exhibited sensitivity after four-weeks of exposure. The resulting 1% effect concentrations for algal biomass and community structure response measures for zooplankton were 1.5 and 2.3 mg L^{-1} magnesium, respectively. The Mg:Ca ratios were less than approximately 10:1 for all except the highest magnesium concentration. Calcium, therefore, did not confer protection to these biological assemblages.

Elsewhere, Ca amelioration in the presence of $MgSO_4$–dominated mine effluents appears evident, including for example, toxicity of $MgSO_4$ measured and inferred for another northern Australian location, where minesite receiving waters (after the addition of mine-derived $MgSO_4$) were hard ($CaCO_3$ >500 mg L^{-1}) (van Dam et al 2014b). Field and laboratory-determined toxicity at this site was in the range 14-18 mg L^{-1} Mg. A leptophlebiid mayfly observed a sharp threshold and was shown to be absent in all receiving waters with Mg concentrations above that range (van Dam et al 2014b; Fig 3A).

Notable amongst recent reviews and studies of effects of saline discharges is the reported complexity associated with organism responses: results for one major ion at one location are not necessarily transferable to other locations because (and amongst other causes) the presence of other major ions can influence toxicity (often amelioration, eg van Dam et al 2010, Prasad et al 2014).

Organisms can also adapt and acclimate under particular conditions. For example, just modest increases in salinity tolerance of the snail, *Physa acuta*, when exposed to greater background salinity led Dunlop et al (2008) to suggest that acquired tolerance to long-term incremental exposure to increased salinity was likely. While Dunlop et al (2008) observed only small differences in the salinity tolerance of the same species collected across large geographical distances (different ecoregions) with different prevailing salinity, greater proportions of salinity-tolerant taxa were present in those ecoregions prone to seasonal and interannual drought. Exemplifying this, the fauna in the agricultural zone of southwestern Australia was noted by Kay et al (2001) to be particularly resilient to high salinities, with some families tolerating salinities orders of magnitude greater than previously reported for streams. They attributed this to pre-existing tolerances for much of the biota in riverscapes receiving naturally saline groundwaters for long geological time

scales. Environmental variability of this type can result in widely varying sensitivities of organisms within the same taxonomic group (eg Rutherford & Kefford 2005, unpubl.).

Constant exposures to salts compared to exposures to short pulses of salts can also affect the salinity tolerance of a species. Exposure to constantly elevated, or slower, incremental increases in salts compared to exposures to short pulses of salts led to greater species tolerance in a mesocosm study by Marshall and Bailey (2004), but reduced the tolerance in toxicity studies by Hogan et al (2013) and mesocosm studies by Cañedo-Argüelles et al (2014). Such discrepancies may possibly be explained by the time scales between pulses: in general, shorter exposures, with shorter pulses, would be expected to decrease toxicity.

Consistent amongst all such studies, however, is the high sensitivity of mayflies (Ephemeroptera) (relative to other invertebrate orders) to salinity. This has been observed amongst mining-related studies in Australia (eg Dunlop et al 2008, van Dam et al 2014b, Dunlop et al 2015), for coal mining effluents of southern Appalachian streams in the USA, where saline discharge waters are dominated by Ca^{2+}, Mg^{2+}, HCO_3^- and SO_4^{2-} ions (eg (Pond 2010, Clements & Kotalik 2016) and anthropogenic settings in other countries (Kefford et al 2012).

Further descriptions of observed or inferred Mg effects on the biota of Magela Creek are presented in other parts of this report.

1.5 Study aims

The hypothesis of this study was that macroinvertebrate communities resident in lentic waterbodies (both artificial waterbodies and natural billabongs[3]), will be altered (relative to reference conditions) following exposure to Ranger mine waters and that these changes can be detected using analyses of the associated field data. The exposure gradient is associated with (mainly) $MgSO_4$, derived from the weathering of exposed waste rock. The study aims were to:

1. Determine whether increased concentrations of Mg ions (or mixture of ions) have contributed to alterations to aquatic macroinvertebrate communities (including reductions in taxa and abundances) in lentic waterbodies of Magela Creek catchment (summary in section 5.1.4, section 5.3).
2. Develop quantitative models of Mg concentration-effects on macroinvertebrate communities using field data (section 5.3.3).
3. Minimise the influence of co-occurring causes (habitat, DO, pH) and potential confounders (sampling design, sampling, sample processing, life histories) that may affect the quantitative models (section 4 and cross-references therein).
4. Based on a weight of evidence assessment of the field data, identify a candidate guideline value that will protect 99% of taxa (sections 5.4 and 6).

3. Beyond this report and to be published or implemented separately, further steps are required to:
   (i) Weigh candidate effects values from field, mesocosm, and laboratory studies to determine a guideline value that will protect 99% of species; and

[3] 'Billabong' is a term applied to any *natural* lentic waterbody in Australia

(ii) Develop and implement methods to assess the effectiveness of the guideline value in achieving 99% of species protection during mine operations and during and post-closure.

4. Cormier et al (2008) proposed a general framework for resolving environmental problems by integrating different types of assessment. The assessment types from Cormier et al (2008) applied in this study include:
   - chemical and physical condition assessment (pre-mining and current, reference and mined sites);
   - causal assessment (in this case, the role of just one cause: is Mg^{2+} responsible for the key changes observed in macroinevertebrate communities in mine-water exposed waterbodies ?); and
   - predictive criterion assessment (in this case, deriving a guideline value for Mg^{2+}).

2 Design of current study and further background

There are several key aspects to the study and its design that are important to consider for drawing inference. These are described in the following subsections.

2.1.1 Lines of evidence

The present study considers a number of lines of evidence in drawing inference about contaminant effects upon macroinvertebrate communities of lentic waterbodies. These include:

1 the significance and nature of relationships between the contaminant(s) of interest and the biological responses measured;
2 the strength of evidence discounting other potential explanations (confounders) for the responses observed (see section 2.1.3); and
3 consistency in observed response to those demonstrated in relevant studies elsewhere.

In turn, the present study provides one broader, collective line of evidence amongst others for which local biological effects information are available on the effects of $MgSO_4$. Other key lines of evidence include laboratory toxicity studies using $MgSO_4$ as a single toxicant (van Dam et al 2010), laboratory and semi-field toxicity assessment of mine water mixtures high in Mg, and a dry season, field mesocosm study conducted in Magela Creek sand channel (McCullough 2006). Consistency in response amongst the different lines of evidence would enhance inferences drawn from this WOE assessment.

Lines of evidence also incorporate different and independent methods of statistical analysis which, if reaching similar conclusions, strengthens inference (sensu Cormier et al 2008).

2.1.2 Sensitive biological assemblages

Macroinvertebrate communities have inherent and traditional virtues for biological monitoring, including the demonstrated sensitivity of this group of organisms to water quality generally (eg Rosenberg & Resh 1993, ANZECC & ARMCANZ 2000). These virtues led to early adoption of this assemblage group for biological monitoring of streams and billabongs in the Alligator Rivers Region of the NT (Humphrey & Dostine 1994). For the present study, pre-mine-impact data for macroinvertebrate assemblages in billabongs were available, a distinct advantage in biological assessment studies. Moreover, over time and with ongoing development, the knowledge base about the sensitivities of specific macroinvertebrate taxa to different water quality types in Australia is expanding (Chessman 2003, Chariton et al 2016, Dafforn et al 2016).

2.1.3 Minimising and accounting for potential confounding

Notable chemical contamination of Ranger onsite ('exposed') waterbodies, including Georgetown, Coonjimba and Djalkmarra Billabongs, and Retention Pond 1 (RP1), commenced in the early 1990s to early 2000s (section 5.1.1.2). While a number of chemical constituents were associated with this contamination, the predominant contaminant was $MgSO_4$ salt, of which the Mg^{2+} ion has been demonstrated to be responsible for toxicity (van Dam et al 2010). As early as the mid 1990s, workers noted departure in macroinvertebrate responses over time in minesite waterbodies relative to similar responses measured in reference waterbodies, suggesting that corresponding increases in Mg concentrations in the onsite waterbodies could be responsible (O'Connor et al 1995).

Field-effects studies used to confirm and establish causal relationships between a contaminant of concern and biological responses are often confounded by stressors or environmental variation that are also correlated with either the CoPC or the response. Several sources of variation and potential confounding associated with season, catchment,

habitat, physical and chemical contaminants (other than $MgSO_4$) and changes in protocol over time are recognised in this study. Potential confounders need to be identified and the extent of their interference in correctly attributing cause and effect between CoPC and response evaluated by weighing all evidence for and against plausible alternative explanations. In the case of plausible confounders, the dataset may be truncated to reduce their effect (Suter & Cormier 2013). Approaches used to quantify, account for and minimise these extraneous factors where possible, are described in section 4. (Statistical routines referred to in these sections are explained in section 3.4 Data analysis).

2.1.4 Change detection and inference

Mine-related changes in macroinvertebrate communities are inferred across a spatial and temporal gradient of exposure to Ranger mine waste waters. Responses of macroinvertebrate communities are compared (i) amongst waterbodies directly exposed to mine waters and reference waterbodies outside of the influence of mining, and (ii) over a time-span from prior to mining (1979) to near-present (2013), a time range over which several exposed waterbodies have become increasingly contaminated with a number of mine-related contaminants, to varying degrees. Prior to minewater contamination (i.e. pre-mining), minesite billabongs maintained a similar water quality to adjacent non-mine-exposed billabongs (Walker & Tyler 1984). Sampling in one mine-exposed waterbody, Georgetown Billabong, has spanned a period of only minor contamination by mine waters for most of the 34 year period (i.e. from 1979 to 2000).

Macroinvertebrate responses have been measured intermittently (several occasions) between 1979 (prior to mining) and 2013. For each sampling year, responses from mine-exposed waterbodies are compared to contemporaneous responses measured in reference waterbodies unaffected by mine water discharges. Inference is mainly drawn from correlation between biological response and water quality measures over time and space, together with association of these changes with known taxa responses to contaminants (e.g. air-breathing taxa tolerant of water quality, or salt-sensitive taxa identified in the literature).

The design of the study and description of the specific configuration of reference and mine-exposed waterbodies are provided in section 3.1 below.

2.1.5 Changes in habitat and water quality over time unrelated to mining

Landscape-wide changes to the savannas and lowland billabongs of the ARR have occurred from the late 1970s, unrelated to mining.

Changes in aquatic plant communities from pre-mining to commencement of mining (1978 and mid 1980s)

Bishop (1987) reported low percent cover of most of the forms of aquatic plants in minesite billabongs over the 1978-79 period, associated with feral Water buffalo wallowing and trampling. Recovery in the density of the macrophyte forms occurred after 1980 in association with local feral animal control measures on the minesite. Comparative plates in Appendix 9 (Plate 1), showing Georgetown Billabong in the wet seasons prior to 1980 and 2012, also highlight the differences in plant cover prior to mining and present. In general, these changes between pre- and post-buffalo-removal were also associated with a decrease in dry season turbidity and an increase in diurnal dissolved oxygen fluxes. Aquatic plants provide primary habitat for macroinvertebrate communities so these changes need to be considered in the study design. Similar changes to aquatic vegetation and water quality were occurring in all other ARR shallow lowland bilabongs and hence the comparison of mine-exposed waterbodies to contemporaneously-sampled reference

billabongs is a key design factor accounting for temporal confounding. These collective observations are relevant mainly to macroinvertebrate sampling and use of associated data from 1979 – discussed in section 5.3.4.2.

Other stressor agents operating at landscape level

Catchment fire regimes and the foraging activities of feral animals (pigs) in the littoral portions of billabongs can also influence habitat and water quality directly. An assessment is made in this report of the potential for either of these stressor agents to affect minesite waterbodies and associated catchments, and not reference billabongs, in any year of sampling. Such differences and associated water quality changes in minesite-only waterbodies would confound interpretation of CoPC effects on macroinvertebrate communities.

2.1.6 Habitats for which billabong effects data are relevant

The effects dataset analysed in the current study differs from laboratory-derived toicity data in key ways: (i) longer exposures in the field (single or multiple generations over years compared to several days in the laboratory), (ii) differences in natural water quality in the dry season including higher water temperature, higher total and dissolved organic carbon and higher concentration of ions (evapo-concentration) compared to laboratory test conditions, (iii) differences in feeding regimes, (iv) exposure of diverse aquatic insect communities, and (v) presence of ecological interactions (e.g. competition, predation) amongst species. Information from both laboratory and field lines of evidence has in common exposure of predominately lentic species, but neither approach includes exposure of insect species that are flow-dependant under natural wet season conditions. Thus we make no claim as to the sensitivity to $MgSO_4$ of organisms in lotic receiving water environments elsewhere, including the extensive sand channels downstream of Ranger.

2.1.7 Standardisation of sampling season

High seasonality in macroinvertebrate community structure in shallow lowland billabongs of the Alligator Rivers Region (ARR) over the annual hydrograph was reported by Marchant (1982b) and Outridge (1988). A common fauna of highest diversity in the billabongs was observed in the late wet-early dry season period, while lowest diversity, associated with poor water quality and shallowing and reduced water volumes, was found during the late dry season. Apart from high diversity, another key advantage in the late wet-early dry season timing of sampling is that it represents the summation and integration of all wet season mine water exposures to aquatic organisms (Humphrey et al 1990). For these reasons, sampling of waterbodies was standardised to the April-May period, ie late wet-early dry season.

While the magnitude of rainfall and stream flow during the antecedent (to sampling) wet season may potentially confound interpretation of results – considered in sections 4.1.1.2 and 5.2.3.2 below – variation in the timing of the seasonal sampling amongst years is not regarded as a contributor to potential misinterpretation of results.

2.1.8 Taxonomic resolution

Biological assessments involving macroinvertebrate communities are generally assumed to provide greater power in impact detection if identifications are conducted at lower taxonomic levels (eg genus or species) than higher taxonomic levels (eg family-level). The assumption of greater sensitivity at lower, especially species, level is based upon the reasonable argument that congeners may vary in their sensitivities to different stressors (eg Cranston 1990). Hence by summarising a response at a higher taxonomic level, critical information may be lost.

Local and national information suggest that while species-level information will provide greater discriminatory power in detecting and assessing water quality changes in local aquatic ecosystems, the use of family-level data in the present study should not compromise this inferential capacity greatly. Information supporting this includes:

1 Results from an experimental manipulation in a small polluted stream in Kakadu National Park (Faith et al 1995) together with other considerations of statistical power associated with Before, After, Control, Impact, Paired difference (BACIP) designs (Supervising Scientist Division 2013).

   Supervising Scientist Division (2013), using local stream data and those of Faith et al (1995), observed that (i) data summarised at lower (e.g. species-level) taxonomic levels could be more variable over time (within either the 'before' or 'after' impact period) than those summarised at higher (e.g. family) taxonomic levels, but (ii) the magnitude of change from 'before' to 'after' impact for data summarised at lower taxonomic levels was greater than that summarised at higher taxonomic levels. These findings imply that the advantage of greater magnitude of change detected at lower taxonomic levels after impact (aspect (ii) from above) can be diminished by loss in statistical power associated with the higher variability in these data. Faith et al's (1995) results support these findings, thus family-level BACIP analyses were not very different from those using species level data.

2 Work elsewhere in Australia on salinity impacts upon aquatic macroinvertebrates has shown, and with few exceptions, that there is little variation in the acute lethal salinity tolerance within families compared to between families (Kefford et al 2003, Kefford et al 2006, Schäfer et al 2011). Kefford et al (2011) noted that species are lost with increasing salinity at a higher rate than families are lost, but concluded that their study "does not imply that families are in general of low indicative power for high or low salinity".

2.1.9 Macroinvertebrate indicator metrics to apply to assessment and threshold determination

Macroinvertebrate indicator metrics applied to assessment and threshold determination in this study included:

- The ANOSIM R statistic compares the mean of ranked (Bray-Curtis) dissimilarities between groups to the mean of ranked dissimilarities within groups. An R value close to '1.0' indicates dissimilarity and marked separation between groups while an R value close to '0' indicates an even distribution of high and low ranks within and between groups, with interspersion of group samples.
- Multivariate similarity including Jaccard and Bray-Curtis (B-C) measures. These are bounded between 0 and 1, where 1 means the two groups (exposed versus reference) sites have the same community composition or (B-C only) structure (that is they share the same taxa and (B-C) relative abundances), and 0 means the two sites do not share common taxa. The B-C measure has been shown to be the most robust to a range of disturbance patterns and so will generally provide the greatest statistical power (Faith et al 1991).
- Taxa number (usually family-level resolution): in this study, generally represented by the mean number of taxa amongst the five replicate locations sampled within a waterbody at any sampling period.

Other candidate metrics *not* used in this study, together with rationale for exclusion:

- Diversity indices, including Shannon–Wiener diversity, convey no information on the actual species composition of a community. These indices combine richness and evenness components into a single measure, even though it is usually more informative to evaluate richness and evenness independently (see critique by (Washington 1984).
- The EPT metric, i.e. % of Ephemeroptera, Plectoptera and Trichoptera (EPT) taxa (noting absence of Plecoptera in Northern Territory freshwater ecosystems) represents purported pollution-sensitive taxonomic groups, and has been commonly applied elsewhere in Australia. Experimental work by Faith et al (1995) in the Alligator Rivers Region showed that subsets of even proven sensitive taxa actually provided less discriminatory power to detect water quality impairment than measures (similarity) of the entire community response. (As a side investigation to the current study, plots of %ET in minesite waterbodies compared to reference billabongs – not included in this report – indicated poor discrimination compared to taxa number and similarity.)
- Biotic indices developed elsewhere in Australia:
    - SIGNAL (Chessman 2003) was developed to detect general biotic impairment by poor water quality, with scores derived from information on pollution tolerances from the better-studied, temperate south east and south west portions of Australia. The scores have not been verified for northern Australia. SIGNAL also has little physiological basis, being derived instead on the basis of distribution across contaminant gradients (and hence correlation).
    - The trait-based, 'species at risk ($SPEAR_{salinity}$) was derived by Schäfer et al (2011) for detecting salinity (NaCl) impacts in south-eastern Australia. The authors advised that a prerequisite to applying $SPEAR_{salinity}$ was the need to verify whether the relative sensitivity ranking for taxa to seawater salts holds both outside of south-eastern Australia and for other ionic proportions.

Information arising from the present study will contribute data towards identifying taxa from soft waters sensitive to magnesium-sulfate-based salinity. This information may contribute to taxa sensitivity-tolerance databases useful for deriving new and regionally-relevant indicator metrics.

3 Methods

3.1 Sampling sites and dates

General features of the field design for this study were described in sections 2.1.4 and 2.1.7 above. Sampling locations, mine-water exposure category, catchment and year of sampling are provided in Table 1.

3.1.1 Sampling sites

Macroinvertebrate communities from between 3 and 14 shallow lowland waterbodies have been sampled by SSB or consultants seven times between 1979 and 2013 (i.e. 1979 (prior to mining), 1995, 1996, 2006, 2009, 2011 and 2013) with sampling conducted during the wet-dry season transition period between April and May. The waterbodies are located in two catchments, Magela and Nourlangie Creeks, and include:

- *Mine exposed waterbodies*: the originally-natural Djalkmara (removed in the 1996 dry season by development of the open pit accessing the Ranger 3 orebody), Coonjimba, Georgetown and Gulungul billabongs and the constructed minesite waterbodies Ranger Retention Pond 1 (RP1) and Retention Pond 2 (RP2). Of these waterbodies, most to least contaminated by Ranger CoPCs followed the order RP2 and Djalkmara (highly contaminated), RP1 (moderately contaminated), Coonjimba (contaminated), Georgetown (low contamination) and Gulungul (negligible contamination).
- *Reference sites, not exposed to Ranger mine waters*:
    - Magela Creek catchment: Baralil, Corndorl and Wirnmuyurr billabongs and Jabiru Lake. (Jabiru Lake, an artificial impoundment, was selected to provide a potentially useful analogue for RP1, a similar man-made structure.); and
    - Nourlangie Creek catchment: Malabanjbanjdju, Anbangbang, Buba and Sandy (shallow) billabongs.

Gulungul Billabong, while downstream of Ranger, has a negligible mine-water signal and for the purposes of the biological analyses conducted hereafter, is included amongst reference waterbodies.

Figures 5 and 6 show the locations of the waterbodies. Djalkmara Billabong was removed in 1996 and hence is not shown on the maps; it occurred in the location now shown as Pit 3 in Figure 6. Coonjimba, Corndorl, Djalkmara, Georgetown, Gulungul, Wirnmuyurr, Anbangbang, Buba and Sandy Shallow billabongs are of the 'backflow' type occurring at the confluence of small tributaries and the main stream (Magela, Wirnmuyurr or Nourlangie creeks), separated from the latter by natural levees. Baralil Billabong is a waterbody lying in the main watercourse of Baralil Creek and is only rarely backfilled by water from Gulungul Creek. Humphrey and Simpson (1985) and Humphrey et al (1990) provide full morphological and hydrological descriptions of the backflow billabongs while Table 1 provides summary information on the morphometry and catchment characteristics of all the waterbodies. At the time of sampling (April-June), the waterbodies were at near maximum depth with macrophytes fringing the margins up to depths of approximately 2 m.

The artificial waterbody, RP1, was constructed in the Coonjimba Creek catchment, upstream of Coonjimba Billabong (Figure 6) between 1979 and 1980. The impoundment affects the hydrology of Coonjimba Billabong, mainly through retention of early wet season runoff in the catchment upstream of the billabong. RP1 also directly affects the

water quality of the billabong through passive or active release of waters over the spillway during the wet season. RP1 contains stored minesite runoff and other mine waste waters, including clean water distillate from brine concentration. Coonjimba Billabong hydrology and water quality are also influenced by backflow from Magela Creek during high wet season flow events in the creek.

3.1.2 Sampling dates

Sampling of waterbodies was conducted in the April-May period. Start and finish dates across all waterbodies for each year are provided in Appendix 1, Table A1.1. The actual dates for sampling of each waterbody are shown in Appendix 1, Table A1.2.

Commencement dates for sampling were influenced by road access to sites so that after a wet season of high rainfall (eg 2011), timing was delayed. The criterion of road access for commencement date also ensured hydrological standardisation of sampling as first-access date generally corresponded to waterbodies of similar water level. The sequence of waterbody sampling was similar amongst years to ensure each waterbody was sampled at a similar hydrological condition.

3.2 Sampling, chemical analysis and compilation of data for environmental variables

In order to appropriately interpret differences in biological assemblage structure and associated community summaries, catchment, morphometric, habitat and water and sediment quality variables associated with each waterbody were measured (Table 2). Total annual rainfall for the wet season immediately preceding macroinvertebrate sampling was taken from the Australian Bureau of Meteorology's Jabiru Airport records (http://www.bom.gov.au/climate/averages/tables/cw_014198.shtml).

3.2.1 Catchment and waterbody morphometry

The catchment area draining to each waterbody, and waterbody surface area at near-maximum (April–May) inundation, were estimated from ArcMap (v. 10) software (ESRI 2013). Waterbodies were ranked for depth (shallowest to deepest) based upon bathymetry (Humphrey & Simpson 1985) and local knowledge acquired from SSB monitoring staff members.

3.2.2 Habitat

Information on macrophyte species composition and relative abundance was collected at each site where macroinvertebrates were collected. A visual assessment was made of the total percentage cover (surface and through the depth profile) and the percentage abundance of each macrophyte species using the methods of O'Connor et al (1995).

For further data analysis, plant forms were grouped according to Sainty and Jacobs (2003) classification, ie 'floating attached' (FA), 'submerged not feathery' (SNF), 'submerged and emergent feathery' (SEF), 'free floating' (FF), 'emergent narrow leaf' (ENL) and 'emergent broad leaf' (EBL). This grouping was performed in order to determine whether gross morphological characteristics of the plants were key features in possible plant-invertebrate relationship (O'Connor et al 1995).

Table 1 List of waterbodies included in the present study together with years of sampling, and locational and morphometric characteristics. Easting and Northing given for UTM Zone 53

Waterbody	Site Code	Sampling years	Catchment	Easting	Northing	Type	Exposure to mine waters	Waterbody area (m^2)	Catchment area (km^2)	Depth rank[1]
Retention Pond 2	RP2	2006, 2011, 2013	Magela	273938	8597335	Artificial	Yes	159703		8
Retention Pond 1	RP1	1995, 1996, 2006, 2009, 2011, 2013	Magela	272215	8598603	Artificial	Yes	162055	2.0	6
Djalkmara	DJKB	1995, 1996	Magela	274085	8598254	Natural	Yes	68668	5.4	1
Coonjimba	CJBB	1979, 1995, 1996, 2006, 2009, 2011, 2013	Magela	272436	8599367	Natural	Yes	40658	3.8	3
Georgetown	GTB	1979, 1995, 1996, 2006, 2009, 2011, 2013	Magela	275323	8597539	Natural	No	42400	28.8	4
Gulungul	GULB	1996, 2006, 2009, 2011, 2013	Magela	270200	8602800	Natural	Yes	33261	77.8	3
Baralil	BARB	1979, 1996, 2006, 2009, 2011, 2013	Magela	269134	8600337	Natural	No	18429	21.3	4
Jabiru Lake	JBL	1995, 1996, 2006, 2011, 2013	Magela	265459	8598260	Artificial	No	167905	2.1	7
Corndorl	CORB	1996, 2006, 2009, 2011, 2013	Magela	269231	8603848	Natural	No	4500	90.4	2
Wirnmuyurr	WINB	2006, 2009, 2011, 2013	Magela	273882	8608754	Natural	No	40951	23.7	3
Malabanjbanjdju	MALB	2006, 2011, 2013	Nourlangie	256302	8587676	Natural	No	20333	40.7	4
Anbangbang	ANGB	1996, 2006, 2011, 2013	Nourlangie	260683	8576735	Natural	No	86940	32.1	2
Buba	BUBB	1995, 1996, 2006, 2009, 2011, 2013	Nourlangie	255055	8578547	Natural	No	53000	73.7	1
Sandy Shallow	SDSB	1995, 1996, 2006, 2009, 2011, 2013	Nourlangie	258500	8572700	Natural	No	124718	8.0	5

1. Depth rank 1 to 8 is shallowest to deepest.

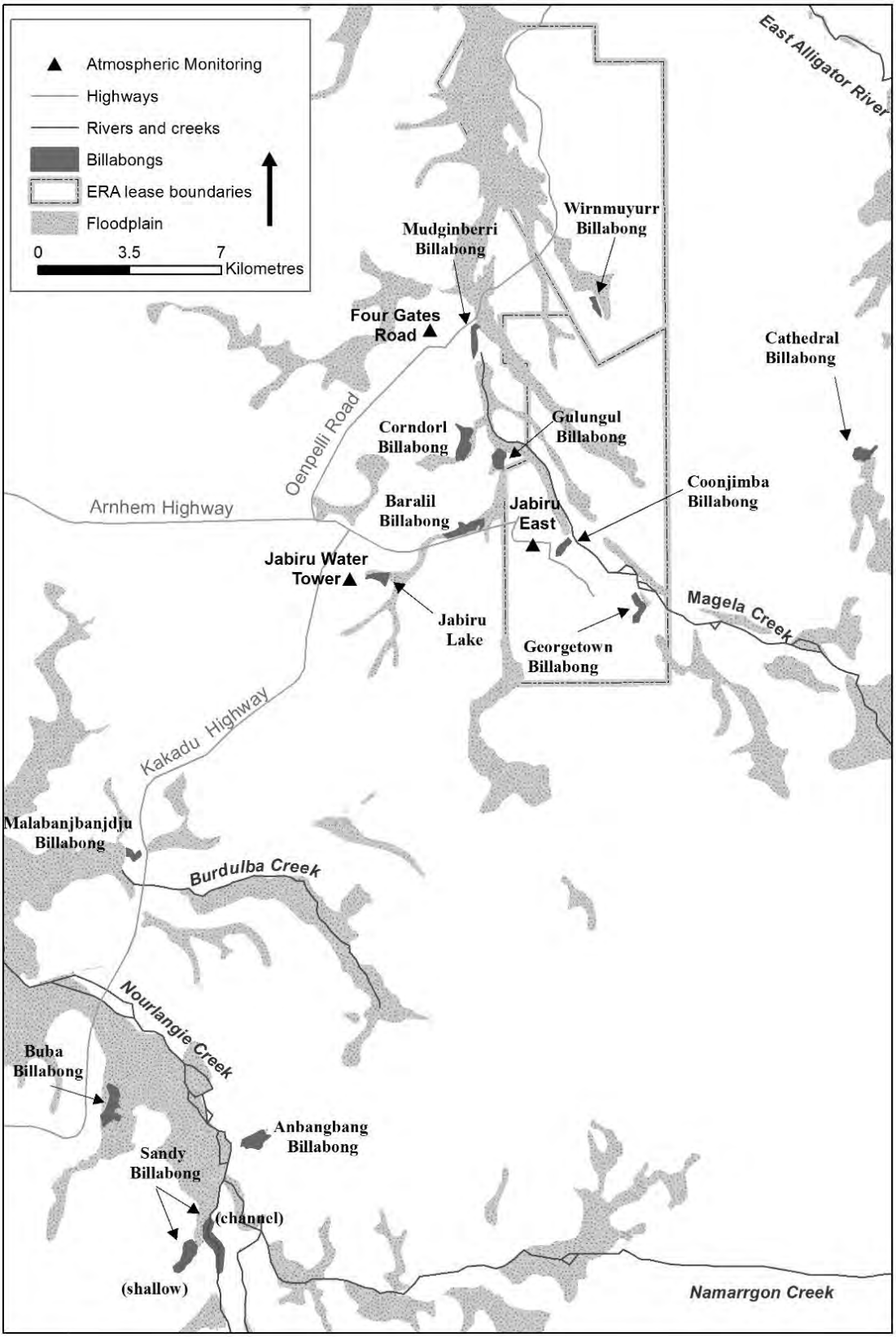


Figure 5 Location of lentic waterbodies sampled for macroinvertebrate communities

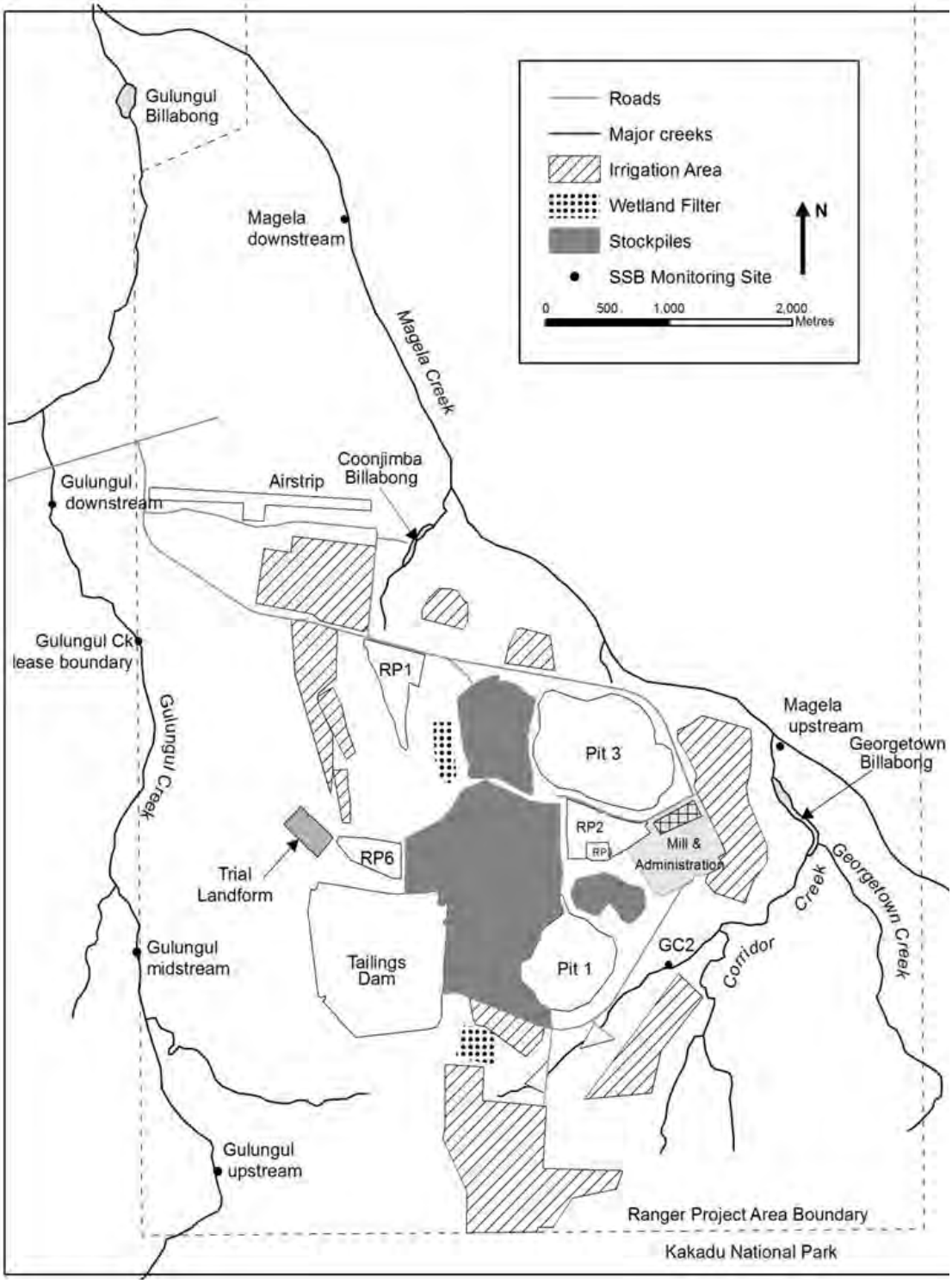


Figure 6 Ranger operational area site map showing RP1, RP2 and on-site billabongs

Table 2 Site variables measured at each site. (Not all variables were measured on each sampling occasion.)

Category	Feature/analyte	Units
Catchment and morphometry	Catchment (Magela = 1, Nourlangie = 2)	–
	Easting	m E
	Northing	m N
	Waterbody catchment area	km^2
	Waterbody surface area (at maximum inundation)	m^2
	Depth rank (1–8, shallowest to deepest)	–
Habitat variables	Total macrophyte cover	%
	Relative abundance of macrophyte genera	%
Water quality variables: field	Temperature	°C
	pH	units
	Electrical Conductivity	$\mu S\ cm^{-1}$
	Dissolved Oxygen	$mg\ L^{-1}$
	Dissolved Oxygen	% sat
	Turbidity	NTU
Water quality variables: laboratory	NO_3, NH_3, PO_4, Ca, Cl, Mg, K, Na, SO_4	$mg\ L^{-1}$
	Al, Cu, Fe, Mn, Pb, U, Zn	$\mu g\ L^{-1}$
Sediment quality variables	Al, Ba, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Rb, S, U, Zn and Total Organic Carbon	$mg\ kg^{-1}$

3.2.3 Water and sediment physico-chemistry

3.2.3.1 Sampling associated with macroinvertebrate collections

3.2.3.1.1 Water physico-chemistry

For 1979 sampling, electrical conductivity, pH, Mg and U data representative of the billabongs at the time of sampling were taken from the Northern Territory Department of Transport and Works datasets (Northern Territory Water Division 1983).

For 1995, field measurements of water temperature, pH, electrical conductivity (EC), dissolved oxygen and turbidity were taken from only one of the five locations in each waterbody using a calibrated Hydrolab water quality meter (O'Connor et al 1995). In 1996, only EC and pH were measured at the five locations in each waterbody (O'Connor et al 1997). No further water samples were collected for chemical analysis for either of these two studies. However, other sources of data were available for these two years for the analyses used in the current report. These included the variables, EC, pH, water temperature, turbidity, metals and major ions, collected at similar time periods for SSB's shallow-billabong fish community monitoring program and the databases of ERA.

For 2006, 2011 and 2013 sampling, field measurements of water temperature, pH, electrical conductivity, dissolved oxygen and turbidity were taken from each of the five locations in each waterbody using a calibrated Hydrolab water quality meter. Water samples from two locations in each waterbody were also collected for chemical analysis. All laboratory analyses were conducted by Envirolab (Sydney) and Northern Territory Analytical Laboratories (NTEL, Darwin), both NATA-accredited laboratories, using a combination of ICP-AES, ICP-MS and FIA methods.

3.2.3.1.2 Sediment chemistry

Sediment samples were collected in 2007, 2011 and 2013. Metals expected in runoff waters from Ranger Mine were of primary interest. Composite samples of surface sediments were collected from the top 5 cm, and for 2011 and 2013 sampling, at the shallow, littoral sites that macroinvertebrates were collected from (i.e. five locations within each waterbody), in water depths of less than 2 m. Chemical characterisation focused on the <63 μm fraction (Table 2).

Each of the sediment samples from above was mixed and wet-sieved, following in-house protocols, to separate < 63 μm and > 63 μm fractions. Both a portion of the total sample and the < 63 μm fraction were dried at 60°C before analysis. The dried fractions were analysed by NTEL. For this, a weak acid (1M HCl) digest method was used where the sample was shaken with 1M hydrochloric acid (HCl) and the elutriate analysed. This represented extraction of the bioavailable fraction of metals (ie weakly bound to the surface of sediment particles and thereby mimicking the gut of benthic organisms).

3.2.3.2 Water chemistry antecedent to sampling and in addition to that measured in association with macroinvertebrate sampling

For the mine water-exposed waterbodies, i.e. RP1 and Coonjimba, Georgetown and Djalkmara billabongs, data for a suite of analytes were compiled from the databases of ERA, Northern Territory Department of Mines & Energy, Northern Territory Department of Transport & Works and SSB relevant to the period 1970 to 2013. All available records for the major ions Ca, Mg, Na, K, Cl, SO_4 and HCO_3 were compiled, together with antecedent (mostly weekly) data for the same analytes, as well as EC and U, for the dry and wet seasons preceding the years sampled for macroinvertebrates. On the basis that macroinvertebrate sampling was conducted in the April-May period, ie at the end of the wet season, data were compiled separately for antecedent wet season (ie wet season just completed), dry season (previous calendar year) and combined wet and dry seasons. Wet season data spanned the period of initial waterbody infilling (usually early January) to May inclusive, while dry season data spanned the period June to December (unless initial waterbody infilling had occurred in December). Median analyte values were calculated for each of wet and dry seasons. Combined wet and dry season antecedent values were calculated using the mean of the wet and dry season medians. Wet and dry season medians, and combined seasons means for the analytes are provided in Appendix 1, Tables A1.5–1.8.

3.3 Macroinvertebrate sampling and sample processing

3.3.1 General methods amongst years

Table 3 provides a summary of sampling and sample processing methods used for each year of study. Family-level (or higher taxonomic level) data were acquired for each year of sampling.

3.3.1.1 Sampling in 1979

Macroinvertebrate communities from three Magela billabongs were sampled in 1979 on a monthly basis (Marchant 1982a), with each monthly sample represented by a single replicate taken from the same general location, and from the same littoral zone as sampled in subsequent years (described below). Each monthly replicate sample represented a composite of macrophyte and sediment habitat (Marchant 1982a). To increase replication for 1979 sampling, the separate data from April, May and June from each billabong were used in analyses. As described in section 2.1.7, these late wet-early dry season months coincided with a common fauna of high diversity in the shallow billabongs, thereby

reducing any seasonal variability. Thus three 'replicate' samples were available for each of the billabongs.

Table 3 Sampling and sample processing methods used for each of the years of study. M and N for reference waterbodies refer to Magela and Nourlangie catchments respectively.

Method	1979	1995	1996	2006	2009	2011	2013
Replicates per waterbody	One each from Apr, May, Jun	5			One from each reference waterbody; Five from each exposed waterbody (incl Gulungul)	5	
Reference waterbodies	1 (M)	1 (M) 2 (N)	4 (M) 3 (N)	4 (M) 5 (N)	3 (M) 3 (N)	4 (M) 5 (N)	
Exposed waterbodies[1]	2	4		3			
Habitat used in analyses	Macrophyte & sediment composite	Separate macrophyte & sediment samples collected but combined prior to processing		Macrophyte & sediment samples collected & processed separately	Macrophyte samples only	Macrophyte & sediment samples collected & processed separately	Macrophyte samples only
Mesh size field (µm)	500	250					
Mesh size laboratory (µm)	N/A	500			250	250	250
Sample processing method	Whole sample	Live-sorted			Laboratory		

1: Refers to data from exposed waterbodies actually used for analysis

3.3.1.2 Sampling in 1995, 1996, 2006, 2011 and 2013

For macroinvertebrate collections commencing from 1995, samples were collected in each waterbody from five locations and with the exception of 2013, at each of these, separate samples were taken from littoral macrophyte and littoral sediment (benthic) habitats (thus 10 samples per waterbody). In 1995 and 1996, benthic and macrophyte samples were combined before processing (O'Connor et al 1995, O'Connor et al 1997), while for 2006 and 2011 samples from each of these two habitats were collected and processed separately. For 2013, the five replicate samples were collected from littoral macrophyte only, as littoral sediment samples from previous years (eg 2011, section 5.2.2) demonstrated very low (natural) diversity in this habitat across all waterbodies, exposed and reference.

3.3.1.3 Sampling in 2009

In 2009, ERA commissioned a number of 'baseline' studies in and around the Ranger minesite in preparation for an EIS seeking approval to build a heap leach facility in the catchment of Georgetown Billabong. A component of those studies included characterisation of aquatic macroinvertebrate communities in Ranger mine-water exposed (including Gulungul Billabong) and adjacent reference billabongs (WRM 2010). The protocol used in that study was the same as used in the 2011 billabong study described above with the following exceptions: (i) macrophyte samples only (and not separate benthic samples) were collected; and (ii) sampling in reference billabongs was unreplicated

(unlike five replicates per reference billabong collected in SSB's 1995 to 2013 sampling). (For Gulungul Billabong, exposed to Ranger mine waters but included amongst reference waterbodies for data analysis, five replicate samples were collected.) With permission of ERA, data arising from April to May 2009 sampling were included in the current analysis and reporting.

3.3.2 Sampling methods for different habitats

For 1979, samples were collected using a standard 500 μm mesh pond net (Marchant 1982b). In each billabong, six 10-second sweeps were made in the littoral zone (< 1 m deep). Marchant (1982b) described the method for collection of each monthly replicate sample as "a vigorous sweep across macrophytes and bottom debris".

Sampling of macroinvertebrates from macrophyte and sediment habitats in subsequent years was undertaken as follows:

3.3.2.1 Macrophyte sampling

Five replicate samples were collected from the littoral zone of each waterbody, with samples taken at regular intervals around the circumference of the waterbody. Sampling of sediment and macrophyte habitat at each location was conducted along a 4 m wide transect perpendicular to the shoreline. For 1995 and 1996 sampling, O'Connor et al (1997) defined the littoral zone as the area at the edge of the billabong of up to 0.7 m water depth. For 2006 onwards, the depth of sampling was extended to <1.5 m to take into account limitations of access, particularly manoeuvring the sampling boat into thick stands of *Eleocharis*.

Samples were collected using a standard 250 μm mesh pond net. At each site, 10 (1995 and 1996) or 5 (subsequent years) broad sweeps over approximately 2 m surface distance were made through submerged macrophyte beds over the depth range of the transect area. The aim of this procedure was to include the broadest range of different aquatic plant types in each sample.

For 2009 sampling conducted by (WRM 2010), macroinvertebrates were collected from macrophyte habitat (only), also using a 250 μm mesh FBA pond net and using the sample method as described above for 1995 to 2013. Replication of sampling within waterbodies is described in section 3.3.1.3, above.

3.3.2.2 Sediment sampling

For 1995, 1996 and 2006, sediment samples were collected using a standard 250 μm mesh pond net, as described in O'Connor et al (1995). Two 1 m sweeps were made through the top 2 cm of sediment, one near the water's edge (depth 0.1 m) and the other in deeper water (0.3-0.4 m). Where the sediment was compacted, it was disturbed and broken up by hand before sampling. Sampling sites were located immediately adjacent to those where macrophyte sampling occurred (described above).

In 2011, a modified Boulton's sampler (Boulton 1984), 27.5 cm in diameter, was used to collect quantitative samples from shallow shoreline areas. Modification involved replacement of the hand pump for sampler evacuation with repetitive bailing of the cylinder using a 2-L plastic beaker. Sampling sites (five per waterbody) were located immediately adjacent to those where macrophyte sampling occurred (described above). At each site, the top 10 cm of sediment within the sampler was collected; this was repeated twice in immediately adjacent areas and the three samples pooled to represent a single replicate sample. Thus the total area of sediment sampled at each of the five locations was 0.18 m^2.

3.3.3 Sample processing and macroinvertebrate identification

Samples from 1979, 2009, 2011 and 2013 were preserved in ethanol for later sample sorting and identification, while samples from 1995, 1996 and 2006 were sorted, live, either in the laboratory or field. The earlier live-sorting method was popularised in national protocols during the mid 1990s (Davies 1994) and was used in the current study because of the reduced time in sorting macroinvertebrates from samples (compared with laboratory processing). Concerns about biases in compositional data arising from this method (Humphrey et al 2000) led to the switch in protocol from 2009 to (traditional) laboratory sorting of subsamples under a dissecting microscope. The implications to this study of the change in protocol are discussed elsewhere (see sections 4.1.5, 5.2.1 and Appendix 7 (A7.1)).

3.3.3.1 Processing and identification in 1979

Samples were taken back to the laboratory and washed with water to remove large debris before being preserved in 90% ethanol. Samples were later sorted, identified and counted (to at least family level) under a dissecting microscope according to the methods of (1982a Marchant (1982b).

3.3.3.2 Processing and identification in 1995 and 1996

Procedures for processing and identification are described in O'Connor et al (1995) and are summarised as follows:

In the field, the fine fraction (<250 μm) of both the sediment and macrophyte samples was washed vigorously through the mesh of the pond net before the sample was emptied into a large plastic bag. Sediment and macrophyte samples were combined in the bag and water added to cover the sample so that invertebrates could be kept alive prior to immediate sorting of specimens, live, in the laboratory.

In the laboratory, samples were washed through nested 8 mm and 500 μm sieves. Material retained in the 8 mm sieve was coarsely sorted for invertebrates then discarded. Material retained in the 500 μm sieve was live-sorted for invertebrates within a period of 6 hours after field collection. Protocols for live-sorting were similar to those prescribed in the Australian Monitoring River Health Initiative River Bioassessment Manual (Davies 1994) except that live-sorting of each sample was carried out for 1 hour instead of 30 minutes. Live-sorting was carried out under constant light conditions in the laboratory using fluorescent desk or 'Magi' lamps. Invertebrates were preserved immediately after sorting using 70% ethanol. The sorted samples were later identified to family-level under a dissecting microscope. The average number of animals sorted for each live-sorted sample was ~200 animals.

3.3.3.3 Processing and identification in 2006

Samples were washed through nested 8 mm and 500 μm sieves in the field. The material retained in the 8 mm sieve was checked for invertebrates then discarded. The material retained in the 500 μm sieve was live-sorted in the field for invertebrates. This was done with two technical staff examining the same sample in the field in a large sorting tray for a time period of 30 minutes in total. The invertebrates were preserved immediately after sorting using 100% ethanol. The sorted samples were later identified to family-level, where possible, in the laboratory. While the average number of animals sorted for each live-sorted sample was ~350, high abundances (>200) were reached through a practice of pipetting, en masse, numerous and swarming Acarina, ostracods, copepods, oribatids and nematodes, or similarly scooping with a small spoon and en masse, heavier bithyniid and planorbid snails, that gathered at edges and corners of the sorting tray. Thus large sample sizes did not result in retrieval of additional taxa to the previous (1995 and 1996) practice of live sorting ~200 animals.

3.3.3.4 Processing and identification in 2009

Samples collected by WRM (2010) were elutriated and washed through 1 mm and 250 μm sieves to remove coarse and very fine organic and inorganic matter. The residue was then preserved in 70% ethanol for laboratory processing. In the laboratory, macroinvertebrates were removed from samples by sorting under a low power dissecting microscope. Consistent with the protocols used in 2011 and 2013, specimens from all replicate samples were identified to family level. Data reported by WRM (2010) were in to log10 scale abundance classes (ie 1 = 1 individual, 2 = 2-10 individuals, 3 = 11-100 individuals, 4 = 101-1000 individuals, 5 = >1000). Abundance classes 3 and 4 were estimated from subsamples and in practice, a target of 200 animals was aimed for in each processed sample.

3.3.3.5 Processing and identification in 2011 and 2013

The samples of both sediment and macrophyte were washed vigorously through nested sieves (8 mm and 250 μm) in the field to remove much of the debris collected, then placed in 1 L sampling pots with 100% ethanol for preservation for later sorting of specimens in the laboratory.. In the laboratory, preserved samples were sub-sampled using a multi-cell subsampler and sorted under a stereo microscope, until a target of 200 animals (or time period of 4 hours) was reached. The sub-sample percentage was recorded so that animal numbers could later be standardised to full sample size. Sorted samples were identified mainly to family-level.

3.4 Data analysis

All results, including macroinvertebrate taxonomic lists and abundance data, rainfall, locational (GPS), catchment, morphometry, habitat, and water and sediment physico-chemistry data, were entered into spreadsheets (Excel, Microsoft). Raw data (as mean values per billabong) are provided in Appendices 1 (environmental data) and 5 (macroinvertebrate data).

Amongst the seven years sampled (between 1979 and 2013), data for additional water quality variables for reference waterbodies were available for three of the latter sampling years, 2006, 2011 and 2013. Therefore, a number of the analyses described below include two general types of analyses, all or most years (1979-2013) but with fewer environmental variables available, and just three years (2006, 2011 and 2013), but with additional environmental variables available.

3.4.1 Analyses of environmental data

Spatial and temporal patterns amongst environmental variables were assessed using multivariate analysis, ie, correlation-based Principal Components Analysis (PCA) using Euclidean distance (Clarke & Warwick 2001) and using the PRIMER (v7) software package (Clarke & Gorley 2015). This ordination technique was used for exploratory assessment. PCA reduces the dimensionality of complex datasets (samples, or sites), to a small number of dimensions to reflect the similarity of their environmental attributes. PCA transforms a number of (possibly) correlated variables into a (smaller) number of primary variables called principal components. The first principal component (PC1) accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible.

The PCA used habitat and water chemistry variables (from Table 2). Variables with missing values, and where the majority of the sites had data that were at or below detection levels (ie variables with no measurable influence on the PCA), were removed prior to analysis. A draftsman plot (scatter plot matrix) was used to determine whether transformation of variables (in this case log(x)) was required for correlation and any subsequent analyses. A

transformation was applied if data were strongly skewed, as the outliers will dominate the PC axes leading to poor quality interpretation of the results. Transformations were not applied to pH nor proportional or percentage data. Prior to running the PCA, all data were normalised to account for different measures and measurement scales (Clarke & Gorley 2006).

Three PCAs were conducted:

1. Environmental data common to each waterbody from 1995, 1996, 2006, 2011 and 2013. Variables included:
    a. Habitat variables: latitude and longitude, rainfall, year of sampling, billabong area (m^2), catchment (Magela or Nourlangie), catchment area (m^2), depth rank, total percent macrophyte cover, macrophyte taxa diversity, relative percent cover of vegetation types – floating attached (FA), emergent broad leaved (EBL), emergent narrow leaved (ENL), submerged and emergent feathery (SEF), submerged not feathery (SNF) and free floating (FF).
    b. Water chemistry variables: EC, pH, Mg and U.
2. Environmental data common to each waterbody from 2006, 2011 and 2013. Variables included:
    a. Habitat variables: latitude and longitude, rainfall, billabong area (m^2), catchment (Magela or Nourlangie), catchment area (m^2), depth rank, total percent macrophyte cover, macrophyte taxa diversity, relative percent cover of vegetation types – floating attached (FA), emergent broad leaved (EBL), emergent narrow leaved (ENL), submerged and emergent feathery (SEF), submerged not feathery (SNF) and free floating (FF).
    b. Water quality variables: EC, pH, turbidity, water temperature, DO ($mg\ L^{-1}$), Al, Cu, Fe, Mn, U, Zn, Ca, Mg, SO_4, NO_3 and NH_3.
3. Using only water quality data common to each waterbody from 2006, 2011 and 2013.

Apart from draftsman and other bi-plots for visual assessment, relationships amongst pairs of environmental variables over time were also analysed and assessed using correlation and regression analyses. Correlation analysis used non-parametric Spearman Rank-order Correlation due to non-normality of the data and in order to assess any monotonic relationships (whether linear or not). (As such, Spearman correlation is relatively insensitive to strong outliers that are in the tails of both samples because Spearman's rho limits the outlier to the value of its rank.). The correlations were not corrected for Type 1 error because their main purpose was visual assessment and comparative assessment of the resulting rho values, not statistical significance per se.

A number of analyses examined relationships between Mg and biological response data, i.e. TITAN, SSDs, and correlations and regressions with taxa abundance, taxa number and aquatic vegetation percent cover and richness. Measurement of Mg did not accompany or coincide with the sampling conducted at all sites and occasions and so, noting the very high correlations between EC (measured on almost all sampling occasions) and Mg, the latter was predicted from derived EC-Mg regression relationships. All available EC and associated Mg data from the waterbodies, representative of the time of macroinvertebrate sampling, were used to derive relationships for the following applications: For EC >50 $\mu S\ cm^{-1}$, Mg was predicted from the regression relationship that used data from all sites and sampling occasions ($R^2 = 0.98$). For all reference waterbodies and Georgetown Billabong for 1995 and 1996, Mg was predicted from the regression relationship using combined data from all reference waterbodies ($R^2 = 0.73$).

3.4.2 Macroinvertebrate community summaries

Community summaries used in this study are listed in section 2.1.9. They are of two types, those not reliant in their calculation on comparison of different exposure types, i.e. number of taxa (mostly families) and total number of organisms (ie total abundance), and those entailing comparison of different exposure types (typically exposed sites to reference sites), i.e. ANOSIM R and Bray-Curtis or Jaccard similarity.

3.4.2.1 Number of taxa and total abundance

Number of taxa and total number of organisms (ie total abundance) were plotted according to waterbody for all years of sampling except 1979 (where only three billabongs were sampled). Where different life forms of the same taxon were present (eg larvae and adults), these were entered as separate 'taxa' for data analysis, as there can be different ecological attributes (including contaminant exposure pathways) of the differing life history stages in many taxa.

Taxa number associated with sampling of macrophyte habitat was derived from the total number of organisms sorted in the sample. For sampling years between 1995 and 2013, a target of ~200 animals was specified or otherwise achieved in applying protocols (section 3.3.1). This fixed target eliminated biases and artefacts in comparing taxa number amongst sites and years, given taxa number is positively correlated with sample abundance (see Gotelli & Colwell 2001).

Total abundance was calculated differently for live-sorted samples from 1995, 1996 and 2006 versus laboratory subsampled and sorted samples from 2009, 2011 and 2013. For laboratory processed samples, abundances of the whole sample were extrapolated from the subsampled abundance, while for live-sorted samples no multiplicative factor was applied. This meant it was not possible to validly compare total abundance between these two broad year groups. (However, this artefact did not affect *relative* abundances as described and defined elsewhere.)

Macroinvertebrate taxa number and total abundance biases were inherent in comparisons of macroinvertebrate communities collected from macrophyte and sediment habitat (sections 3.3.2 and 5.2.2). Cumulative taxa number versus abundance for the replicates from each habitat were plotted to interpret the results from these comparisons.

3.4.2.2 Similarity-based summaries

ANOSIM R and Bray-Curtis similarity were calculated between the community structure data of exposed and reference sites.

Similarity of taxa compositional data was also calculated between all possible pairs of derived classes of site contamination (viz EC gradient), and plotted with EC, using the method of Kefford et al (2010). For this the following steps were undertaken:

- The EC gradient observed amongst all waterbodies and all years except 1979 and 2009 was divided into 7 classes – <20, 20–29.9, 30–39.9, 40–49.9, 50–99.9, 100–299.9 and >300 $\mu S\ cm^{-1}$.
- Within the EC classes, biological samples and corresponding EC observations were randomly allocated into replicate lots of 17 observations, this value corresponding to the smallest number of observations available in an EC class. Thus for EC classes containing >17 observations, replicate lots of 17 observations were created, discarding any residual data for which n <17 (insufficient to create a new replicate lot).

- Using compositional data, Bray-Curtis and Jaccard similarity was calculated (i) *between* all EC classes using pairwise replicate samples within each replicate lot, and (ii) *within* the replicate lots of each of the (seven) EC classes (ie all pairwise combinations of 17 observations).
- Similarity was averaged amongst replicate lots within an EC class. For approach (ii), averaging amongst replicate lots was undertaken after averaging the similarities derived within each replicate lot.
- Similarity for adjacent EC classes was plotted separately for each EC class across the available pairwise comparisons; progressively fewer pairwise comparisons were available to plot with increasing EC class.

3.4.2.3 Correlation analysis of selected community summaries

Correlation of number of taxa, B-C similarity and ANOSIM R of exposed sites relative to reference sites with environmental data was conducted using Spearman Rank-order Correlation (see rationale in section 3.4.1). Regression analysis was performed on selected significant correlations to further examine the nature of the relationships. Regressions were performed on non-ranked data.

3.4.3 Correlations involving individual macroinvertebrate taxa

Relationships between relative abundance (percent of total sample abundance) of individual macroinvertebrate taxa and each of aquatic plant percent cover, aquatic plant species number and magnesium concentration were derived using Spearman Rank-order Correlation or regression, thus:

- For macroinvertebrate taxon relative abundance versus aquatic plant percent cover and magnesium concentration, (Spearman Rank-order) correlations were derived for both Type I error corrected and uncorrected data. Corrected correlations used the Bonferroni correction applied to 45 relationships. Plotted (significant) relationships found to be not statsitsically significant after Bonferroni correction were still retained for visual assessment.
- Plots of macroinvertebrate taxon relative abundance and aquatic plant taxa number indicated non-monotonic (unimodal) relationships for which non-parametric correlation was not applicable. Instead, an in-house polynomial quantile regression method was applied to determine statistical significance. Thus and in lieu of a readily available statistical analysis package to calculate 2nd order polynomial quantile regression, a pseudo-calculation was performed. Each stage of the analysis was performed manually in excel. The 0.9 quantiles for Y-values (macroinvertebrate taxon relative abundance) corresponding to an X-value (aquatic plant taxon number) distribution were calculated using the PERCENTILE function in excel. Multiple regression (2nd order polynomial) analysis was performed on the subsequent 0.9 quantile values.

3.4.4 Analysis of macroinvertebrate assemblage data using multivariate (similarity-based) analyses

Hereon, the terms macroinvertebrate 'community composition' and 'community structure' refer to taxa presence-absence and taxa total or relative (proportional/percentage) abundance lists, respectively. Unless otherwise indicated, community structure data were used in analysis of macroinvertebrate assemblage data.

Macroinvertebrate community structure data, comprising taxa relative abundance lists, for all waterbodies and years were analysed using multivariate procedures from the PRIMER (v7) software package and Clarke and Gorley (2006) manual. As performed for community

summaries, different life forms of the same taxon were separated for data analysis. Six levels of multivariate analysis were applied to the data:

1 Patterns amongst the assemblage data were visualised using the ordination method of Multidimensional Scaling (MDS) (Clarke & Gorley 2006), based on Bray-Curtis similarity matrices. Ordinations were depicted as two-dimensional plots based on the site by sites similarity matrices. For the combined-years ordination (1995, 1996, 2006, 2011 and 2013), within-waterbody replicate data were averaged to create mean taxa abundance data per waterbody.

2 For comparison of different *a priori* groupings, for example, exposed versus reference sites, Analysis of Similarity (ANOSIM) – effectively an analogue of the univariate ANOVA – was conducted to determine if these were significantly different from one another, and more importantly, the extent of group separation. The ANOSIM test statistic compares the observed differences between groups (e.g. exposed waterbodies (RP1, Coonjimba and Georgetown billabongs) versus reference billabongs) with the differences amongst replicates within the groups[4]. The test is based upon rank similarities between samples in the underlying Bray-Curtis similarity matrix. The degree of separation between groups is denoted by the R-statistic, where R-statistic > 0.75 = groups well separated, R-statistic > 0.5 = some group overlap but clearly different, and R-statistic < 0.25 = groups barely separable (Clarke & Gorley 2001). A significance level < 5% denotes significant effect/difference.

3 The PERMDISP routine tests the homogeneity of multivariate dispersions on the basis of any resemblance measure (Anderson et al 2008). In this study, PERMDISP was used to examine within-group (eg replicate samples within a minesite waterbody, or replicates from all reference waterbodies) dispersion in multivariate space. The metric is a measure of the "spread" around central tendency measurement (centroid or spatial mean). Group centroids for groups of samples were calculated using the 'distance from centroids' procedure (Anderson & Walsh 2013).

4 The SIMPER routine was used to examine which taxa were contributing to the difference of groups that were found to be different according to the ANOSIM procedure.

5 The relationship between the environmental and biotic data was assessed using the BIOENV routine in PRIMER (v6). This routine produces similarity matrices of the environmental variables, selecting those that "best explain" patterns in the biological community data. In particular the procedure takes combinations of the environmental variables, *k* at a time, and derives the best matches of biological and environmental similarity matrices for each *k*, as measured by (in this case) Spearman Rank-order Correlation. The suite of variables included in the BIOENV routine were the same as those used for the PCA routine, listed in section 3.4.1.

6 Canonical analysis of principal coordinates (CAP) is a routine in the PERMANOVA add-on for PRIMER. The purpose of CAP is to find axes through the multivariate cloud of points generated by the taxonomic data that either (i) are the best at discriminating among *a priori* groups (discriminant analysis) or (ii) have the strongest

[4] A PERMANOVA test of the BA x CI interaction provides a powerful test of the hypothesis "mean response before the event (or the period of interest) is consistent with mean response after event (or the period of interest) between reference and exposed waterbody type". While the test accommodates asymmetric designs – in this case unequal numbers of sites representing C and I terms, and times representing B and A terms – the very limited number of sites representing B (1979 only) and C (see Table 1) precluded meaningful application of this routine.

correlation with the environmental variables (canonical correlation) (Anderson et al 2008). For this study, high success in allocating sites to pre-defined exposure groups, together with high correlation between primary CAP and environmental gradients, provide additional support to the biological-water quality relationship being assessed. CAP was applied in two ways:

a Examining the community data similarity matrix versus the *a priori* exposure groups of EC > 100 μS cm^{-1} (predominantly mine-exposed waterbodies), Georgetown Billabong and EC < 100 μS cm^{-1} (*referenc*e waterbodies). CAP removes one sample at a time and applies the canonical model from all the other samples to the left-out sample in order to place it into the canonical space and allocate it to a particular community group, thereby, providing a correct classification rate of *a priori* samples; and

b Examining how well the multivariate data can predict positions of samples along a gradient (Anderson et al 2008)) by using the principal component scores from the PCA of all available environmental variables as a proxy variable for an overall contamination gradient (specifically for PC1 axis – the distribution of which was primarily driven by mine derived variables eg Mg).

While not the primary intent of CAP, its ability to identify taxa variables distinguishing the *a priori* groups provides additional information for other taxa-environment relationships that are sought in SIMPER, SSDs and TITAN. Taxa variables correlated with the CAP axes were determined using PRIMER vector overlays.

To account for the method-specific biases in estimates of taxa abundances (ie live-sorted samples from 1995, 1996 and 2006 versus laboratory subsampled and sorted samples from 2009, 2011 and 2013 – see section 3.4.2), wherever (and only when) data from different years were *combined for data analysis* (combined-years: ordination, BIOENV, SIMPER and CAP), a standardisation (ie relative abundance) was applied.

For *analyses conducted separately for each year* (ie Paired-site (exposed-reference waterbody) similarity, ordinations, ANOSIM and SIMPER), PRIMER's dispersion weighting was applied to abundance data, to downweight counts from clumped species. The resulting proportional abundance data were then log(x+1) transformed. The exception to this was (infrequent) addition of 1979 and 2009 data: 1979 data were log(x+1) transformed but received no prior dispersion-weighting; this was because the three samples representative of each site in 1979 are from 3 consecutive sampling months with no replication, therefore dispersion weighting cannot be applied. The 2009 data were derived from a processing method with inherent (built-in) data standardisation (sorted counts are performed on a log scale, see section 3.3.3.4) and received no dispersion-weighting nor log transformation.

Otherwise and unless indicated above, default values or procedures recommended in the PRIMER User Manual were employed for PRIMER routines.

3.4.5 Threshold determination across macroinvertebrate responses

The concept of hazardous concentration to N% of the biological response (HCN) was introduced in section 1.2.2. The biological response measures associated with 'hazardous' for this report include change in numbers of taxa or similarity, relative to reference waters. Thus the HC5 or HC1 are the concentrations at which (i) five or one percent of the taxa (respectively) exhibit an effect, or (ii) there has been a five or one percent change in similarity (respectively) in community composition or structure, relative to reference waters.

Four methods were used to explore or determine protective hazardous or threshold concentrations for magnesium:

3.4.5.1 Community concentration-response relationships

The similarity of macroinvertebrate community structure (viz Bray-Curtis similarity measure or ANOSIM R) and number of taxa in mine water-contaminated waterbodies relative to the same measures in reference waterbodies were plotted against corresponding antecedent contaminant concentrations. (The method for calculating concentrations of water chemistry variables antecedent to sampling is described in section 3.2.3.2.) Exposed-reference site comparisons were performed as follows:

- For calculation of (Bray-Curtis) similarity in each mine water-contaminated waterbody (RP1, Coonjimba, Georgetown) relative to reference waterbodies, each of the five replicates from a mine water-contaminated waterbody was randomly paired with a replicate from each of the reference billabongs for each year. That resulted in 5 (mine water-contaminated waterbody replicates) x *N* (total number of reference billabongs) similarity values. These values were averaged, with mean and/or associated error used in plots and analyses.
- For calculation of ANOSIM R groupwise comparisons between each mine water-contaminated waterbody (RP1, Coonjimba, Georgetown) relative to reference waterbodies, the ANOSIM routine was performed on the five replicates from a mine water-contaminated waterbody and all replicates from all reference billabongs for each year.
- For calculation of the proportion of taxa number in each mine water-contaminated waterbody (RP1, Coonjimba, Georgetown) relative to the taxa number in reference billabong, number of taxa for each of the five replicates from a mine water-contaminated waterbody was calculated as a percent of the mean of taxa number from the 5 (reference billabong replicates) x *N* (total number of reference billabongs) reference billabong values available. These five values were averaged, with mean and/or associated error used in plots and analyses.

For selected community summaries, non-linear 3-parameter sigmoidal regressions were conducted on pooled response data to derive contaminant concentration-response curves (using SigmaPlot v13). These concentration-response curves were used to determine HC5 and HC1 values. Regressions derived from these concentration-response data violate the assumption of statistical independence because the same data (typically reference site data viz "percent of control") are used for more than one observation used in regression analysis. While this would result in biased confidence intervals around the regression (associated with positive autocorrelation), predictions and derivations of hazardous concentrations themselves would not necessarily be affected (K McGuinness, Charles Darwin University, pers. comm.).

3.4.5.2 Relative family retention

A method of calculating and comparing similarity of taxa compositional data within and between all possible pairs of derived classes of site contamination (viz EC gradient), using large numbers of pooled samples per contaminant class, was described above (section 3.4.2.2). From these similarity data, relative family retention (RFR) rates were calculated using the method of Kefford et al (2010). Both Bray-Curtis and Jaccard similarities were derived but for illustrative purposes, just the Jaccard measure is described. From the 7 contaminant categories (least to most contaminated, section 3.4.2.2) then Jaccard $(j)_{x,y}$ with $x \neq y$ is the mean Jaccard Index between categories x and y, and $j_{x,x}$ and $j_{y,y}$ are the mean JI's within categories x and y, respectively. The RFR between contaminant categories x and y

is $j_{x,y}/j_{x,x}$ (Kefford et al 2010). So, a RFR of 0.85, for example, would indicate that across 17 samples, 85% of families are common to both EC categories but 15% are only found in one or the other EC category and thus there is a 10% turnover of families.

Small decreases in RFR – eg up to 5% – from those values derived between EC categories reflecting reference condition were assessed as potentially indicative of EC (Mg)-related change to community composition.

3.4.5.3 Field species sensitivity distributions (SSDs)

The relationship between a contaminant and the probability of observing each taxon – in this case the upper-most concentration at which it occurred in waterbodies – was modelled using a cumulative distribution function. A HC value was estimated from various (generally) protective percentiles (1 and 5%) of the distribution of taxa in the modelled relationship. (Elsewhere (e.g. (Cormier & Suter 2013a), the concentration that results in near-extirpation (ie XC) of each taxon (e.g. XC95), as determined from field concentration-abundance relationships for each taxon, and not absence, are applied to field SSDs. Hence the approach used in this study, while easier to derive, was less protective than one based upon near-extirpation.)

For the field SSDs derived in this study, data from 1995, 1996, 2006, 2011 and 2013 were used, with taxa with less than three occurrences removed from the analyses, as per Baker and King (2010). Taxa were also excluded if they were considered terrestrial or semi-aquatic (eg Limnichidae adults). Two SSDs were derived, one using all waterbodies and the other using data from just Georgetown Billabong. Model selection for the curve of best fit was determined on the basis of the Anderson-Darling statistic (Minitab 2010).

3.4.5.4 TITAN indicator value scores

In order to detect thresholds along environmental (including water chemistry) gradients, the Threshold Indicator Taxa ANalyis (TITAN) method was applied to the standardised macroinvertebrate community structure data, whereby abundance of each taxon was calculated as a proportion of the total abundance for that sample (Baker & King 2010). The method is based on the indicator values scores developed by Dufrêne and Legendre (1997) and integrates occurrence, abundance and directionality of taxa responses, to produce change points (thresholds) for individual species and the community as a whole. It does this for both species disappearing and species that are appearing along an environmental gradient.

The method uses bootstrap sampling to assess the uncertainty of the change point and individual species indicator values, estimating confidence limits around those values. From this, the quality of the response for each taxon is determined viz purity and reliability. Purity is the proportion of response directions (increasing or decreasing) when passing the change point that agree with the observed response. Pure indicators are consistently assigned the same response direction. Reliability is estimated by the proportion of bootstrap change points that consistently result in the significant grouping of a taxon. Only taxa with high reliability ($\geq$0.95) can be considered as indicator taxa.

TITAN is limited to using only one key environmental predictor and therefore does not account for other influencing environmental predictors.

TITAN assessed taxa responses from all sites, across Mg and EC gradients, i.e. Mg and EC values representative of the time of sampling (measured or predicted). Standardised macroinvertebrate data from 1995, 1996, 2006, 2011 and 2013 were used, with taxa with less than three occurrences removed from the analyses, as recommended by Baker and King (2010).

4 Procedures to minimise confounding and to isolate the key influences of macroinvertebrate responses

As discussed in section 2.1.3, several sources of variation and potential confounding associated with season, catchment, habitat, physical and chemical contaminants (other than $MgSO_4$), changes in habitat and water quality over time unrelated to mining and changes in protocol over time were recognised in this study. Approaches to quantify, account for and minimise variation and potential confounding where necessary and where possible, are described in the following sections. (Statistical routines referred to in these sections are explained in section 3.4.)

4.1 Influence of other key environmental variables and factors

4.1.1 Catchment/landscape-scale influences, including climate or unidentified landscape drivers

4.1.1.1 Catchment

There was potential for spatial confounding because all of the mine water-exposed waterbodies are found in Magela Creek catchment only, so that separation of mine water-exposed waterbodies from reference waterbodies in Nourlangie Creek catchment in analyses could simply be an artefact of different catchments. For this reason it is necessary to ensure there were sufficient reference waterbodies sampled in Magela Creek to compare with, and that changes in the macroinvertebrate communities of mine water-exposed waterbodies over time, attributed to change in water quality, are reflected in corresponding separation from waterbodies sampled in Magela Creek.

Artefacts and confounding could arise as follows:

1 Potential for minesite waterbodies in Magela Creek, prior to significant contamination, to be different in biological characteristics to reference waterbodies nearby in Magela Creek or in adjacent Nourlangie catchment; and
2 More Nourlangie Creek reference waterbodies have been incorporated into the sampling design over time (Table 1). If these reference sites are naturally different from reference waterbodies in Magela catchment, greater departure in macroinvertebrate responses over time relative to similar responses measured in reference waterbodies may be an inadvertent consequence of differences in the reference condition, unrelated to mining.

4.1.1.2 Climate influences

Differences in annual rainfall may lead to natural differences in macroinvertebrate communities amongst waterbodies. Such climate-related, biological variation could be reflected in (natural) differences in amongst-site multivariate measures of dispersion, in turn, potentially affecting similarity and other multivariate response measures (see Anderson & Walsh 2013). Such climate-related patterns in between-site similarity are evident, for example, amongst lotic stream sites in the (same) Alligator Rivers Region (Supervising Scientist Division 2013).

A scenario possible for lentic waterbodies in low rainfall wet seasons is more variable habitat due to patchiness in regional rainfall patterns, while dispersal opportunities for invertebrates may be more stochastically driven. In this scenario (low rainfall years) higher multivariate dispersion and lower within-group similarity of organism communities amongst waterbodies may be observed. Care is required in interpreting macroinvertebrate responses in minesite waterbodies over time relative to similar responses measured in reference waterbodies. Thus greater dispersion of site or group replicates in low rainfall

years could result in less discriminatory resolution in metrics of group difference between macroinvertebrate communities of minesite and reference waterbodies and potential for Type II error (a real impact is masked). Conversely, in other natural (climate-related) scenarios where greater homogeneity and reduced multivariate dispersion is observed – but where group centroids are similar as those occurring in the former scenario – greater discriminatory resolution between macroinvertebrate communities of minesite and reference waterbodies could result in potential for Type I error (inferring mine impact when there is none).

4.1.1.3 Analytical approaches to investigate catchment and climate influences

Analytical approaches used to investigate catchment and climate influences included use of ordination, ANOSIM and multivariate dispersion (PERMDISP). Absence of confounding would be evident if:

1 All waterbodies grouped together in multivariate space by year, rather than by catchment and exposure type. Moreover, any pattern amongst years in ordination space was found to be unrelated to other method artefacts, including change in sample processing method over time. Such demonstration would indicate that landscape/climate-level drivers are key influences of interannual variability, and that catchment and waterbody morphometry are less influential.
2 Magela and Nourlangie reference waterbodies in any sampling year grouped together, demonstrating similarity in biological characteristics. Separation of reference waterbodies between catchments should not be coincidental with concurrent putative impacts in minesite waterbodies.
3 Prior to notable chemical contamination in minesite waterbodies, these exposed waterbodies were similar to reference condition.
4 Indices of multivariate dispersion calculated for different waterbody types (reference and exposed) amongst years were found to be unrelated to sample processing method.
5 Multivariate dispersion and other multivariate response measures that are climate-related are unrelated to key contaminant-response (cause-effect) relationships.

4.1.2 Habitat influences

Outridge (1988) and earlier work undertaken in the present study (described elsewhere in this report) found much higher macroinvertebrate diversity (number of taxa and relative abundances) amongst macrophytes (aquatic plants) in the littoral zones of shallow lowland billabongs than in the sediments of the billabongs. Hence, macroinvertebrates amongst all sites and years were sampled predominately from littoral macrophytes though in most years, samples from sediments were combined with macrophyte samples. Aquatic plant composition, structure and cover would be expected to influence resident macroinvertebrate communities. To this end, community structure of macrophytes themselves was measured to determine whether this habitat measure accounted for any variation in macroinvertebrate community structure amongst sites and years. Artefacts and confounding when inferring water quality-related changes to macroinvertebrates could arise as follows:

1 Sediment fauna strongly influenced macroinvertebrate community structure of samples when combined with macrophyte sample components;
2 Onsite waterbody contaminants other than Mg in sediment were responsible for the observed biological responses; and
3 Changes in macrophyte community structure and cover occurred just in onsite and not reference waterbodies, coincident with a period of inferred effects attributed to changes in water quality. (If Mg adversely affected macrophytes, in turn indirectly

affecting macroinvertebrates (via changed habitat), such indirect effects still need to be identified.)

Analytical approaches used to resolve this included use of ordination, ANOSIM and BIOENV. Absence of artefacts and confounding would be evident if:

1 Naturally low macroinvertebrate diversity was found in sediment habitat compared with macrophyte habitat and the sediment faunal contribution to the combined sediment-macrophyte sample was negligible in multivariate space;
2 Contaminants in sediments of onsite waterbodies are below concentrations known to adversely affect aquatic ecosystems;
3 Macrophyte community structure was similar between reference and exposed waterbodies generally;
4 Community structure of the sediment fauna of minesite waterbodies was similar to the sediment fauna of reference waterbodies;
5 Macrophyte cover and associated community structure were less influential in accounting for macroinvertebrate community patterns in ordination space than water quality variables (BIOENV); and
6 Changes to macrophyte cover and community structure in onsite waterbodies over time, relative to the same responses measured in reference waterbodies, was unrelated to both corresponding exposed-reference waterbody macroinvertebrate responses and changes in water quality.

4.1.3 Water and sediment physico-chemistry

Increases in $MgSO_4$ in onsite waterbodies over time has been associated with changes to the concentrations of major ions other than Mg and SO_4 (including K and HCO_3) as well as other COPCs for Ranger (including U and Mn) and (low) pH. Attributing changes in macroinvertebrate responses to Mg, and deriving thresholds of change for this contaminant, could be confounded if other contaminants present in waters and sediment were (also) responsible and/or interacted with the effect of Mg in a synergistic or antagonistic manner[5].

Analytical approaches used to resolve this included use of ordination (PCA), biplots, correlation and regression, BIOENV and CAP. Absence of, and/or negligible, artefacts and confounding would be evident if:

1 Mine-derived contaminants other than Mg present in waters and sediment were less influential in PCA ordination space compared with $MgSO_4$;
2 The ionic strength of surface waters of minesite waterbodies was observed to be dominated by Mg and SO_4 ions;
3 Mine-derived contaminants other than Mg present in waters and sediment were below concentrations known to adversely affect aquatic ecosystems;
4 Mine-derived contaminants other than Mg present in waters and sediment were less influential in accounting for macroinvertebrate community patterns in ordination space than Mg (BIOENV, CAP); and
5 Changes to macroinvertebrate community summaries and structure in onsite waterbodies over time, relative to the same responses measured in reference

[5] Synergism refers to toxic effects which may exceed the total additive effects of the separate constituents, while antagonism refers to an effect which is less than the sum of the separate constituents

waterbodies, was unrelated to, or poorly correlated with, mine-derived contaminants other than Mg present in waters and sediment.

4.1.4 Changes in habitat and water quality over time unrelated to mining

Catchment fire regimes and the foraging activities of feral animals (pigs) in the littoral portions of billabongs were considered earlier in this report (section 2.1.5) as factors that influence habitat and water quality directly, and thereby may indirectly affect littoral macroinvertebrate communities. The rooting, trampling and wallowing activity of pigs in littoral zones destroys aquatic plant habitat and alters water quality, in particular, raising turbidity levels. Intense fires in wet-dry topical savannas of northern Australia have been linked to increased richness and abundances of stream macroinvertebrate communities (Andersen et al 2005); reduced riparian shading and release of soil nutrients in burnt catchments are thought to enhance primary and secondary production in aquatic ecosystems.

A detailed assessment of the potential of fire regimes and pig damage to affect minesite waterbodies and associated catchments, and not reference billabongs, in any year of sampling, was beyond the scope of this study. Instead, a qualitative (fire) and semi-quantitative (pigs) assessment of just intense 'activity' of each of these agents adjacent to minesite waterbodies and in their catchments, coinciding with putative water quality-related impact (especially Georgetown Billabong, 2011 sampling, section 5.4.1) was undertaken. This entailed: examination of satellite-derived fire history for the Ranger project area for years associated with macroinvertebrate sampling (section 5.2.4.1); and assessment of aquatic plant cover and water quality amongst replicate sites in Georgetown Billabong during 2011 sampling (section 5.2.4.2).

4.1.5 Differences in sample processing

For the waterbodies sampled in the present study, different sample processing methods were applied over time. In 1995, 1996 and 2006, live macroinvertebrates were extracted from samples by eye in the field – so-termed live-sorting. In 2009, 2011 and 2013, the processing method differed; samples were preserved in the field and later subsampled and sorted in the laboratory under a microscope, i.e. laboratory-processed samples. Community structure data arising from these two approaches differ from one another (Humphrey et al 2000). As a consequence, there is a need to ensure that any change in macroinvertebrate community structure attributed to mining over the full time series is not mistakenly attributed to this change in methodology.

Potential artefacts of this type were assessed by comparing analyses of community structure datasets derived (i) from samples first live-sorted in field, then (ii) from the same sample residues preserved and later subsampled and sorted in the laboratory. A number of samples were available for which these two different datasets were available for the same sample. If relative differences in macroinvertebrate community summaries and structure between two arbitrarily-selected groups of samples were the same for analyses based on each of the two (live-sorted and laboratory processed) datasets, it would indicate the difference in sample processing method was not confounding the exposed-reference waterbody comparisons made in the current study. The analyses reported in Appendix A7.1 were used to assess the implications of method changes to this results of the present study.

Apart from different sample processing methods, mesh size for retaining macroinvertebrates also differed over time with 500 μm used in 1995, 1996 and 2006, and 250 μm used in 2009, 2011 and 2013 (Table 3). An earlier study conducted in the ARR

(Humphrey et al 1997) investigated the differences in results for community structure analysis arising from different mesh sizes. In that study, nested sieves were employed during sample processing (500 and 250 μm) with the additional macroinvertebrate component retained on the 250 μm sieve collected and the data arising from 500 μm and 250 μm components compared. The results of that comparison are provided in section 5.2.1.

4.2 Other supporting evidence and final weight of evidence evaluation

There is accruing knowledge in Australia and elsewhere of the tolerances and sensitivities of specific macroinvertebrate taxa to water quality, including salts (eg Horrigan et al 2005, Dunlop et al 2008, Schäfer et al 2011). The responses observed in the current study were compared with salinity databases as diagnostic, supporting information used to interpret results.

Consideration and elimination of alternative explanations for the observed macroinvertebrate responses, together with an assessment of consistency in response of specific macroinvertebrate taxa to salt gradients, form the basis of the ensuing Results. The various aspects raised in this section are summarised in a weight of evidence evaluation in section 6.

5 Results and Discussion

Hereafter, abbreviations for the key minesite waterbodies are used in the text, i.e. GTB Georgetown Billabong), DJKB (Djalkmara Billabong), CJBB (Coonjimba Billabong), RP1 (Retention Pond 1) and RP2 (Retention Pond 2).

5.1 Analyses of environmental data

All water and sediment quality data are summarised in Appendix 1, Tables A1.3–A1.9, while habitat variables are summarised in Appendix 1, Table A1.2. Correlations amongst all environmental variables are tabulated in Appendix 3.

As noted in section 3.1, the hydrology and water quality of CJBB are directly affected by: 1) backflow from Magela Creek during high wet season flow events in the creek; and 2) the artificial waterbody, RP1, located in the Coonjimba creek-line upstream of the billabong and from which waters may passively or actively be released over the spillway during the wet season.

5.1.1 Water quality in surface waterbodies

5.1.1.1 Water quality conditions pre-mining or in reference billabongs

At the end of the wet season (April-May), all natural shallow billabongs sampled in this study had relatively uniform water quality (summarised below). In 1979 and prior to any minewater contamination (i.e. pre-mining), GTB, CJBB and DJKB also maintained a similar water quality to the adjacent (current) reference billabongs during this period (Walker & Tyler 1984). Humphrey and Simpson (1985) reviewed the various sources of water quality information for Magela catchment billabongs available at the time, and the following summary from that report is provided here. Typically, surface water characteristics for end of wet season conditions include:

- Low turbidity (typically < 20 NTU);
- High water temperatures (up to 30°C) and under-saturation by dissolved oxygen in the early morning (below 50% saturation (3.5 to 4 mg L^{-1}));
- A generally uniform water chemistry, i.e. very dilute (EC typically below 25 $\mu S\ cm^{-1}$), near neutral pH (typically between 6 and 7) and dominated by sodium bicarbonate (cationic dominance Na > Mg > Ca > K and anionic dominance HCO_3 > Cl > SO_4); and
- Phytoplankton productivity and nutrient levels are low, while macrophyte production has peaked after wet season growth.

Because some aquatic macroinvertebrate taxa persist into the dry season, some description of dry season water quality is also necessary as context, even though sampling was not conducted in this season. This dry season water quality description uses the reports of Walker and Tyler (1984) and Humphrey and Simpson (1985). Considerable divergence in water quality occurs over the dry season (in the previous calendar year, antecedent to sampling) and by the late dry season, surface water quality is generally poor as a consequence of evapo-concentration and groundwater input of solutes, increase in ambient temperatures, and shallowing and water volume loss. The late dry season characteristics for these same billabongs may include:

- With shallowing of billabongs, wind-induced re-suspension of sediments and subsequent senescence of macrophytes, turbidity levels may be moderate (30-70 NTU)

to high (>100 NTU), depending upon overall water depth and orientation of billabong to prevailing south-east trade winds;

- High water temperatures (up to 40ºC) and ongoing under-saturation by dissolved oxygen in the early morning (below 50% saturation (3.5 to 4 mg L^{-1}));
- Variable water chemistry, with most billabongs becoming sodium chloride dominated (sulfate dominated in Corndorl Billabong), high in EC (30 to >200 μS cm^{-1}), and acidic (pH declining over the dry season to values as low <5 in the early morning);
- High nutrient levels, variable phytoplankton productivity (1-150 μg L^{-1} chlorophyll a, depending upon turbidity, which inhibits phytoplankton at sustained turbidity >50 NTU) and low macrophyte production or senescence in the shallower billabongs (i.e. most of those billabongs listed in Table 1).

The water quality characteristics of Magela Creek billabongs described above, also apply to Nourlangie Creek billabongs (Walker & Tyler 1984), though the ionic composition of late dry season surface waters of Nourlangie billabongs is not known.

5.1.1.2 Major ions and contaminants of potential concern in minesite waterbodies (GTB, DJKB, CJBB and/or RP1)

Marked increases since the early 2000s in Mg, SO_4 and Ca are evident in three minesite waterbodies with evidence for some increases over the same time period in Na and HCO_3 as well (Figure 7). The decline in Mg, SO_4 and Ca in RP1 and CJBB after 2009 coincided with improved water management around the western stockpile (Figure 6) (ie re-direction of runoff) and introduction of brine concentrator water treatment at Ranger and the associated discharge of the clean water distillate to RP1.

Strong correlations (rho > 0.85) are observed amongst Mg, SO_4 and Ca and also between Na and Cl (Figure 8 and Table 4). Based upon correlations between the various ions and EC, the ionic composition of surface waters of the minesite waterbodies is dominated by Mg and SO_4 ions (rho > 0.98), as well as Ca (rho = 0.92) (Figure 8 and Table 4 and 5).

Contaminants of potential concern (CoPCs) for Ranger receiving (surface) waters were listed in section 1.3, i.e. magnesium and sulfate (with EC a surrogate measure), uranium, and manganese. Three mine-derived CoPCs have approached concentrations in the minesite waterbodies that may exceed locally-derived, biological-effects guideline values, i.e. Mg (GV of 3 mg L^{-1}), U (GV of 2.8 μg L^{-1}) and Mn (76 μg L^{-1}).

For Mg, consistent GV exceedances appeared progressively later in the time sequence (shown in Figure 9–11) for RP1, CJBB and GTB, i.e. the entire time series (since 1991, RP1), 1993 (CJBB) and 2001 (GTB). For U, GV exceedances were rarely observed in GTB and only occasionally in CJBB where median values were only exceeded in the 2002 to 2003 period (Figures 9 and 10). The GV for U was exceeded consistently in RP1 in the period 1999 to 2010 (Figure 11). For DJKB, the GVs for Mg, Mn and U were exceeded consistently prior to sampling in 1995 and 1996 (Appendix 1, Table A1.8). Mg and U values in the minesite waterbodies, in the context of GV exceedances prior to macroinvertebrate sampling, are discussed below (section 5.1.4). The general improvement in water quality in the three waterbodies after 2009 (Figure 9–11) was explained above.

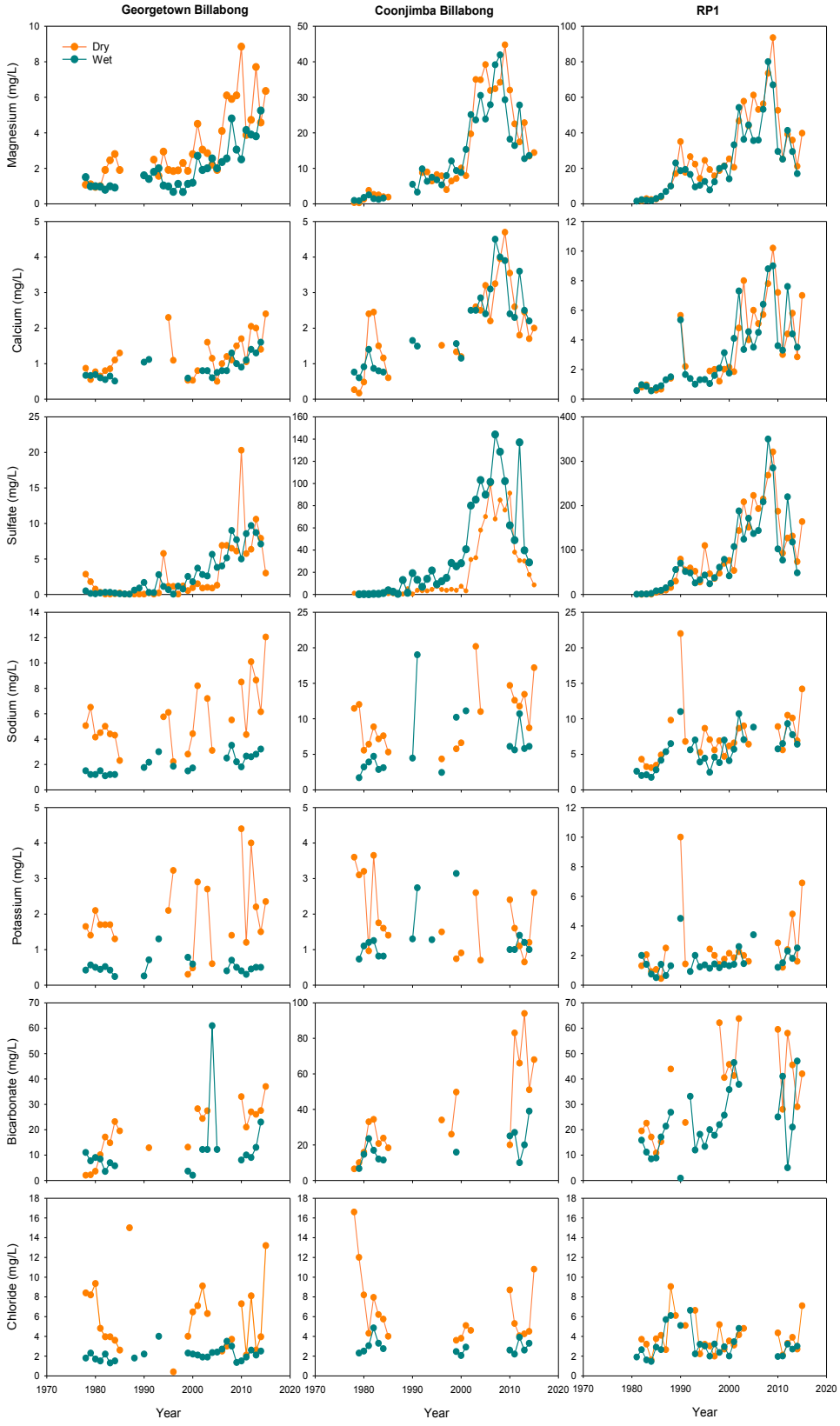


Figure 7 Median concentrations of generally weekly major cations (Ca, Mg, Na, K) and anions (SO_4, HCO_3, Cl) measurements for GTB, CJBB and RP1 between 1979 and 2016. Data from Energy Resources of Australia, NT Dept of Mines & Energy, NT Dept of Transport & Works and the Supervising Scientist Branch. Wet season – January to May. Dry season – June to December

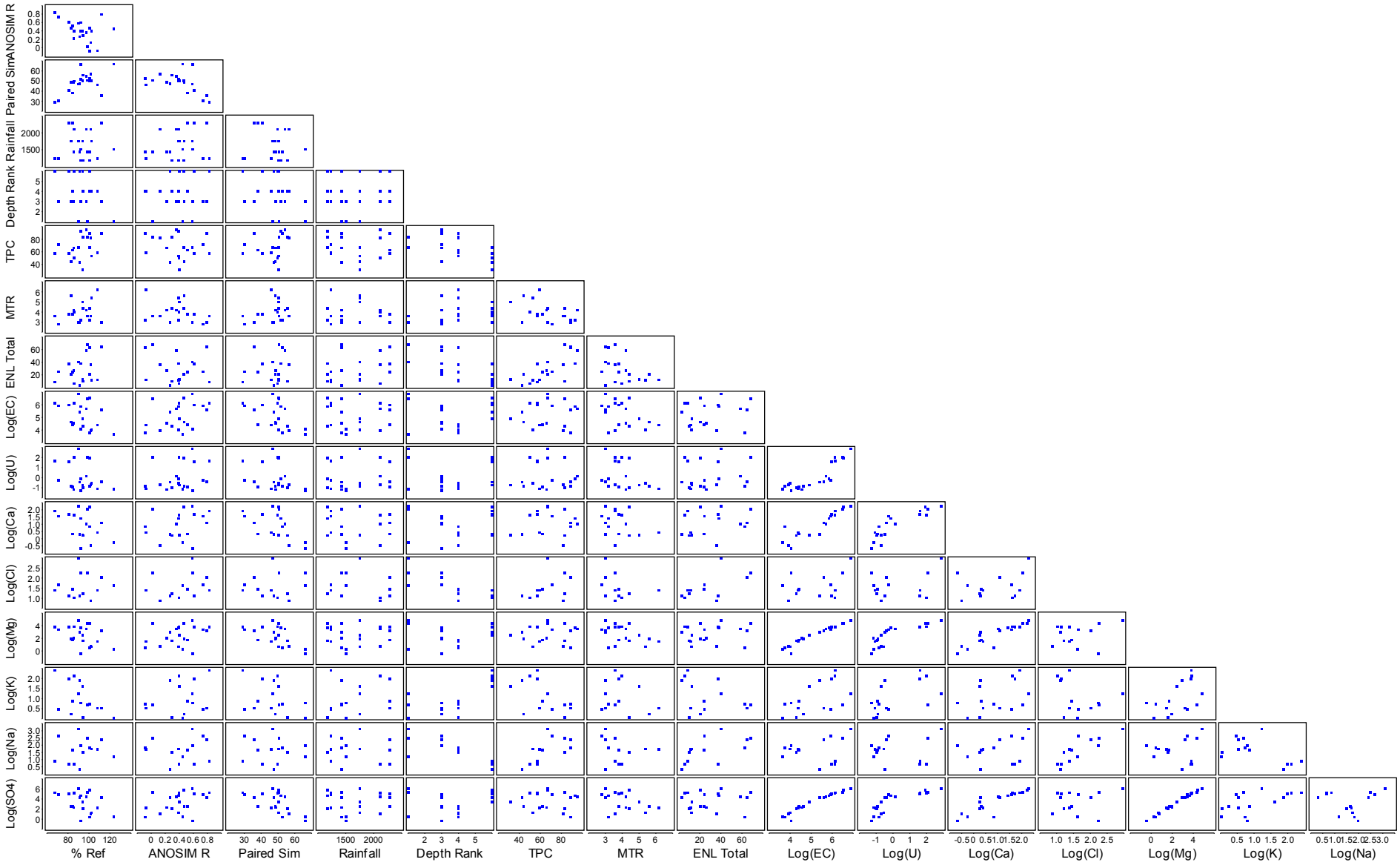


Figure 8 Draftsman plots for macroinvertebrate community summaries and selected environmental and antecedent water chemistry (logged) variables for GTB. DJKB CJBB and RP1, 1979-2013

Table 4 Spearman Rank correlation results for selected macroinvertebrate community summaries and selected environmental and antecedent water chemistry variables for GTB, DJKB, CJBB and RP1, 1979-2013. For a given sampling year, %REF taxa is the mean number of macroinvertebrate taxa observed in each of GTB, DJKB, CJBB and RP1 as a percent of mean number of taxa in reference billabongs, while ANOSIM R is the groupwise comparison between the five replicates from each minesite waterbody and reference billabongs. Macrophyte abbreviations are TPC = Total percent cover, MTR = Macrophyte taxa richness, ENL = emergent narrow leaves.

	% Ref	ANOSIM R	Paired Sim	Rainfall	Depth Rank	TPC	MTR	ENL Total	EC	U	Ca	Cl	Mg	K	Na
% REF taxa															
ANOSIM R	-0.441*														
Paired Sim	0.519*	-0.493*													
Rainfall	-0.0217	0.213	-0.107												
Depth Rank	-0.187	0	-0.243	0											
TPC	0.361	-0.174	0.386	-0.163	-0.57**										
MTR	0.192	-0.26	0.255	-0.0421	0.257	-0.379									
ENL Total	0.224	-0.0301	0.18	0.277	-0.687***	0.714***	-0.4								
EC	-0.432	0.389	-0.494*	-0.0668	0.078	-0.0181	-0.312	0.11							
U	-0.195	0.235	-0.317	0.0622	0.123	0.111	-0.419	0.215	0.862***						
Ca	-0.332	0.284	-0.567*	-0.115	0.02	0.00859	-0.233	0.27	0.918***	0.896***					
Cl	0.0945	0.327	-0.0901	-0.0512	-0.819***	0.488	-0.426	0.615*	0.275	0.138	0.305				
Mg	-0.375	0.304	-0.45*	-0.0542	0.095	0.0369	-0.316	0.138	0.982***	0.892***	0.923***	0.209			
K	-0.502*	0.178	-0.34	0.295	0.677**	-0.49	-0.115	-0.315	0.54*	0.537*	0.397	-0.338	0.503*		
Na	0.193	0.0876	-0.0286	-0.0471	-0.792***	0.68**	-0.395	0.751**	0.122	0.136	0.255	0.88***	0.0822	-0.434	
SO_4	-0.394	0.378	-0.52*	-0.0565	0.0892	-0.0158	-0.323	0.108	0.985***	0.89***	0.93***	0.235	0.989***	0.496*	0.0769

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Table 5 Spearman Rank correlations for selected macroinvertebrate community summaries and selected antecedent water chemistry (logged) variables for GTB, DJKB, CJBB and RP1, 1979-2013. Only sites and years with complete data are included. For a given sampling year, %REF taxa is the mean number of macroinvertebrate taxa observed in each of GTB, DJKB, CJBB and RP1 as a percent of mean number of taxa in reference billabongs, while ANOSIM R is the groupwise comparison between the five replicates from each minesite waterbody and reference billabongs.

	% Ref	ANOSIM R	Paired Sim	EC	U	Ca	Cl	Mg	K	Na
ANOSIM R	-0.489									
Paired Sim	0.593*	-0.588*								
EC	-0.467	0.17	-0.401							
U	-0.451	0.17	-0.368	0.989***						
Ca	-0.429	0.203	-0.484	0.956***	0.945***					
Cl	0.242	0.253	0.033	0.137	0.115	0.192				
Mg	-0.451	0.17	-0.368	0.989***	1***	0.945***	0.115			
K	-0.652*	0.223	-0.333	0.591*	0.545	0.498	-0.338	0.545		
Na	0.182	0.14	-0.11	0.187	0.154	0.25	0.88***	0.154	-0.449	
SO_4	-0.462	0.198	-0.44	0.989***	0.989***	0.973***	0.148	0.989***	0.564*	0.173

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

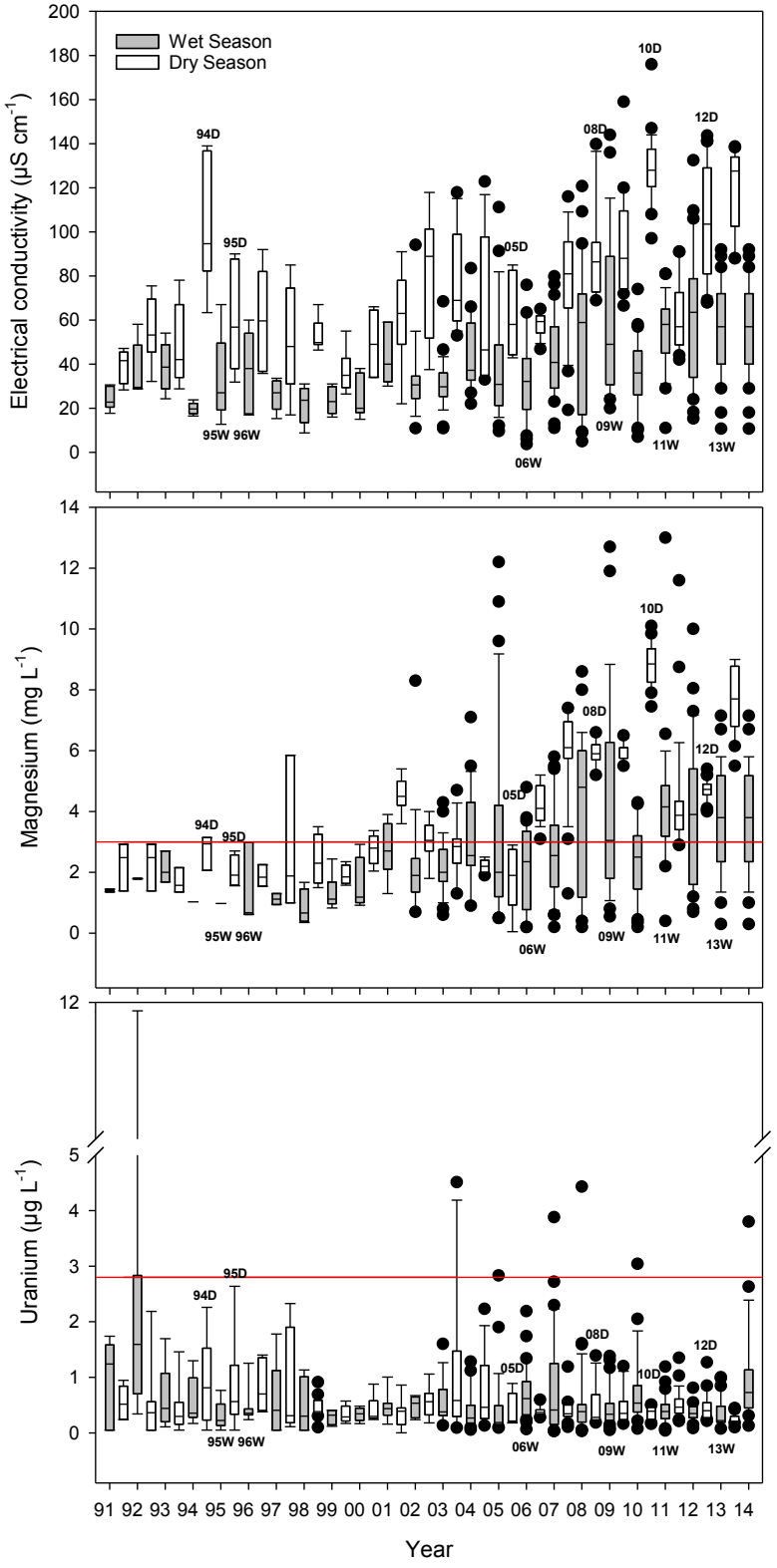


Figure 9 Summary box plots of generally weekly electrical conductivity (EC), magnesium and uranium values measured in Georgetown Billabong (GTB) between 1991 and 2014. Box plots show median, range, 25th and 75th percentiles and outliers (points). Periods relevant to macroinvertebrate sampling are indicated by year and season (wet (W) or dry (D)). Data from Energy Resources of Australia, NT Dept of Mines & Energy, and Supervising Scientist. Wet season – January to May. Dry season – June to December. Red lines indicate water quality GVs for Mg (3 mg L-1) and U (2.8 µg L-1).

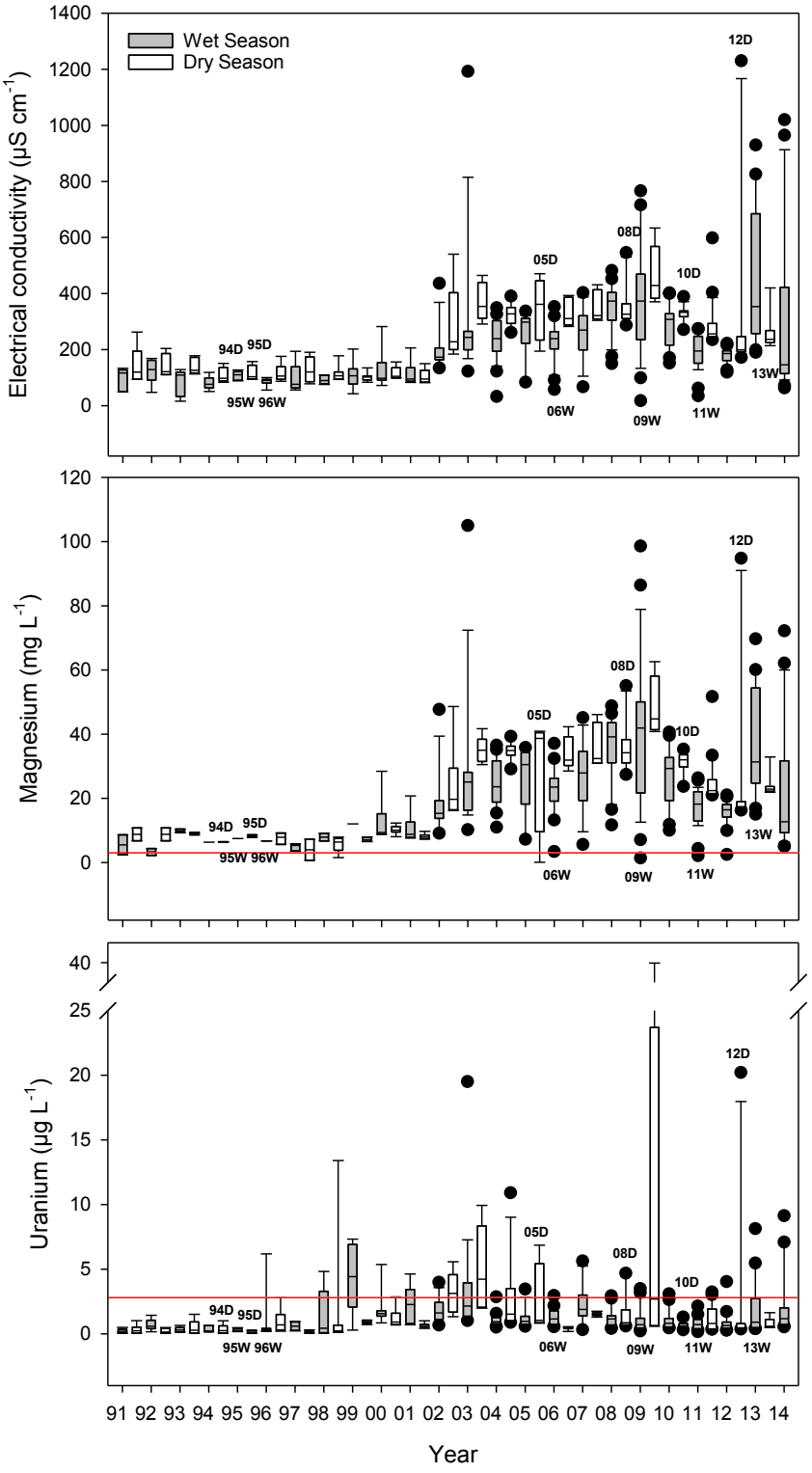


Figure 10 Summary box plots of generally weekly electrical conductivity (EC), magnesium and uranium values measured in Coonjimba Billabong (CJBB) between 1991 and 2014. Box plots show median, range, 25th and 75th percentiles and outliers (points). Periods relevant to macroinvertebrate sampling are indicated by year and season (wet (W) or dry (D)). Data from Energy Resources of Australia, NT Dept of Mines & Energy, and Supervising Scientist. Wet season – January to May. Dry season – June to December. Red lines indicate water quality GVs for Mg (3 mg L-1) and U (2.8 µg L- 1).

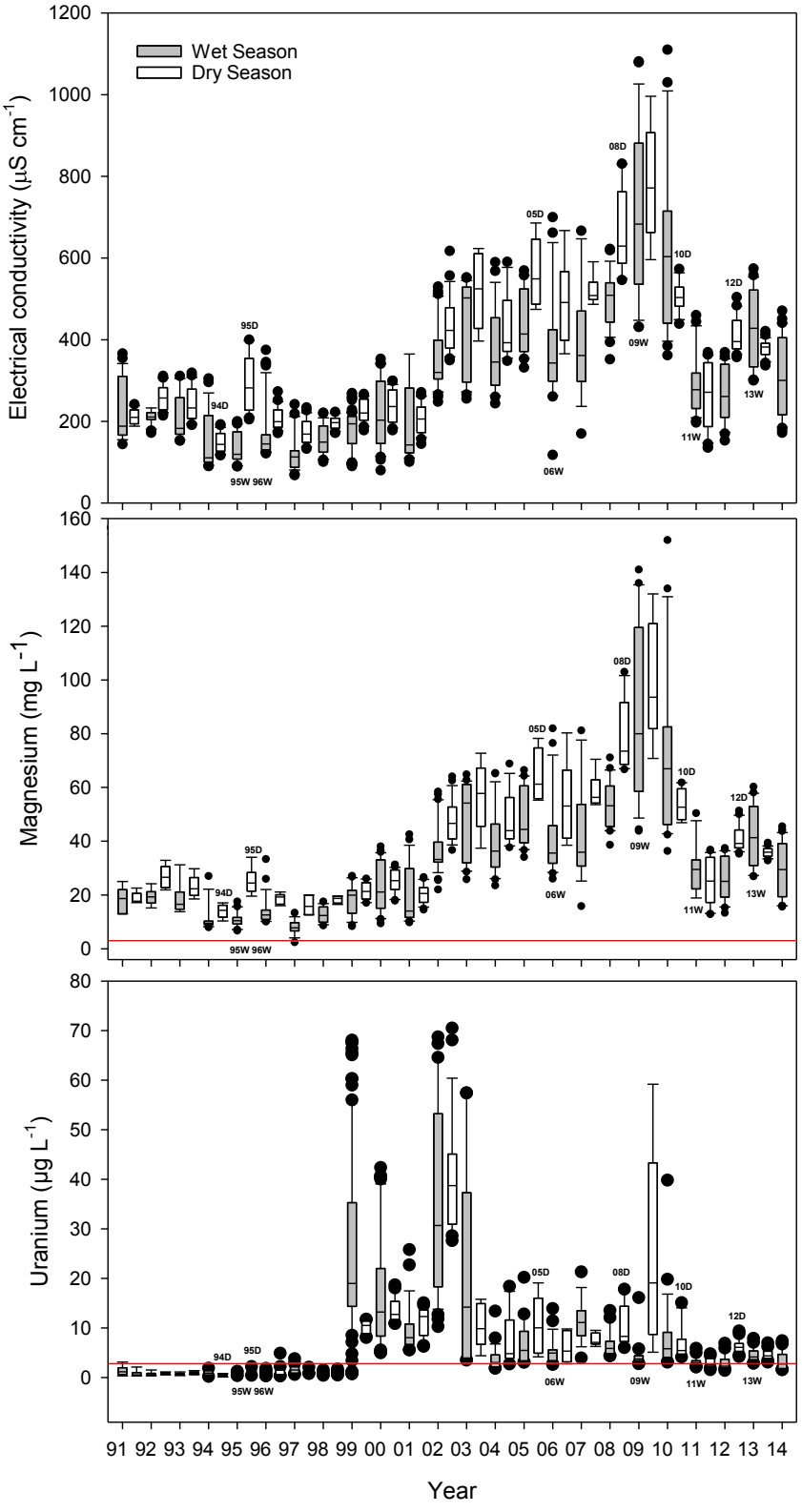


Figure 11 Summary box plots of generally weekly electrical conductivity (EC), magnesium and uranium values measured in Retention Pond 1 (RP1) between 1991 and 2014. Box plots show median, range, 25th and 75th percentiles and outliers (points). Periods relevant to macroinvertebrate sampling are indicated by year and season (wet (W) or dry (D)). Data from Energy Resources of Australia, NT Dept of Mines & Energy, and Supervising Scientist. Wet season – January to May. Dry season – June to December. Red lines indicate water quality GVs for Mg (3 mg L^{-1}) and U (2.8 µg L^{-1}).

Antecedent dry season EC is typically much higher than wet season values in the waterbodies (Figures 8–10) due to evapo-concentration and groundwater inputs of solutes during this period. For GTB (Figure 9), solutes in surface runoff from the minesite (via Corridor Creek, see Figure 6) are diluted by wet season surface water contributions from the rest of the GTB catchment, as well as backflow from Magela Creek. For CJBB, less wet season dilution of mine-derived solutes is available because the only upstream source of runoff is (mine-contaminated) RP1. This billabong is also set back some distance from adjacent Magela Creek and as such is flushed less by backflow events in Magela Creek compared to GTB. Prior to 1982 (GTB) and 1980 (CJBB, ie before construction of RP1), EC in both billabongs naturally reached median dry season values of 43 (GTB) and 68 μS cm^{-1} (CJBB) but in this period Na, K, Cl and alkalinity were the main contributors to total solute concentration and not Mg nor SO_4 (median Mg value only 1.0 mg L^{-1} (GTB) and 0.6 mg L^{-1} (CJBB), ERA LIMS database and Northern Territory Water Division 1983).

For GTB, the average of the antecedent wet and dry season median Mg and EC values for 1995, 1996 and 2006 did not exceed the site-specific Mg guideline value of ~3 mg L^{-1} (van Dam et al 2010, Sinclair et al 2014) (equivalent EC guideline of 42 μS cm^{-1}). However, in the sampling periods for 2009, 2011 and 2013, the average of the antecedent wet and dry season median Mg and EC values in GTB (Figure 9) exceeded the Mg and EC guideline values (average of the medians of 5.1, 5.7 and 4.3 mg L^{-1} Mg for 2009, 2011 and 2013 respectively). For CJBB and RP1, the average of the antecedent wet and dry season median Mg and EC values for all sampling years exceeded the local Mg guideline value (Figure 10 and 11).

Uranium in the surface waters of the Ranger RPA is effectively attenuated within short distances of contaminant sources due to its high affinity for binding to dissolved organic matter and sediment. Thus, the concentrations of mine water derived U in GTB and CJBB for the antecedent periods (ie wet and/or dry season months) for all macroinvertebrate sampling years were below the site-specific GV of 2.8 μg L^{-1} (van Dam et al 2017) (Figure 9 and 10). The average of the antecedent wet and dry season median U values in RP1 exceeded the local guideline value for 2006, 2009, 2011 and 2013 sampling (average values respectively of 7.3, 6.8, 4.9 and 5.1 μg L^{-1}) (but see section 5.1.4, footnote 6).

5.1.1.3 Relationship between Mg and EC in waterbodies

Very high correlations (r = 0.982, Table 4) were observed between Mg and EC in the minesite waterbodies (Figure 12). To acquire additional Mg data where this major ion was not measured, but where EC was measured, regression equations were derived between Mg and EC and from which Mg could be predicted (section 3.4.1). Wet and dry season regression relationships for GTB, CJBB and RP1 are shown in Appendix 2, Figures A2.1, A2.2 and A2.3 respectively. The coefficients of determination were high ($R^2 > 0.9$, Figure 12 and Table 4) for all waterbodies and seasons except for GTB dry season events where turbidity exceeded 50 NTU (Figure A2.1). Under these high turbidity, late dry season conditions, predicted Mg is lower for a given EC, presumably due to re-suspension of additional ions (eg ammonia) from sediments that also contribute to surface water ionic strength. High dry season turbidity is not usually observed in CJBB or RP1.

For predictive purposes:

- For EC > 50 μS cm^{-1}, Mg was predicted from the regression relationship that used data from all sites and sampling occasions (Figure 12). (Note that the regression line does not pass through the origin because other ions are also contributing to EC. The regression is for predictive purposes, and hence was not forced through the origin which would result in a poorer fit.)

- For all reference waterbodies and GTB for 1995, 1996 and 2006, Mg was predicted from the regression relationship using combined data from all reference waterbodies (Figure 13).

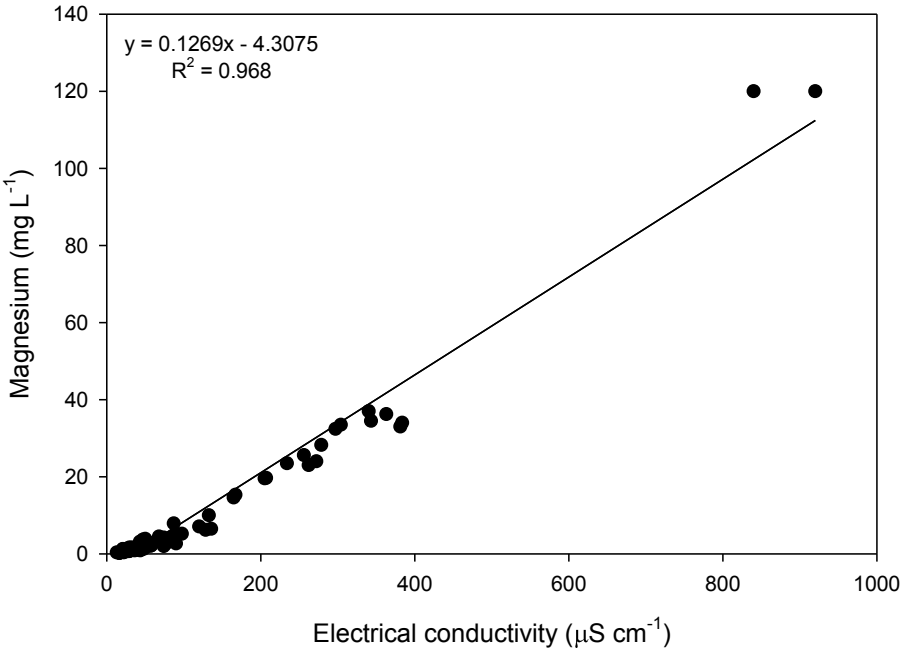


Figure 12 Regression relationship between (filtered) Mg and EC, using data from all sites and sampling occasions

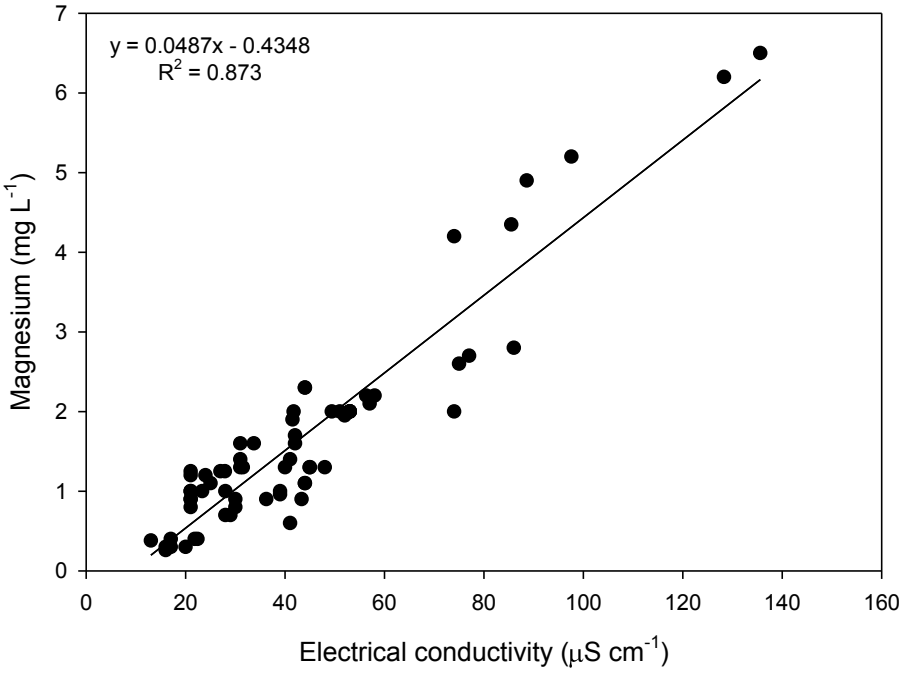


Figure 13 Regression relationship between (filtered) Mg and EC, using combined data from all reference waterbodies

Results of multivariate analyses of the water quality data are provided in section 5.1.3.

5.1.2 Sediment quality of waterbodies

Illes et al (2010) and Parry (2015) compared sediment quality data from reference and onsite waterbodies – in particular, for the period 2003–2006 and 2007–2013 respectively

– against national (ANZECC & ARMCANZ 2000), international (Sheppard et al 2005) and/or locally-derived (Harford et al 2015a) sediment quality GVs. Years for which comprehensive data were available included 2006, 2007, 2011 and 2013 (see Table A1.9 of Appendix 1). For the metals, Mg, Mn, Fe, Cu, Pb, Cd, Zn, Cr and V listed as CoPCs by Brown et al (1985b), no GV exceedances were found or (where no guidelines could be found) inferred.

Uranium in sediment was compared against an international GV of 100 mg kg^{-1} derived by (Sheppard et al 2005). A locally-derived GV is being determined on the basis of field experimental work by (Harford et al 2015a), and is likely to be a similar value to that reported by (Sheppard et al 2005) (A Harford, pers. comm.). The highest sediment U concentrations were found in onsite waterbodies during 2011. The results for sediment U in minesite waterbodies from 2011 and 2013 sampling programs are summarised in Table **6**.

Parry (2015) found no evidence of trends in sediment U over time in any of the minesite waterbodies. Of the four comprehensive sampling campaigns (listed above), sediment U guideline exceedances were only observed in RP1:

- In 2006, two out of 20 cores in the pond exceeded the local GV, though these locations were in the centre of the pond and not in littoral zones from which macroinvertebrate sampling has been conducted; and
- In 2011, for three out of the five sediment samples corresponding to macroinvertebrate sampling locations, the GV was exceeded (102–149 mg kg^{-1}).

Further assessment of the RP1 sediment U GV exceedances is made in section 5.2.2.

Table 6 Resullts of sediment chemistry analysis (1M HCl extraction on the <63 µm fraction) for uranium and sulfur for samples collected in 2011 and 2013. Units are in mg kg^{-1}.

Waterbody	Analyte	Mean	SD	Min	Max	N
2011						
RP1	Uranium	74.1	34.0	32.3	111	5
CJBB		19.9	6.2	13.4	30.1	5
GTB		15.7	8.3	5.5	40.8	34
Reference		2.7	1.2	0.9	7.47	40
2013						
RP1	Uranium	33.9	16.1	16.5	56.9	5
CJBB		9.9	4.5	7.2	17.9	5
GTB		16.6	10.0	7.9	33.6	5
Reference		2.4	0.9	0.9	4.8	40
2013						
RP1	Sulfur	1050.5	786.8	220	2250	5
CJBB		1250	673.9	585	2380	5
GTB		33.5	14.3	25	52.5	5
Reference		31.6	11.9	10	70	35

In section 1.3, potential for build-up of sulfate in sediments of waterbodies, with subsequent mobilisation of contaminants through sediment-surface water fluxes, was raised as a potential risk to ecosystem health. The sediments of CJBB and RP1 have accumulated significant concentrations and loads of sulfate (Table 6). Since 2002-2003, CJBB in particular, under (natural) wetting and drying conditions of the organic-rich, billabong sediments, has experienced brief, early wet season oxidation of sulfides, and subsequent acidification events as the billabong begins to refill with early rains and catchment runoff – see pH data shown in Figure 14. The ERA database from which much of the pH data were extracted also show mobilisation of Mn (and other metals) during these episodic events (data not provided here). The early wet season events usually last for less than about one month and occur in a period when CJBB (at least) is reduced to a chain of small shallow pools.

Further assessment of these acidic events in CJBB and RP1 is made in section 5.1.4.

5.1.3 Principal Components Analysis of environmental data from all waterbodies

Three PCAs were conducted: most years but fewer environmental variables, just three years but with all variables, and three years but with just water quality variables (see variable combinations in section 3.4.1 above).

5.1.3.1 Environmental data for each waterbody from most years (1995, 1996, 2006, 2011 and 2013)

The results for Axes 1 and 2 of the 'most-years' PCA analysis, accounting for 46% of the total sample variance respectively, are shown in Figure 14, with results for the key influential variables for each axis provided in Appendix 4.

The PC Axis 1 (PC1) accounts for 30% of the total sample variance and depicts a water quality gradient (from right to left) associated with the mine-derived contaminants Mg and U, as well as EC and pH. (Sulfate data were not available for this analysis, section 3.4.1.) PC2 accounts for 16% of the total sample variance, and is most strongly driven by geographical and geomorphic factors. From bottom to top, PC2 depicts location, Nourlangie (south) to Magela (north) (latitude, longitude and catchment (Magela or Nourlangie)) and larger to smaller waterbody size. PC3, accouting for 11% of the total sample variance is associated with a number of aquatic plant types (emergent narrow leaf, submerged not feathery and floating attached), depth rank and year (Appendix 4).

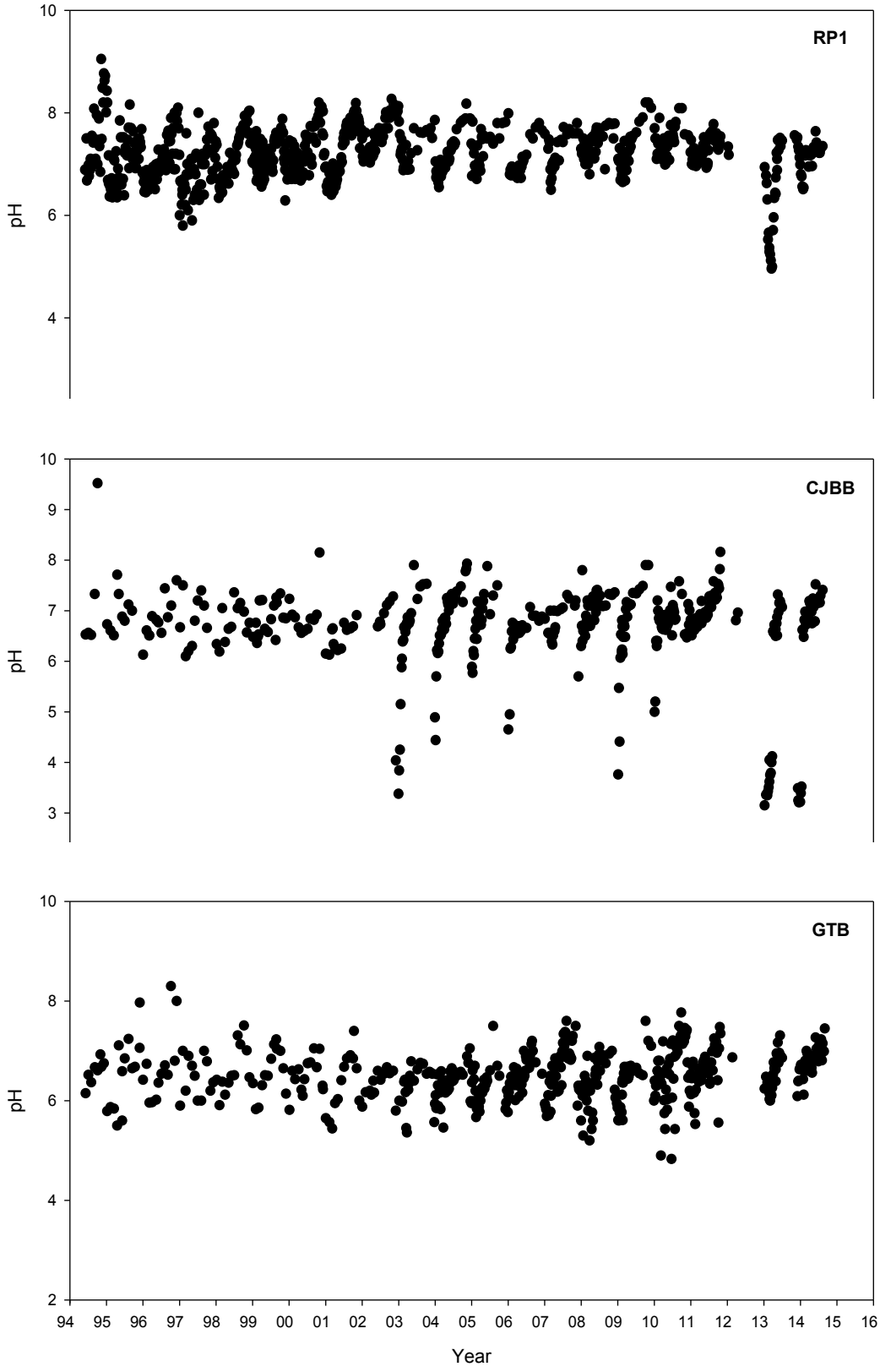


Figure 14 Measured pH for RP1, Coonjimba and Georgetown billabongs since 1994.

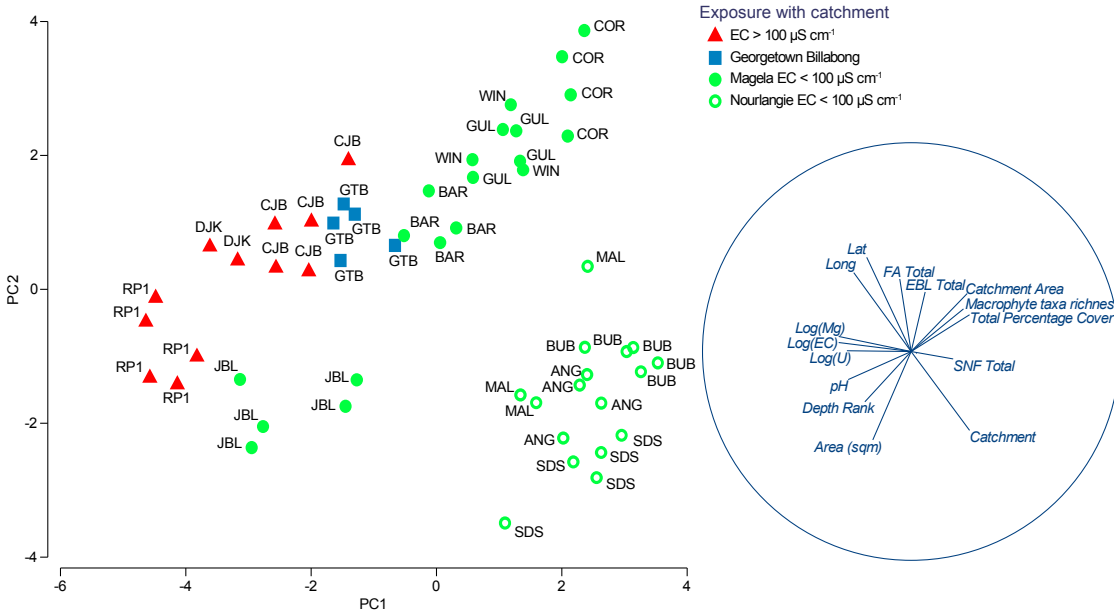


Figure 15 Two-dimensional Principal Components Analysis ordination of environmental data for most years (1995, 1996, 2006, 2011 and 2013). Axes PC1 and PC2 together account for 46% of the total sample variance. Vectors shown if correlation > 0.2. Site codes are provided in Table 1.

5.1.3.2 Environmental data for each waterbody from 2006, 2011 and 2013

The results for PC1 and PC2 of the PCA analysis for 'three years but all variables', accounting for only 39% of the total sample variance, are shown in Figure 16, with results for the key influential variables for each axis provided in Appendix 4.

PC1 accounts for 25% of the total sample variance and, like the most-years PCA, depicts a water quality gradient (from right to left) associated with the mine-derived contaminants Mg and U, with associated EC, SO_4 and the Mg/Ca ratio. PC2 accounts for 14% of the total sample variance, and is most strongly driven by waterbody size and additional water quality variables. From bottom to top, PC2 depicts decreasing waterbody size and dissolved oxygen, and increasing Mn, though there is no separation of these variables along the axis between Nourlangie and Magela, and mine-exposed and reference, waterbodies. Thus the axis does not depict any mine-related gradients. PC3 accounting for 10% of the total sample variance, is associated with emergent narrow-leafed aquatic plants and rainfall, while PC4 accounting for 8% of the total sample variance, is associated with turbidity and dissolved copper (Appendix 4).

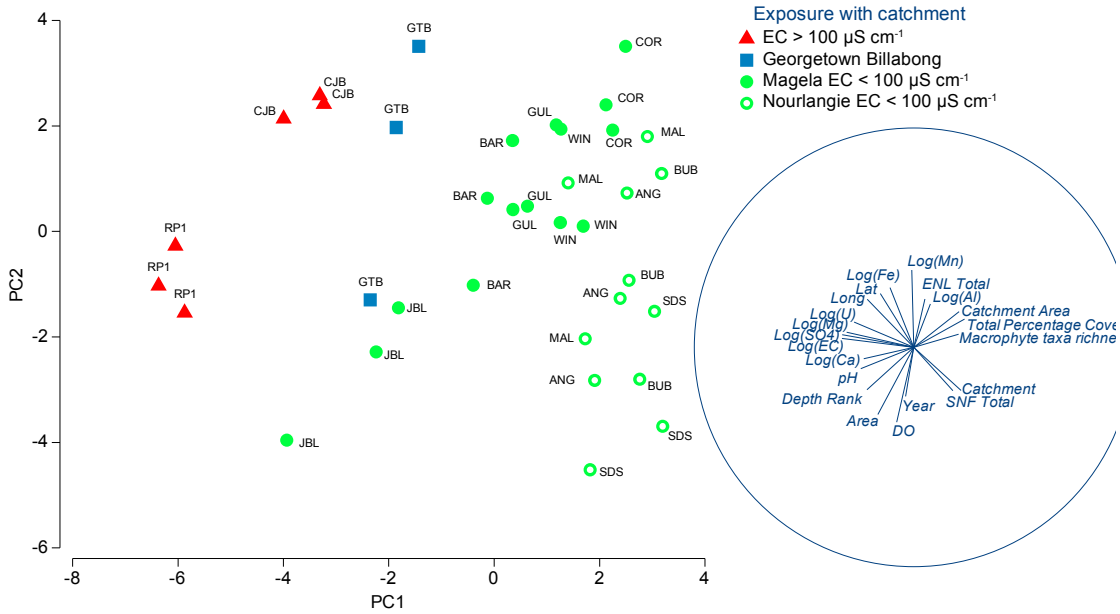


Figure 16 PCA analysis on all available environmental data for 2006, 2011 and 2013. Axes PC1 and PC2 together account for 39% of the total sample variance. Vectors shown if correlation > 0.2. Site codes are provided in Table 1.

5.1.3.3 Water quality data (only) for each waterbody from 2006, 2011 and 2013

PC1 and PC2 of the PCA analysis for 'three years, water quality only' variables, accounting for 47% of the total sample variance, are shown in Figure 17 with results for the key influential variables for each axis provided in Appendix 4.

The PCA based on just water chemistry variables from 2006, 2011 and 2013 (Figure 17) is very similar to the PCA based on all environmental variables from these years (Figure 16). PC1 accounts for 31% of the total sample variance and, like the previous two PCAs, depicts a water quality gradient (from right to left) associated with the mine-derived contaminants Mg, with associated EC and SO_4 and U. This PCA better explains PC2, accounting for 16% of the total sample variance; from top to bottom this axis depicts increasing turbidity and associated sediment-derived metals, including Al, Mn and Fe. Some macrophyte decomposition may also be associated with the increasing turbidity because there is also a gradient in decreasing dissolved oxygen in this same direction (but not increasing ammonia which might support this observation – see Appendix 4). As for the PCA based on all environmental variables (2006, 2011 and 2013), there is no separation of these variables along PC2 between Nourlangie and Magela, and mine-exposed and reference, waterbodies (i.e. non-mine-related).

PC3 accounting for 12% of the total sample variance, is associated with turbidity and dissolved copper, while PC4 accounting for 10% of the total sample variance, is associated with pH, dissolved iron and calcium (Appendix 4).

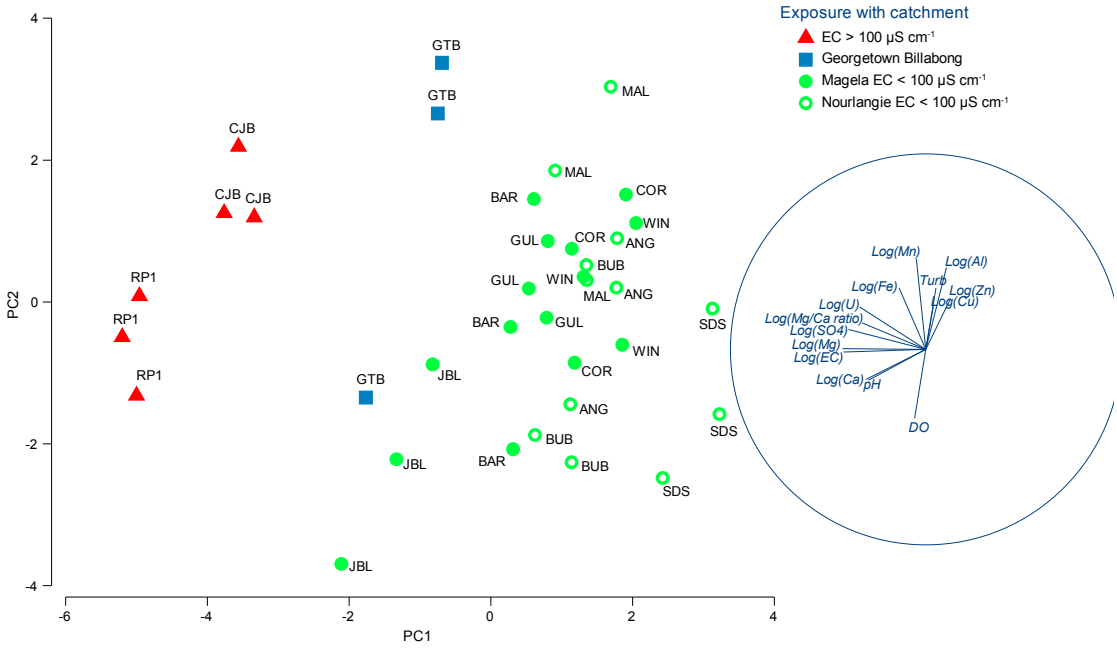


Figure 17 PCA analysis on water physico-chemistry data for 2006, 2011 and 2013. Axes PC1 and PC2 together account for 47% of the total sample variance. Vectors shown if correlation > 0.2. Site codes are provided in Table 1.

5.1.4 Implications of water and sediment physico-chemistry for ensuing biological assessments

No exceedances of the ammonia guideline value occurred in minesite waterbodies antecedent (12 months) to the macroinvertebrate sampling described in this report (section 1.3). Exceedance of guideline values for surface water CoPCs, i.e. Mg, U and Mn, amongst minesite waterbodies for water quality antecedent to all years of sampling is shown in Table **7**. The derivation method for calculating average annual values for the CoPCs is described in section 3.2.3.2 with the values provided in Tables A1.5–A1.8 (Appendix 1).

Table 7 Exceedance of CoPC guideline values (99% species protection) for surface amongst minesite waterbodies for water quality antecedent to all years of sampling

Analyte	Guideline value	GV Exceedances		
		Waterbody	Sampling years	Av. annual value
Mg	3 mg L^{-1}	GTB	2009, 2011, 2013	4.3-5.7
		CJBB	1995, 1996, 2006, 2009, 2011,2013	7.0-37.4
		DJKB	1995, 1996	79.5-126
		RP1	1995, 1996, 2006, 2009, 2011,2013	11.9-82.9
U	2.8 µg L^{-1}	DJKB	1995, 1996	7.8-17.4
		RP1	2006, 2009, 2011,2013	4.9-7.3
Mn	73 µg L^{-1}	CJBB	2013	244
		DJKB	1995, 1996	259-729

GV exceedances commonly occurred for Mg amongst all waterbodies. The U GV was commonly exceeded in DJKB and RP1, though ambient mean concentrations were mostly within the same order of magnitude as the GV, while most of the values fall within the GV for 95% protection (8.3 µg L^{-1}L U). Antecedent U concentrations in CJBB are deemed not sufficiently high to confound interpretation of any Mg effects in this billabong.

However, because DJKB U and Mn data in particular are potentially confounded in interpreting Mg effects, none of the analyses associated with threshold and GV determination for Mg include data from this billabong. (However, see footnote below[6].)

In addition to the CoPCs, antecedent water quality conditions to sampling also indicated high acidity in the early wet season of 2013 in both CJBB and RP1 – see section 5.1.2 and Figure 14. The acid sulfate event in CJBB in early 2013 was also likely responsible for the Mn GV exceedance in the 2013 wet season (Tables 7 and A1.6). There is a high likelihood that these events resulted in impact to the biota of both waterbodies (particularly CJBB), prior to sampling a few months later. Thus, biological data for CJBB and RP1 in 2013 are potentially confounded in interpreting Mg or U effects upon macroinvertebrate communities[7]. Any analyses associated with threshold and GV determination for Mg, therefore, consider both inclusion and removal of 2013 CJBB and RP1 data.

As described in section 5.1.2, for three out of the five sediment samples corresponding to macroinvertebrate sampling locations in RP1 in 2011, the GV of 100 mg kg^{-1} was exceeded (102–149 mg kg^{-1}). As reported in section 5.2.2, there is no evidence that the exceedances adversely affected the benthic macroinvertebrate fauna of RP1.

5.2 Analyses of macroinvertebrate data

All macroinvertebrate data are provided in Appendix 5.

5.2.1 Consequences of different sample processing methods

Section 3.3.3 described different macroinvertebrate sample processing methods applied between years 1995, 1996 and 2006 (live-sorting) and 1979, 2009, 2011 and 2013 (laboratory processing). To ensure that any change in macroinvertebrate community structure attributed to mining over the full time series was not mistakenly attributed to this change in methodology, an investigation was undertaken, comparing the results of live-sorting and laboratory processing conducted on the same samples – see section 4.1.5 for general design.

The results of the comparative analyses described in section 4.1.5 are provided in Appendix 7, Part A7.1. The results showed that relative differences in macroinvertebrate community summaries and structure were the same for analyses based on each of the two comparative (live-sorted and laboratory processed) datasets. Even when data from live-sorted and laboratory components were ordinated together, the separation between the methods was minor (see Appendix 7, Figure A7.1.3). Thus, the difference in sample processing methods was not confounding the data for combined (all years) or exposed-reference waterbody comparisons made in the current study.

The implications of differences in mesh size (500 μm used in 1995, 1996 and 2006, and 250 μm used in 2009, 2011 and 2013) was also assessed. An earlier study by Humphrey et

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[6] At the time of publishing this report, speciation modelling conducted by Dr Scott Markich (Aquatic Solutions International) for SSB showed that filtered uranium occurring in dry season billabong and Ranger retention pond waters, even at concentrations >100 μg/L, has very low predicted bioavailability due to competing ions or association of the uranyl ion with organic matter (unpublished data). This also applies generally to aluminium, copper and zinc concentrations in the waterbodies, but not to magnesium nor manganese.

[7] Such acidity and mobilisation events are a consequence of acid sulfate sediments and oxidation as the waterbodies refill with early wet season rains and runoff (see sections 1.3 and 5.1.2). If $MgSO_4$ salts as a collective were being assessed for ecosystem and landscape-level effects then the inclusion of CJBB and RP1 2013 data would be reasonable additions to the assessment.

al (1997) investigated the differences in results for community structure analysis arising from different mesh sizes. In that study, nested sieves were employed during sample processing (500 and 250 μm) with the additional macroinvertebrate component retained on the 250 μm sieve collected and the data arising from 500 μm and 250 μm components compared. (Community structure data from the 500 μm component was added to the data arising from the 250 μm component to derive the 250 μm sample data.) The 1997 study observed small increases in total abundance and an average 10% increase in number of taxa in the 250 μm sample data, but negligible differences in similarity-based analyses. In the present study, number of taxa in mine site waterbodies was always calculated as a percent of (concurrently-sampled) reference billabong number of taxa, and for key analyses never compared between years as absolute values. Hence no artefacts arose from the use of different mesh sizes between years.

Notwithstanding, where data from different years were pooled for analyses (see section 2.5.3), the data were standardised to provide *relative* abundance, and then log transformed, to eliminate any abundance biases that were evident between the two sample processing methods and mesh sizes employed.

5.2.2 Comparison of macroinvertebrate communities collected from macrophyte and sediment habitats

As discussed in section 4.1.2, while macrophyte has been the primary habitat sampled in the present study (Table 3), in some years samples from sediments have been combined with macrophyte samples. This process may have confounded the ability to attribute Mg to a response if, 1) sediment fauna strongly influenced macroinvertebrate community structure of samples when combined with macrophyte sample components, or if 2) onsite waterbody contaminants other than Mg in sediment were responsible for observed biological responses in sediments. Differences in relative sampling effort between the two habitats would be evident if the sediment fauna was (naturally) high in diversity compared to macrophyte fauna and/or community structure of the sediment fauna of minesite waterbodies differed naturally from the sediment fauna of reference waterbodies (section 4.1.2). These aspects were assessed in the analyses described below.

Macroinvertebrate samples were collected from macrophyte and from sediments in 2006 and 2011, with samples processed separately. Sampling in 2011 coincided with a putative adverse macroinvertebrate response in GTB, thereby allowing an assessment of the extent to which the sediment fauna also contributed to (or confounded interpretation of) this response.

Macroinvertebrate community structure data (taxa and their abundances) from replicate sites of the different waterbodies from 2006 and 2011 were summarised and analysed using community summaries and MDS ordination. Data for macrophyte and sediment habitat were treated separately in the analyses. Community summaries reported here were based on taxa (usually family) number and total abundance.

5.2.2.1 Sampling in 2006

Humphrey et al (2008b) reported results of the comparison of macroinvertebrate communities sampled from sediment and macrophyte habitat in 2006. Relevant findings included:

1 Mean total abundance and mean number of taxa of macroinvertebrates in the sediment habitat for all waterbodies (reference and exposed), were much lower compared to the equivalent macrophyte habitat (Figure 2 from Humphrey et al (2008b));

2 Separate datasets for the two habitats sampled in 2006 were combined for analysis, thereby simulating the approach adopted in previous years (1995 and 1996) where sediment and macrophyte samples were composited before sample processing. In multivariate analyses, habitat-composited macroinvertebrate community structure data were the same (in MDS space, ANOSIM) as macrophyte habitat-only data (Figure 1 from Humphrey et al (2008b));

3 The ordination based upon sediment habitat type only, and for different exposure types, showed, in comparison to the macrophyte-only ordination, much greater scatter with little separation of higher EC mine-exposed waterbodies (CJBB and RP1) from other waterbodies (Figure 1 from Humphrey et al (2008b)). This was accompanied by greater within-waterbody scatter of replicate sites reflecting higher biological variability. Thus sediment habitat did not discriminate between different exposure types, in contrast to macrophyte habitat.

5.2.2.2 Results for 2011

Amongst all waterbodies, exposed and reference, total abundance and number of taxa for the sediment and equivalent macrophyte habitat from 2011 were plotted as waterbody means (Figure 18A) and as cumulative sample abundance and number of taxa for successive replicates (or 'accumulation curves') (Figure 18B).

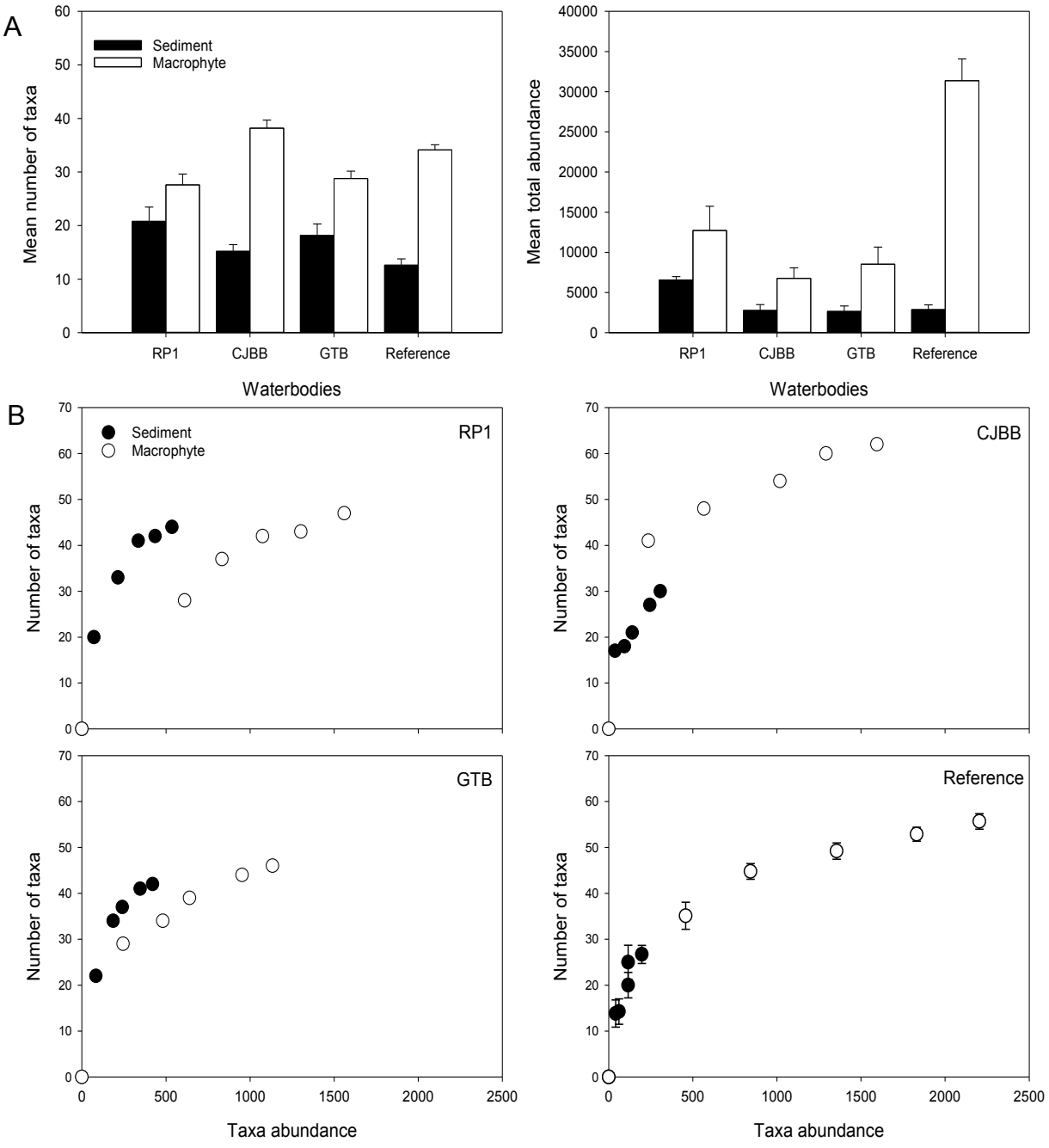
Accumulation curves were constructed because processing of sediment samples resulted in much lower abundances than abundances derived from respective macrophyte samples; because taxa number is positively correlated with sample abundance (Gotelli & Colwell 2001), mean values of taxa number across replicate samples may not accurately depict true taxon number in different habitats.

The accumulation curves (Figure 18B) show the low abundances of macroinvertebrates present in sediment habitat. While retrieval of 200 animals was the target for sample processing, this was never achieved for a total sample processing effort of 4 hours per sample. Thus benthic organisms were in low density in sediment samples. Comparing number of taxa between sediment and macrophytes, the accumulation curves show that sediment taxa number was (i) lower in CJBB, (ii) equivalent in reference billabongs, and (iii) higher in RP1 and GTB, compared to macrophyte habitat, but with indications in RP1 and GTB at least, that sediment taxa number could asymptote earlier than in macrophyte habitat.

The MDS ordination of community structure data for the separate macrophyte and sediment habitats, without identification of waterbody exposure type, is shown in Figure 19.

Sediment samples were well separated from macrophyte samples in ordination space (Figure 19), confirmed with ANOSIM testing (ANOSIM $R = 0.75$, $p = 0.0001$). SIMPER results are shown in Table 7, and indicate from most to least (top to bottom) influential taxa discriminating between macroinvertebrate taxa of macrophyte and sediment. All taxa occurred in higher abundance in macrophyte habitat.

A sediment habitat-only ordination is shown in Figure 20, according to different exposure types, reference (EC $< 100\ \mu S\ cm^{-1}$), GTB, and CJBB and RP1 (EC $> 100\ \mu S\ cm^{-1}$) waterbodies. The sediment habitat ordination showed little separation of exposure groups (ANOSIM Global $R = -0.06$, $P = 0.727$). Pairwise ANOSIM tests, tabled below, showed no separation between any of the two minesite exposure groups (GTB and CJBB/RP1) and reference sites.


A
Sediment
Macrophyte
Mean number of taxa
RP1
CJBB
GTB
Reference
Waterbodies
Mean total abundance
B
RP1
CJBB
GTB
Reference
Number of taxa
Taxa abundance

F
a
b

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or
),

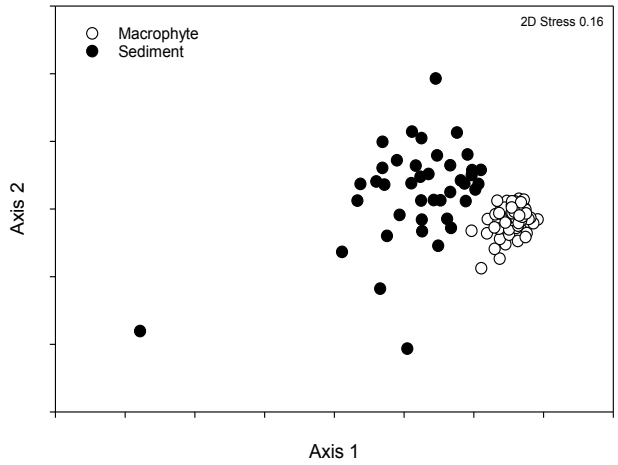


Figure 19 MDS ordination of 2011 macroinvertebrate community structure, grouped by habitat type and across all waterbodies

Table 8 SIMPER results on transformed data, discriminating macroinvertebrate taxa occurring in macrophyte and sediment habitat in 2011, for waterbodies of all exposure types.

Taxon	Group Macrophyte	Group Sediment				
	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Caenidae	1.36	0.19	4.02	1.75	4.73	4.73
Chironomidae (L)	1.50	0.52	3.64	1.50	4.28	9.01
Ceratopogonidae (L)	1.22	0.24	3.59	1.41	4.22	13.22
Oligochaeta	1.08	0.08	3.36	1.63	3.95	17.17
Anisoptera	1.03	0.07	3.31	1.61	3.89	21.06
Oribatida	1.03	0.18	3.02	1.50	3.54	24.61
Zygoptera	0.87	0.03	2.74	1.49	3.22	27.83
Planorbidae	0.86	0.28	2.65	1.14	3.12	30.94
Acarina	0.90	0.19	2.63	1.36	3.09	34.03
Atyidae	0.77	0.07	2.62	0.83	3.08	37.11
Leptoceridae (L)	0.79	0.05	2.56	1.44	3.01	40.13
Chironomidae (P)	0.79	0.09	2.54	1.28	2.99	43.12
Coenagrionidae	0.76	0.06	2.53	1.32	2.97	46.09
Baetidae	0.71	0.06	2.27	1.31	2.67	48.76
Hydroptilidae (L)	0.61	0.00	2.00	1.20	2.35	51.11
Pleidae	0.64	0.03	1.93	1.09	2.27	53.38
Nematoda	0.65	0.26	1.77	1.14	2.08	55.45
Mesoveliidae	0.52	0.02	1.69	0.92	1.98	57.44
Bithyniidae	0.52	0.06	1.59	0.83	1.86	59.30
Libellulidae	0.54	0.06	1.58	0.96	1.85	61.15
Dytiscidae (A)	0.38	0.21	1.54	0.83	1.81	62.96
Hydrophilidae (A)	0.46	0.04	1.51	0.84	1.78	64.74
Naucoridae	0.45	0.00	1.49	0.76	1.75	66.49
Veliidae	0.44	0.00	1.37	0.81	1.61	68.10
Ecnomidae (L)	0.36	0.00	1.36	0.75	1.60	69.70
Hydrophilidae (L)	0.37	0.01	1.34	0.70	1.58	71.28

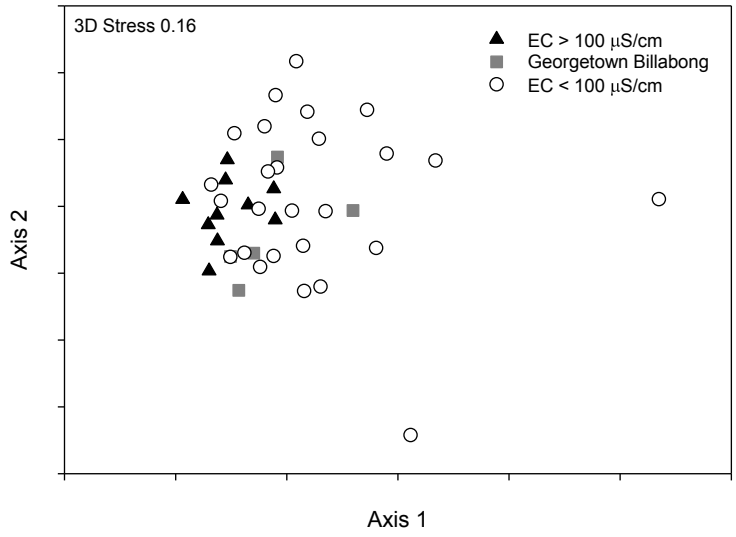


Figure 20 MDS ordination of 2011 macroinvertebrate community structure for sediment habitat only. Waterbodies are classified according to different degrees of exposure to mine waters indicated by electrical conductivity (EC).

This result contrasts with 2011 macroinvertebrate community structure of macrophyte habitat, where GTB communities, at least, differed significantly from the same responses measured in macrophyte habitat of reference waterbodies.

5.2.2.3 Conclusions

Investigations conducted on both 2006 and 2011 datasets showed similar results:

1 Macroinvertebrate density (yield per unit effort) in sediments of all waterbody exposure types is low compared to density in macrophyte habitat. This result has been commonly observed in other studies as well (ie (Storey & Figa 1998, Della Bella et al 2005). In the present study, and as also attributed in the other studies cited, the result is likely, in part at least, to be a consequence of fine-grained sediment particles (poor habitat for many macroinvertebrate forms) and lower dissolved oxygen concentrations at the greater depths (compared to macrophyte sampling) from which sediment samples were collected.
2 Macroinvertebrate community structure of sediment habitat was similar for all exposure types.

Finding 2 above could inadvertently lead to Type II error, i.e. combining invertebrates from the sediment habitat to those from the macrophyte habitat could potentially tend to diminish the influence of any true difference between contaminated and uncontaminated sites. (Thus if a difference is found between contaminated and uncontaminated sites, it is unlikely to be because of the inclusion of invertebrates from the sediment habitat. However, if no differences between the contaminated classes are observed, it may be (partly) because of the 'dilution' effect of non-responding sediment invertebrates.) However, because sediment sample abundances are so low compared to macrophyte sample abundances, the risk of false negatives is deemed very small, an observation borne out by the results of the 2006 study summarised above.

These findings show that either intentional or incidental inclusion of macroinvertebrate samples from sediment in the study design is inconsequential to impact assessment. The low abundance fauna of this habitat is insensitive to the ambient water quality measured

over the period of this study. Given the independence of macroinvertebrate communities in sediment and macrophyte habitat further, it is highly unlikely that contaminants bound and elevated in sediments (e.g. U and SO_4) would influence the fauna resident in the macrophyte habitat in the water column above the waterbody substrates. (The exception here would be acid sulfate events which would release these contaminants from sediments into the water column.) In 2011, U in the sediments of three of the 5 RP1 replicates was above the GV (section 5.1.2). There is no evidence that this contamination adversely affected the benthic fauna of RP1 given the interspersion of RP1 replicates amongst reference billabong replicates and non-significance of the corresponding ANOSIM comparison (Figure 19 and Table 9).

Table 9 ANOSIM pairwise test for the different exposure groups when examining only the 2011 sediment macroinvertebrate community data. (Negative R statistics are explained in section 5.3.3.)

Group comparison	R Statistic	P
EC > 100 µS cm^{-1}, Georgetown Billabong	0.543	0.001
EC > 100 µS cm^{-1}, EC < 100 µS cm^{-1}	-0.078	0.772
Georgetown Billabong, EC < 100 µS cm^{-1}	-0.077	0.634

5.2.3 Broad-scale, long-term and climate-related patterns in macroinvertebrate communities

5.2.3.1 Broad-scale and long-term patterns

The design of the study and selection of waterbodies serving as reference sites are critical to drawing correct inference. As described in section 4.1.1.1, minesite waterbodies in the absence of water quality impairment, may differ naturally from adjacent reference waterbodies in the same (Magela) catchment, and these reference waterbodies may in turn differ naturally from additional reference waterbodies from the adjacent (Nourlangie) catchment. To ensure such artefacts were not confounding the study, it was necessary to demonstrate: i) biological similarity amongst reference sites from both catchments, and ii) biological similarity of exposed waterbodies to other reference waterbodies prior to significant contamination and putative impact. These aspects were assessed in the analyses described below.

An MDS ordination based on standardised log transformed community data was derived using the mean of replicate data for each waterbody sampled in 1979, 1995, 1996, 2006, 2011 and 2013, without identification of waterbody exposure type. The ordination is shown in Figure 21 together with antecedent rainfall totals for sampling years as a proxy for climate-based, landscape drivers.

The ordination shows strong fidelity of waterbodies along Axis 1 to year of sampling, regardless of catchment, exposure type (mine-exposed, reference), waterbody morphometry and tributary catchment size (variables provided in Table 1). Along Axis 1, there was no clear relationship evident between rainfall and 'annual location' in the ordination. Differences in sample processing methods between 1995, 1996 and 2006 and 2011 and 2013 cannot explain the result either (section 5.2.1). Thus the general shift in macroinvertebrate composition from left to right along Axis 1, corresponding to early (1995, 1996) and progressively later years (2006, 2011, 2013), is driven by unknown, overriding landscape-wide factors affecting all waterbodies similarly.

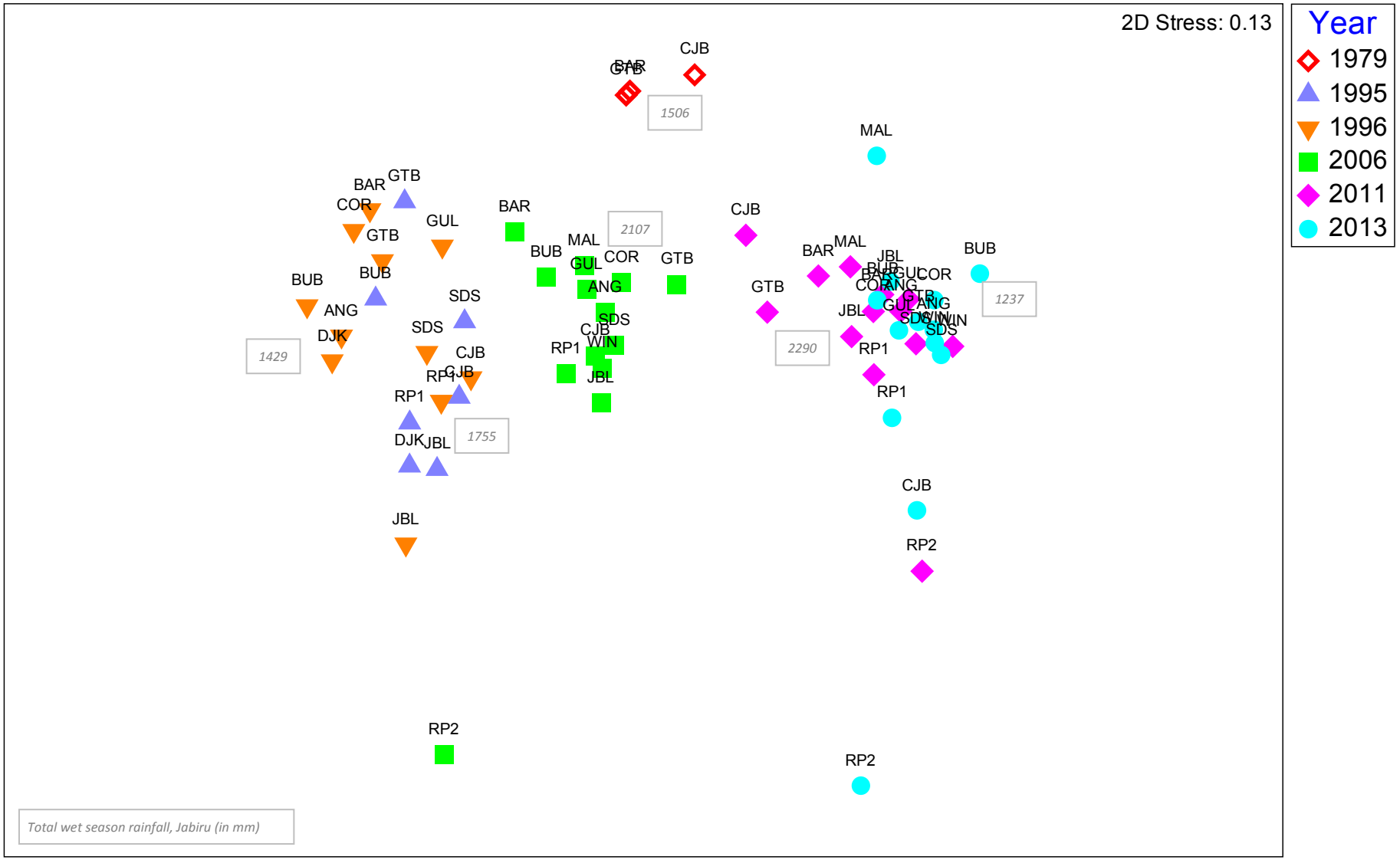


Figure 21 MDS ordination conducted on log transformed taxa data for 1979, 1995, 1996, 2006, 2011 and 2013, in relation to rainfall (mm) in the wet season immediately prior to sampling. Site codes shown in Table 1.

Axis 2 of the ordination generally represents a gradient, separating mine-water contaminated waterbodies (RP2, DJKB, CJBB, RP1) in the lower portion of the ordination, from references billabongs in the upper portion of the ordination. RP2 is a highly contaminated waterbody with Mg, U, Mn, Cu, Pb and Zn concentrations in 2006, 2011 and 2013, all well above local and national (ANZECC & ARMCANZ 2000) guideline values. While RP2 is accordingly well separated from other sites along Axis 2, its location in ordination space alsong Axis 1 still tracks with other waterbodies. Thus changes in macroinvertebrate communities of RP2 are as much driven by landscape-wide, non-mining related factors as they are by poor water quality.

The strong landscape patterns demonstrated in lentic waterbody invertebrates of this study are consistent with the concept of temporal coherence or spatial synchrony. This is "the tendency of population, community or ecosystem dynamics to behave similarly among locations through time as a result of spatially-correlated environmental stochasticity (Moran effect), dispersal or trophic interactions" (Huttunen et al 2014). The phenomenon is well documented for boreal ecosystems, though is not confined to this climatic biome, and has been reported for freshwater macroinvertebrate (Huttunen et al 2014) and zooplankton (Rusak et al 2008) communities, as well as fish populations (Phelps et al 2008) – amongst other biological communities and populations. The observations from the present study also accord with those from other broadscale studies on freshwater ecosystems of the wet-dry tropics of northern Australia. Thus Warfe et al (2011) highlighted spatially concordant distributions of biological (including invertebrate) communities across the landscape, primarily driven by the region's characteristic hydrological seasonality.

Possible regional-level drivers of the temporal coherence observed in the present study can only be speculated upon, e.g. climate change unrelated to rainfall, fire regimes, invasive species. Regardless and for bioassessment, temporal coherence has advantages including (Huttunen et al 2014) (i) replication demands upon long-term monitoring locations may be lessened, and (ii) the regional, climatic forcing of variability provides a useful basis for assessing any differences amongst sites sampled at the same time.

ANOSIM was used to compare, for each sampling year, macroinvertebrate composition between reference sites in the Magela and Nourlangie catchments. For this analysis, replicate data (5 locations within each waterbody) were used. Despite significant differences between catchments in most years, the ANOSIM R values are low (mostly <0.3, Table 10) indicating little separation of macroinvertebrate composition in reference waterbodies between catchments (see guiding criteria for interpreting ANOSIM R in Clarke and Gorley (2001) and section 3.4.4).

The ordination and ANOSIM results confirm the adequacy of the field design in important respects (as raised in section 4). In particular:

1 The grouping together of all waterbodies in multivariate space by year, rather than by catchment, exposure type and other morphometric and local catchment properties, indicates an overarching similarity amongst diverse lentic waterbodies. Such 'landscape control' increases confidence in attributing macroinvertebrate responses to changes in water quality; and
2 Magela and Nourlangie reference waterbodies in any sampling year group together, demonstrating similarity in macroinvertebrate community composition. Thus, differences in the relative numbers of reference waterbodies from each catchment used over time did not give rise to potential artefacts.

Table 10 ANOSIM summary statistic results for each year comparing reference waterbodies between Magela and Nourlangie catchments. 1979 and 1995 reference waterbodies are only from Magela and Nourlangie catchments respectively, hence no data. (Jabiru Lake not used as a reference waterbody for this analysis.)

Year	R Statistic	P	Number of Magela reference waterbodies	Number of Nourlangie reference waterbodies
1996	0.376	0.0001	3	3
2006	0.279	0.0001	4	4
2009 [1]	0.293	0.2	4	2
2011	0.06	0.041	4	4
2013	0.112	0.0008	4	4

1: 2009 data for comparison included, Magela reference: Gulungul (5 replicates), Baralil (1), Corndorl (1), Wirnmuyurr (1) and Nourlangie reference: Sandy (1), Buba (1).

Elsewhere (section 5.2.5) it was demonstrated that for sampling occasions prior to 2011 (i.e. before significant mine-related, water quality contamination) macroinvertebrate communities in GTB were similar to reference waterbodies. This indicates that the choice and role of reference waterbodies in the study was suitable for inferring impact.

The collective results increase confidence that natural waterbody differences were not contributing alternative explanations to those based on water quality.

5.2.3.2 Interannual variability in macroinvertebrate communities

Differences in annual wet season rainfall may lead to natural differences in macroinvertebrate communities amongst waterbodies in ways not depicted in the all-years ordination (Figure 21). It was hypothesised in section 4.1.1.2, for example, that for the lentic waterbodies sampled in this study, lower rainfall wet seasons might result in higher multivariate dispersion and lower within-group similarity of organism communities amongst waterbodies due to more variable habitat (ie arising from rainfall patchiness) and stochastic invertebrate dispersion opportunities. Discriminatory resolution in metrics of group difference between macroinvertebrate communities of minesite and reference waterbodies might be affected by such natural variation.

Differences amongst years in multivariate dispersion were examined, and these metrics were then related to rainfall, sample processing method and water quality changes in the mine site waterbodies to assess the potential for misinterpreting contaminant-response relationships described in later sections of this report. For selected years, PERMDISP was used to estimate mean distance of group replicates from the group centroid for reference billabongs, and the replicates from each of GTB, CJBB and RP1. The years of comparison included only those for which the reference billabongs were common, i.e. 1996, 2006, 2011 and 2013. The results are plotted in Figure 22.

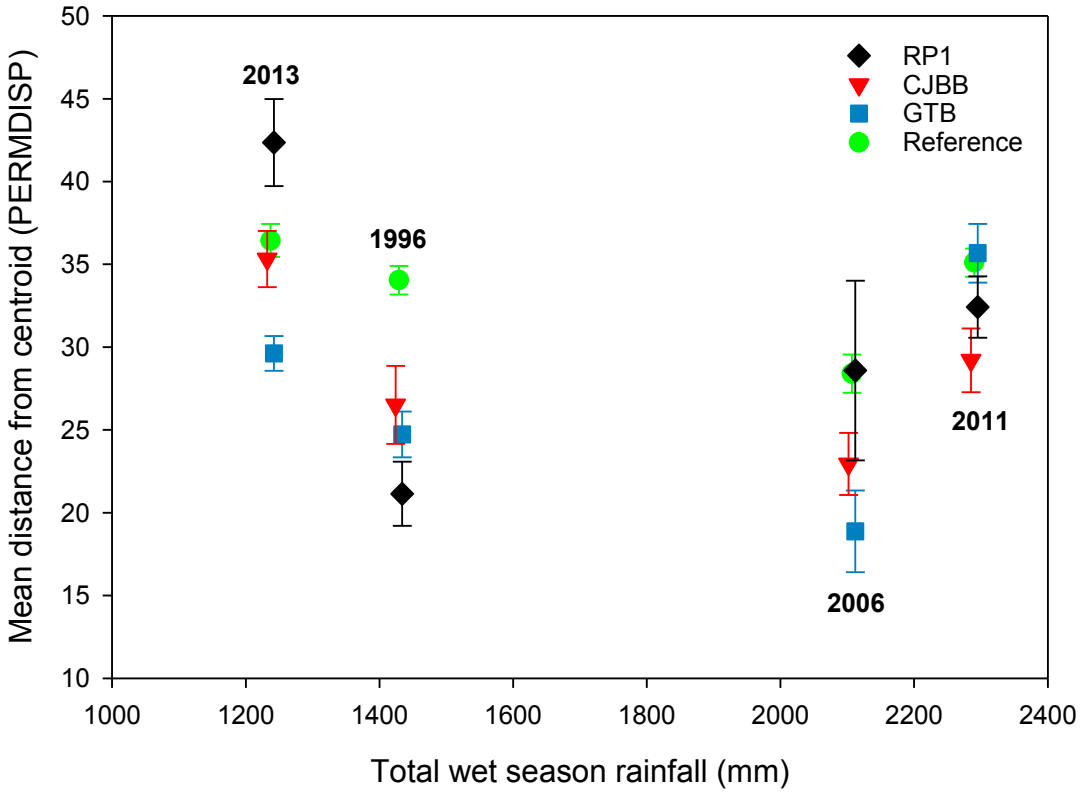


Figure 22 Variability in community structure, as depicted using the mean distance from the centroid with PERMDISP among all RP1, CJBB, GTB and all common reference sites within four of the annual sampling occasions. (2009 and 1995 sampling occasions excluded as reference sites were not all common to other years).

From Figure 22, it is evident that dispersion within reference billabong groupings for the four years did not fluctuate greatly amongst years (also evident in Figure 21). Dispersion within each of the mine site waterbodies generally followed the same pattern as reference billabongs, though in general, dispersion – and amongst replicate variability – was less than the corresponding reference-billabong dispersion. This lesser dispersion would be expected for single versus multiple waterbody comparison, where the multiple (reference) waterbody dispersion incorporates greater variability associated with different billabong types, from different catchments.

PERMDISP associated with reference billabongs provides the best measure for ascertaining natural, annual rainfall-related variability in macroinvertebrate communities in these lentic waterbody types. From Figure 22, it appears that dispersion within reference billabong groupings for the four years is unrelated to rainfall.

Live sorted samples (1996 and 2006) would be expected to have higher within-group dispersion than laboratory-processed samples (2011 and 2013) because of operator biases (see Appendix 7.1, Figure A7.1.3). However, in this study, live-sorted groups have lower dispersion than laboratory-processed groups, and so dispersion for the four years is unrelated to sample processing method as well.

The pattern of dispersion within reference billabong groupings for the four years did not (coincidentally) correspond to the increasing mine water contamination observed in the minesite waterbodies over time (evident in Figure 9–11). Dispersion was relatively even over the four progressive sampling occasions (Figure 22). Thus there are no time-related (natural) differences in amongst-site variation that could, in turn, potentially affect

reference-exposed waterbody comparisons based upon similarity and other multivariate response measures.

In conclusion, there is no evidence that the natural, amongst-site variation observed across years in this study would lead to misinterpretation of the key contaminant-response (cause-effect) relationships found elsewhere in this report.

Variability in aquatic communities has been associated with stress and disturbance in the associated ecosystems (eg Warwick & Clarke 1993). While dispersion within each of the mine site waterbodies was generally less than the corresponding reference-billabong dispersion (see above), three sampling events, i.e. RP1 in 2006 and 2013, and GTB in 2011, show similar or higher variability than within-reference waterbody-dispersion measured in the same years (Figure 22). These observations could provide evidence of water quality-related change for the two waterbodies on these sampling occasions. This is considered further in section 5.4.1 below.

5.2.4 Changes in habitat and water quality over time unrelated to mining

5.2.4.1 Fire regimes around minesite waterbodies and associated catchments

The potential for fire regimes to affect minesite waterbodies and associated catchments, and not reference billabongs, in any year of sampling, was raised in section 4.1.4 as a potential confounding factor in interpreting water-quality related changes in minesite waterbodies. Reduced riparian shading and release of soil nutrients in burnt catchments may enhance primary and secondary production in aquatic ecosystems (Andersen et al 2005). Putative water quality-related impact in Georgetown Billabong in 2011 (section 5.4.1) is of particular interest as responses in this year of sampling are used as the basis for deriving hazardous concentrations for guideline derivation (section 5.4.1).

A qualitative assessment of fire intensity adjacent to minesite waterbodies and in associated catchments, coinciding with or antecedent to sampling, was undertaken. For this, fire history data and imagery were acquired from the Darwin Centre for Bushfire Research, Charles Darwin University. Metadata for this mapping are provided in Appendix A7.4, Table A7.4.1. Fire intensity mapping for the Ranger minesite and adjacent landscape by way of early dry season (early June) and late (intense) dry season burning were obtained for the years of macroinvertebrate sampling and the year antecedent to sampling. This mapping is provided in Appendix A7.4 with the Georgeown Creek catchment outlined.

The mapping shows regular annual burning of minesite catchments. Most intensive burning occurred in the GTB catchment in the late dry seasons, antecedent to sampling in 2009 and 2013. Further, the fire history preceding sampling of GTB in 2011 did not appear unusual compared to other years. These observations suggest no obvious link between fire history and putative impacts to macroinvertebrate communities of GTB in 2011.

5.2.4.2 Potential for feral pig impacts upon littoral zone of Georgetown Billabong in 2011

The feeding activity of pigs in littoral zones of billabongs can destroy aquatic plant habitat and alter water quality, in particular, raising turbidity levels. Assessment of aquatic plant cover and water quality amongst replicate sites in GTB during 2011 sampling was used to address the potential for pig damage to have confounded Mg-inferred change to GTB macroinvertebrate communities in that year.

Figure 24 shows an ordination plot of macroinvertebrate communities from GTB and other waterbodies on or near the Ranger minesite for 2011 sampling. Two locations in GTB, replicates 3 and 5, are well separated from replicates from all other waterbodies. Field notes from sampling in that year did not report evidence of feral animal damage to

littoral vegetation and localised increase in turbidity; these observations are borne out by high aquatic plant cover at both sampling locations with no evidence of elevated turbidity (Table 11). Thus there is no evidence of pig-associated impacts to GTB in 2011 coincident with macroinvertebrate sampling.

Table 11 Water quality and habitat characteristics of Georgetown Billabong sampling locations in 2011

Environmental variable	Replicate				
	1	2	3	4	5
Electrical conductivity (µS cm^{-1})	51	50	49	47	45
Turbidity (NTU)	11.7	8.1	12.8	28.7	14.5
Aquatic plant cover (%)	10	90	65	65	80

5.2.5 Changes in community summaries and community structure over time

As described in section 2.1.4, spatial and temporal gradients of exposure to Ranger mine waters may be used to infer mine-related changes in macroinvertebrate communities. The temporal gradient includes a time-span from prior to mining (1979) to near-present (2013), over which several exposed waterbodies have become increasingly mine-water contaminated to varying degrees. The results from the previous sections, 5.2.1–5.2.4, validate the comparison of mine-exposed waterbodies to reference waterbodies, for each year of sampling (thereby eliminating interannual variability), as the basis for detecting change amongst the minesite waterbodies over time.

Macroinvertebrate community summaries for littoral macrophyte habitat in the exposed and reference waterbodies are shown in Figure 23 for each year of sampling (1995–2013). As noted in section 5.2.1, there were differences in sampling processing methods for years 1995, 1996 and 2006 versus 2009, 2011 and 2013. Abundances for the latter years (2009-2013) were scaled up from a processed subsample of the whole sample. Therefore, where mean total abundance is being compared amongst years, this comparison should be confined to those years *within* each of the two groups.

Apart from Ranger RP2, the mean number of taxa and mean total abundance did not vary markedly amongst the waterbodies in 1995, 1996, 2006 and 2009 (Figure 23). However, number of taxa relative to reference waterbodies declined in 2011 in GTB and RP1, and again in CJBB and RP1 in 2013.

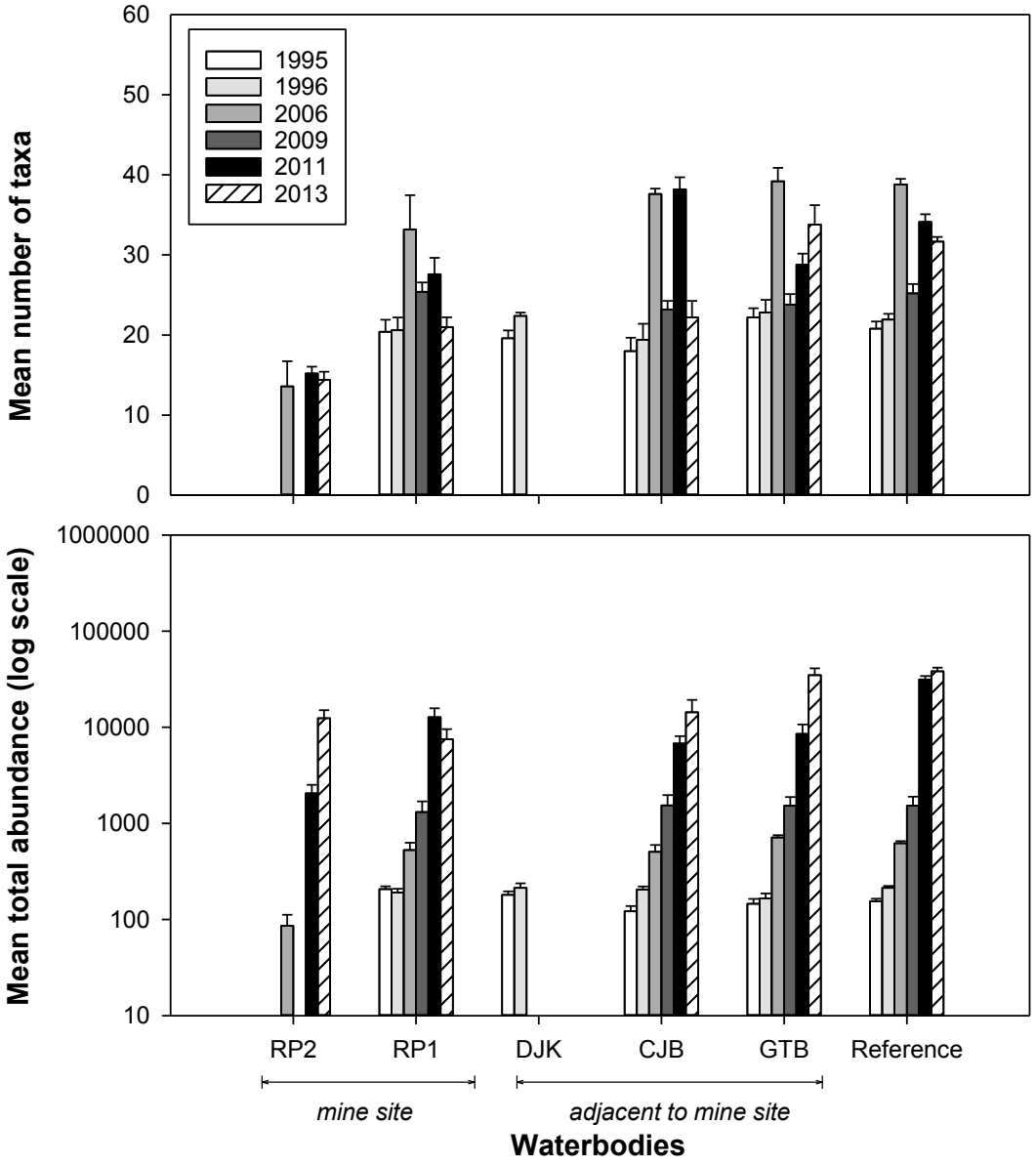


Figure 23 Histograms of mean (±SE) taxa richness (=number) and macroinvertebrate abundance amongst waterbodies on or near Ranger uranium mine site for the six years of sampling.
Site codes are Ranger Retention Pond 2 (RP2), Ranger Retention Pond 1 (RP1), Coonjimba (CJB), Georgetown (GTB) and Djalkmara (DJK) billabongs. Reference waterbodies are Gulungul, Baralil, Corndorl, Wirnmuyurr, Malabanjbanjdju, Anbangbang, Buba and/or Sandy Shallow billabongs and Jabiru Lake.

MDS ordinations for each sampling year are shown in Figure 24. For each year, apart from 1979, ANOSIM comparisons were also undertaken between each of GTB, CJBB and RP1 and reference waterbodies; these results are shown in Table 12.

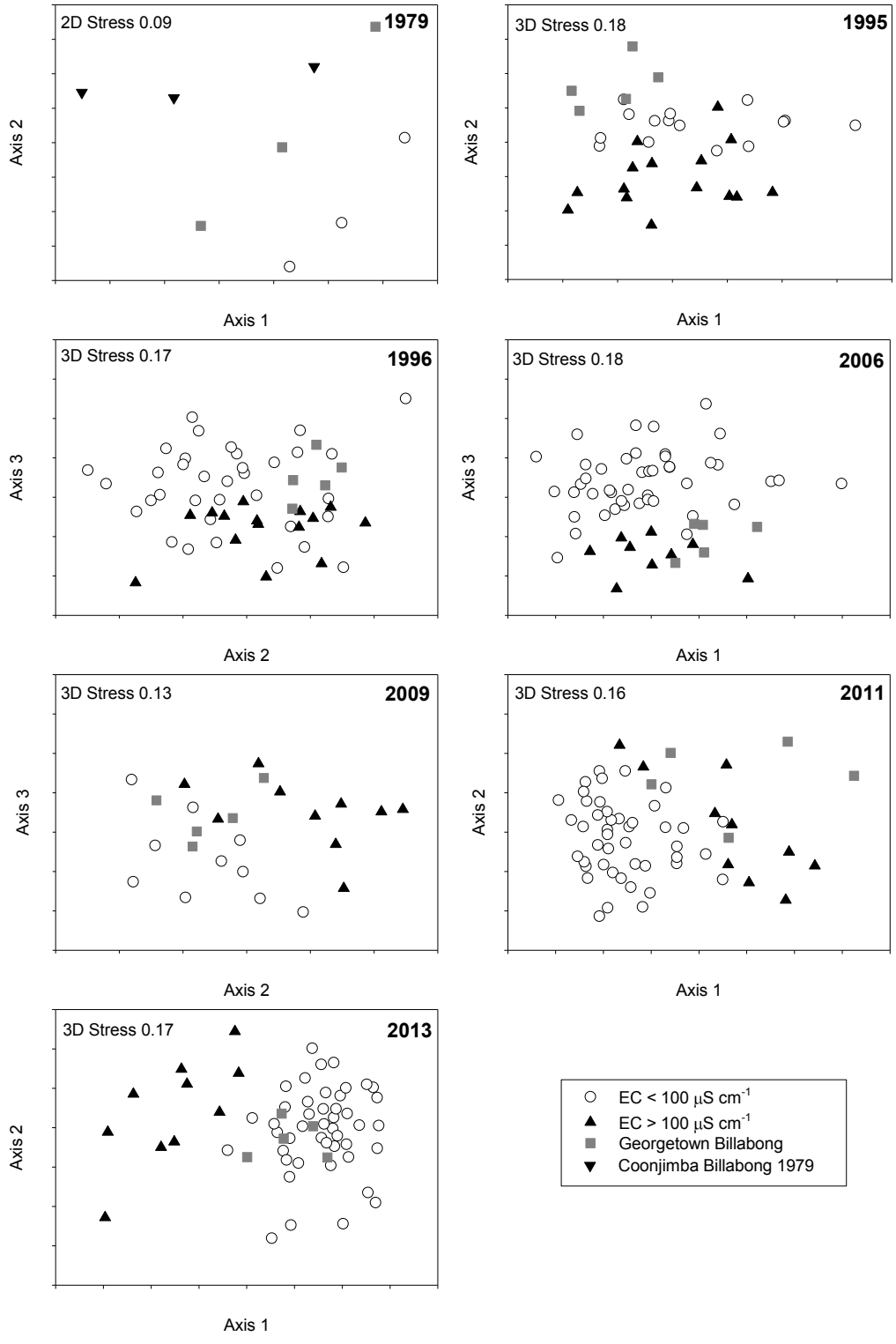


Figure 24 Ordination plots of macroinvertebrate communities from different waterbodies on or near the Ranger minesite for six sampling years. Waterbodies are classified according to different degrees of exposure to mine waters indicated by electrical conductivity (EC): high for two waterbodies (RP1 and Coonjimba Billabong) and low for all other waterbodies including Georgetown Billabong.

Table 12 ANOSIM summary statistic results for each year comparing GTB, CJBB and RP1 with reference waterbodies (excluding Jabiru Lake).

Year	R Statistic	P	Global R	Global P
Retention Pond 1				
1995	0.399	0.005	0.605	0.0001
1996	0.270	0.016	0.29	0.0001
2006	0.392	0.012	0.312	0.0001
2009	0.470	0.003	0.513	0.0001
2011	0.608	0.0002	0.582	0.0001
2013	0.827	0.0001	0.455	0.0001
Coonjimba Billabong				
1995	0.468	0.003	0.605	0.0001
1996	0.225	0.038	0.29	0.0001
2006	0.362	0.011	0.312	0.0001
2009	0.393	0.005	0.513	0.0001
2011	0.792	0.0001	0.582	0.0001
2013	0.737	0.0002	0.455	0.0001
Georgetown Billabong				
1995	0.391	0.007	0.605	0.0001
1996	-0.08[1]	0.742	0.29	0.0001
2006	0.129	0.177	0.312	0.0001
2009	0.295	0.023	0.513	0.0001
2011	0.522	0.0009	0.582	0.0001
2013	-0.076[1]	0.685	0.455	0.0001

1: Negative ANOSIM R values are explained in section 5.3.3.1

The MDS plots show, generally, interspersion of replicate samples from GTB among reference waterbody samples in 1995, 1996, 2006, 2009 and 2013 but separation of GTB samples from reference waterbody samples in 2011 (Figure 24). For CJBB and RP1 (EC > 100 μS cm^{-1}), interspersion of replicates with those of reference waterbodies is evident between 1995 and 2006, but increasing separation is evident from 2009. ANOSIM test statistics generally support these observations (Table 10), with R values >0.5 denoting some group overlap but clear differences (Clarke & Gorley 2001). This departure in macroinvertebrate community structure between minesite and reference waterbodies over time may infer mine-related effects, especially changes to water quality. Such inference is considered in the following section.

5.3 Environmental relationships with community structure and community summaries

5.3.1 Relating biological assemblage data to environmental data

5.3.1.1 BIOENV

The BIOENV routine was used to calculate the smallest subset of environmental variables explaining the greatest percentage of variation in the multivariate ordination patterns. The best matches of biological and environmental similarity matrices are denoted by Spearman rank correlation. Two BIOENV analyses were undertaken, based on (i) most years (1995, 1996, 2006, 2011 and 2013) (with a reduced environmental dataset) and (ii) three years 2006, 2011 and 2013 (with an expanded environmental dataset).

5.3.1.1.1 BIOENV for most years (1995, 1996, 2006, 2011 and 2013)

The high correlation between year of sampling and the ordination space was the overwhelming, dominant influence of the BIOENV (*ρ* value of 0.82) (Table 13). All other environmental variable combinations produced lower correlations, and a different covariable with 'year', for each of the top 10 results (Table 13). Year of sampling is strongly

correlated along Axis 1 of the ordination (Figure 21), separating the distinct annual groupings (regardless of waterbody exposure type and catchment) accordingly. In this case, year of sampling is a proxy for the unknown, landscape/climatic drivers of interannual variability in macroinvertebrate community structure that was discussed in section 5.2.3.1. Note that this BIOENV analysis did not include RP2 samples from 2006, 2011 and 2013. With inclusion of those data, it is likely that mine-derived CoPCs would have provided higher correlation given the relatively strong gradient along Axis 2 of the ordination (Figure 21), of mine-water contaminated waterbodies.

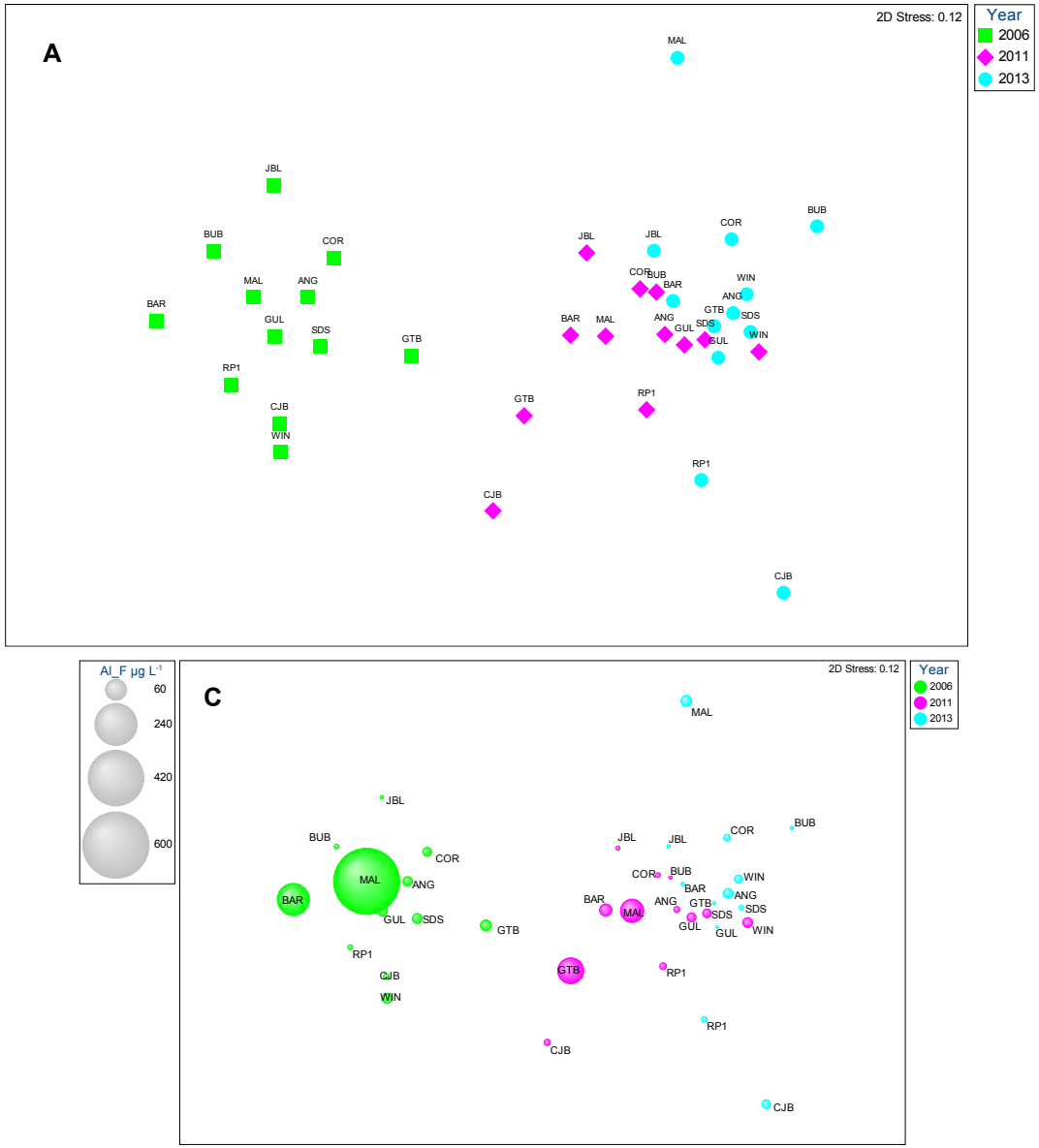
Table 13 Best ten BIOENV results between environmental and log transformed standardised macroinvertebrate community data for all years (1995, 1996, 2006, 2011 and 2013)

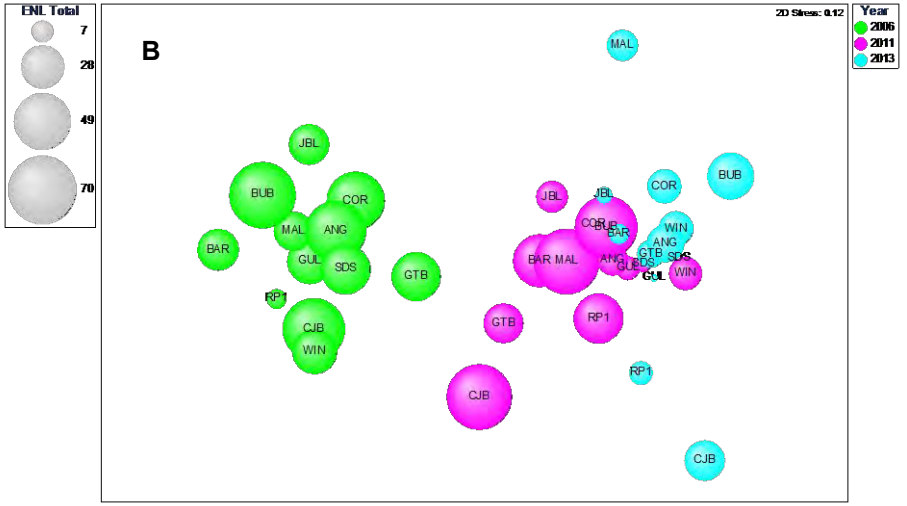
Spearman rank correlation (p)	Variable combinations
0.82	Year
0.710	Year, Free floating macrophyte
0.640	Year, Macrophyte taxa richness
0.633	Year, Depth rank
0.619	Year, Log (EC)
0.619	Year, Macrophyte total percentage cover
0.618	Year, Log(U)
0.617	Year, Log(Mg))
0.615	Year, Emergent narrow leaved macrophyte
0.603	Year, Floating attached macrophyte

5.3.1.1.2 MDS ordination and BIOENV for years 2006, 2011 and 2013

An MDS ordination based on log transformed, standardised macroinvertebrate community data for years 2006, 2011 and 2013 was derived using the mean of replicate data for each waterbody. The ordination, without identification of waterbody exposure type, is shown in Figure 25A, and, like the comparable all-years ordination (Figure 21), shows strong fidelity of waterbodies to year of sampling, regardless of catchment, exposure type (mine-exposed, reference), waterbody morphometry and tributary catchment size. As with the all-years ordination, the shift in macroinvertebrate composition from year to year, mainly from left to right along Axis 1, is driven by unknown, overriding landscape factors affecting all waterbodies similarly. Axis 2 of the ordination generally represents a gradient, separating mine-water contaminated waterbodies (CJBB, RP1) in the lower portion of the ordination, from references billabongs in the upper portion of the ordination.

The best 10 BIOENV results between environmental and community patterns of the ordination are shown in Table 14. None of the environmental variable combinations produced high correlations with the ordination space (maximum *p* value of 0.343), with the top 10 results consistently including emergent narrow-leafed (ENL) aquatic plants (e.g. *Eleocharis* sp.), measures of suspended sediment, i.e. Fe and Al, and measures of mine salts, i.e. SO_4 and Mg/Ca ratio.





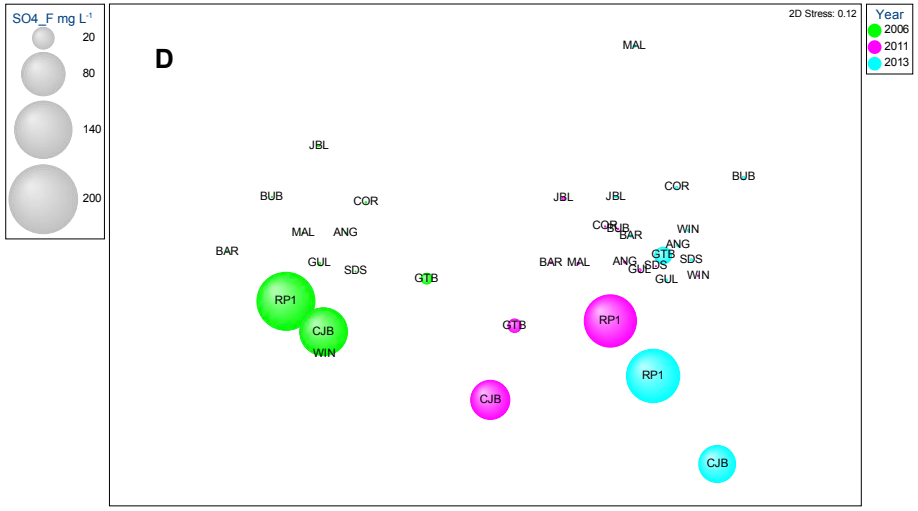


Figure 25 MDS ordination conducted on A. log transformed standardised macroinvertebrate community data for years 2006, 2011 and 2013. Bubble plots show B. emergent narrow-leaf macrophyte, C. Aluminium, and D. sulfate, highlighted by BIOENV routine. Waterbody codes explained in Table 1

Table 14 Best ten BIOENV results between environmental and *log transformed* standardised macroinvertebrate community data for years 2006, 2011 and 2013

Spearman rank correlation (p)	Variable combinations
0.343	ENL total, Dissolved Oxygen, Log(Al), Log(Fe), Log(SO_4)
0.339	ENL total, Turbidity, Dissolved Oxygen, Log(Fe), Log(SO_4)
0.338	FA total, ENL total, Log(Al), Log(Fe), Log(SO_4)
0.335	Rainfall, ENL total, Log(Al), Log(Fe), Log(SO_4)
0.333	ENL total, Log(Al), Log(Fe), Log(SO_4), Log(mg/Ca ratio)
0.333	ENL total, Dissolved Oxygen, Log(Al), Log(Fe), Log(Mg/Ca ratio)
0.332	ENL total, Log(Al), Log(Fe), Log(SO_4),
0.331	ENL total, Log(EC), Log(Al), Log(Fe), Log(Mg/Ca ratio)
0.330	FA total, Dissolved Oxygen, Log(Al), Log(Fe), Log(SO_4)
0.333	ENL total, Turbidity, Log(Al), Log(Fe), Log(SO_4)

The influence of ENL, Al and SO_4 were indicated by way of bubble plot overlays upon the three-year ordination (Figure 25). ENL (Figure 25b) showed interannual differences in relative abundance across exposure types, having reduced abundances in 2013 (i.e. unknown climate/landscape drivers of change, though this sampling year was preceded by the lowest rainfall wet season of those years sampled (Figure 21). Aluminium was consistently high in one or two 'reference' waterbodies, associated with early dry season turbidity (Malabanjbanjdju) or possible runoff from Jabiru sewage works (Baralil) (Figure 25c). Sulfate, strongly associated with mine water contamination, was in high concentrations in CJBB and RP1 (Figure 25d). Sulfate is highly correlated with Mg and U amongst minesite waterbodies and over time (rho ~0.99, Table 5) so it is unlikely that its dominance in the BIOENV results over Mg and U is particularly meaningful.

5.3.1.2 Canonical analysis of principal components (CAP)

Canonical analysis of principal coordinates (CAP) was applied to find axes through the (biological) multivariate cloud of points that either (i) were the best at discriminating among the three *a priori* (EC) exposure groups, or (ii) had the strongest correlation with the primary environmental Principal Component axis corresponding to the same biological samples (as described in section 3.4.1). CAP was applied to data for all years (1995, 1996, 2006, 2011, 2013) (reduced number of water quality variables) and data for three years (2006, 2011, 2013) (additional water quality variables).

5.3.1.2.1 All years analysis

CAP showed significant differences amongst exposure groups by permutation tests (P = 0.0001), with CAP1 (first canonical axis; squared canonical correlation of 0.62) separating macroinvertebrate communities in mine-exposed waterbodies from those in reference waterbodies. CAP2 distinguished GTB and similar reference billabong communities from the other waterbodies, although the squared canonical correlation for this second axis was not strong (0.34) (Figure 26). The three EC groups, CJBB-RP1, GTB and reference waterbodies, were correctly classified for 75, 60 and 81% of samples respectively, with an overall correct classification for the samples of 67%. Misclassifications for individual samples are indicated in Table 15.

Table 15 CAP results for all years, showing individual samples that were misclassified

Sample	Original Group	Classification Group
RP11996	EC > 100 µS cm^{-1}	EC < 100 µS cm^{-1}
CJB1996	EC > 100 µS cm^{-1}	EC < 100 µS cm^{-1}
CJB2011	EC > 100 µS cm^{-1}	Georgetown Billabong
GTB1995	Georgetown Billabong	EC > 100 µS cm^{-1}
GTB2013	Georgetown Billabong	EC < 100 µS cm^{-1}
JBL1995	EC < 100 µS cm^{-1}	EC > 100 µS cm^{-1}
SDS1995	EC < 100 µS cm^{-1}	Georgetown Billabong
GUL1996	EC < 100 µS cm^{-1}	Georgetown Billabong
BAR1996	EC < 100 µS cm^{-1}	Georgetown Billabong
JBL1996	EC < 100 µS cm^{-1}	EC > 100 µS cm^{-1}
BAR2006	EC < 100 µS cm^{-1}	Georgetown Billabong
WIN2011	EC < 100 µS cm^{-1}	EC > 100 µS cm^{-1}

These misclassifications are not surprising given the interspersion for a number of sampling years of minesite waterbodies amongst reference sites in the biological ordination space (see Figure 21). GTB in 1996, 2006 and 2013, and CJBB in 1996, were similar to reference condition (Table 12) which explains at least 6 of the 12 two-way misclassifications (i.e. GTB or CJBB 'predicted' as reference, or reference predicted as GTB). Taxa correlations in the CAP axes, depicted as vector overlays of Figure 26, are discussed in section 5.5.

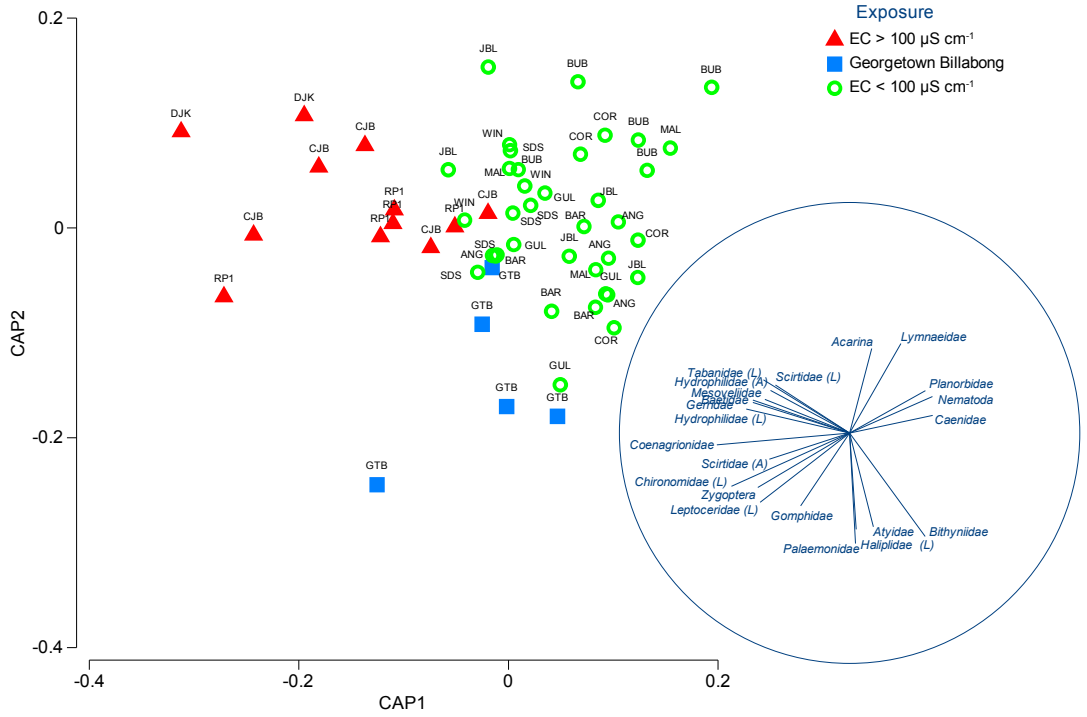


Figure 26 Canonical ordination for the discriminant analysis of all years macroinvertebrate community data with *a priori* exposure groups

CAP was also applied to the primary (biological) CAP and equivalent (environmental) PCA axes to assess goodness of fit and correct allocation of the *a priori* exposure groups to (in this case) the mainly water quality gradient arising from the PCA described in section 5.1.3.1. The first PCA axis (from Figure 15) explains 31% of the variability with mine-derived variables (EC, U and Mg) having the highest contributions; this axis thereby provides a convenient proxy mine-exposed, water quality gradient. The resulting CAP-PCA plot is shown in Figure 27. The relationship between the *a priori* biological classification (mine-water exposure viz EC) and environmental gradient is high (squared canonical correlation 0.63) providing strong support for water quality effects upon macroinvertebrate communities. The plot also identifies biological communities of Jabiru Lake as water-quality impaired, supporting the decision to remove this location from reference waterbodies in other inferential analyses.

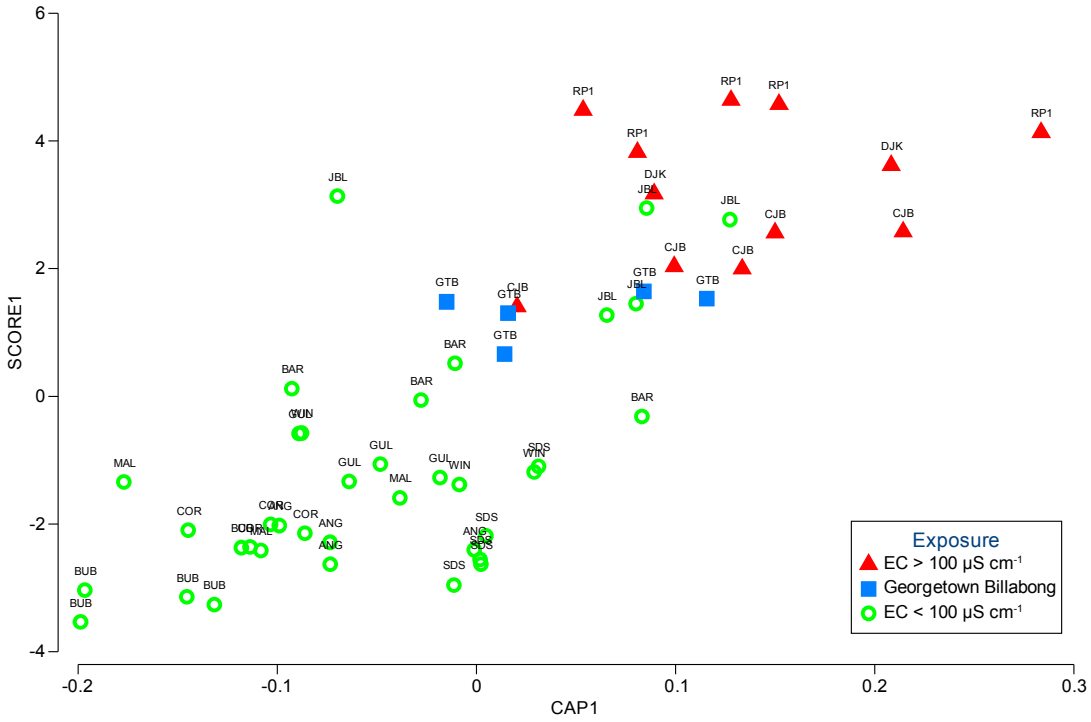


Figure 27 CAP analysis relating all-years macroinvertebrate community data to a (mainly) mine-related water quality gradient

5.3.1.2.2 Three-years analysis

CAP again showed significant differences amongst exposure groups by permutation tests (P = 0.001), with the best separation along CAP Axis 1 (squared canonical correlation of 0.73, cf 0.62 for all-years CAP 1 axis). CAP1 and CAP2 (squared canonical correlation 0.18, cf 0.34 for all-years CAP 2 axis) separated macroinvertebrate communities in mine-exposed waterbodies from those in reference waterbodies (Figure 27) in the same manner as found for the all-years CAP.

The three Exposure (viz EC) groups, CJBB-RP1, GTB and reference waterbodies, were correctly classified for 50, 67 and 89% of samples respectively, with an overall correct classification for the samples of 81%. Thus compared to the all-years CAP, there was improved allocation of samples overall, including GTB and reference waterbodies, to assigned *a priori* groups, but poorer prediction of CJBB-RP1 waterbodies to the high EC group. Misclassifications for individual samples are indicated in Table 16.

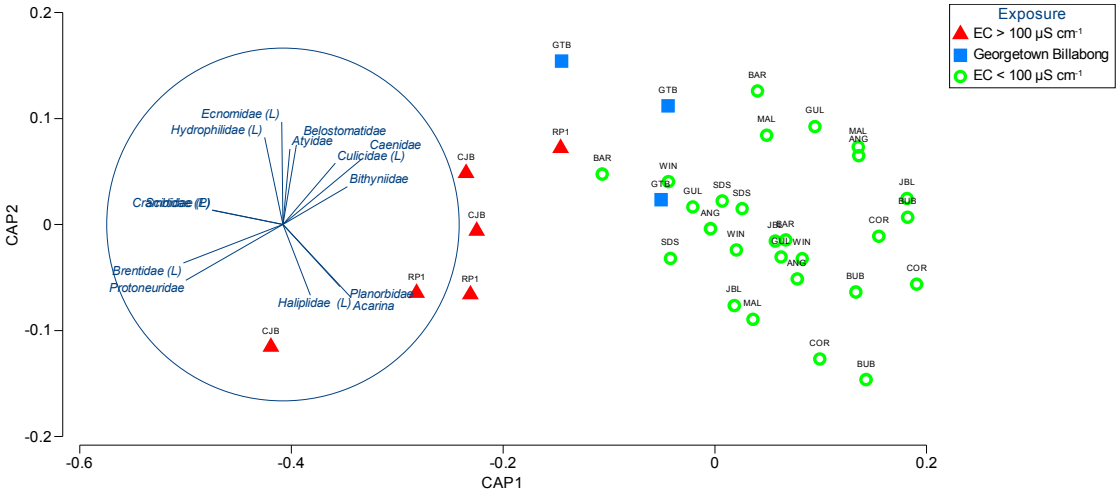


Figure 28 Canonical ordination for the discriminant analysis of three-years macroinvertebrate community data with *a priori* exposure groups

Table 16 CAP results for 2006-2013 sites, showing individual samples that were misclassified

Sample	Original Group	Classification Group
CJB2006	EC > 100 µS cm^{-1}	Georgetown Billabong
RP12011	EC > 100 µS cm^{-1}	Georgetown Billabong
CJB2011	EC > 100 µS cm^{-1}	Georgetown Billabong
GTB2013	Georgetown Billabong	EC < 100 µS cm^{-1}
BAR2006	EC < 100 µS cm^{-1}	Georgetown Billabong
BAR2011	EC < 100 µS cm^{-1}	Georgetown Billabong
WIN2011	EC < 100 µS cm^{-1}	Georgetown Billabong

The misclassifications shown in Table 16 reflect the interspersion for a number of sampling years of either minesite waterbodies or some reference billabongs with GTB, the latter billabong reflecting biological condition intermediate between reference sites and other minesite waterbodies in the biological ordination space (see Figure 28). Taxa correlations in the CAP axes, depicted as vector overlays of Figure 28, are discussed in section 5.5.

CAP was again applied to the primary CAP and equivalent PCA axes to assess goodness of fit and correct allocation of the *a priori* exposure groups to the (same) mainly water quality gradient arising from the PCA described in section 5.1.3.2. The first PCA axis (Figure 15) explains 25% of the variability and as for the all-years CAP provides a proxy mine-exposed, water quality gradient. The resulting CAP-PCA plot is shown in Figure **29**. The relationship between the *a priori* biological classification (Exposure viz EC) and environmental gradient is relatively high (squared canonical correlation 0.62, cf 0.63 for all-years), again providing strong support for water quality effects upon macroinvertebrate communities.

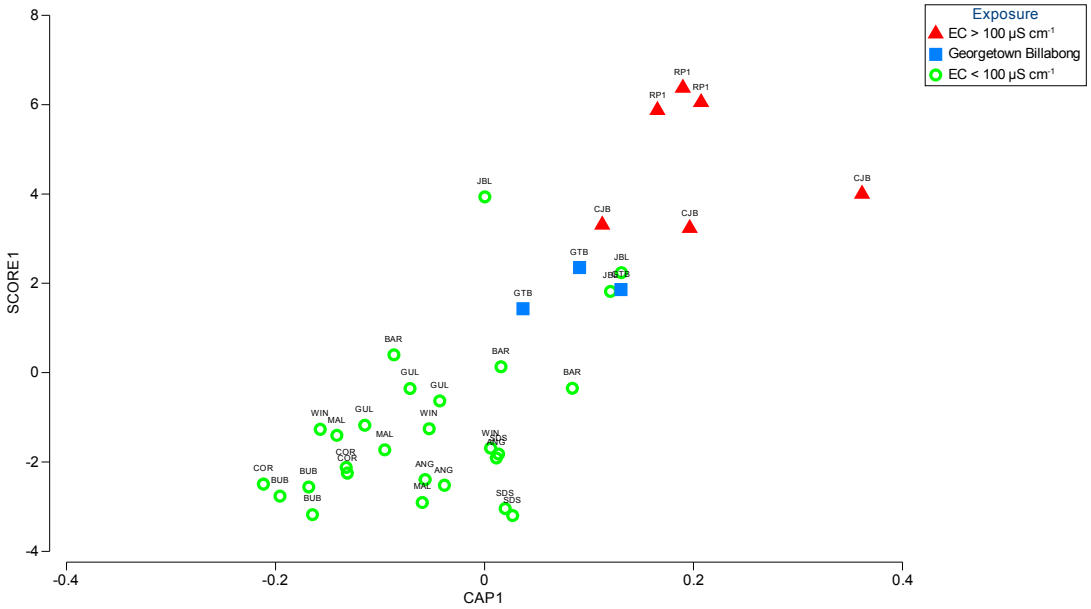


Figure 29 CAP results for 2006-2013 sites using the PCA scores 1 for all available environmental data. EC, Mg, SO_4 and to a lesser extent U main driving factors for this PC1 axis

5.3.2 Correlations between individual taxa and key environmental variables

Correlation analyses and PCA conducted on environmental variables (section 5.1) together with BIOENV and CAP used to seek or corroborate (CAP) environmental correlates of biological assemblage patterns (section 5.3.1), identified several key water quality and habitat variables as possible contributors to the macroinvertebrate responses observed amongst waterbodies. While magnesium, sulfate, EC and U were variously intercorrelated in analyses, Mg is used here as a surrogate for these key minesite water quality contaminants. (Isolating or apportioning the contribution of these CoPCs to biological responses is assessed in section 5.3.3). Habitat structure and complexity were also correlated with biological assemblage patterns in BIOENV, and hence percent aquatic plant cover and number of plant taxa are also considered further.

Spearman rank correlations were performed between standardised macroinvertebrate abundance of each taxon for each sampling occasion (using data from 1995, 1996, 2006, 2011 and 2013), and percent aquatic plant cover, aquatic plant taxa number and Mg. Results and plots of just the significant relationships are provided in this report (see plots in Appendix 6). The abundances of 60 taxa were significantly correlated with one or more of the three environmental variables. A further 48 taxa, many uncommon amongst all samples, observed no correlation at all with any of the variables (plots for these were not included in Appendix 6). Of the 60 significantly correlated taxa, 17 were either terrestrial, rare in occurrence or included pupal forms for which larvae of the same taxon also showed significant correlation. These 17 taxa were not considered further, and just the remaining 43 were plotted and assessed. Of these taxa, 28 observed significant correlations between abundance and aquatic plant cover, 23 between abundance and number of aquatic plant taxa, and 24 between abundance and Mg concentration. There were 7 significant correlations unique to plant percent cover, 6 unique to plant taxa number and 4 unique to Mg.

After Bonferroni correction for Type I error, the abundances of only 25 taxa were significantly correlated with one or more of the three environmental variables.

There were some typical patterns in the biological response-environmental factor plots (Appendix 6), exemplified in Figure 30 below for the pulmonate snail family, Planorbidae. In general, most taxa increased in occurrence and abundance with increasing macrophyte cover (or at least towards high but not total (100%) cover), often reached optimal abundances at an intermediate macrophyte number of taxa and showed various patterns of abundance for Mg concentration.

Despite the significance of many of the correlation plots shown in Appendix 6, considerable heterogeneity in response was observed, indicating that a number of factors other than the independent variable were responsible for the biological responses observed. Quantile regression may be used to identify different relationships within heterogeneous responses (eg Cade & Noon 2003) such as those observed in Appendix 6. For example, Cormier et al (2008) used quantile regression to estimate a water quality stressor level for a proportional reduction in a high (e.g. 90^{th}) percentile of biological response. However, examination and modelling of individual taxon responses to environmental factors were not undertaken in the present study, as the patterns appeared to be interpretable on the basis of empirical ecological knowledge on occupancy-abundance relationships and spatial distribution. Thus it is commonly observed that there is a positive relationship between the number of sites or areas in which a species in a taxonomic assemblage occurs regionally and its local abundance (eg Holt et al 2002).

Occupancy-abundance relationships between macroinvertebrate taxon abundance and macrophyte percent cover, macrophyte species number and Mg concentrations would be demonstrated wherever taxon abundance matched the frequency of sampling according to these environmental factors. Frequency of sampling according to macrophyte percent cover, macrophyte species number and Mg concentrations (0.1 log bins) across sites and times is shown in Figure 30D-F respectively. For planorbid snails (Figures 30A-C) and many other taxa (Appendix 6), the patterns of abundance match the frequency of sampling for the respective habitat or water quality characteristic (Figure 30D-F). Occurrence of planorbid snails (Figure 30C) corresponding to the frequency at which samples were collected (Mg concentration being a proxy for sampling frequency) indicates this taxon is *not* responding to Mg and thus is not sensitive to Mg at the ambient concentrations sampled.

Occupancy abundance has been used to identify niche and habitat characteristics of different aquatic macroinvertebrate species (Verberk et al 2010). It can also potentially assist in identifying the sensitivity ranges of taxa in the present study across a Mg gradient. An advantage of the approach is normalisation of taxon occurrence, ie expected occurrence if there was *no* relationship between abundance and Mg concentration. A qualititative ranking is provided here using the rationale of matching each taxon abundance profile for Mg concentration (Appendix 6), to corresponding expectations of abundance based on frequency of sampling. For example, taxa potentially sensitive to Mg would be expected to be absent or demonstrate low abundances when they would be expected to be high based on frequency of sampling. A qualititative, visual assessment based on inspection of the taxon plots in Appendix 6 suggests the following taxa responses:

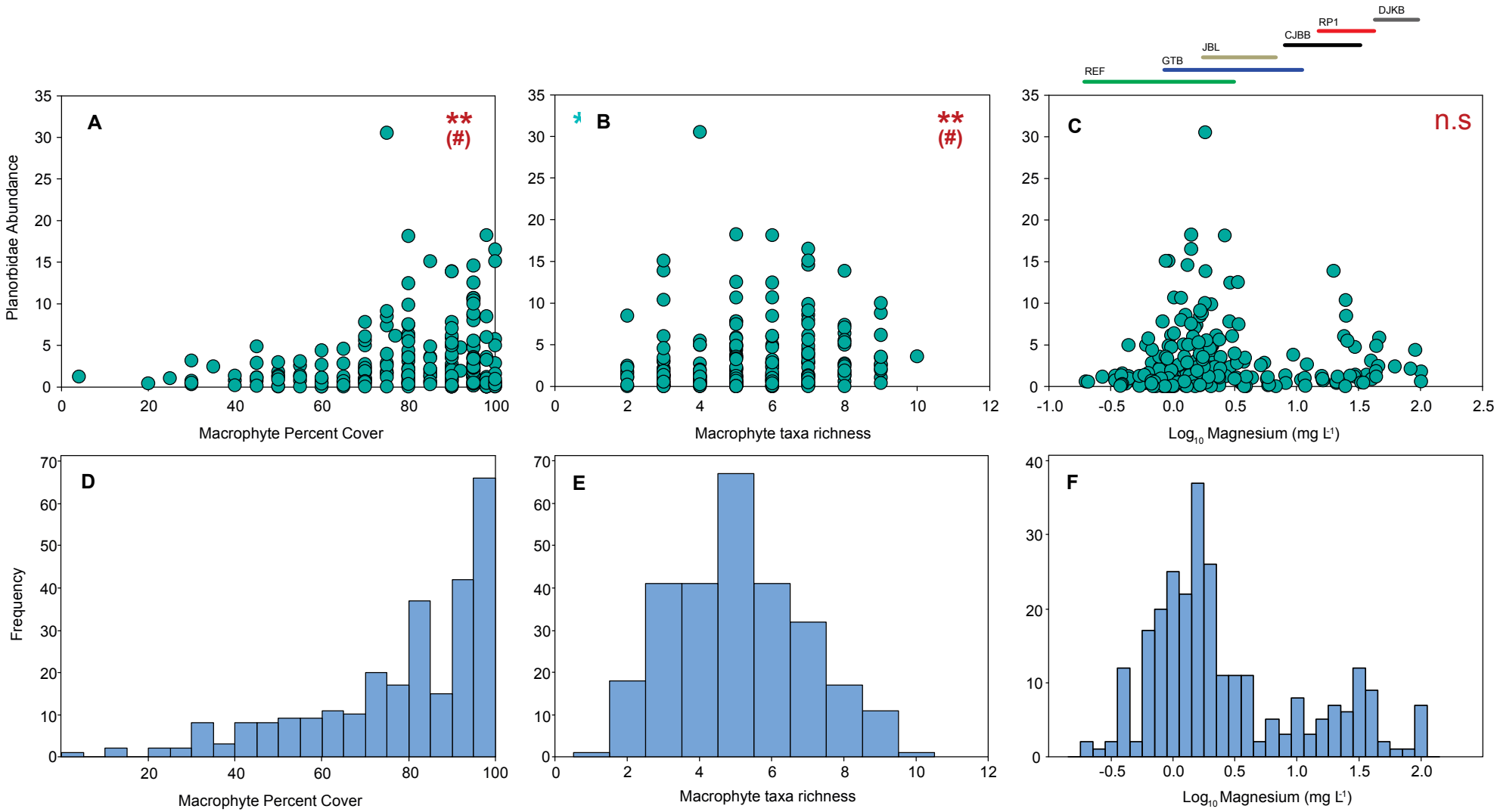


Figure 30 Relationships beween planorbid snail abundance and (A) macrophyte percent cover, (B) macrophyte taxa richness and (C) Mg, for all waterbodies sampled in 1995, 1996, 2006, 2011 and 2013. (D-F) Histograms for macrophyte percent cover, macrophyte taxa richness and Mg (respectively) observed in different waterbodies over the time series indicated in exemplary taxon plot. Waterbody code above Plot C: REF is 'Reference' while other codes provided in Table 1. ** and (#) = Spearman rank correlation significance at P <0.01 and significance after Bonferroni Type 1 error correction, respectively. Green asterisk in Plot B indicates significant quantile (90%) polynomial regression

- *Sensitive taxa*: Bithyniidae, Lymnaeidae, Glossophonidae, Acarina, Hebridae
- *Partial sensitivity*: Palaemonidae, Atyidae
- *Indifferent (no response)*: Nematoda, Ancylidae, Planorbidae, Oribatida, Baetidae, Caenidae, Anisoptera, Naucoriidae, Dyticidae
- *Preference (favoured)*: Hydridae, Oligochaeta, Conchostraca, Zygoptera, Mesoveliidae, Leptoceridae, Nepidae, Hydroptilidae, Scirtidae

Further quantitative assessments of taxon responses were outside the scope of the present study but are clearly warranted. Taxa sensitivities to Mg and other salts are considered further in section 5.5.

Given macrophytes provide important habitat for most feeding guilds of macroinvertebrates, it is unsurprising that such habitat structure and complexity enhances abundances of many taxa (Appendix 6). The extent to which macrophyte richness and cover can account for changes in macroinvertebrate communities of minesite waterbodies over time (see potential for confounding in section 2.1.5) is considered in section 5.3.4.

5.3.3 Relationships between key macroinvertebrate community responses and Mg, EC and U

5.3.3.1 Macroinvertebrate responses in minesite waterbodies relative to reference billabongs

Relationships between key macroinvertebrate response measures and associated Mg, EC and U water quality variables, amongst all minesite waterbodies and all sampling occasions, were examined. Macroinvertebrate response measures included similarity of macroinvertebrate community structure (viz Bray-Curtis similarity measure and ANOSIM R values) between mine water-contaminated and reference waterbodies and number of taxa in mine water-contaminated waterbodies expressed as a proportion the number found in reference waterbodies. Annual antecedent contaminant concentrations, as described in section 3.2.3.2, were used for these relationships as these were deemed to represent the full period of biological exposure. Specifically, responses observed in the April-May period (at sampling) were the consequences of antecedent wet and dry season water quality since macroinvertebrates comprise long-term residents (e.g. molluscs, worms) and short-term colonists (e.g. many aquatic insect life stages).

The ANOSIM R value is sensitive to the degree of dissimilarity within and between groups being compared, and for some exposed-reference comparisons negative R values were observed. This denotes greater dissimilarity among replicates within samples than between samples (see Chapman & Underwood 1999). These occurrences are deemed artifacts that diminish the ability to derive (ANOSIM R) contaminant exposure- response relationships. Nevertheless, the relationships are plotted to indicate general associations.

Number of taxa, similarity and ANOSIM R response measures were plotted with antecedent Mg, EC and U, and regression equations derived for significant relationships. Site DJKB is shown in the plots but as discussed in section 5.1.4, was not included in regression analyses involving EC and Mg because of elevated antecedent U and Mn concentrations in the billabong. (However, see footnote 6 in section 5.1.4.) As also described in section 5.1.4, 2013 biological data for CJBB and RP1 may have been affected by low pH events in the months leading up to sampling so regression analyses were undertaken with and without these data (Mg and U only). Table **17** shows the outputs for the various combinations of regressions undertaken between biological response variables and CoPCs.

Table 17 Linear regression outputs for significant relationships between exposed-reference waterbody-comparisons of number of taxa and similarity and (log) antecedent Mg, EC and U for GTB, CJBB and RP1

Analyte	Biological response	Site combination	Regression parameters			
			Slope	Intercept	R^2	P
Mg	Similarity	With CJBB/RP1 2013	-6.57	55.17	0.158	<0.0001
		Without CJBB/RP1 2013	-4.63	54.67	0.115	0.0007
	Number of taxa	With CJBB/RP1 2013	-6.403	100	0.066	0.008
		Without CJBB/RP1 2013				NS
EC	Similarity	With CJBB/RP1 2013	–8.893	67.849	0.136	<0.0001
	Number of taxa	Without CJBB/RP1 2013	–10.429	116.541	0.069	0.0066
U	Similarity	With CJBB/RP1 2013	–3.943	47.938	0.045	0.0289
		Without CJBB/RP1 2013				NS
	Number of taxa	With CJBB/RP1 2013				NS
		Without CJBB/RP1 2013				NS

For the combination of all sites and times (ie with CJBB and RP1 2013), taxa number, similarity and ANOSIM R response measures are plotted with antecedent Mg, EC and U in Figure 31-33 respectively, with regression equations included where significant relationships were observed. As expected noting the high correlation between Mg and EC (e.g. Appendix 2), plots for these two variables are near-identical (Figure 31 and 32), and hereafter, only Mg is considered.

With increasing Mg concentration and relative to reference waterbodies since 1979, number of taxa declined and community structure became less similar (Figure 31). For the same study duration and with increasing U concentration, number of taxa remained unchanged while community structure became less similar (Figure 33). For both Mg and U, change to community structure (viz similarity) over the contaminant gradient is a more responsive measure than number of taxa. This is not surprising given that number of taxa is also affected by replacement with water quality tolerant taxa (e.g. DJKB see Figure 42). In their Queensland study and review of other Australian studies, Horrigan et al (2005) similarly observed the greater sensitivity of taxa composition to salinity gradients compared to number of taxa.

Removal of relevant CJBB and RP1 2013 data made little difference to the similarity-Mg relationship, while the number of taxa versus Mg relationship became non-significant (Table 17).

Associations between biological responses and U were weak or non-existent, particularly after removal of relevant 2013 data (Table 17). This would indicate that this CoPC was not contributing to the change in exposed-reference waterbody similarity and therefore was not confounding Mg-inferred change. (See also footnote 6 in section 5.1.4.)

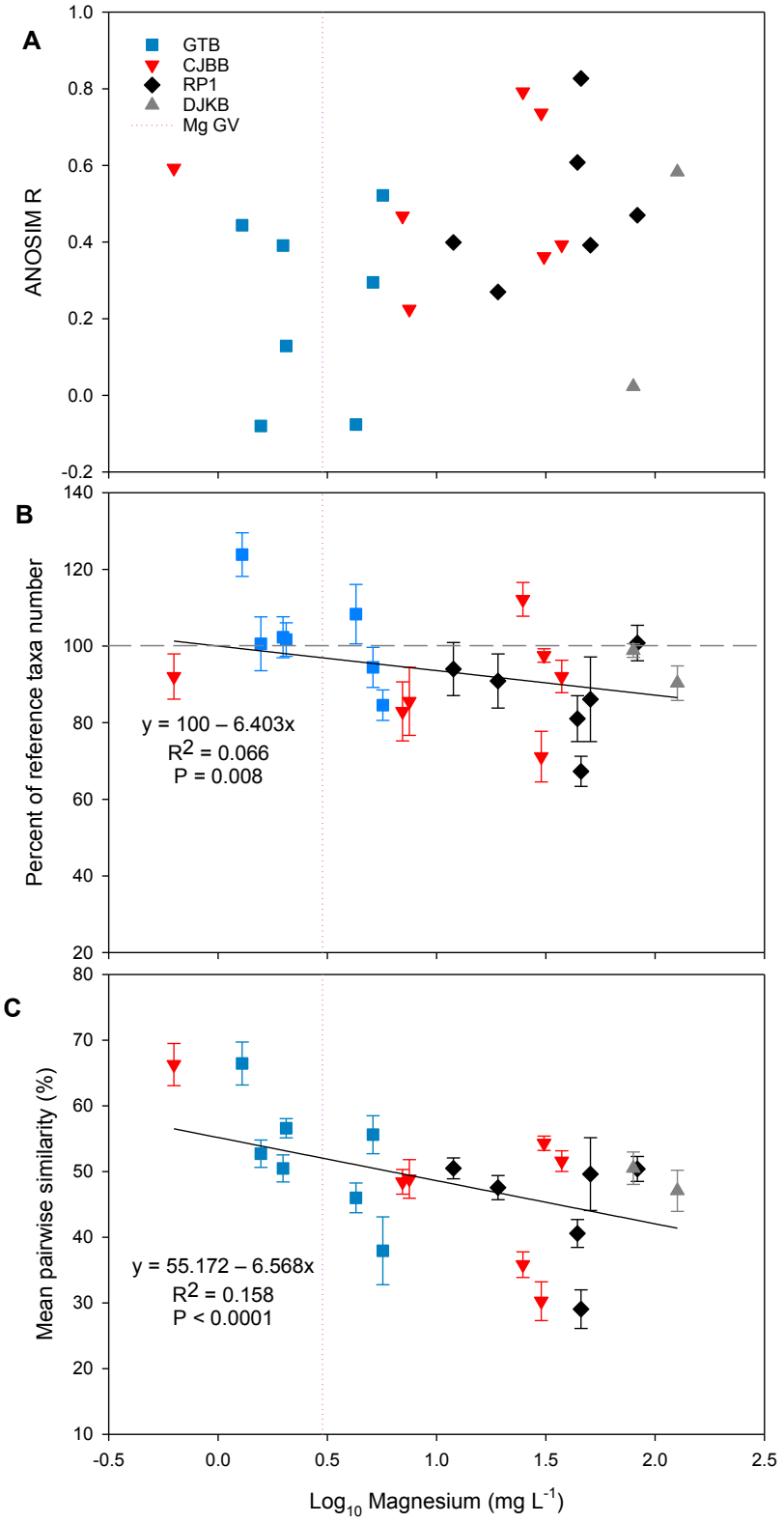


Figure 31 Georgetown Billabong (GTB), Coonjimba Billabong (CJBB) and Ranger Retention Pond 1 (RP1) vs reference waterbodies (A) ANOSIM R values (B) number of taxa, as a percent of control waterbodies number of taxa and (C) mean pairwise similarities (versus reference), in relation to the log10 of the median antecedent wet and dry season Magnesium concentration. Lines fitted according to linear regression where relationship was significant. Laboratory determined guideline value for Mg = 3 mg L^{-1} or log value of ~0.48 (dotted line)

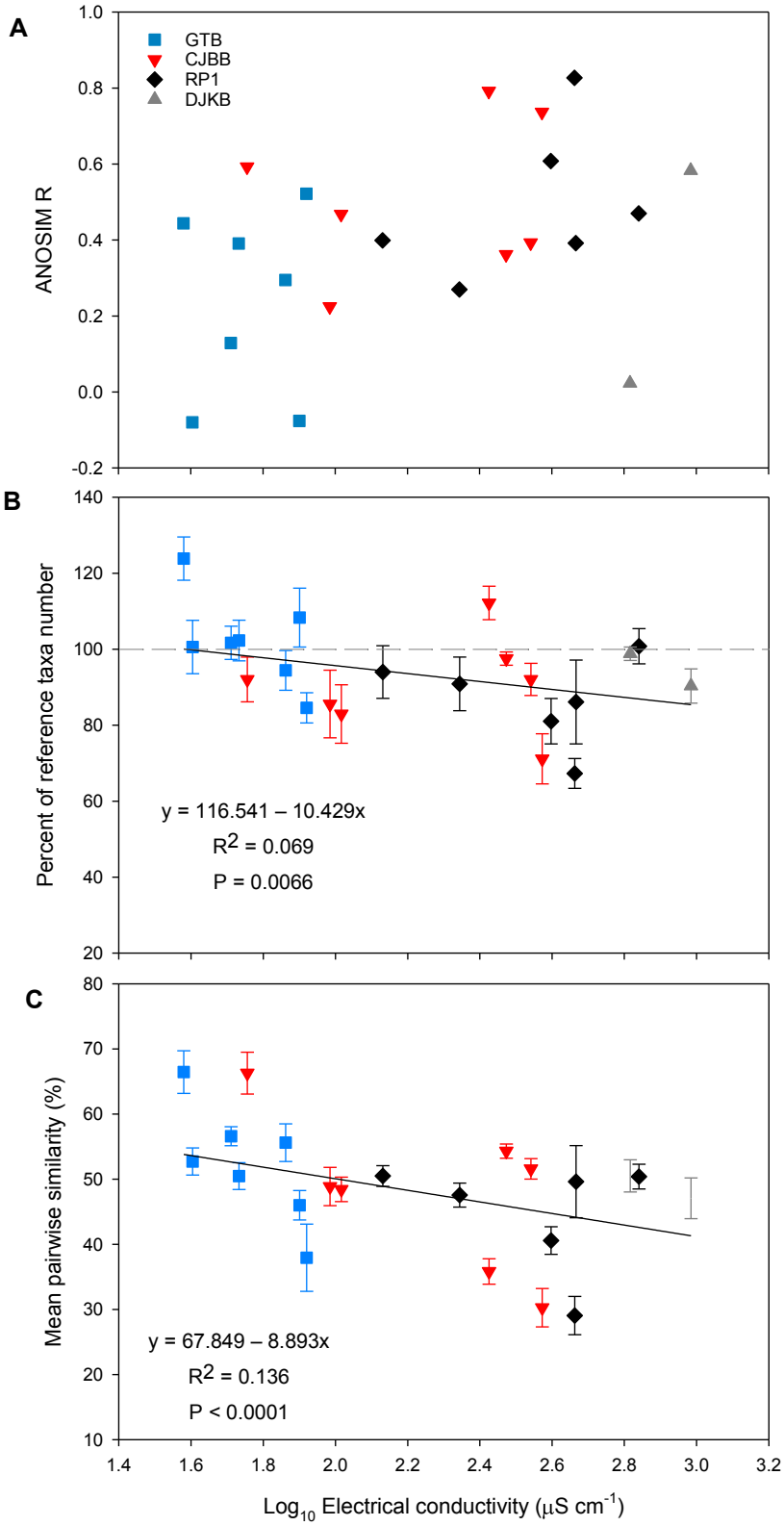


Figure 32 Georgetown Billabong (GTB), Coonjimba Billabong (CJBB) and Ranger Retention Pond 1 (RP1) vs reference waterbody (A) ANOSIM R values (B) number of taxa, as a percent of control waterbodies number of taxa and (C) mean pairwise similarities (versus reference), in relation to the log10 of the median antecedent wet and dry season electrical conductivity. Lines fitted according to linear regression where relationship was significant.

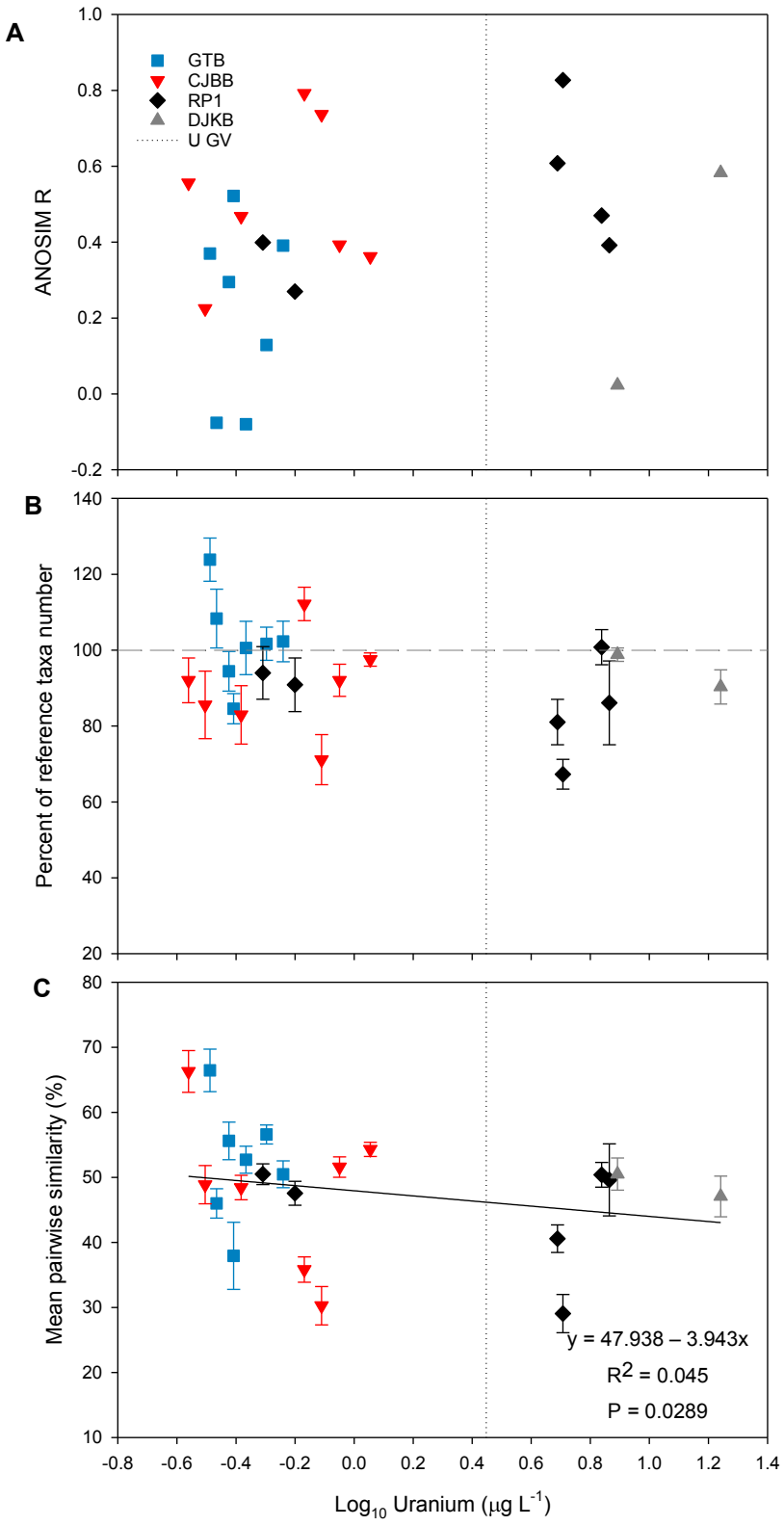


Figure 33 Georgetown Billabong (GTB), Coonjimba Billabong (CJBB) and Ranger Retention Pond 1 (RP1) vs reference waterbody (A) ANOSIM R values (B) number of taxa, as a percent of control waterbodies number of taxa and (C) mean pairwise similarities (versus reference), in relation to the log10 of the median antecedent wet and dry season Uranium concentration. Lines fitted according to linear regression where relationship was significant. Laboratory determined guideline value for U = 2.8 µg L^{-1} or log value of ~0.45 (dotted line)

The derived regression equations for the relationships between biological responses and magnesium have characteristically low R-squared values, with at best, only 16% of the variation in biological response explained by Mg (Table 17). Apart from (unknown) environmental factors other than Mg contributing to the relationships, the large amounts of unexplained variation can result simply from relatively low responsiveness of organisms to Mg arising from exposures to comparatively low ambient concentrations. This is evident when field results are compared to laboratory toxicity exposures to Mg. Full concentration-response relationships for the laboratory species tested (Table 18) required maximum concentrations of between 300 (*Moinodaphnia macleayi*) and 4000 mg L^{-1} Mg (*Mogurnda mogurnda*) (van Dam et al 2010); Mg:Ca ratio of 9:1). These values contrast with the much lower maximum antecedent concentrations of Mg occurring in the waterbodies of just 160 mg L^{-1}. When the laboratory exposures are constrained to a maximum concentration similar to field exposures (ie 160 mg L^{-1}), the amount of unexplained variation in derived concentration-response models increases markedly with similar low association between response and toxicant, as shown in Table 18 (see reduction in R^2 between full concentration range and constrained maximum concentration).

While the derived field regression equations have low predictive precision, the very significant P values (Table 17) and trend (high regression slopes compared to laboratory regression slopes, Table 18) still support a real relationship between Mg and the response variables.

Table 18 Regression outputs for significant linear and non-linear relationships for (i) field results between exposed-reference waterbody-comparisons of number of taxa and similarity and (log) antecedent Mg for GTB, CJBB and RP1, and (ii) laboratory toxicity test species responses to Mg exposure (from van Dam et al 2010). NR = not relevant.

Biological response		**Regression parameters**		
		Concentrations <160 mg L^{-1} (linear)		**All concentrations (non-linear)**
		Slope	**R^2**	**R^2**
Field	**Site combination**			
Similarity	With CJBB/RP1 2013	-6.57	0.158	NR
	Without CJBB/RP1 2013	-4.63	0.115	NR
Number of taxa	With CJBB/RP1 2013	-7.03	0.066	NR
Laboratory	**Species tested**			
Population growth	*Chlorella* sp	-0.07	0.43	0.76
Population growth	*Lemna aequinoctialis*	-0.126	0.21	0.87
Reproduction	*Amerianna cumingi*	-0.3	0.36	0.77
Reproduction	*Moinodaphnia macleayi*	-0.303	0.37	0.88
Population growth	*Hydra viridissima*	-0.05	0.14	0.98
Survival	*Mogurnda mogurnda*	0	No reponse	0.87

5.3.3.2 Macroinvertebrate compositional similarity amongst classes of site EC contamination

Similarity of taxa compositional data between all possible pairs of derived classes of site contamination – viz EC and calculated Mg – are plotted in Figure 34 with underlying data shown in Table 19. (See method of derivation in section 3.4.2.2.) Jaccard and Bray-Curtis similarity was calculated and found to be virtually identical in results, so just Bray-Curtis similarity is plotted and discussed, for consistency with other multivariate results reported

elsewhere in this study. Sample sizes used in similarity calculations are shown in Table 19. The four low EC categories shown in Figure 34 (≤50 µS cm^{-1}) represent virtually all non-minesite waterbodies (i.e. inclusion of just 5 GTB samples).

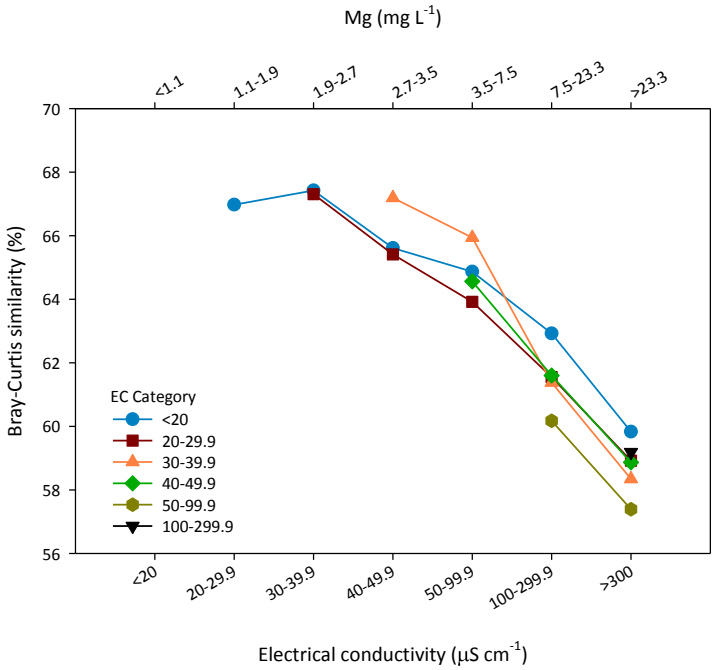


Figure 34 Mean pairwise similarity of macroinvertebrate community composition between EC/Mg categories, all years except 1979 and 2009 and all sites combined.

Table 19 Top right triangle: Bray-Curtis similarity of taxa compositional data between all possible pairs of site EC/Mg classes. Analysis of similarity (ANOSIM) pairwise R statistics and significance shown in brackets. Bottom left triangle (italics): Results of relative family retention (RFR) across 17 samples (per category) between all pairs of EC/Mg categories. (ANOSIM significance denoted * <0.05, ** <0.01, *** <0.001)

EC Category (µS cm^{-1})	**<20**	**20-29.9**	**30-39.9**	**40-49.9**	**50-99.9**	**100-299.9**	**>300**
Mg range (mg L^{-1})	**<1.1**	**1.1-1.9**	**1.9-2.7**	**2.7-3.5**	**3.5-7.5**	**7.5-23.3**	**>23.3**
n (samples)	17	58	32	42	53	45	23
Mean Within similarity	70.6	66.0	69.2	65.7	63.5	61.3	58.5
<20		67 (-.02)	67.4 (0.12**)	65.6 (0.08)	64.9 (-0.04)	62.9 (0.01)	59.8 (0.29***)
20-29.9	*0.95*		67.3 (-0.05)	65.4 (0.04)	63.9 (0.05**)	61.5 (0.18***)	58.9 (0.37***)
30-39.9	*0.96*	*1.02*		67.2 (-0.05)	65.9 (-0.12)	61.4 (0.1)	58.3 (0.34***)
40-49.9	*0.93*	*0.99*	*0.97*		64.6 (-0.03)	61.6 (0.12***)	58.9 (0.33***)
50-99.9	*0.92*	*0.97*	*0.95*	*0.98*		60.2 (0.15**)	57.4 (0.32***)
100-299.9	*0.89*	*0.93*	*0.89*	*0.94*	*0.90*		59.2 (0.09*)
>300	*0.85*	*0.89*	*0.84*	*0.90*	*0.90*	*0.97*	

As described in section 3.4.2.2, the similarity plots of Figure 34 depict large scale – spatial and temporal – change in community composition along an EC gradient, using pooled, multiple samples from within similar levels of EC. Unlike the comparison of community structure of minesite waterbodies relative to reference waterbodies for each year of sampling and the relationship between derived similarity and EC/Mg – as shown in the previous section (ie Figures 31 and 32), the multiple pooled samples shown in Figure 34 are not constrained to years and waterbody exposure type (minesite vs reference). Kefford et al (2010) reasoned that pooling of large numbers of samples in this way is more likely to lessen confounding by other factors because habitat and other factors should be represented across the contaminant gradient. Such reasoning is supported in the present study with clear responses observed across the contaminant gradient. Thus samples with similar EC in the similarity plots shown in Figure 34 have higher similarity than samples with dissimilar concentrations, providing evidence that the main contributor to EC, Mg, is affecting taxa composition.

ANOSIM was performed on the underlying dataset used to derive the similarity plots to determine the strength of compositional differences between all pairwise contaminant comparisons (Table 19). Significant separation ($P<0.05$) was consistently found for all comparisons involving EC >100 $\mu S\ cm^{-1}$. However, at best the degree of separation (ANOSIM R value) was only moderate (maximum value of 0.37 for categories 20–29.9 vs >300 $\mu S\ cm^{-1}$, Table 19). These results are not surprising given the exposures to comparatively low ambient concentrations of Mg – discussed above (section 5.3.3.1).

5.3.4 Consequences of aquatic plant – macroinvertebrate relationships

In section 5.3.2 significant (positive) relationships were reported between macrophyte cover and taxa number and the abundances of a large number of macroinvertebrate taxa. The causal association is not in dispute given that aquatic plants offer important habitat for macroinvertebrate communities. When inferring water quality-related changes to macroinvertebrate communities, however, there is a need to assess whether, and/or the extent to which, macroinvertebrate responses are (also) related to plant community structure. There are two possibilities:

1 Aquatic plants themselves are sensitive to water quality and have either responded in a similar manner to macroinvertebrates, or macroinvertebrates have not responded but their loss is due to loss of habitat (indirect effect). In these cases, changes over time in the aquatic plant communities of minesite waterbodies relative to reference waterbodies could explain similar shifts over time in macroinvertebrates.

2 Aquatic plants are relatively indifferent to water quality, but nevertheless, macroinvertebrate responses in onsite waterbodies (relative to reference waterbodies) are collinearly (with Mg) responding to plant structure and composition.

Together with some historical information on aquatic plant communities of the minesite billabongs, these aspects are considered in turn below.

5.3.4.1 Responses of aquatic plant communities to changes in water quality

Appendices A7.2 and A7.3 provide results of studies examining changes in aquatic plant communities of minesite and reference waterbodies of the ARR. Key findings from these studies, together with additional analyses conducted here, include:

1 Aquatic plant compositional differences between minesite and reference waterbodies were small while relative differences in composition, plant form and number of taxa

over a time series between 1993 and 2014 have remained essentially unchanged (Appendices A7.2 & A7.3).

2 While significant negative relationships were found between both (1) plant percent cover and Mg and (2) number of plant taxa and Mg across combined reference and minesite waterbodies (1995-2013) (Figure 35A), no significant relationships were found between:

   a. Both plant percent cover and number of taxa, and Mg, using just data from minesite waterbodies where strong Mg gradients occur (Figure 35B). (However, there are indications that the upper limits of number of plant taxa are constrained by Mg – likely revealed in quantile regression of the 90th regression quantile); and

   b. Both plant number of taxa and similarity relative to reference waterbodies, and Mg, in GTB (even though macroinvertebrate responses changed over the same time series) (Appendix A7.3, Figures A7.3.2 & A7.3.3).

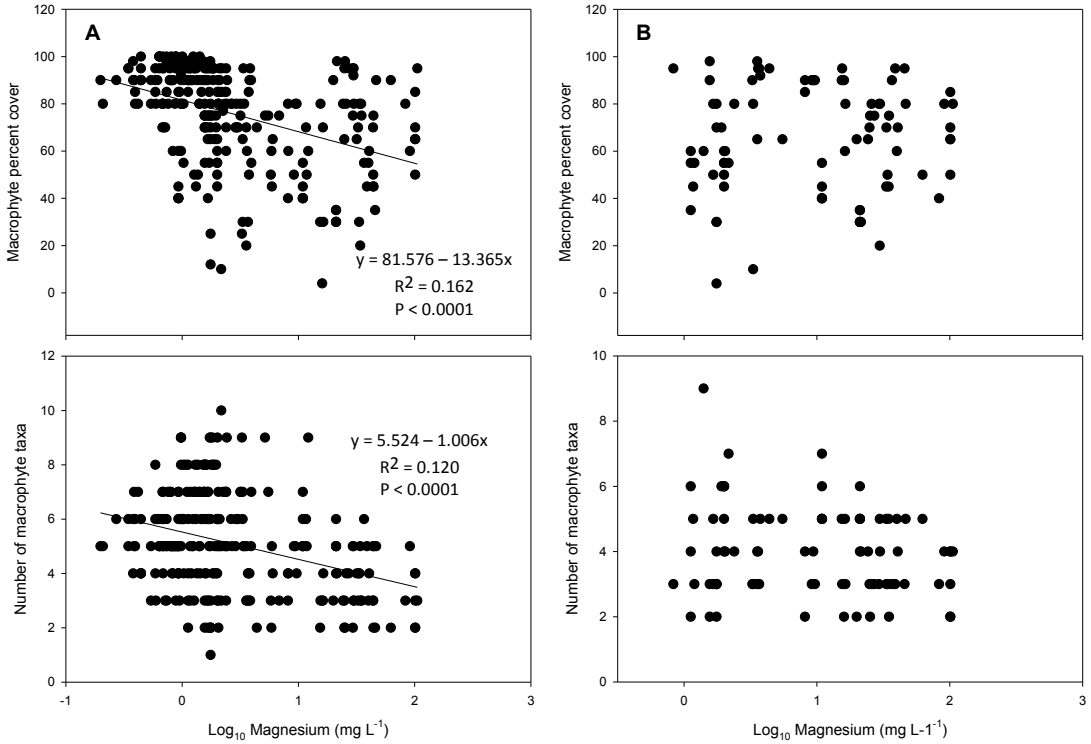


Figure 35 Macrophyte percent cover and number of taxa in relation to magnesium concentration for (A) all sites and years and (B) all years but no reference sites.

5.3.4.2 Macroinvertebrate responses in minesite waterbodies in relation to aquatic plant cover

Macroinvertebrate number of taxa and similarity relative to reference waterbodies are plotted with macrophyte percent cover for the minesite waterbodies in Figure 36. (Data for CJBB and RP1 2013 were removed – see rationale in section 5.3.3.)

No relationship was found between macroinvertebrate similarity and plant percent cover, though a significant positive relationship was found between macroinvertebrate number of taxa (relative to reference waterbodies) and plant percent cover (Figure 35). Scrutiny of the number of taxa versus plant percent cover relationship shows its significance is largely driven by data from CJBB. Data for 1979 CJBB and GTB were not included in the plot because no associated aquatic plant data were gathered at the times of sampling. However, given the observations from section 2.1.5 above ('Changes in aquatic plant communities

from pre-mining to commencement of mining'), it can be assumed that plant percent cover would have been low in 1979 for both waterbodies. Thus, had high relative macroinvertebrate number of taxa in 1979 (92% for CJBB and 122% for GTB) been included with associated low plant percent cover in Figure 36, no significant relationship would have resulted.

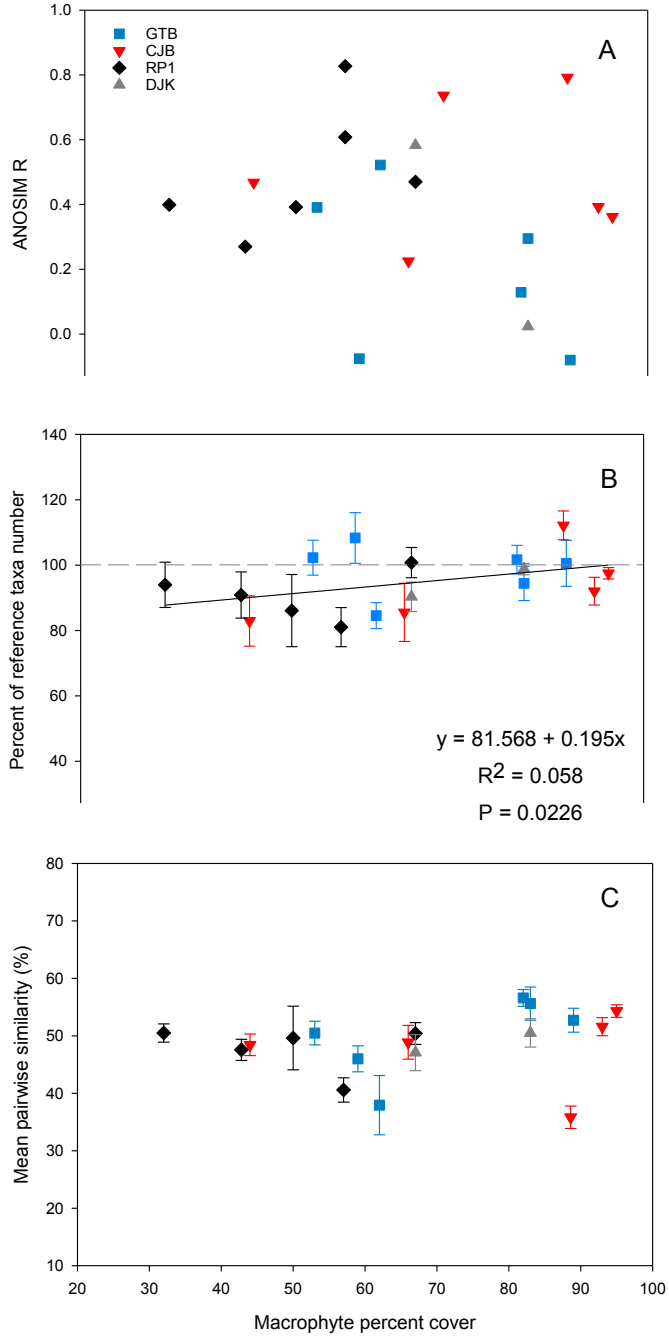


Figure 36 Georgetown Billabong (GTB), Coonjimba Billabong (CJB) and Ranger Retention Pond 1 (RP1) vs reference waterbody (A) ANOSIM R values (B) number of taxa, as a percent of control waterbodies number of taxa and (C) mean pairwise similarities (versus reference) for macroinvertebrates, in relation to the percent cover of macrophytes. CJBB and RP1 data for 2013 removed. Lines fitted according to linear regression where relationship was significant.

Nevertheless, it is not unreasonable to assume a relative increase in macroinvertebrate diversity (viz number of taxa) with increase in available habitat and for now, plant cover cannot be eliminated as a co-contributor, with Mg, to change in macroinvertebrate number

of taxa *amongst all mine site waterbodies* over time. However and regardless of whether or not relative macroinvertebrate number of taxa in minesite waterbodies was enhanced by increasing aquatic plant cover, macroinvertebrate number of taxa was not a particularly sensitive biological response measure. As noted in section 5.3.3.1, number of taxa may remain unchanged when water quality sensitive taxa were replaced with tolerant taxa; such taxa may be favoured by aquatic plants. Moreover, conditions in CJBB and RP1 favouring tolerance to salts may inure otherwise water quality sensitive taxa (section 3.3.2); this is considered further in section 5.6.

5.4 Threshold analysis applied to macroinvertebrate community data

Results to this point in the report indicate that changes in water quality, particularly increase in $MgSO_4$ in minesite waterbodies over time, provide the most plausible explanation for associated changes to macroinvertebrate communities of minesite waterbodies relative to reference billabongs. In this section, a threshold biological response to Mg is determined across the water quality gradient using data from all minesite waterbodies and from GTB alone. The results for GTB, in particular, provide a useful opportunity to determine a threshold biological response to Mg. This is because:

1 When combined with other sites, GTB biological responses were seen to be part of a continuum across a gradient of Mg (i.e. least contaminated end of the gradient);

2 The 34-year sampling period in GTB has spanned a period of negligible contamination by mine wastewaters for most of the record, to more significant contamination in recent sampling, with an associated biological response over this gradient. Thus, the observations encompass desirable experimental design principles of Before, After, Control, Impact (BACI);

3 GTB was not confounded by other water and sediment CoPCs, including U in water and sediment, Mn, and had no acid sulfate sediments (with an absence of early wet season acid events). Macroinvertebrate responses were also unrelated to aquatic plant habitat and other catchment stressors; and

4 GTB fauna appeared not to display tolerance, unlike the fauna of CJBB and RP1 (see section 5.6), providing more conservative estimates of adverse response.

In using GTB for threshold analysis, it is accepted that the results for this billabong alone (described below) hold limited inferential power in attributing increases in Mg to changes in macroinvertebrate communities because the effects upon biota are confined to just one year of sampling (2011). Similarly, concentration-response relationships depend upon sufficient representation of negligible to large effects for full characterisation, and hence accurate hazardous concentration determination. Collective results from minesite waterbodies (ie combined) provide the main line of evidence in water-related causation. HC determination from just GTB also requires evaluation and assessment against the wider concentration-response relationships available from all minesite waterbodies.

5.4.1 Concentration-response relationships in GTB

ANOSIM R, number of taxa and similarity for GTB macroinvertebrate communities relative to reference waterbodies since 1979, are plotted with antecedent Mg concentration in Figure 37.

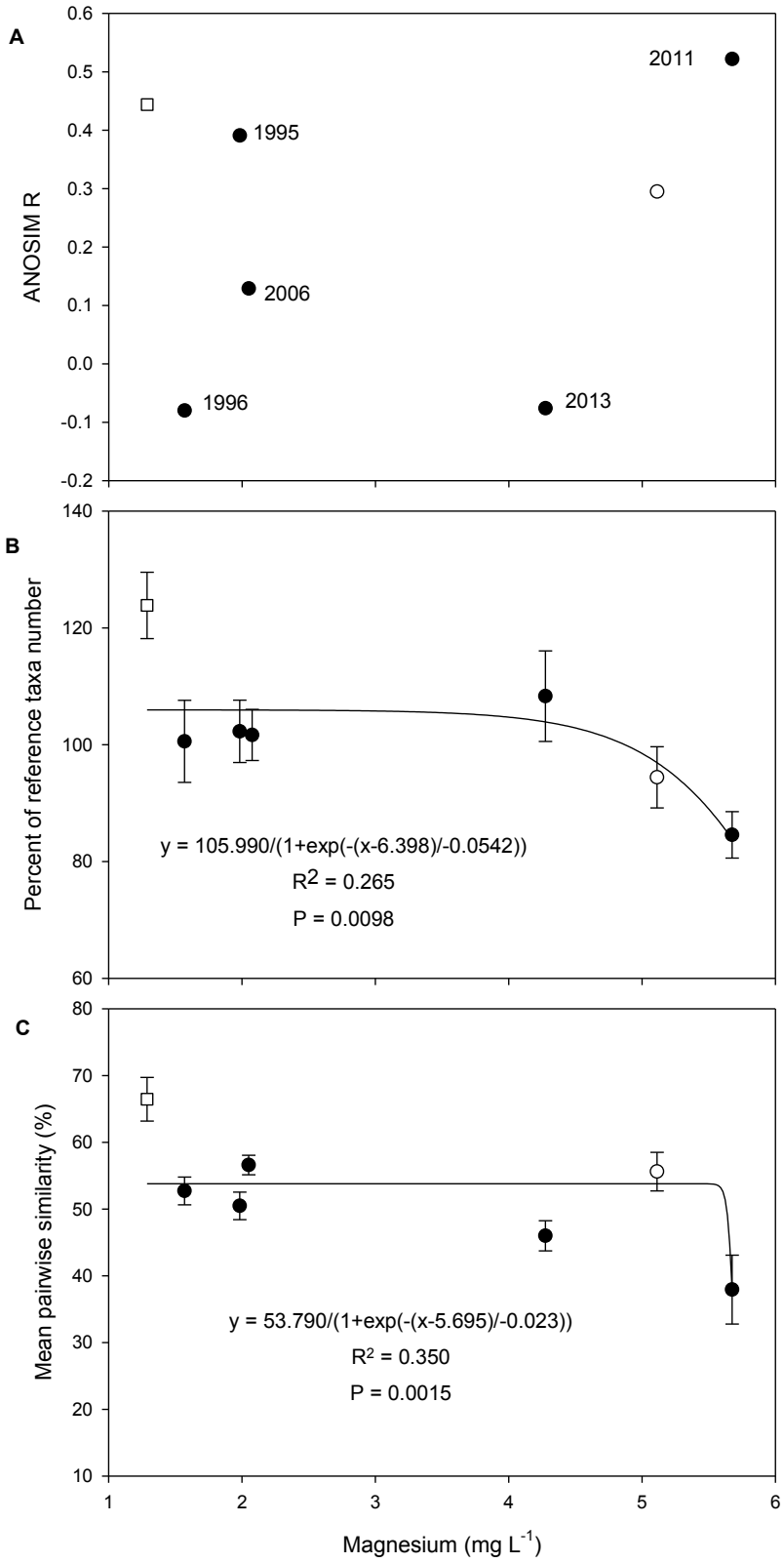


Figure 37 (A) ANOSIM R, (B) Mean (±SE) macroinvertebrate number of taxa (as % of mean reference waterbody number of taxa) and (C) Mean pairwise similarity for Georgetown Billabong sites in relation to median antecedent wet and dry season Mg concentration. (Open symbols indicate 1979 (square) and 2009 (circle) sampling where sampling effort in reference waterbodies was reduced compared to other years. Lines fitted for all displayed data according to sigmoidal model where relationship was significant.

GTB macroinvertebrate communities were similar to reference waterbodies prior to the 2009-2011 period. However, in 2011 GTB macroinvertebrate responses became well separated from those in reference billabongs (number of taxa, community structure) in association with an increase in antecedent Mg concentrations. In 2013, some recovery was apparent with improvement in water quality in GTB and a return to similar biological responses as in reference billabongs (Figure 37). A water quality-related impact to the biota in 2011 provides the best explanation for the 2011 observations. HCs for 5% and 1% for the response-Mg relationships are provided in Table 20.

Table 20 'Hazardous' concentrations (HCx) for 5% and 1% in the logistic fit for the response-Mg relationships.

Threshold criterion	Threshold (mg L^{-1})
GTB percent of reference number of taxa:	
HC5	4.8
HC1	3.9
GTB community similarity to reference:	
HC5	5.6
HC1	5.6

5.4.2 Relative family retention

Calculation of Relative species (or family) retention (RFR) was described in section 3.4.5.2. It is derived from taxa compositional similarity data within and between all possible pairs of derived classes of site contamination, as shown in Table 19. RFR between two contaminant categories is calculated as between-group similarity / within-group similarity for the lower concentration group being compared. RFR results are shown in Table 19. Both Jaccard and Bray-Curtis similarity was used for RFR calculation and found to be virtually identical in results, so just Bray-Curtis-derived RFR results are shown (Table 19) and discussed.

As described in section 5.3.3.2, very few minesite waterbody replicates were associated with EC <50 μS cm^{-1}. Hence small and consistent decreases in RFR from those values derived between pairwise EC groups less than this EC value are presumed to be indicative of EC/Mg-related change to community composition.

From Table 19, at the lowest EC associated with minewater contamination, ie 50-100 μS cm^{-1} (or 3.5-7.5 mg L^{-1} Mg) there is a consistent decline in similarity between corresponding EC groups <50 μS cm^{-1} representing non-minesite waterbodies of between 1 and 5%. Thus 1-5% of taxa are only found in one or other EC category and so there is up to a 5% turnover of families as EC reaches these EC values. This EC range (50-100 μS cm^{-1}) is consistent with that derived for GTB above (section 5.4.1).

Modelling of the relationship between similarity data from all possible pairs of classes of site contamination and EC/Mg (Figure 34) may better quantify hazardous concentrations. Such regressions will violate the assumption of statistical independence, as discussed in section 3.4.5.1, but would not necessarily affect predictions and derivations of hazardous concentrations themselves. The data in Figure 34 were used to derive a non-linear 3-parameter sigmoidal regression, using the median of the calculated Mg values within each contaminant class to represent concentration values. The resulting regression equation is:

$$\text{Similarity} = 132.53/(1+\exp(-(\text{Mg}-9.88\text{E}-09)/-126.63)),\ R^2 = 0.834,\ P = <0.0001$$

HC5 and HC1 values were 14.3 and 4.1 mg L^{-1} respectively. This regression has marked improvement in the R^2 value over those derived from GTB-only data (section 5.4.1) because higher maximum contaminant values are available with the inclusion of CJBB and RP1 data.

5.4.3 Field-based species sensitivity distributions (SSDs)

The approach and methods for deriving empirical cumulative distribution functions were provided in section 3.4.5.3. Two SSDs, using data from 1995, 1996, 2006, 2011 and 2013, were derived, one using data from all waterbodies and the other using data from just GTB. Log logistic models were fitted to the SSDs, the model for all waterbodies shown in Figure 38 and that for GTB shown in Figure 39. The all-waterbodies SSD excluded CJBB and RP1 from 2013, with low pH measured a few months prior to sampling (see section 5.1.4).

Taxa are plotted against the maximum concentration of Mg that they have been observed/recorded, with the contaminant concentration values for each taxon ordered from lowest to highest. 'Hazardous' concentrations (HCx), were determined at the 5th and 1st percentile of the distribution of taxa. For all waterbodies, the HC5 and HC1 were estimated at 11.2 and 4.7 mg L^{-1} respectively and for GTB at 7.6 and 5 mg L^{-1} Mg respectively (Figure 38 and 39 respectively). From section 5.6 below, there was evidence for contaminant-induced tolerance in the fauna of CJBB and RP1, not evident in GTB. Thus, even though the all-waterbodies model was a better fit of the data, the EC5 and EC1 for GTB were regarded as the more protective and useful estimates of threshold adverse response.

In general, the SSDs constructed in this study are not deemed particularly useful for GV derivations. Limitations are alluded to in the sections above. Firstly, apparent tolerance acquired by some taxa in the most contaminated mine site waterbodies (section 5.6) adds complexity to the effect-concentration relationships. Secondly, the contaminant gradient was incomplete as a large number of taxa occurred at the maximum concentration measured in the study, while there was low representation of concentration and associated effects data for some intermediate Mg concentrations across the contaminant range (see Figure 30F). Following from the second limitation, selecting the upper-most concentration at which a taxon occurred in waterbodies did not take into account any reduction in abundance across the contaminant range. The method of Cormier & Suter (2013a), estimating the concentration that results in near-extirpation (e.g. XC95) of each taxon, would improve modelling by reducing error and variability evident in the tail-end of the concentration-response relationship and enabling extrapolation beyond maximum field concentrations at which taxa are still viable.

5.4.4 TITAN indicator value scores

The Threshold Indicator Taxa ANalyis (TITAN) method, described in section 3.4.5.4, was used to detect change in taxa distributions along the Mg gradients. The method was applied to all sites and attempted for GTB alone, using standardised abundance data from 1995, 1996, 2006, 2011 and 2013. TITAN distinguishes taxa losses (z-) and gains (z+) with increasing Mg concentrations, with synchrony amongst taxa change points providing evidence for community thresholds.

Results for TITAN analysis of all-sites data for Mg are provided in Table 21, while a plot showing significant indicator taxa change points is shown in Figure 40.

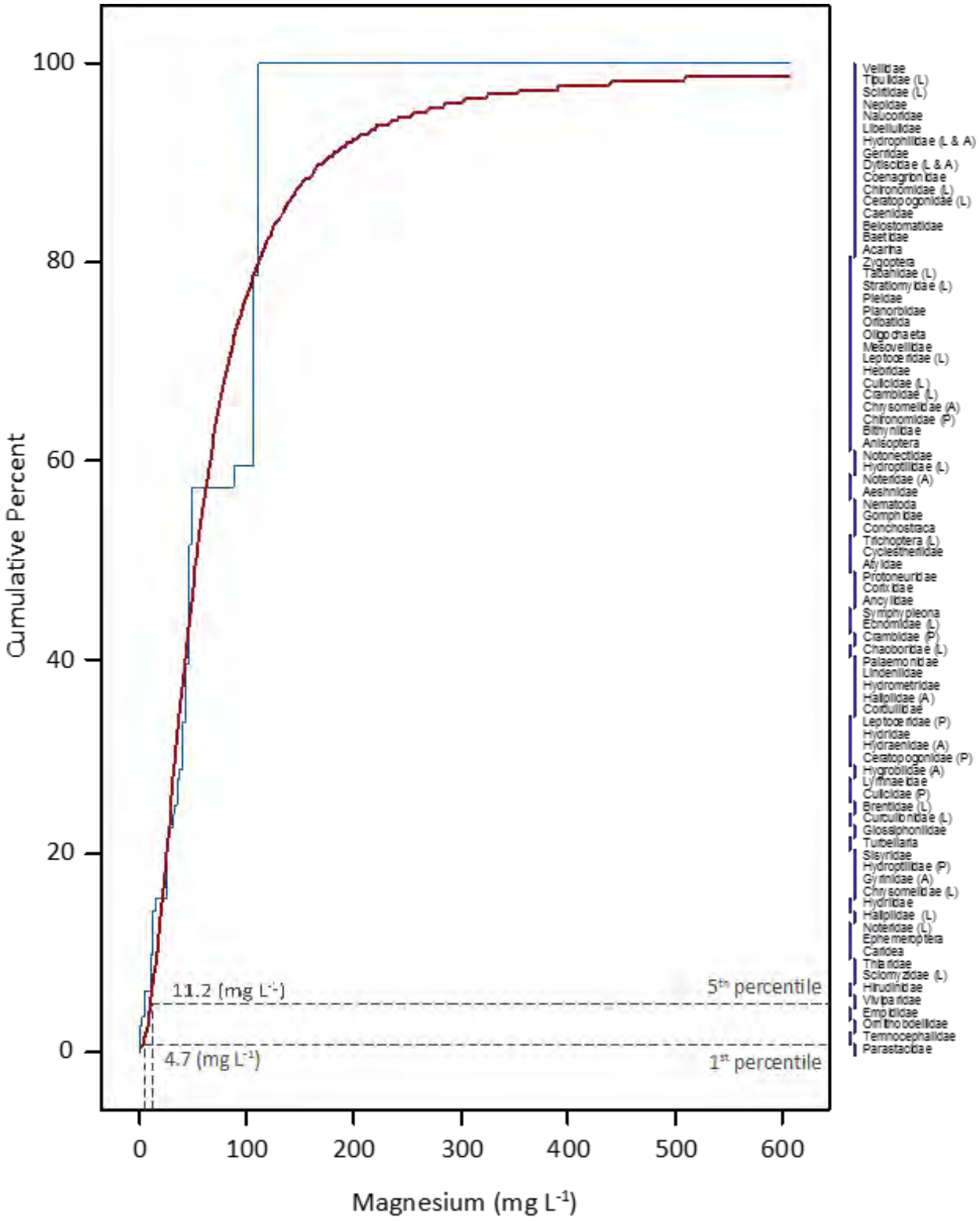


Figure 38 Empirical cumulative distribution functions (blue line) and fitted (redline, log logistic distribution) based on extirpation concentrations (upper magnesium tolerances) for taxa sampled across all years in all billabongs. The dotted line at the 1st and 5th percentile (HC1 and HC5) indicates the 99% and 95% species protection level. Taxa used are listed along the side from those with the highest extirpation values to the lowest.

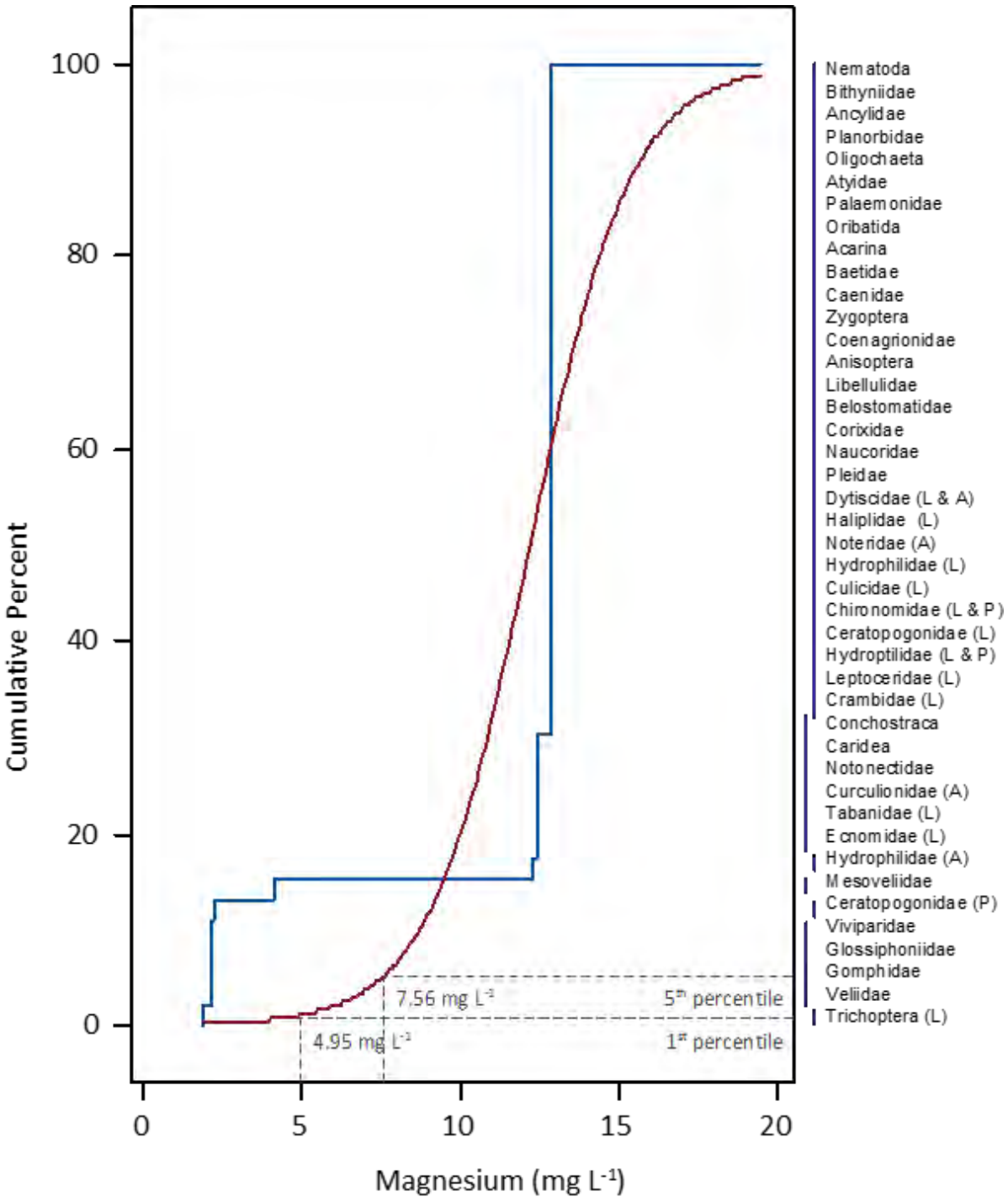


Figure 39 Empirical cumulative distribution functions (blue line) and fitted (redline, logistic distribution) based on extirpation concentrations (upper magnesium tolerances) for taxa sampled across all years in Georgetown Billabong. The dotted line at the 1st and 5th percentile (HC1 and HC5) indicates the 99% and 95% species protection level. Taxa used are listed along the side from those with the highest extirpation values to the lowest.

Table 21 TITAN calculated change points for magnesium applied to macroinvertebrate data from all sites and years 1995, 1996, 2006, 2011 and 2013.

Analysis	Change Point (mg L^{-1} Mg)	Confidence level				
		0.05	0.1	0.5	0.9	0.95
sumz-	**2.37**	0.69	0.7	1.18	2.41	2.46
sumz+	22.53	4.27	11.7	22.52	24.32	24.52
fsumz-	**1.34**	1.17	1.25	1.34	2.51	5.63
fsumz+	22.32	12.62	18.98	22.32	24.2	24.52

sumz- = taxa disappearing from samples with increasing Mg concentrations;
sumz+ = taxa appearing in samples with increasing Mg concentrations;
fsumz- = pure and reliable taxa disappearing from samples with increasing Mg concentrations;
fsumz+ = pure and reliable taxa appearing from samples with increasing Mg concentrations.

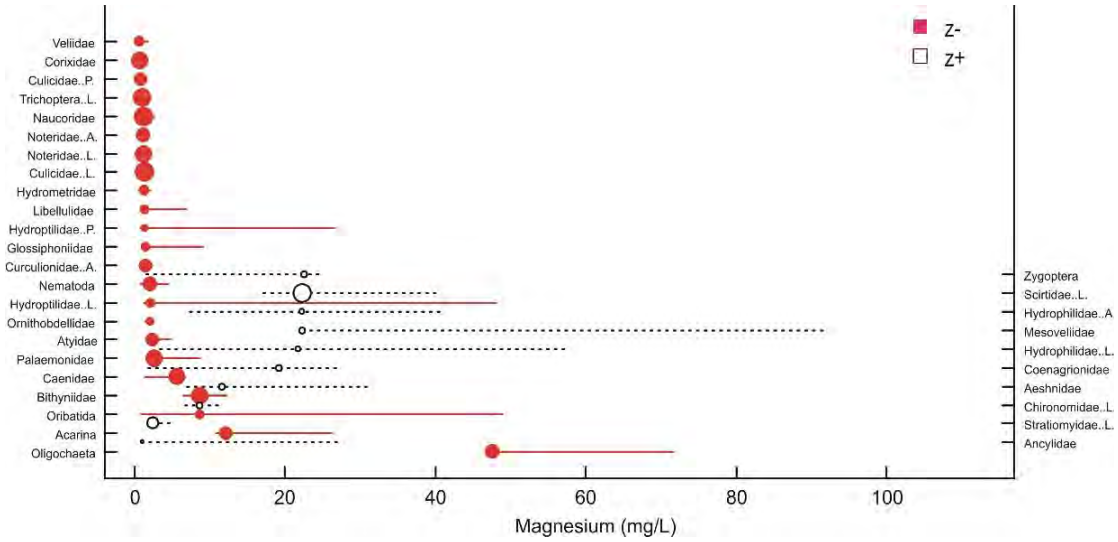


Figure 40 TITAN plot showing significant indicator taxa change points for magnesium for all sites and most years

The rows of Table 21 include the change points for declining taxa (sumz-), increasing taxa (sumz+), and corresponding scores using filtered versions of both sums. Filtering is based on the sum(z) scores using only those taxa that are determined to be "pure and reliable indicators" (https://cran.r-project.org/web/packages/TITAN2/vignettes/glades.TITAN.html). Filtered scores are provided as a way to determine whether "impure or unreliable indicator taxa are contributing substantially to the pattern of sum(z) scores" (Baker & King 2010). Such contribution is evident in Table 21 where the filtered sumz- is equivalent to the median confidence interval (C.I), suggesting the value is more stable, and that the thresholds should be based on the filtered (fsumz) scores.

Using data for all waterbodies and all years, the TITAN Mg change point for taxa disappearing is near background concentrations (1.34 mg L^{-1}) (Table 21). Examining the pattern of disappearance and appearance of taxa, there were 23 pure and reliable indicator taxa disappearing across the Mg gradient (z-) and 10 appearing (z+) (Figure 40). These indicator taxa of change are considered further in section 5.5.

The TITAN community-level output is also provided in Figure 41. Different peaks in taxa loss or gain indicate different change points. These change points are not dissimilar to the individual taxon versus Mg plots shown in Appendix 6. In section 5.3.2 polymodality in the taxa plots was attributed mainly to missing (unmeasured) effects and concentration

data for some intermediate Mg concentrations across the contaminant range. Thus at an assemblage level, taxa occurrence may be responding predominately to the frequency at which samples were collected and hence the different peaks may simply be artefacts of sampling intensity across the Mg concentration range. In turn, such bi- or polymodality in the peaks in taxa loss or gain (Figure 41) may have resulted in an overly-conservative TITAN Mg change point for taxa disappearing, limiting the usefulness of the method.

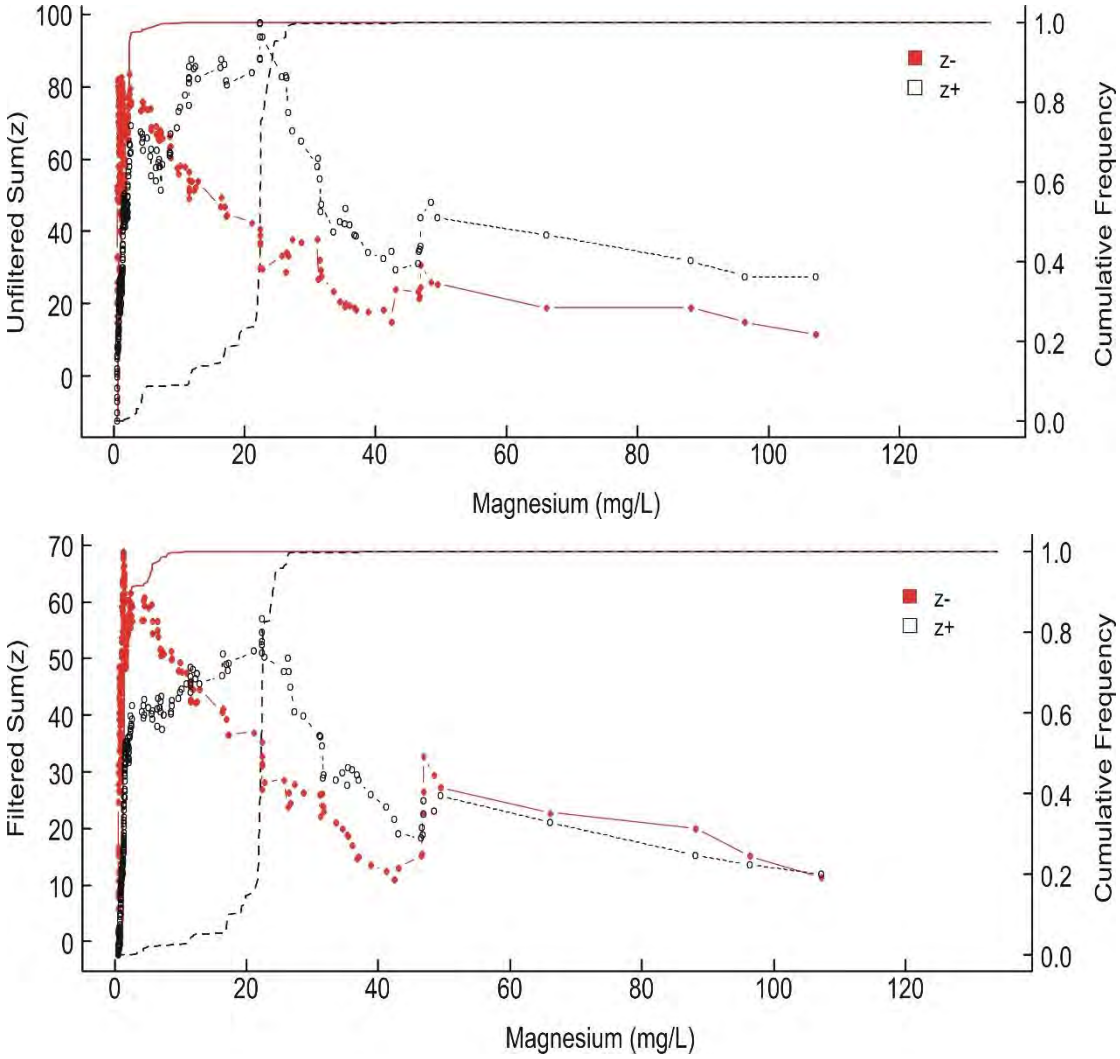


Figure 41 TITAN2 sum(z−) and sum(z+) values for all possible change points in response to magnesium. Unfiltered (upper plot) and Filtered (lower plot). The filled and hollow symbols denote the magnitude of summed z scores of increasing (z+) or decreasing (z-) taxa. Peaks in the values indicate points along the environmental gradient that produce large amounts of change in community composition and/or structure. Plateaus denote regions of change. Solid and dashed lines are cumulative frequency distributions (CFDs) of sum(z-) and sum(z+) maxima (respectively) across bootstrap replicates. Vertical CFDs indicate narrow uncertainty about where the maximum chnage occurs, sloping or stair-step CFDs suggest broad uncertainty regarding the location of maximum change (from https://cran.r-project.org/web/packages/TITAN2/vignettes/ glades.TITAN.html).

TITAN analysis for GTB provided a filtered change point of 1.37 mg L^{-1}. However, there was a low number of pure and reliable taxa, and so this analysis is not considered sufficiently robust to report. This may be related to the Mg concentration gradient in GTB not being sufficiently strong enough to identify pure and reliable indicator taxa disappearing across the gradient.

5.4.5 Summary of threshold analysis applied to macroinvertebrate community data

Hazardous concentrations and thresholds from the preceding analyses are summarised in Table 22. HCxs based on relative number of taxa and similarity data for GTB, and similarity amongst classes of site contamination ("All sites and times similarity"), are regarded as the most reliable field-effects estimates and lines of evidence informing closure criteria, amongst other laboratory and field data used for this purpose.

Table 22 Thresholds and effect concentrations for magnesium applied to macroinvertebrate data from all sites and GTB alone.

Threshold response and calculation	HC_X or threshold for Mg (mg L^{-1})			
	20%	10%	5%	1%
GTB similarity	5.6	5.6	5.6	5.6
GTB number of taxa	5.6	5.2	4.8	3.9
All sites and times similarity	51.4	26.9	14.3	4.1
SSD: All sites	25.6	16.7	11.2	4.7
SSD: GTB	10	8.7	7.6	5.0
TITAN: All minesites, filtered (5-95% CI)	1.34 (1.17– 5.63)			
TITAN: All minesites, unfiltered (5-95% CI)	2.37 (0.69–2.46)			

5.5 Taxa distinguishing water quality gradient

In weight of evidence evaluations, consistency in response of specific taxa for similar classes of contaminants reported elsewhere, regionally or nationally, may potentially provide diagnostic support to inferences being made. Comparative assessments are of two general types, using information from (i) experimentally-derived, dose response relationships, or (ii) from field observed, maximum concentrations.

There are some important limitations in such comparative assessments:

1. Low taxonomic resolution, including family-level, may mask the different responses of congenerics or conspecifics. Notwithstanding and as reviewed in section 2.1.8, local and national information suggest that while species-level information will provide greater discriminatory power in assessing water quality changes in local aquatic ecosystems, the use of family-level data in the present study should not compromise this capacity greatly.

2. As noted in this study (section 5.4.3) and also by Rutherford and Kefford (2005, unpubl.) some field observations derived from local and national databases are from low salinity sites, so some taxa may not have encountered their true maximum tolerance. Therefore, estimates of field observed, maximum concentrations may be biased. On a related note, field data that are biased in the intensity of observations generally, or sampling of different habitat types, across the salinity gradient, may also bias probability of occurrence of taxa, irrespective of true physiological sensitivity – see section 5.3.2.

A number of other limitations are embodied under the concept of "context dependency", ie factors related to, or responsible for, "variation in ecological patterns and processes across environmental or spatiotemporal gradients" (Clements et al 2012). Clements et al (2012) noted that regional differences in how communities respond to natural and anthropogenic stressors are a result of environmental (including natural water quality), historical, biogeographical or climatic factors. Adapting some of these key factors to the present study:

1. The fauna of lotic and lentic systems vary and are naturally distinctive from one another. For the present study, few of the comparative national and international studies report results for lentic waterbodies. For example, representation of the families of mayflies known elsewhere to be salt-sensitive (e.g. Leptophlebiidae) is poor in the waterbodies sampled in the present study because these are most often flow-dependant. Sensitivities to toxicants of organisms are also known to differ between adjacent habitat types (Clements et al 2012).
2. Other national experimental and field observational databases are strongly focused on NaCl as the source of salinity. It is not known whether exposure to $MgSO_4$ would affect organisms differently than exposure to NaCl.
3. Magnesium and other salts present particular challenges in such comparisons because the presence of other major ions can influence toxicity (eg in the case of Mg, amelioration by Ca (van Dam et al 2010)). Moreover, Magela Creek receiving waters are extremely soft by Australian standards, increasing organism sensitivity to solutes.
4. As reported in section 5.1.1.1, dry season ECs in the lentic waterbodies sampled in this study can reach natural values of up to 250 $\mu S\ cm^{-1}$ through evapo-concentration and groundwater influences, compared to typical values at the time of sampling in this report of <25 $\mu S\ cm^{-1}$. There is a natural decline in diversity of the fauna over the dry season. Organisms either have dry season aerial or terrestrial dormant stages, dormant aquatic stages that can withstand stress, while elements persist throughout the year and must, therefore, acclimate to higher EC waters over the dry season period. Seasonal tolerances or sensitivities of the fauna to these changes in EC (and associated changes in relative ionic composition) are not known though clearly these species have evolved life histories that accommodate the extremes of the dry season, organisms can adapt to saline waters under particular conditions (Marshall & Bailey 2004, Dunlop et al 2008) or be pre-adapted in naturally-saline ecosystems (eg Kay et al 2001), while specific responses and relative and varying taxon sensitivities to different ions (eg Na vs Mg) are unknown.
5. The pollution-induced community tolerance (PICT) hypothesis (Blanck & Wängberg 1988, Tlili et al 2008) states that increased tolerance can occur when either (i) sensitive species are eliminated from a community after long-term exposure to stressor, (ii) the populations of taxa that persist under sublethal concentrations of a stressor undergo genetic adaptation, or (iii) individuals within a taxon acclimate. In the present study, community structure appeared more resistant to change from increasing Mg concentration when exposed to Mg – see section 5.6. Other examples from Australia were described in section 1.4 on adaptation, tolerance and acclimatation under particular conditions of short-term to very long-term (geological time scale) saline exposure durations. These observations, and the unknown effects arising from ecological interactions,

including predation and competition amongst species at local scales, complicate regional comparisons of species sensitivities to salts.

Notwithstanding these limitations, the taxa responses observed in the current study were compared amongst different analyses (within the study) and to salinity databases acquired from elsewhere in Australia.

Taxa responses across different waterbody exposure types were derived in CAP, SIMPER, SSDs and TITAN analyses in the present study. Patterns of occurrence amongst individual taxa and ambient Mg concentration, described in section 5.3.2, also contribute information about taxa sensitivities.

SIMPER results, determining the distinguishing taxa between GTB and reference waterbodies and between all minesite waterbodies and reference waterbodies, from year to year, are shown in Table 23 and 24 respectively. Taxa correlations (R >0.35) in the canonical ordination for the discriminant analysis of macroinvertebrate community data are shown in Figure 26 (all-years) and Figure 28 (three-years). Of the top 10 taxa influential in separating GTB macroinvertebrates from reference waterbodies in multivariate space (SIMPER, Table 23), 7 were reduced in abundance in GTB compared to reference billabongs in 2011, but only 3 to 4 out of 10 were reduced in any other year between 1995 and 2013. Comparing all mine site waterbodies to reference billabongs in this same way (Table 24), there was an increasing proportion of influential taxa that were reduced in abundance in the mine site waterbodies (relative to reference billabongs) over the time series, 1995 to 2013 (from 6 to 10 taxa). This supports a water quality impact in GTB just in 2011 but increasing impact associated with water quality in the mine site waterbodies generally, over time.

From the collective SIMPER and CAP results, separation of mine site waterbodies and reference billabongs was associated with reduced abundances of caenid mayflies, mites (Acarina and Oribatida) and planorbid snails. A number of hemipteran families Naucoridae, Pleidae and Corixidae, and decapod crustaceans, Atyidae and Palaemonidae, were also underrepresented in CJBB and RP1 with increasing mine water contamination, and in the latter years (2011 and 2013) also caddisfly families, Hydroptilidae and Leptoceridae. Conversely, hydrophilid, scirtid and dytiscid beetles, mesoveliid bugs and coenagrionid damselflies were generally in higher proportions in mine site waterbodies compared to reference billabongs. Patterns amongst individual taxa and Mg (from section 5.3.2) supported these results generally though baetid and caenid mayflies, oribatid mites and planorbid snails appeared indifferent to water quality, in contrast to SIMPER results.

To examine specific taxon responses to salinity further, a comparative analysis was undertaken between northern Australian $MgSO_4$-effects information, and information acquired in other salinity-effects databases in Australia. The datasets and analyses were summarised to (mainly) family-level, and were prepared according to the methods described in sections 5.5.1 and 5.5.2 below.

5.5.1. Other Australian salinity-effects databases

Four databases were used:

1 Laboratory toxicity data using south-eastern Australia and Queensland species. Dunlop et al (2008) examined the acute response (LC_{50} concentrations) of 31 species of macroinvertebrates over 72 h to a standard synthetic marine salt.

2 Field salinity database from Victoria and South Australia. Rutherford and Kefford (2005) compiled their salinity database from records of the maximum salinity at which species were observed in the field.

3 Field salinity database using AUSRIVAS macroinvertebrate data from QLD 'edge habitat', acquired from Horrigan et al (2005).

4 The trait-based, 'species at risk' ($SPEAR_{salinity}$) database was derived by Schäfer et al (2011) for detecting salinity (NaCl) impacts in south-eastern Australia.

Table 23 SIMPER results for Georgetown Billabong and reference billabongs

Group comparison and Summary Statistics	Dominant taxa (top 10) in decreasong order contributing to separation of groups
1. Georgetown versus Reference sites (all years log transformed data) ANOSIM R Statistic = 0.024. Significance level = 33.9%	*Oribatida*[1], *Nematoda*[1], Coenagrionidae[2], Bithyniidae[2], Atyidae[2], *Oligochaeta*[1], Libellulidae[2], *Acarina*[1], Ceratopogonidae[3], *Hydrophilidae*[1]
Average inter-group dissimilarity (%)	43.00
Cum. % of overall dissimilarity (top 10) contributed by dominant taxa	40.09
2. Georgetown versus Reference sites **1995**	Bithyniidae[2], Atyidae[2], *Acarina*[1], *Belostomatidae*[1], Palaemonidae[2], *Chironomidae (L)*[1], Coenagrionidae[2], Baetidae[2], Curculionidae (A)[2], Pleidae[2]
Average inter-group dissimilarity (%)	50.05
Cum. % of overall dissimilarity (top 10) contributed by dominant taxa	36.04
3. Georgetown versus Reference sites **1996**	Palaemonidae[2], *Naucoridae*[1], *Planorbidae*[1], Ceratopogonidae (L)[2], *Caenidae*[1], *Acarina*[1], Dytiscidae (L)[2], Chironomidae (L)[2], Belostomatidae[2], Dytiscidae (A)[2]
Average inter-group dissimilarity (%)	47.52
Cum. % of overall dissimilarity (top 10) contributed by dominant taxa	37.41
4. Georgetown versus Reference sites **2006**	Atyidae[2], Oribatida[2], *Acarina*[1], *Naucoridae*[1], *Planorbidae*[1], Oligochaeta[2], Coenagrionidae[2], *Veliidae*[1], Curculionidae (A)[2], Corixidae[1]
Average inter-group dissimilarity (%)	42.95
Cum. % of overall dissimilarity (top 10) contributed by dominant taxa	29.04
5. Georgetown versus Reference sites **2011**	Atyidae[2], *Caenidae*[1], *Oribatida*[1], *Ceratopogonidae (L)*[1], *Chironomidae (L)*[1], *Planorbidae*[1], Naucoridae[2], Palaemonidae[2], *Oligochaeta*[1], *Acarina*[1]
Average inter-group dissimilarity (%)	61.90
Cum. % of overall dissimilarity (top 10) contributed by dominant taxa	34.54
6. Georgetown versus Reference sites **2013**	Atyidae[2], *Acarina*[1], *Planorbidae*[1], Chironomidae (P)[2], Caenidae[2], Oribatida[2], Corixidae[2], Hydrophilidae (L)[2], Ceratopogonidae (L)[2], *Leptoceridae (L)*[1]
Average inter-group dissimilarity (%)	54.02
Cum. % of overall dissimilarity (top 10) contributed by dominant taxa	35.52

1 Reduced abundance at Georgetown Billabong compared to reference sites (italicised for ease of visual distinction); 2 Increased abundance at Georgetown Billabong compared to reference sites; 3 Absent from all reference sites. Jabiru Lake not considered reference.

Table 24 SIMPER results for 'exposed' waterbodies and reference billabongs.

Group comparison and Summary Statistics	Dominant taxa (top 10) in descending order contributing to separation of groups
1. Exposed versus Reference sites (all years log transform data) ANOSIM R Statistic = 0.21. Significance level = 0.09%	*Nematoda*[1], *Oribatida*[1], Coenagrionidae[2], Hydrophilidae (A)[2], Libellulidae[2], Dytiscidae(A)[2], *Acarina*[1], *Caenidae*[1], *Planorbidae*[1], *Bithyniidae*[1]
Average inter-group dissimilarity (%)	46.10
Cum. % of overall dissimilarity (top 10) contributed by dominant taxa	43.75
2. Exposed versus Reference sites **1995**	Dytiscidae (A)[2], *Pleidae*[1], *Libellulidae*[1], Coenagrionidae[2], *Curculionidae (A)*[1], *Caenidae*[1], Hydrophilidae (A)[2], Chironomidae (L)[2], *Belostomatidae*[1], *Crambidae (L)*[1]
Average inter-group dissimilarity (%)	51.51
Cum. % of overall dissimilarity (top 10) contributed by dominant taxa	36.78
3. Exposed versus Reference sites **1996**	*Pleidae*[1], *Naucoridae*[1], *Planorbidae*[1], Chironomidae (L)[2], *Dytiscidae (L)*[1], *Caenidae*[1], Dytiscidae (A)[2], Ceratopogonidae (L)[2], Coenagrionidae[2], Oligochaeta[2]
Average inter-group dissimilarity (%)	51.41
Cum. % of overall dissimilarity (top 10) contributed by dominant taxa	36.52
4. Exposed versus Reference sites **2006**	*Naucoridae*[1], *Caenidae*[1], Coenagrionidae[2], *Acarina*[1], *Libellulidae*[1], *Pleidae*[1], *Baetidae*[1], *Corixidae*[1], *Planorbidae*[1], Hydrophilidae (A)[2]
Average inter-group dissimilarity (%)	58.21
Cum. % of overall dissimilarity (top 10) contributed by dominant taxa	27.73
5. Exposed versus Reference sites **2009**	*Chironomidae (L)*[1], *Palaemonidae*[1], Baetidae[2], *Caenidae*[1], *Atyidae*[1], *Naucoridae*[1], Hydrophilidae (A)[2], Cyclestheriidae[2], *Planorbidae*[1], *Dytiscidae (L)*[1]
Average inter-group dissimilarity (%)	46.25
Cum. % of overall dissimilarity (top 10) contributed by dominant taxa	31.30
6. Exposed versus Reference sites **2011**	*Caenidae*[1], *Acarina*[1], *Ceratopogonidae (L)*[1], *Chironomidae (L)*[1], *Oribatida*[1], *Oligochaeta*[1], *Planorbidae*[1], *Anisoptera*[1], *Atyidae*[1], *Hydroptilidae (L)*[1]
Average inter-group dissimilarity (%)	70.12
Cum. % of overall dissimilarity (top 10) contributed by dominant taxa	35.17
7. Exposed versus Reference sites **2013**	*Acarina*[1], *Planorbidae*[1], *Nematoda*[1], *Chironomidae (L)*[1], *Oribatida*[1], *Ceratopogonidae (L)*[1], *Leptoceridae (L)*[1], *Caenidae*[1], *Oligochaeta*[1], *Pleidae*[1]
Average inter-group dissimilarity (%)	71.12
Cum. % of overall dissimilarity (top 10) contributed by dominant taxa	36.69

1 Reduced abundance at exposed sites compared to reference sites (italicised for ease of visual distinction); 2 Increased abundance at exposed sites compared to reference sites; 3 Absent from all reference sites. Jabiru Lake not considered reference.

Data from thesefour sources were tabulated in decreasing order of taxon sensitivity (or occurrence) to salts (Table 25). The nature of the salts is also indicated in Table 25. For the

Queensland dataset analysed by Horrigan et al (2005), streams in the east of the state were dominated by NaCl, while those to the west were often high in $Ca(HCO_3)_2$ and SO_4 (indicated in Table 25). Horrigan et al (2005) also provided a 'salinity sensitivity score' for each taxon, ascribed 'sensitive', 'tolerant' or 'very tolerant'. These scores are indicated in Table 25.

5.5.2. Northern Australian $MgSO_4$-effects information

SIMPER-derived rankings of macroinvertebrates were calculated for Mg-contaminated versus reference sites in (a) Limestone Creek (Argyle region, Kimberleys, WA; 2006, 2007 and 2008 data combined) (Humphrey et al 2008a) and (b) waterbodies from this study (i.e. GTB, CJBB and RP1 data combined versus reference billabongs, for 2011). (Just 2011 data for (b) were selected as that year was identified as one where putative impacts were observed for all three mine site waterbodies – see sections 5.3.3 and 5.4.1.)

SIMPER distinguishes taxa, in decreasing order of influence, that best separate *a priori* groups. A method of assigning a sensitivity/tolerance ranking to SIMPER results was developed just for this study. Amongst the outputs of SIMPER for each taxon, are average abundance in Mg-contaminated sites and reference sites, and the percent contribution of the taxon to the dissimilarity measure derived between the two groups (highest percentage contribution for the most influential taxa). The difference between average abundance in Mg-contaminated sites and reference sites was multiplied by the "percent contribution" value to derive a 'sensitivity score', from minus (sensitive) to positive (tolerant) (i.e. a lower taxon abundance in Mg-contaminated compared to reference locations gives rise to a negative value etc.) The sensitivity scores were ranked (lowest 'minus' to maximum 'plus') and tabulated accordingly (Table 25). Limestone Creek sensitivity codes were assigned 'sensitive' for scores that were all minus across the three years of data, 'tolerant' where scores ranged from minus to positive across the three years, and 'very tolerant' where scores were all positive across the three years. No such assignment was applied to the 2011 mine site waterbodies analysis as only one year of sampling was included

TITAN results from the all-sites and years analysis described in section 5.4.4 also provide an independent approach to ranking taxon sensitivity. TITAN produces change points for both taxa disappearing (taxa losses (z-)) and taxa that are appearing (gains (z+)) along the Mg gradient. These results are shown in Figure 40. Taxa were ranked from most to least sensitive based on the change points for 'pure and reliable' taxa shown in Figure 40. A sensitivity score of 'sensitive' or 'tolerant' was assigned on the basis of z- (taxa loss) or z+ (taxa gain) scores. These results are also shown in Table 25.

Consistent amongst the field-based sensitivity ratings of Table 25 (i.e. any of the studies tabled except that of Dunlop et al (2008) was the early disappearance of Hirudinea groups (leeches; Glossiphonidae, Ornithobdellidae) (see also section 5.3.2) and the increasing prevalence of the hemipteran (bug) family, Mesoveliidae, adults of the coleopteran (beetle) family, Hydrophilidae, and odonate groups including damselfly larvae from the family, Coenagrionidae, across a Mg or other salt gradient. In the most contaminated waterbody of the present study (DJKB), air-breathing beetle and bug groups dominated the fauna compared to other mine site waterbodies (Figure 42), consistent with known tolerances of such forms to poor water quality elsewhere (eg Chessman 2003), including salts (Table 25).

Schäfer et al (2011)		Dunlop et al (2008)		Rutherford & Kefford (2005, unpubl)		Horrigan et al (2005)			Limestone Ck (2006-08)			Minesite waterbodies (2011)		All sites, all years, TITAN			
NaCl		NaCl		NaCl (mainly)		Dominant ions NaCl (E), CaHCO3 (W)			MgSO4			MgSO4		MgSO4			
Taxon	SPEAR	Taxon	LC50 (mS/cm)	Taxon	Mean MFS (g/L)	Taxon	Mean MFS (µS/cm)	Sens/ty	Taxon	Mean Sens	Sens/ty Score	Taxon	Sens	Taxon	CP Mg (mg/L)	Sens/ty Score
Temnocephalidae	S	Leptophlebiidae	6.9	Microcrustacea	*0.7*	Helicopsychidae	1387	S	Leptophlebiidae	-11.72	S	Caenidae	-4.23	Veliidae	0.6	S
Lymnaeidae	S	Baetidae	8.7	Lymnaeidae	*2.7*	Dugesiidae	2460	S	Philapotamidae (L)	-6.07	S	Acarina	-2.60	Corixidae	0.7	S
Ancylidae	S	Notonectidae	10.8	Hirudinea	4	Simuliidae	2460	S	Dytiscidae (L)	-2.90	S	Oribatida	-2.47	Culicidae (P)	0.8	S
Oligochaeta	S	Baetidae	11.7	Lepidoptera	*4*	Hydropsychidae	2780	S	Leptoceridae (L)	-2.17	S	Oligochaeta	-2.18	Trichoptera (L)	1.3	S
Hyrudinea	S	Corixidae	12.8	Elmidae	8.8	Corduliidae	2980	S	Dytiscidae (A)	-1.43	S	Chironomidae (L)	-2.02	Naucoridae	1.2	S
Acarina	S	Caenidae	13.1	Ecnomidae	4.4	Tipulidae	2980	S	Ceratopogonidae (L)	-1.28	S	Hydroptilidae (L)	-1.54	Noteridae (A)	1.2	S
Baetidae	S	Baetidae	13.2	Palaemonidae	*5*	Temnocephalidea	3040	S	Baetidae	-1.26	S	O-Anisoptera	-1.39	Noteridae (L)	1.2	S
Caenidae	S	Calamoceratidae	13.9	Planorbidae	5.3	Elmidae	3100	S	Ecnomidae	-0.91	S	Ceratopogonidae (L)	-1.36	Culicidae (L)	1.3	S
Leptophlebiidae	S	Chironomidae	14.7	Ancylidae	6.2	Pyralidae	3200	S	Nepidae	-0.88	S	Planorbidae	-1.31	Hydrometridae	1.3	S
O-Lestidae	S	Leptoceridae	15.1	Baetidae	7.1	Leptophlebiidae	3910	S	Hydroptilidae (L)	-0.84	S	Nematoda	-0.97	O-Libellulidae	1.4	S
O-Aeshnidae	S	Physidae	15.7	Hydroptilidae	7.2	Hydroptilidae	5990	S	Trichoptera (P)	-0.78	S	Pleidae	-0.96	Hydroptilidae (P)	1.4	S
O-Gomphidae	S	Corixidae	17.3	O-Gomphidae	*7.8*	O-Gomphidae	12000	S	Hydraenidae (A)	-0.78	S	Chironomidae (P)	-0.70	Glossiphonidae	1.4	S
O-Corduliidae	S	Leptoceridae	18.4	Leptophlebiidae	7.9	Corbiculidae	2150	T	Tipulidae	-0.55	S	O-Zygoptera	-0.69	Curculionidae (A)	1.5	S
O-Libellulidae	S	Corbiculidae	18.4	O-Aeshnidae	9	Ancylidae	2560	T	O-Isostictidae	-0.55	S	Culicidae (L)	-0.53	Nematoda	2.1	S
Veliidae	S	O-Gomphidae	21	O-Libellulidae	10.2	O-Aeshnidae	4500	T	Caenidae	-0.53	S	Bithyniidae	-0.50	Hydroptilidae (L)	2.1	S
Gerridae	S	Acariformes	21.5	Simuliidae	10.3	Calamoceratidae	5570	T	Prostigmata	-0.41	T	Veliidae	-0.41	Ornithobdellidae	2.1	S
Hebridae	S	Corbiculidae	23.1	Caenidae	10.3	Stratiomyidae	5570	T	Culicidae (L)	-0.31	T	Naucoridae	-0.31	Atyidae	2.4	S
Corixidae	S	Psephenidae	23.4	Corixidae	10.7	Gerridae	5600	T	Trichoptera (L)	-0.21	T	O-Libellulidae	-0.29	Palaemonidae	2.7	S
Notonectidae	S	Leptoceridae	28.2	Scirtidae	10.9	Gyrinidae	5600	T	Pleidae	-0.20	T	Crambidae (L)	-0.28	Caenidae	5.7	S
Pleidae	S	Leptoceridae	28.5	Platyhelminthes	11.8	O-Isostictidae	5600	T	Oribatida	-0.19	T	Turbellaria	-0.19	Bithyniidae	8.6	S
Sisyridae	S	Hydrophilidae	29	Oligochaeta	13.3	Psephenidae	5600	T	Collembola	-0.17	T	Tabanidae (L)	-0.17	Oribatida	8.7	S
Carabidae (A)	S	Thiaridae	30.6	O-Coenagrionidae	13.4	Scirtidae	5600	T	Cladocera	-0.12	T	Leptoceridae (L)	-0.14	Acarina	12.1	S
Haliplidae (L/A)	S	Atyidae	33.1	Leptoceridae	13.6	Hydrophilidae	6010	T	Hydrochidae (A)	-0.11	T	Hebridae	-0.14	Oligochaeta	47.7	S
Noteridae (L/A))	S	Parastacidae	33.5	Notonectidae	14	Notonectidae	6010	T	O-Coenagrionidae	-0.10	T	Hydroptilidae (P)	-0.12	Ancylidae	1.1	T
Hydrophilidae (L/A)	S	O-Coenagrionidae	34.1	Veliidae	16	Veliidae	8700	T	Mesoveliidae	-0.10	T	Sisyridae	-0.10	Stratiomyidae (L)	2.5	T
Hydraenidae (A)	S	Atyidae	34.2	Parastacidae	*16.1*	Acarina	11730	T	Ancylidae	-0.03	T	Lymnaeidae	-0.10	Chironomidae (L)	8.7	T
Staphylinidae (L/A)	S	Atyidae	34.2	Cnidaria	16.3	Baetidae	11730	T	Planorbidae	-0.01	T	Corixidae	-0.07	O-Aeshnidae	11.6	T
Scirtidae (L/A)	S	Dytiscidae	37.4	Chironomidae	17.6	Caenidae	11730	T	Corixidae	0.00	T	Ceratopogonidae (P)	-0.07	O-Coenagrionidae	19.2	T
Elmidae (L/A)	S	O-Zygoptera	40.1	Hydropsychidae	18.3	Ceratopogonidae	11730	T	Veliidae	0.04	T	Curculionidae (A)	-0.07	Hydrophilidae (L)	21.7	T
Culicidae (L/P)	S	Atyidae	41.3	Atyidae	*18.7*	Corixidae	11730	T	Lymnaeidae	0.09	T	Dytiscidae (L)	-0.07	Mesoveliidae	22.3	T
Chironomidae (L/P)	S	Palaemonidae	42.5	Acariformes	18.8	Ecnomidae	11730	T	Copepoda	0.23	T	Belostomatidae	-0.04	Hydrophilidae (A)	22.3	T
Ceratopogonidae (L/P)	S	O-Coenagrionidae	34.1	Collembola	*25*	Leptoceridae	11730	T	Gerridae	0.29	T	Symphypleona	-0.04	Scirtidae (L)	22.3	T
Psychodidae (L)	S			Dytiscidae	31.6	O-Libellulidae	11730	T	Simuliidae (L)	0.42	T	Mesoveliidae	-0.02	O-Zygoptera	22.5	T
Tipulidae (L/P)	S			Ceratopogonidae	35	Oligochaeta	11730	T	Chironomidae (P)	0.43	T	Culicidae (P)	-0.02			
Empididae	S			Hydrophilidae	37	Orthocladiinae	11730	T	Hydropsychidae (P)	0.43	T	Palaemonidae	0.00			
Ecnomidae (L)	S			Nematoda	64	Pleidae	11730	T	Hydrophilidae (A)	0.58	T	Gyrinidae (A)	0.03			
Hydroptilidae (L/P)	S			Hydraenidae	*119*	Tanypodinae	11730	T	Hydropsychidae (L)	0.66	VT	Hydridae	0.03			
Leptoceridae (L/P)	S					Atyidae	12000	T	Atyidae	0.67	VT	Ancylidae	0.05			
Philopotamidae (L)	S					Cladocera	12000	T	Empididae	0.73	VT	O-Aeshnidae	0.05			
Polycentropodidae (L)	S					Palaemonidae	12000	T	Corbiculidae	0.75	VT	Noteridae (A)	0.07			
Crambidae (L/P)	S					O-Protoneuridae	12000	T	Corixidae	0.82	VT	Cyclestheriidae	0.07			
Nematoda	T					Nepidae	5570	VT	Dolichopodidae	0.85	VT	Baetidae	0.09			
Planorbidae	T					Hydrometridae	5990	VT	O-Anisoptera spp.	0.86	VT	Nepidae	0.09			
Atyidae	T					Naucauridae	5990	VT	Simuliidae (P)	0.87	VT	Crambidae (P)	0.17			
Palaemonidae	T					Staphylinidae	5990	VT	Thiaridae	0.98	VT	O-Coenagrionidae	0.25			
O-Coenagrionidae	T					Tabanidae	5990	VT	Hydroptilidae (P)	1.06	VT	Notonectidae	0.28			
O-Protoneuridae	T					Lymnaeidae	6010	VT	Ostracoda	1.08	VT	Atyidae	0.28			
O-Isostictidae	T					Mesoveliidae	6010	VT	Tabanidae	1.22	VT	Brentidae (L)	0.37			
Hydrometridae	T					Ostracoda	6010	VT	Pyralidae	1.66	VT	Ecnomidae (L)	0.39			
Mesoveliidae	T					Culicidae	11730	VT	Palaemonidae	1.76	VT	Gerridae	0.45			
Nepidae	T					Hydraenidae	11730	VT	Limnichidae (A)	1.90	VT	Dytiscidae (A)	0.50			
Belostomatidae	T					Planorbidae	11730	VT	O-Libellulidae	2.20	VT	Hydrophilidae (A)	0.63			
Naucoridae	T					O-Coenagrionidae	12000	VT	Hydrophilidae (L)	2.46	VT	Hydrophilidae (L)	0.71			
Dytiscidae (L/A))	T					Copepoda	12000	VT	Oligochaeta	3.64	VT					
Hydrochidae	T					Dytiscidae	12000	VT	O-Gomphidae	3.77	VT					
Tabanidae (L)	T					Parasticidae	12000	VT	Elmidae (L)	4.45	VT					
Stratiomyidae (L)	T					Thiaridae	12000	VT	Elmidae (A)	4.80	VT					

Table 25 Rank of sensitivity (most to least sensitive) of aquatic macroinvertebrate taxa to salinity gradients amongst several Australian studies.

Details for the 7 studies are described in the text.

Higher taxon by colour codes:
- Ephemeroptera (mayflies)
- Trichoptera (caddisflies)
- Hemiptera (true bugs)
- Coleoptera (beetles)
- Diptera (true flies)
- Mollusca
- Crustacea: Decapoda
- "O-" = Odonata (damsel- and dragonflies)

A and L after taxon name, Adult and Larvae respectively.

Sensitivity codes:
- S = Sensitive,
- T = Tolerant,
- VT = Very Tolerant

LC50 = Lethal concentration at which 50% of population killed

Mean sensitivity ("Mean Sens") explained in text.

MFS = Maximum salinity observed in the field.

CP (TITAN) = Change Point

Italicised numbers refer to just one field observation.

On the other hand, while caenid mayflies appeared sensitive on the basis of SIMPER results in the present study (ie lower relative abundances in minesite waterbodies, Table 24, see also Figure 42C), their occurrence and abundances corresponded to the frequency at which samples were collected across years and sites (section 5.3.2). This suggests this taxon is *not* responding to Mg and thus is not sensitive to Mg at the ambient concentrations sampled. Even prior to mining or prior to significant contamination (i.e. < log Mg 0.5, Figure 42), relative abundances of caenids were low in GTB and CJBB (Figure 42C). In south-eastern Australia (all data sources) and Queensland (laboratory data) caenid mayflies are sensitive to salts although Queensland field observations do not support this (Horrigan et al 2005) (Table 25). Amongst all of the NaCl-dominated salinity studies represented in Table 25, laboratory and field, there are other very general consistencies, including the sensitivity of most Ephemeroptera (mayflies) families and to a lesser extent, a number of Trichopteran (caddisfly) families. Most (but not all) mollusc groups are generally salt tolerant, and Coleoptera and Decapoda (crustaceans) more so. Hemiptera species tested in the laboratory were relatively more sensitive than observations made of Hemiptera in the field.

Sensitivity rankings for sites in mine water-exposed Limestone Creek vs reference sites (Table 25) are of particular interest in that $MgSO_4$ is the predominant contaminant (14-18 mg L^{-1} Mg) (unlike mostly NaCl for the other comparative studies) and Ca amelioration (high hardness, $CaCO_3$ >500 mg L^{-1}) and tolerance are apparent (Humphrey et al 2008a). Yet while number of taxa between exposed and reference sites was similar, community structure between contaminated and reference sites differed. The taxa responses observed in Limestone Creek are consistent with NaCl-dominated salinity studies from elsewhere: mayfly and caddisfly sensitivity, and general tolerance of molluscs, beetles (except Dyticidae) and shrimps (Table 25).

Determining taxa sensitivities across a Mg contaminant gradient in this study was beset with many of the limitations that were described in the introduction to this section. These include the following:

Habitat differences. As described above, comparisons made across lentic and lotic databases are limited by natural faunal differences. For example, the most salt-sensitive mayfly family found interstate and in northern Australia (e.g. Table 25; Humphrey et al 2008a), Leptophlebiidae, does not usually occur in lentic waterbodies such as those sampled in the present study.

Potential artefacts in analytical approaches

1. TITAN analysis performed in the current study highlighted several Hemipteran families that exhibited amongst the lowest change points (Table 21, Figure 40). Further, the rank order of taxa losses indicated in TITAN (Figure 40) appeared to be biased towards early loss of a number of hemipteran families, more consistent with laboratory toxicity results (Dunlop et al 2008, Table 25) than with other field data comparisons. However and as discussed in section 5.4.4, taxa occurrence may be responding predominately to the frequency at which samples were collected and hence the change points may simply be artefacts of sampling intensity across the Mg concentration range.

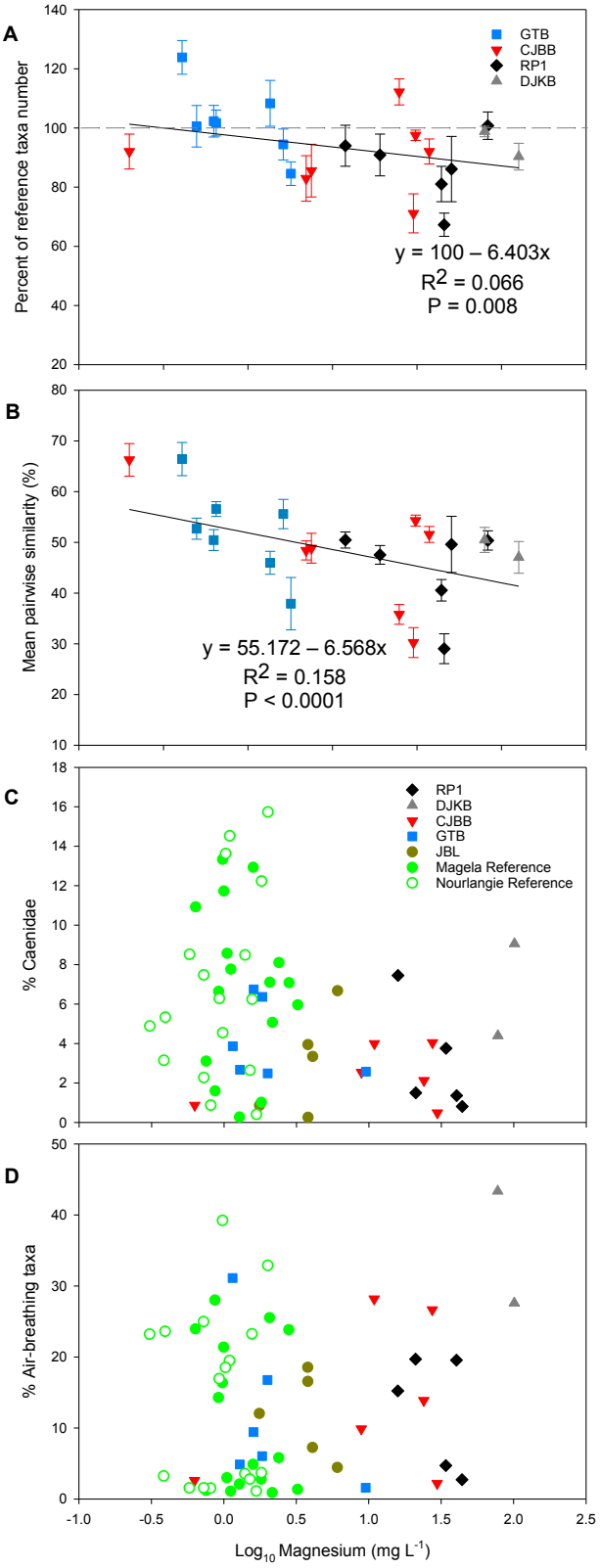


Figure 42 (A) GTB, CJBB, DJKB and RP1 number of taxa, as a percent of reference waterbody number of taxa, in relation to Mg concentration. (B) Mean pairwise similarities (versus reference) for GTB, CJBB, DJKB and RP1. (C) Relative percent abundance of Caenidae and (D) relative percent taxa richness of air breathing taxa (Coleoptera and Hemiptera) in relation to magnesium concentrations

2. *Temporal comparisons*: Using SIMPER 2011 results as a basis for determining taxa sensitivities does not account for taxa losses, gains or shifts over time.
3. *Unequal sampling effort across the Mg gradient*. SIMPER 2011 analyses used in this evaluation may also have been biased by unequal sampling intensity across the Mg concentration range, ie greater representation of reference billabong data (see section 5.3.2).
4. *Incomplete contaminant gradient*. Higher Mg concentrations than those encountered in the present study may have better defined taxa sensitivities. For example, taxa defined as tolerant in the present study may be intolerant to the higher EC waters that are more typical of other compiled databases. (See also section 5.4.3.)

Occupancy abundance analysis may provide an improved approach to identifying the sensitivity ranges of taxa in the present study across a Mg gradient, as discussed in section 5.3.2. The approach removes much sampling bias and normalises taxon occurrence, ie expected occurrence if there was no relationship between abundance and Mg concentration. Using this approach, occupancy abundance analysis by way of assessment of the taxa plots representing all years of sampling (Appendix 6), indicates some new information or difference in conclusions from those indicated in other analyses, including potential sensitivity of bithyniid and lymnaeid snails, partial sensitivity of palaemonid and atyid shrimps and tolerance at the Mg concentrations encountered of planorbid snails, oribatid mites, caenid mayflies and naucoriid bugs (section 5.3.2).

5.6 Acclimation and tolerance

McCullough et al (2008) noted the presence of *Hydra* (and other freshwater species used in the Supervising Scientist's toxicity testing program) in CJBB and RP1, at concentrations well above the laboratory-determined guideline, and sought to determine the basis for this persistence. They noted the same responses to Mg in *Hydra* sourced from CJBB and a reference billabong (i.e. suggesting, but not proving, lack of genetic adaptation in CJBB *Hydra*) and with further work, concluded that CJBB tolerance was due to Ca amelioration (i.e. Mg:Ca ratio <9:1 in CJBB and thereby protective).

Reduced Mg toxicity due to Ca amelioration is one explanation for this result but high tolerance in a population or community generally, may have other causes associated with pollution-induced community tolerance (PICT) (Blanck & Wängberg 1988, Tlili et al 2008). PICT can occur through several processes: (i) acclimation, ie increasing tolerance as a result of prior exposure within an organism's life-span, (ii) physiological adaptation (ie phenotypic plasticity of an individual), (iii) selection of tolerant genotypes within a population over time, and (iv) replacement of species with more tolerant species within a community.

Manifestation of PICT may depend upon exposure duration. Constant exposures to salts compared to exposures to short pulses of salts can affect the salinity tolerance of a species though there are contradictory results associated with these investigations (see review in section 1.4).

Evidence of PICT in CJBB and RP1 macroinvertebrate communities from the field data examined in this study is apparent in Figure 37 showing ANOSIM R values for GTB, CJBB and RP1 comparisons with reference billabongs, with associated antecedent Mg concentrations. The non-linear relationship observed over the Mg concentration range indicates that group separation in CJBB and RP1 is elicited at increasingly higher Mg concentrations, respectively, than for GTB. Thus, the fauna of these waterbodies has a

higher tolerance to Mg than GTB fauna. Mg:Ca ratios in GTB, CJBB and RP1 are, respectively, ~3.5:1, 8:1 and 7.5:1. Thus, Ca amelioration (i.e. Mg:Ca ratio <9:1) cannot explain this greater tolerance because the prevailing Mg:Ca ratio in GTB has been less than CJBB and RP1 for all sampling years and hence, and from the results of van Dam et al (2010), should actually confer *greater* protection. (Lack of Ca protection, despite Mg:Ca ratio <9:1, was also reported for biological communities colonising mesocosm tubs, deployed in adjacent Magela Creek channel in 2002 and impaired by $MgSO_4$ additions; see section 1.4.) There are several possibilities:

(i) there may be Mg toxic modes of action that are independent of Ca;

(ii) Ca amelioration varies greatly amongst species (i.e. laboratory results for Mg:Ca incorporating just two invertebrate species, are just a subset of responses);

(iii) the derived laboratory Mg:Ca ratios protective of organisms may not apply under the much longer-term Mg exposures observed in the field;

(iv) other (unknown) ionic interactions may be interfering with the protective role of Ca or may be additive in toxicity (e.g. K, HCO_3);

(v) toxicants other than major ions are contributing to toxicity;

(vi) indirect ecological effects, including species and trophic interactions, not possible to be predicted from laboratory studies are operating; and/or

(vii) different exposure regimes amongst the minesite waterbodies operate to promote PICT in CJBB and RP1 but not GTB.

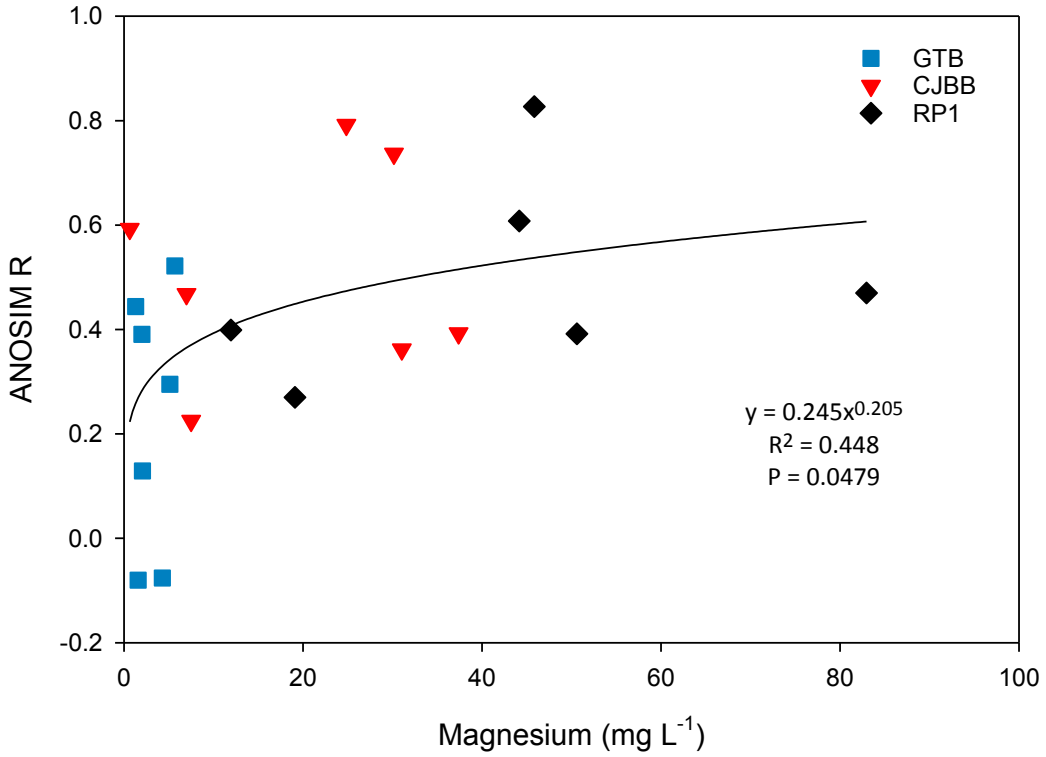


Figure 43 GTB, Coonjimba Billabong (CJB) and Ranger Retention Pond 1 (RP1) vs reference waterbody ANOSIM R values, in relation to median antecedent wet and dry season Mg concentration.

While all these possibilities require further study, there is some information to support (vii) above, ie that the different exposures to mine waters for the three waterbodies confer different tolerances of resident macroinvertebrate fauna. Greatest to least spatial connectivity of waterbodies to Magela Creek follows the order GTB, CJBB then RP1. RP1

has no direct connectivity to Magela Creek while CJBB backflows occasionally at high creek flows. GTB both backflows regularly and becomes a channel of Magela Creek itself at high flow events due to its very close proximity to the adjacent creek channel. (Figure **6** 6 shows the configuration of the waterbodies in relation to Magela Creek.) GTB's exposure to mine-derived waters in the wet season is intermittent due to its closer contact and exchange with flow in Magela Creek, though since 2006, these 'partial-cleansing' events during backflow and high creek-flow events have reduced in frequency. Conversely, CJBB and RP1 have constant exposure to mine waters with negligible flushing events. These patterns of almost constant exposure to 'high' Mg concentrations in CJBB, even in the wet season, but exposure to much lower Mg concentrations in GTB, with more frequent and intermittent 'pulsing' during the wet season, are illustrated for the 2010–2011 period in Figure 44.

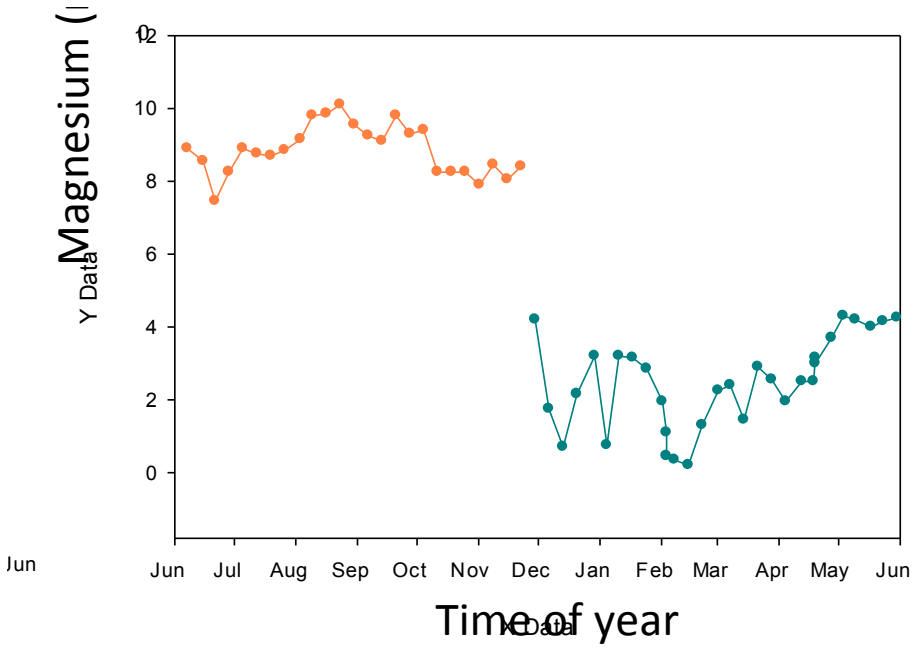


Figure 44 Magnesium concentrations in Coonjimba and Georgetown Billabongs in the 2010–2011 period.

It is possible that PICT of the biota in CJBB and RP1 is only maintained by constant exposure to mine wastewaters and this is the explanation for the biological responses

observed (Figure 43). The pulse-type delivery of Mg to the wet season GTB fauna in 2011 in particular, may have exposed the fauna to cumulative 'ecosystem shock', reminiscent of the experimental findings of Marshall and Bailey (2004). These latter authors conducted field experiments that showed delivery of salts (NaCl) in mesocosms in short pulses of high salt concentrations was more detrimental to components of macroinvertebrate communities than delivering the same salt load at a low concentration over a longer period of time (Marshall & Bailey 2004). (However, other authors have reported contrary experimental findings – see review in section 1.4.)

A possible hypothesis of development of tolerance and acclimation in the minesite waterbodies is that of initial impact with exposure to an elevated, threshold concentration of $MgSO_4$, followed by recovery with continuing and increasing contaminant concentrations. This possible evolution is depicted in Figure 45 where, for CJBB at least, early in the period of increase in $MgSO_4$ to the billabong, biological impairment (viz low relative number of taxa) was evident (two close 1995 and 1996 points near EC value of (log) 2.0), with 'recovery' thereafter, despite increase in salt concentrations. From Figure 45, such 'early exposure' to increasing concentrations of salts corresponded to low relative number of taxa in GTB 2011 as well. The range of antecedent salt concentrations for GTB 2011 and CJBB 1995 and 1996 was narrow: EC range (log) 1.9 to 2.0 (or EC 83 to 104 µS cm^{-1}), equivalent to Mg concentrations between 5.7 and 7.5 mg L^{-1}. It is possible that these narrow Mg ranges represent an early threshold of adverse biological effect; this would require further investigation to resolve.

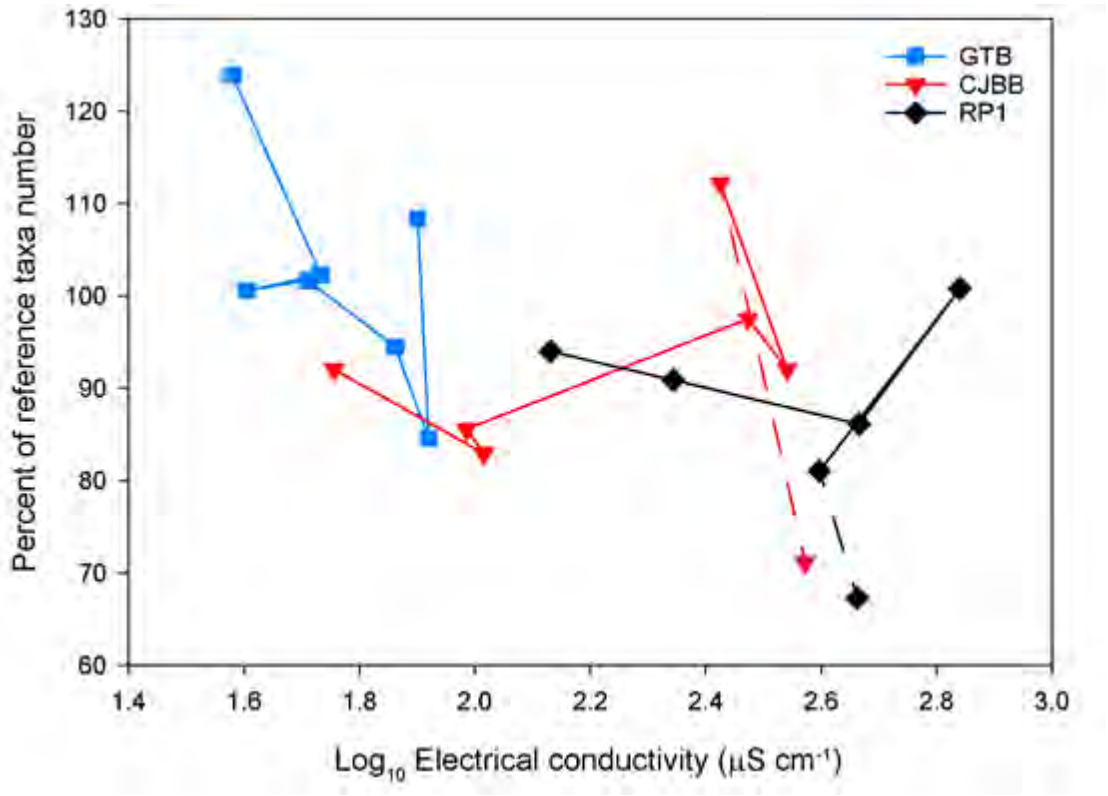


Figure 45 Time sequence of change in mean number of taxa relative to reference waterbodies, in relation to median antecedent wet and dry season electrical conductivity, in Georgetown (GTB) and Coonjimba (CJBB) Billabongs, and Ranger Retention Pond 1 (RP1). The lines joining the points follow the time series from 1979 (GTB and CJBB) or 1995 (RP1) to 2013. Dashed lines to 2013 data for CJBB and RP1 indicate possible effects unrelated to salts (see section 5.3.3).

Because taxonomic identification of macroinvertebrates in this lentic waterbody study was conducted at the family-level, another explanation in PICT for the higher tolerances observed in CJBB and RP1 is species or genus replacement, i.e. sensitive taxa being replaced by more tolerant taxa from the same family. Only limited species-level identifications have been conducted, most notably Chironomidae in 1995 sampling (O'Connor et al 1995). This study demonstrated a preference of a few species from the genera *Tanytarsus*, *Larsia* and *Polypedilum* for high EC waters, but none of the 45 chironomid species were unique to either waterbodies receiving mine wastewaters or reference waterbodies with no mine influence. Persistence of taxa in waterbodies receiving Ranger mine wastewaters appears best explained by other mechanisms in PICT.

6 Weight of evidence evaluation and conclusions

6.1 Types of evidence for evaluating confounding and inferring causation

This field-effects study has focused in large part on quantifying changes in lentic macroinvertebrate communities in Ranger mine site waterbodies over time and providing evidence that such changes can predominately be attributed to Mg increase in the waterbodies over the same time period. As described in sections 1.5 and 2.1.1, outside of this report, candidate effects values from field, mesocosm, and laboratory studies will be considered in a separate weight of evidence evaluation to both determine a guideline value for ecosystem protection and, in combination, strengthen the evidence for decisions made. This final section also uses results from these other lines of evidence to strengthen inference (section 6.2). However, its main purpose is to summarise and evaluate the collective lines of evidence supporting Mg-related effects in *this* field study, with an emphasis on eliminating (or apportioning the contribution of) alternative explanations for the observed changes. As noted by Suter et al (2000) typically WOE evaluations are used to provide greater confidence in the causal relationship that is best supported by the available evidence.

Suter and Cormier (2013) provide a useful framework for evaluating confounding and describe 10 types of evidence for assessment. These are listed in Table 26. There are three characteristics of causation represented amongst these 10 types of evidence, adapted from Hill (1965) (see Cormier and Suter (2013b) for further descriptions):

1. Types 1 to 4 relate to *co-occurrence*, i.e. evidence that the cause co–occurs with the unaffected entity in space and time. Co-occurrence considers size of the correlation between the effect and cause/confounders and evidence that the association between the effect and cause has been repeatedly observed in different places, circumstances, and times.
2. Types 5 to 8 relate to *sufficiency*, i.e. evidence that the intensity, frequency, and duration of the cause are adequate, and the entity is susceptible to produce the type and magnitude of the effect. Biological gradients and laboratory dose-response relationships may be invoked here.
3. Types 9 to 10 relate to *alteration*, i.e. the degree of specificity of the effects of the putative cause compared to confounders.

Six broad classes of potential confounding were evaluated in this study:

1. Appropriateness of the reference billabongs used (i.e. appropriate experimental design);
2. Interannual patterns in macroinvertebrate community composition that may have coincidentally corresponded with putative mine water associated biological change;
3. Variations in sample processing protocol, including inclusion or exclusion of sediment-dwelling macroinvertebrates;
4. Habitat (aquatic plants and sediment);
5. Changes in habitat and water quality over time unrelated to mining; and
6. Water and sediment CoPCs other than Mg (major ions, metals including U, ammonia, nitrate and turbidity, low pH and associated release of metals from binding surfaces (including from sediments)).

One other diagnostic line of evidence was invoked to determine whether it supported the cause-effect relationship:

- Consistency of taxa responses to those observed in similar salt studies.

Only confounder classes 4, 5 and 6 require evaluation against WOE causation precepts, such as listed in Table 26. Elements 1 to 3 were assessed in dedicated investigations reported in this study. The aims of these investigations (elements 1 to 3) were to assess the degree, if any, to which these potential sources of error and confounding contributed, ultimately, to uncertainty in deriving a field-effects Mg GV.

Table 26 Types of evidence to assess confounding proposed by Suter and Cormier (2013). Each evidence type is described and is then followed by an explanation. In the descriptions, "the cause" refers to the cause of concern (e.g. Mg) and "the confounder" refers to any potential confounder of the causal relationship.

Evidence type	Description and explanation
Type 1	Correlation of confounder and cause: Confounders are correlated with the cause of interest. A low correlation coefficient is evidence against the potential confounder.
Type 2	Correlation of confounder and effect: Confounders are correlated with the effect of interest. A low correlation coefficient is evidence against the potential confounder.
Type 3	Influence of the confounder at extreme levels: Even when the confounder is not correlated with the cause of interest, it may be influential at extreme levels. A lack of influence at extreme levels of the potential confounder is evidence against the potential confounder.
Type 4	Influence of the presence of the confounder: If the frequency of the effect does not diminish when the potential confounder is never present or is present in all cases, it can be discounted in that subset.
Type 5	Occurrence of confounder at sufficient levels: The magnitude of the potential confounder (e.g., concentration of a co-contaminant) may be compared to exposure–response relationships from elsewhere (e.g., laboratory toxicity tests) to determine if the exposure to the potential confounder is sufficient to influence the effect. If it is not sufficient, that is evidence that it is not acting as a confounder.
Type 6	Influence of removing a confounder where it is at sufficient levels: If the confounder is estimated to be sufficient in a subset of cases, those cases may be removed from the data set and the remaining set reanalysed to determine the influence of their removal on the results. If the cause–effect relationship is unchanged, the confounder was not causal or influential. Note that this evidence of confounding may also identify a treatment for confounding
Type 7	Influence of the confounder in multivariate correlations: Multiple regression and other multivariate statistical techniques may be used to estimate the relative degree of association of the cause and potential confounders with the effect
Type 8	Frequency of occurrence of the confounder: If the potential confounder occurs in a sufficiently small proportion of cases, it can be ignored. That is because if it occurs rarely, it cannot significantly influence the causal relationship
Type 9	Occurrence of characteristic effects of the confounder: If a potential confounder has characteristic effects that are distinct from those of the cause of concern, then the absence of those effects can eliminate the potential confounder as a concern in either individual cases or the entire data set.
Type 10	Occurrence of characteristic effects of the cause: If the effects are characteristic of the cause of concern and not of the potential confounder, then the potential confounder can be eliminated as a concern in either individual cases or the entire data set.

In addition to those evidence types listed above (Table 26), Hill (1965) included other epidemiological precepts that usefully assist in inferring causation. These are:

1. Presence of stressor in tissues, i.e. measurement parameters of exposure (e.g. residues, breakdown products) must be present in the tissues of affected organisms;
2. Temporality or timing, i.e. exposure to the cause must precede the effect in time;
3. Biological plausibility, i.e. there is a biologically plausible explanation for causality, even if the precise mechanism is unknown;
4. Coherence, i.e. the causal interpretation should not seriously conflict with existing knowledge about the natural history of the organism and the behaviour of any substances associated with the disturbance; and
5. Analogy, similar disturbances cause similar effects.

A number of these additional criteria are allied to one another, and to earlier criteria listed above (in Table 26). The collective criteria are considered against the potential confounders identified in this study in the following section.

6.2 Evaluating confounding and inferring causation

This WOE section presents a summary tabulation and associated narrative, evaluating the alternative explanations for the observed macroinvertebrate responses examined in this report (Table 27). This evaluation is accompanied by an assessment of the collective evidence against Hill's (1965) *full* precepts of epidemiology, where external (to this study) supportive evidence is also considered (Table 28). This latter evaluation considers the additional 5 precepts listed above (from Hill 1965) to those provided by Suter and Cormier (2013) (listed in Table 26).

Often, qualititative scoring systems of from one to three (**+**) or (–) symbols are used to indicate the weight of a piece of evidence. Suter and Cormier (2013) describe such an example where *plus* (**+**) indicates evidence suggesting that the potential confounder is actually causing the effect, or *minus* (–) indicates evidence that the potential confounder does not contribute to the effect:

(**+ + +**) or (– – –) indicates convincing support or weakening,
(**+ +**) or (– –) indicates strong support or weakening,
(**+**) or (–) indicates some support or weakening, and
0 indicates no effect on the hypothesis of confounding.

Such scoring was not applied in this study as most of the weighting of evidence appeared binary, i.e. either supportive of Mg as the main contributor to change and the effects-based threshold derived, with or without censoring of confounding data, or indicating a need to undertake more investigation to confirm the conclusions. Instead, a two-colour qualitative scoring system was used in Table 27 to weight and assess the evaluation:

- No potential for the confounder to contribute to observed change, or
- Potential to contribute to the observed change and requiring data censoring or further investigation.

Table 27 Evaluation of potential for different factors to contribute to observed macroinvertebrate community responses in Ranger minesite waterbodies over time. Colour coding: Green = Null hypothesis of non-confounding of water-quality-related change supported; Orange = Null hypothesis not supported with confounders potentially contributing to observed change and requiring further study. NR = Not required.

Potential for confounding (null statement)	Analysis undertaken and result (with section addressed)	Potential to contribute to observed change	Subsequent assessment
Study design			
Minesite waterbodies in Magela Creek are biologically comparable to reference waterbodies in all respects other than effects of mine water contaminants	Sect 5.2.3.1: In multivariate ordination space, waterbodies are associated with, and group by, year, rather than waterbody exposure type and catchment (MDS ordination).	No potential, null statement supported.	NR
Nourlangie reference waterbodies are biologically comparable to those in Magela catchment. Differences in the relative numbers of reference waterbodies from each catchment used over time did not give rise to potential artefacts.	Sect 5.2.3.1: Magela and Nourlangie reference waterbodies in any sampling year were similar to one another in community structure (MDS ordination and ANOSIM).	No potential, null statement supported.	NR
Before, or outside of periods of, significant mine water contamination, minesite billabongs were similar to reference	Sect 5.2.5: GTB was similar to reference up to 2006. When water quality improved after 2011, GTB returned to reference condition (MDS ordination and ANOSIM)	No potential, null statement supported.	NR
Natural biological variation amongst waterbodies from year to year, associated with differences in rainfall and hydrology, do not lead to errors (Type I or II) in interpreting mine-associated impacts.	Sect 5.2.3.2: There are no time- or climate (rainfall)-related differences in amongst-site variation in community structure in reference billabongs that could, in turn, potentially affect reference-exposed waterbody comparisons based upon similarity and other multivariate response measures. Similarly, natural variability observed amongst reference billabongs was unrelated to sample processing method (PERMDISP).	No potential, null statement supported.	NR
Variations in protocols			
Changes in macroinvertebrate community structure attributed to mining over the full time series are not associated with coincidental changes in macroinvertebrate sample processing methods	Sect 5.2.1: Simulated paired exposure-reference site comparisons showed that relative differences in macroinvertebrate community summaries and structure were the same for analyses based on each of the two (live-sorted and laboratory processed) datasets. Even when data from live-sorted and laboratory components were ordinated together, the group separation was minor. Thus, the difference in sample processing methods was not confounding the data for combined (all years) or exposed-reference waterbody comparisons made in the current study (ANOSIM, paired-site similarity, MDS ordination).	No potential, null statement supported.	NR

Potential for confounding (null statement)	Analysis undertaken and result (with section addressed)	Potential to contribute to observed change	Subsequent assessment
Habitat influences: Aquatic plants			
Hypothesis I: Macroinvertebrate assemblages are not influenced by habitat viz key aquatic plant measures	Sects 5.3.1, 5.3.2, 5.3.4: Many macroinvertebrate taxa increased in occurrence and abundance with increasing macrophyte cover (a proxy for habitat structure and complexity), and various aquatic plant measures were also correlated with macroinvertebrate assemblage patterns in multivariate space. Macroinvertebrate number of taxa but not similarity (relative to reference billabongs) across minesite waterbodies and all years, was positively correlated with plant percent cover (BIOENV, Correlaton and regression)	Null statement not supported. Relationships between biological responses and key aquatic plant habitat are confimed	
Hypothesis II: Relationships between biological responses and key aquatic plant habitat do not affect threshold determination for Mg	Sects 5.1.1, 5.1.3, 5.3.1.2, 5.3.4: Correlations between environmental variables and macroinvertebrate responses were always stronger for water quality variables. Amongst all water quality and habitat variables, separation of waterbodies amongst years for abiotic factors and biological communities was most strongly water quality driven (Axis 1: PCA, CAP). Correlations between aquatic plant measures and Mg were also poor. Macroinvertebrate number of taxa (relative to reference waterbodies) correlation with plant percent cover across minesite waterbodies and all years was influenced by CJBB only. No such relationship occurred in GTB and RP1 and the data did not include 1979, a (pre-mining) period when plant cover was known to be lower than post 1985. No potential for confounding by aquatic plant factors was found for GTB.		No potential, null statement supported.
Habitat influences: Sediment fauna			
Incidental or intentional inclusion of sediments in samples to be processed (i.e. in addition to primary macrophyte habitat) does not result in errors (Type I or II) in interpreting mine-associated impacts because of differences in community structure of sediment-dwelling organisms	Sect 5.2.2: Macroinvertebrate diversity in sediments of all waterbody exposure types is low compared to diversity in macrophyte habitat. Macroinvertebrate community structure of sediment habitat is similar for all exposure types and so when low diversity data or samples from sediment habitat are combined with those of macrophyte habitat, the results are the same as for macrophyte habitat alone (MDS ordination, ANOSIM).	No potential, null statement supported.	
Water quality and habitat influences: Fire regimes and feral pigs			
Hypothesis I: The fire regime in GTB catchment antecedent to sampling in 2011 did not affect habitat and water quality and was not the reason for impacts upon macroinvertebrate communities	Sect 5.2.4.1: The fire history preceding sampling of GTB in 2011 did not appear unusual compared to other years. There is no obvious link between fire history and putative impacts to macroinvertebrate communities of GTB in 2011.	No potential, null statement supported	
Hypothesis II: Pig damage to the littoral zone of GTB in 2011 was not sufficiently intense to have resulted in loss of aquatic plant cover and increase in turbidity, and these factors were not the cause of impacts upon macroinvertebrate communities	Sect 5.2.4.2: The most biologically impacted locations in GTB in 2011 did not suffer any littoral vegetation loss, nor was turbidity higher in these locations than other locations sampled. Thus there no evidence of pig-associated impacts to GTB in 2011 coincident with macroinvertebrate sampling.	No potential, null statement supported.	

Potential for confounding (null statement)	Analysis undertaken and result (with section addressed)	Potential to contribute to observed change	Subsequent assessment
Consistency of taxa responses to those observed in similar studies			
Taxa responses are consistent with those reported in other salt-related studies	Sect 5.5: This study observed sensitivity of particular insect groups, including Hemiptera, Ephemeroptera and Diptera, and tolerance of some Gastropoda, Odonata, decapod Crustacea and Coleoptera. In general, these patterns were consistent with other Australian and USA (U.S.EPA 2016) salt studies. However, preferences of specific macroinvertebrate taxon goups for particular aquatic plant forms is not well known, so an evaluation of water quality sensitivityversus habitat preference is not possible. The conclusion in the table cell to the right, therefore, is somewhat muted.	No evidence available to *reject* the null statement.	
Effects of water and sediment CoPCs other than Mg			
Hypothesis I: Macroinvertebrate assemblages are not strongly correlated with water and sediment CoPCs other than Mg	Sects 5.1.1.2, 5.1.2, 5.3.3: (i) Macroinvertebrate responses are correlated with major ions other than Mg and SO_4, i.e. Ca and K. Insufficient data to examine HCO_3 for similar correlation, but possible relationship as well (Spearman rank correlation); (ii) Antecedent wet and dry season median U values in RP1 (only) exceeded the local GV for 2006, 2009, 2011 and 2013 sampling. Correlation was found between U and exposed-reference site macroinvertebrate similarity (but not number of taxa) (correlation and regression) (iii) Amongst mine site waterbodies, sediment U GV exceedance observed in RP1 in 2011. (iv) Extreme acidity events occurred in the early wet season of 2013 in both CJBB and RP1	Null statement not supported. Relationships between biological responses and water and sediment CoPCs other than Mg confirmed.	
Hypothesis II: Relationships between biological responses and water and sediment CoPCs other than Mg do not affect threshold determination for Mg	Sects 5.1.1.2, 5.1.2, 5.2.2, 5.3.3:		
	(i) SO_4 (apart from accumulation in sediments – see below) and Ca not toxic to aquatic organisms (Mount et al 1997, 2016; van Dam et al 2010). K had highest correlation with macroinvertebrate relative number of taxa but no correlation with exposed-reference similarity; Mount et al (1997) found K and HCO_3 the most toxic of the major ions. K not a strong contributor to ionic strength in mine site waterbodies (cf $Mg/SO_4/Ca$).		Additional laboratory testing required to assess K and HCO_3 toxicity interaction with Mg and SO_4.
	(ii) Water-borne CoPCs, manganese, ammonia, nitrate and turbidity associated with mine water inputs did not approach concentrations in the minesite waterbodies exceeding locally-derived, biological-effects GVs		No potential, null statement supported.
	(iii) Sediment-borne metal CoPCs, Mg, Mn, Fe, Cu, Pb, Cd, Zn, Cr and V (Brown et al 1985b), no GV exceedances found in mine site waterbodies. In 2011, U in the sediments of some sampling locations in RP1 was above the GV. However, no adverse biological effects observed (MDS ordination, ANOSIM).		No potential, null statement supported.

Potential for confounding (null statement)	Analysis undertaken and result (with section addressed)	Potential to contribute to observed change	Subsequent assessment
	(iv) U in waters: Concentrations of U in GTB and CJBB for the antecedent periods (ie wet and/or dry season months) for all macroinvertebrate sampling years were below the site-specific GV. After removal of CJBB and RP1 2013 data (see (v) below), U was no longer correlated with any biological response measure.		No potential, null statement supported.
	(v) Extreme acidity events preceding CJBB and RP1 sampling in 2013	Inclusion of 2013 CJBB and RP1 data have potential to confound results	2013 CJBB and RP1 data removed.

Table 28 Criteria to formalise the use of independent lines of evidence in inferring causation of magnesium in Ranger mine site waterbodies (from Hill 1965)

Name of criterion	Description of criterion	Mg in Ranger mine site waterbodies
Strength of association	Size of the correlation between the intensity of the disturbance and the response of the measurement parameter	Sites and times with high concentrations of Mg have less similar community structure and often, reduced number of taxa, compared with sites and times with low concentrations of the toxicant. Non-linearity in response across the gradient may be explained by pollution-induced community tolerance (PICT).
Consistency of association	The association between the disturbance and the measurement parameter has been repeatedly observed in different places, circumstances, and times	The reduced similarity was a response gradient observed across three separate waterbodies. For another $MgSO_4$-affected receiving water (Limestone Creek, Kimberleys, WA) (Humphrey et al 2008a), macroinvertebrate community structure also differed from communities measured in adjacent reference waterbodies but there was strong evidence of Ca amelioration and PICT. Salts studied elsewhere in Australia and overseas elicit similar adverse responses at high concentrations.
Specificity of association	The observed effect is diagnostic of exposure to the disturbance	In this case, a change in biological response is not diagnostic of the disturbance because such responses may be altered by other, natural, processes. (With further investigation, the ranking of Mg-sensitive to tolerant taxa may be found to conform more with responses found in other salt studies – in turn different from what might be expected from other stressors (chemical or otherwise).)
Presence of stressor in tissues	Measurement parameters of exposure (e.g. residues, breakdown products) must be present in the tissues of affected organisms	Not applicable; Mg does not bioaccumulate
Temporality or timing	Exposure to the disturbance must precede the effect in time	Macroinvertebrate communites in mine water exposed billabongs were similar to one another and similar to adjacent references waterbodies prior to mining or prior to 'significant' mine water contamination. Decrease in similarity amongst the waterbodies coincided with increase in Mg concentrations in minesite waterbodies. When Mg concentrations decreased in GTB in 2013 (after the putative impact in 2011), biological responses in GTB returned to reference condition.
Biological gradient	A dose–response relationship exists (i.e. response of measurement parameter is a function of increases in magnitude of disturbance)	Laboratory toxicity tests (van Dam et al 2010) and mesocosm studies have established a dose–response relationship. From local and overseas toxicity testing, Mg has been shown to be amongst the most toxic of the common cations, explaining the high sensitivity of Mg observed in the field and laboratory in the absence of significant Ca amelioration.
Biological plausibility	There is a biologically plausible explanation for causality, even if the precise mechanism is unknown	Mg has similar physiological and ecological actions as other salts, i.e. increases in salinity of freshwater ecosystems may affect aquatic organisms through: 1) direct toxicity through physiological changes (particularly associated with disruption of ionoregulation (ionic homeostasis), acid-base regulation (pH homeostasis) and potentially membrane integrity), and 2) indirect effects associated with changes in community structure, and changes to species interactions and ecological processes
Coherence	The causal interpretation should not seriously conflict with existing knowledge about the natural history of the organism and the behaviour of any substances associated with the disturbance	Mg-sensitive to tolerant taxa are consistent with taxa responses measured in other salt-related studies. PICT and Ca amelioration may account for variances.
Experimental evidence	A valid experiment provides strong evidence of causation or corroborates variances in observed response	Field macroinvertebrate responses are corroborated with extensive laboratory toxicity testing (van Dam et al 2010) and field mesocosm results (McCullough 2006). Lack of Ca protection (GTB) also shown in mesocosm studies.
Analogy	Similar disturbances cause similar effects	Other salts related to Mg have shown similar dose–response curves and responses in field experiments with different but related species. Ionic amelioration and PICT are also documented in accounting for variances.

6.2.1 Assessing potential confounders

Each of the five broad classes of potential confounding identified in this study was evaluated as follows:

6.2.1.1 Appropriateness of the reference billabongs used (i.e. appropriate experimental design)

The assessment summarised in Table 27 concluded that no catchment or waterbody differences amongst the study locations had potential to confound and contribute to misinterpretation of the observed Mg-biological response relationships.

6.2.1.2 Interannual patterns in macroinvertebrate community composition

Time-, climate (rainfall) or protocol-related differences in amongst-site variation in community structure in reference billabongs over time did not have the potential to coincidentally correspond with putative mine water associated biological change (Table 27).

6.2.1.3 Variations in sample processing protocol

A change in sample processing methods after 2006 did not confound the data for combined (all years) or exposed-reference waterbody comparisons made in the study. Protocol variations that incorporated or excluded sediment-dwelling macroinvertebrates did not confound the data either (Table 27).

6.2.1.4 Habitat (aquatic plants)

The evidence considers Types 1, 2 and 7 from Table 26 with the assessment summarised in Table 27. A number of macroinvertebrate taxa increased in abundance with increasing macrophyte cover while various aquatic plant measures were also correlated with macroinvertebrate assemblage patterns in multivariate space. However, correlations between aquatic plant measures and macroinvertebrate responses were not as strong as those derived between Mg and macroinvertebrate responses. Correlations between aquatic plant measures and Mg were also poor. No relationships between aquatic plant measures and macroinvertebrate responses were found in GTB where the Mg threshold and GV was derived.

6.2.1.5 Water quality and habitat influences: Fire regimes and feral pigs

The evidence considers Types 1, 2, 7 and 9 from Table 26 with the assessment summarised in Table 27. The fire history preceding sampling of GTB in 2011 did not appear unusual compared to other years. These observations suggest no obvious link between fire history and putative impacts to macroinvertebrate communities of GTB in 2011. Most intensive burning occurred in the GTB catchment in the late dry seasons antecedent to sampling in 2009 and 2013. The most biologically impacted locations in GTB in 2011 did not suffer littoral vegetation loss, nor was turbidity higher in these locations than other locations sampled. Thus there was no evidence of pig-associated impacts to GTB in 2011 coincident with macroinvertebrate sampling.

6.2.1.6 Water and sediment CoPCs other than Mg

The evidence considers Types 1, 2, 3, 5, 6 and 7 from Table 26, with the assessment summarised in Table 27. The CoPCs considered are other major ions (noting EC and SO_4 are not considered as confounders as they are surrogates for the same salt), metals including U, ammonia, nitrate, turbidity and low pH. Various CoPCs were correlated with macroinvertebrate responses, Ca and K, with higher correlation than Mg. Correlations between Mg and both U and Ca were also high. Additional potential confounding was associated with above-GV values for U in waters and sediment of RP1 (only), and acidity events that occurred in the early wet season of 2013 in both CJBB and RP1, prior to 2013

sampling. A number of approaches were taken to discount, reduce or eliminate potential confounding:

1 Water-borne CoPCs, manganese, ammonia, nitrate and turbidity, as well as Ca, and sediment-borne metals (Mg, Mn, Fe, Cu, Pb, Cd, Zn, Cr and V) were noted not to exceed relevant GVs.
2 CJBB and RP1 data from 2013 removed from Mg and U relationships with macroinvertebrate responses, thereby eliminating low pH confounding.
3 After 2, U was no longer correlated with any biological response measure amongst waterbodies.
4 Above-GV for U in sediment of RP1 (2011) found not to contribute to any adverse effects.

GTB, where the Mg threshold and GV was derived, did not observe significant (e.g. above relevant GV) elevations in other water and sediment CoPCs. While it is assumed that this assessment provided strong support for the Mg GV, the possible presence of elevated, mine-derived K and HCO_3, ions which were not regularly measured in the mine sites waterbodies over time, places a caveat on the derived GV. Additional laboratory testing is required to assess toxicity interaction of these ions with Mg and SO_4.

6.2.2 Evaluating other lines of evidence

Whilst not explicitly described in section 6.1 above, Type 5 evidence from Table 26 considers Hill's (1965) criteria of 'biological gradient' and 'experimental evidence'. Table 28 summarises the findings of complementary laboratory toxicity testing (van Dam et al 2010) and field mesocosm results (McCullough 2006)[8]; the laboratory-derived GV (section 1.4) and mesocosm assemblage EC5 values (see footnote) are similar to, and therefore, corroborate, the field-effects-derived GV.

Hill's (1965) criterion of 'temporality' is well met in the current study. As described in Table 28, departure in macroinvertebrate community structure in mine water exposed billabongs from the reference condition occurred over time and was coincident with increasing Mg in the mine site waterbodies. Improved water quality in the time series in GTB corresponded with a return to reference condition in macro invertebrate responses.

Hill's (1965) criteria of 'consistency of association' and 'analogy' are considered in Table 28. Types 9 and 10 evidence from Table 26 also consider characteristic effects typically associated with the putative cause and not the confounder. Other salt-effects studies confirm the sensitivity of a number of aquatic invertebrate groups to salts, including Mg (reviewed in section 1). Otherwise, the diagnostic attribute that has potential to assess these lines of evidence is consistency of taxa responses to those observed in similar studies. This aspect is evaluated in Table 27. Taxa responses observed in this study were generally consistent with those found or reported in other salt-effects studies though plant habitat preferences of taxa must also be considered for the ecosystem types sampled in this study. This requires literature review and further research.

Consistency, analogy and characteristic effects must also consider physiological acclimation. The circumstances which may lead to the evolution of such tolerance (section

[8] Results of this mesocosm work, including recent re-analyses (SSB Unpublished data), demonstrated sensitivity in $MgSO_4$-dosed tubs after four-week exposure of phytoplankton (algal biomass viz chlorophyll a) and zooplankton). The 5% hazardous concentrations (EC5) for algal biomass and community structure response measures for zooplankton were <2 and 2.2 mg L^{-1}Mg respectively (where Mg:Ca ratios were 2.5:1 for ambient creek water control and between 4-25:1 for successive four Mg treatments).

5.6) may create difficulties in evaluating these lines of evidence against other salt-effects studies. Several factors were considered in section 5.6 that may be responsible for explaining the complex patterns of taxon occurrences amongst the different mine site waterbodies of this study, and why laboratory predictions may not necessarily match field information, even though the derived Mg GV is consistent between laboratory and field.

6.3 Conclusions

The hypothesis that changes in lentic macroinvertebrate communities in Ranger mine site waterbodies over time can predominately be attributed to Mg increase is confidently supported after considering sources of potential confounding and alternative explanations for the observed biological responses. While the collective data from the three mine site waterbodies represented a continuum in reponse to Mg, the data from GTB represented contaminant-response information unaffected by any of the identified potential confounders. Thus, the information from this site was used to confidently determine a Mg threshold and GV for closure and to verify as well the appropriateness of the current GV for mine operations.

The caveat to this Mg GV derivation is the possibility that K and/or HCO_3 ions, known in the literature to be more toxic than Mg, interacted with Mg to affect toxicity and the associated indirect ecological interactions observed in the field. This aspect requires further investigation. Laboratory confirmation of interaction in the combination of ions to toxicity would indicate the mixture of ions is the cause of the observed changes and that ionic strength, viz, EC, would constitute a more appropriate basis of GV derivation. However, the very strong relationship between EC and Mg in the mine site waterbodies (Table 4) would render such a derivation redundant.

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Appendix 1 Environmental data

Table A1.1. Sampling commencement and completion dates of waterbodies

Year	Commencement	Completion
1979 [1]	2 April	13 June
1995	10 May	8 June
1996	13 May	30 May
2006	17 May	3 June
2009	6 May	11 May
2011	27 May	17 June
2013	21 May	12 June

1 See Marchant (1982a) for actual sampling dates for each billabong

Table A1.2. Summary of habitat results (means) for all years of sampling. Vegetation groups are given as relative percent totals (rounded to 1 decimal place). Groups are Floating Attached (FA), Emergent Broad Leaf (EBL), Emergent Narrow Leaf (ENL), Submerged & Emergent Feathery (SEF), Submerged Not Feathery (SNF), Free Floating (FF) and Charophyta (Ch). Blank cells indicate site was not sampled. See Table 1 for site codes

Variables	Units	Waterbody													
		RP2	RP1	DJKB	CJBB	GTB	GULB	BARB	JBL	CORB	WINB	MALB	ANGB	BUBB	SDSB
2013		**28 May**	**29 May**		**24 May**	**23 May**	**21 May**	**31 May**	**3 June**	**22 May**	**6 June**	**5 June**	**30 May**	**4 June**	**12 June**
Total Cover	%	51	57		71	59	85	80	59	94	98.4	66	95	82.6	74
Taxa Richness		2.2	3.6		2.8	6.2	5.4	6	3.4	8.4	6.6	4.6	4.8	7.4	4.8
FA Total	%	0	46.2		37.3	28.3	42.2	46	33.4	47.9	23.4	1.5	28	9.3	27.4
EBL Total	%	21.8	0.2		0	0	1.9	0	0	3.9	0.8	0	0	0	0
ENL Total	%	29.2	8.3		24.4	11.5	0.8	5.7	3.96	17.6	18.3	14.6	21.4	33	0
SEF Total	%	0	1.9		9.1	7.2	11	27.5	0	11.1	6.9	0	6	9.8	17.9
SNF Total	%	0	0.5		0.3	9.9	29.1	0.7	2.8	8.5	49.1	30.1	38.8	27.7	21.8
FF Total	%	0	0		0	0	0	0.2	0	5	0	1.4	0.8	0	0.2
Ch Total	%	0	0		0	2.3	0	0	19	0	0	18.4	0	2.8	6.7
2011		**9 June**	**10 June**		**8 June**	**7 June**	**27/30 May**	**6 June**	**17 June**	**1 June**	**15 June**	**2 June**	**3 June**	**14 June**	**16 June**
Total Cover	%	25	57		88.6	62	55	78	87	83	98.2	76	88	92.2	90.6
Taxa Richness		2	3.8		3	3.8	6.6	6.2	5	6.8	5.2	4	4	5.4	5.4
FA Total	%	12.8	13.4		12.6	36.5	26.7	19.5	20.7	29	27.9	6.9	26.2	6.3	30.7
EBL Total	%	8.6	0		0	0	1.2	0	0.8	7.4	9.3	0	0	3.6	0
ENL Total	%	3.6	37.3		64.6	23.9	8.5	42	15.2	15.4	16.4	64.7	14.4	59.2	3.6
SEF Total	%	0	1.9		10.7	0.7	4.8	12.1	43.2	21.5	2.4	0.8	0	2.9	1.1
SNF Total	%	0	4.2		0.8	1	13.8	4.4	7.2	0	42.2	0.9	46.9	18.9	54.7
FF Total	%	0	0		0	0	0	0	0	9.8	0	2.8	0.5	1.4	0.5
2006		**25 May**	**22 May**		**23 May**	**17 May**	**26 May**	**29 May**	**30 May**	**31 May**	**2 May**	**19 May**	**24 May**	**1 June**	**3 June**
Total Cover	%	56.6	50		95	82	92	86	53	89	92.6	89	98	95	91
Taxa Richness		1.2	4		4.2	3.6	6.6	5.8	6.8	7.4	5.6	8.4	4.8	6	5.4
FA Total	%	0	38.5		28.1	45	32.5	26.6	4	24.3	40.7	45.1	15.7	8.2	24.3

Variables	Units	Waterbody												
		RP2	RP1	DJKB	CJBB	GTB	GULB	BARB	JBL	CORB	WINB	MALB	ANGB	BUBB	SDSB
EBL Total	%	6	0		0.2	0	0	0	0.2	0	0	0.7	0	0	0
ENL Total	%	50.6	5.7		58.4	36.2	31.1	26.2	25	51.31	30.7	23.5	55.6	66.7	37.9
SEF Total	%	0	5.4		2.8	0.1	2.6	15	0	5.6	2.6	10.9	23.4	3.2	5.1
SNF Total	%	0	0.4		5.5	0.6	25.9	18.2	23.9	5.8	18.6	1.4	2.9	16.5	22.9
FF Total	%	0	0		0	0	0	0	0	2	0	7.4	0.4	0.4	0.9
1996			**20 May**	**13 May**	**15 May**	**14 May**	**16 May**	**21 May**	**17 May**	**27 May**			**28 May**	**30 May**	**22 May**
Total Cover	%		42.8	83	66	89	86	76	46	92			90	82.2	86
Taxa Richness			3	3.6	4.2	3.2	6.4	5.6	3	5.2			3.6	6.7	5.4
FA Total	%		27.0	13.3	28.8	23.8	29.7	29.3	12	17.9			6.5	11.6	12.7
EBL Total	%		0	0	9.2	0	5.2	0	0.6	0			0	0.6	0
ENL Total	%		3.2	68.3	26.4	62.6	20.4	19.3	32	36.4			57.8	36.0	38.8
SEF Total	%		7.364	0	0	0	4.2	19.6	0	5.8			25.4	21.3	16.9
SNF Total	%		5.3	1.5	1.6	2.7	26.5	7.8	1.4	0			0.2	10.4	13.3
FF Total	%		0	0	0	0	0	0	0	31.9			0.2	2.2	4.3
1995			**7-8 June**	**18-19 May**	**12 May**	**10-11 May**			**23 May**					**26 May**	**29 May**
Total Cover	%		32	67	44	53			50.4					70	52
Taxa Richness			5	3	5.6	5.4			2					6.4	4.4
FA Total	%		11.2	24.3	20.9	19.6			9.3					20.25	2.4
EBL Total	%		0	0	0	0			0					0.3	0
ENL Total	%		12.5	40.1	20.8	11.8			41.1					27	23.5
SEF Total	%		6.0	0	0.4	2.8			0					14.99	15.6
SNF Total	%		2.3	2.7	1.9	18.9			0					7.32	10.6
FF Total	%		0	0	0	0			0					0.14	0

Table A1.3. Summary of general parameter results (means) for billabong replicate sites for all years of sampling. Blank cells indicate parameter not recorded for that site. See Table 1 for site codes

Variables	Units	Waterbody													
		RP2	RP1	DJKB	CJBB	GTB	GULB	BARB	JBL	CORB	WINB	MALB	ANGB	BUBB	SDSB
2013															
Temperature	**°C**	27.83	27.09		27.69	29.68	28.11	26.31	28.36	28.61	25.26	26.50	27.72	26.58	26.38
Conductivity	**µS cm^{-1}**	2417.2	381.1		224.1	109	53.2	75.2	133.7	46.1	35.2	40	25.5	43.3	23.8
pH	**Units**	7.07	6.42		6.07	6.20	5.64	6.35	7.82	5.54	5.69	6.43	5.94	6.22	5.47
Turbidity	**NTU**	7.74	1.18		10.14	7.94	7.22	11.22	4.08	8.76	7.10	15.50	8.12	3.62	3.08
Dissolved Oxygen	**mg L^{-1}**	6.01	4.52		1.29	4.02	0.50	1.81	7.80	0.65	1.83	6.34	6.71	1.92	2.95
Dissolved Oxygen	**%**	77.22	57		16.98	53.00	6.50	17.52	100.56	8.40	22.32	79.18	87.26	23.96	36.84
2011															
Temperature	**°C**	26.97	26.13		22.85	23.76	24.60	22.81	22.76	21.96	20.26	22.55	22.21	21.23	**21.91
Conductivity	**µS cm^{-1}**	2064	302.2		188.2	48.4	31.8	58	92.9	41.6	24.4	46.2	20.8	37.6	**16.9
pH	**Units**	8.74	7.19		6.40	6.15	5.99	6.39	7.80	5.98	5.65	6.58	6.06	6.10	**5.34
Turbidity	**NTU**	10.28	1.56		6.54	15.16	1.24	3.06	2.22	2.98	3.14r	9.18	5.38	10.62	**0.90
Dissolved Oxygen	**mg L^{-1}**	9.98	5.93		2.38	3.86	4.79	7.14	8.67	2.70	4.62	5.31	7.22	5.40	**7.87
Dissolved Oxygen	**%**	120.14	72.66		26.72	44.76	56.7	70.28	100.10	33.18	51.92	62.77	80.70	58.68	**87.60
2006															
Temperature	**°C**	28.32	27.912		27.0	26.68	27.20	23.40	24.80	21.76	23.46	26.95	26.19	22.8	22.57
Conductivity	**µS cm^{-1}**	1323.6	352		205.2	41.8	29.4	51.4	86.8	29	22	30	23.8	31.4	17
pH	**Units**	6.568	6.76		6.33	6.31	5.97	6.47	7.22	5.97	5.92	6.38	6.02	6.38	5.39
Turbidity	**NTU**	0.52	0.36		5.16	3.82	1.44	6.22	3.66	10.5	3.6	*30.2	4.5	9.26	5.88
Dissolved oxygen	**mg L^{-1}**	7.352	4.41		1.51	3.52	2.94	3.30	6.74	1.69	2.35	2.13	2.82	3.28	4.28
Dissolved oxygen	**%**	94.56	56		18.66	43.64	37.1	39.22	88.57	19.36	27.54	26.98	35.1	35.5	50.76
1996															
Temperature	**°C**														
Conductivity	**µS cm^{-1}**		159.2	646	104	32.5	27.8	66.6	86.9	26.7			29.0	50.2	15.2

Variables	Units	Waterbody												
		RP2	RP1	DJKB	CJBB	GTB	GULB	BARB	JBL	CORB	WINB	MALB	ANGB	BUBB	SDSB
pH	**Units**		6.81	6.92	6.18	6.21	6.38	6.81	7.30	6.16			6.33	6.44	5.98
Turbidity	**NTU**														
Dissolved oxygen	**mg L^{-1}**														
Dissolved oxygen	**%**														
1995															
Temperature	**°C**		26.5	25.2	25.7	25.1			27					25.8	26.2
Conductivity	**µS cm^{-1}**		200	830	120	50			45					41	28
pH	**Units**		7.5	6.9	7.3	7.1			7.3					5.8	5.6
Turbidity	**NTU**														
Dissolved oxygen	**mg L^{-1}**		6.58	0.85	2.58	4.18			7.02					3.97	5.18
Dissolved oxygen	**%**		79.7	10	30.7	49.2			85.6					47.2	63
1979															
Temperature	**°C**				25.67	27.67									
Conductivity	**µS cm^{-1}**				38.67	35									
pH	**Units**				6.13	6.77									
Turbidity	**NTU**				5.6	14.77									
Dissolved oxygen	**mg L^{-1}**														
Dissolved oxygen	**%**														

** field instrument communication failed during measurement for replicate sites

* field instrument not reading turbidity values

Table A1.4. Summary of water chemistry results (means). Blank cells indicate variable was not recorded for that site. See Table 1 for site codes. Symbols *,** and *** indicate data collected from ERA, Fish pop-netting and Magnesium calculated from EC:Mg relationship respectively. Analyte suffix "_F" denotes filtered sample.

Variables	Units	Waterbody													
		RP2	RP1	DJKB	CJBB	GTB	GULB	BARB	JBL	CORB	WINB	MALB	ANGB	BUBB	SDSB
2013															
Turbidity	**NTU**	10.8	1.9		9.55	4.05	9.65	1.1	1.15	3.1	10.4	12.85	3.85	4.75	0.3
EC	**µS cm^{-1}**	2448.5	382.3		267.2	100.2	52.9	76	132	46.5	32.6	39.8	25.6	23.1	22.1
Temperature	**°C**	27.70	26.92		26.87	30.08	28.29	25.90	28.26	28.13	25.08	26.88	27.49	27.97	26.34
pH	**Units**	7.17	6.65		6.08	6.08	5.69	6.36	7.67	5.5	5.81	6.28	6.15	6.27	5.77
DO	**mg L^{-1}**	6.13	6.79		0.88	4.32	1.18	1.80	7.71	0.70	4.16	4.57	7.09	2.95	5.69
DO	**%**	78.45	85.1		10.8	56.9	15.25	22.05	98.9	8.85	50.65	57.1	91.8	37.45	70.65
Al_F	**µg L^{-1}**	23	4.5		10.5	1.5	1	2	1.5	6.5	9.5	16.5	13.3	1.5	3
Cu_F	**µg L^{-1}**	2	0.14		0.1	0.55	0.2	0.1	0.15	0.15	0.15	0.7	0.23	0.55	0.2
Fe_F	**µg L^{-1}**	1.5	215		190	16	30	26	130	184	43	31	37	8	9
Mn_F	**µg L^{-1}**	270	14.5		41	1.5	15.7	0.5	0.2	4.9	18	3.5	1.4	0.3	1.4
Pb_F	**µg L^{-1}**	0.3	0.02		<0.01	<0.01	0.02	0.01	0.02	0.03	<0.01	0.03	0.02	<0.01	0.02
U_F	**µg L^{-1}**	3150	3.45		0.35	0.15	0.015	0.01	0.01	0.007	0.006	0.03	0.02	0.004	0.003
Zn_F	**µg L^{-1}**	19	0.13		0.5	0.6	0.9	2.5	0.3	2.0	0.5	2.3	0.8	0.4	0.3
Ca_F	**mg L^{-1}**	68	6.7		2.6	1.9	1.4	2.6	5.8	1.9	0.4	1.8	0.9	1.9	0.7
K_F	**mg L^{-1}**	9.6	5		1.3	0.6	0.6	1.0	1.7	1.5	0.8	1.7	0.2	0.7	0.1
Mg_F	**mg L^{-1}**	285	33.5		23.5	7.3	2.1	2.7	6.4	1.3	1.5	0.9	1.1	2.0	0.4
Na_F	**mg L^{-1}**	53.5	9.2		10.0	5.0	4.8	8.5	9.0	3.8	3.6	4.2	1.9	3.4	2.5
SO4_F	**mg L^{-1}**	1100	120		57.5	12	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Cl_F	**mg L^{-1}**	14	3		3.5	2	4	8.5	16	3	4.5	4.5	2.5	3	3.5
OH	**mg L^{-1}**	<1	<1		<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
HCO3	**mg L^{-1}**	75	37		52	33	23	30	51	23	9	20	11	22	4
CO3	**mg L^{-1}**	<1	<1		<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Total Alkalinity	**mg L^{-1}**	75	37		52	33	23	30	51	23	9	20	11	22	4

Variables	Units	Waterbody												
		RP2	RP1	DJKB	CJBB	GTB	GULB	BARB	JBL	CORB	WINB	MALB	ANGB	BUBB	SDSB
Total N	**mg L^{-1}**	3.9	0.6		0.9	0.5	0.8	0.5	0.4	1.0	1	0.7	0.67	0.9	0.3
NO3_N	**mg L^{-1}**	3.15	<0.005		0.008	<0.005	0.007	<0.005	<0.005	0.006	<0.005	<0.005	<0.005	<0.005	<0.005
NH3_N	**mg L^{-1}**	0.16	0.016		0.018	<0.005	0.007	<0.005	0.018	0.025	0.007	<0.005	<0.005	0.023	0.010
PO4_P	**mg L^{-1}**	<0.005	0.008		0.008	<0.005	<0.005	<0.005	<0.005	<0.005	0.013	<0.005	0.007	<0.005	0.008
Total P	**mg L^{-1}**	0.3	<0.05		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
TOC	**mg L^{-1}**	4.26	9.32		15.67	6.98	8.00	5.59	4.35	9.60	9.32	9.52	7.46	7.24	5.68
DOC	**mg L^{-1}**	3.77	9.18		14.19	6.58	5.45	5.08	4.46	8.17	6.70	8.50	6.80	5.62	4.75
2011															
Turbidity	**NTU**	6.1	0.15		1.85	12.6	0.55	1.25	1.3	0.6	2.5	8.8	1.95	2.35	0.9
EC	**µS cm^{-1}**	2060	300.5		220.5	48.5	31	57.5	93.1	42.5	23	44	21	44	16.9
Temperature	**°C**	26.63	25.48		21.92	22.81	23.74	22.62	22.06	22.95	20.86	23.59	21.29	22.09	21.91
pH	**Units**	8.83	7.64		6.45	6.15	6.06	6.38	7.30	6.07	5.87	6.28	5.90	6.27	5.34
DO	**mg L^{-1}**	10.17	7.24		1.72	3.76	5.08	5.18	7.63	2.97	7.26	4.75	5.80	5.76	7.87
DO%	**%**	125.55	88		19.45	42.75	59.75	59.3	87.15	36.95	79.6	51.4	63.05	63.2	87.6
Al_F	**µg L^{-1}**	19.2	6.4		5.5	92.9	11.1	20.7	2.2	3.2	13.3	74.0	5.1	1.6	10.9
Cu_F	**µg L^{-1}**	6.96	0.41		0.14	0.64	0.28	0.19	0.17	0.135	0.30	0.71	0.21	0.13	0.22
Fe_F	**µg L^{-1}**	4	40		139	170	140	160	218	100	27	170	18	74	26
Mn_F	**µg L^{-1}**	1.0	0.8		32.3	10.1	4.5	3.8	0.7	7.8	1.7	23.0	6.2	2.7	0.8
Pb_F	**µg L^{-1}**	9.12	0.05		<0.01	0.05	0.02	0.03	0.02	0.01	<0.01	0.13	0.02	<0.01	0.01
U_F	**µg L^{-1}**	3810	3.93		0.303	0.590	0.034	0.017	0.011	0.007	0.039	0.036	0.046	0.004	0.015
Zn_F	**µg L^{-1}**	23.8	0.4		0.5	0.6	0.4	0.5	0.5	0.7	0.4	0.7	3.1	0.5	1
Ca_F	**mg L^{-1}**	74.2	3.8		2.3	1	0.8	2.3	4.5	1.7	0.3	2	0.3	2.4	0.6
K_F	**mg L^{-1}**														
Mg_F	**mg L^{-1}**	307.8	33.0		21.6	3.8	1.4	2.2	5.1	1.3	1.0	1.1	1.2	2.3	0.4
Na_F	**mg L^{-1}**														
SO4_F	**mg L^{-1}**	1407.5	115.5		64.6	7.6	0.3	0.2	0.4	<0.1	<0.1	0.2	0.2	0.1	<0.1

Variables	Units	Waterbody												
		RP2	RP1	DJKB	CJBB	GTB	GULB	BARB	JBL	CORB	WINB	MALB	ANGB	BUBB	SDSB
Cl_F	**mg L^{-1}**														
OH	**mg L^{-1}**														
HCO3	**mg L^{-1}**														
CO3	**mg L^{-1}**														
Total Alkalinity	**mg L^{-1}**														
NO3_N	**mg L^{-1}**	1.394	0.008		<0.005	0.006	<0.005	0.006	0.014	<0.005	0.007	<0.005	0.006	0.01	<0.005
NH3_N	**mg L^{-1}**	0.033	<0.005		0.013	0.005	0.006	0.008	<0.005	<0.005	0.007	0.023	0.009	<0.005	<0.005
PO4_P	**mg L^{-1}**	0.029	<0.005		0.008	0.005	0.006	0.006	0.006	0.01	0.006	0.013	<0.005	<0.005	<0.005
TOC	**mg L^{-1}**														
DOC	**mg L^{-1}**														
2006															
Turbidity	**NTU**	0.3	1.2		3.9	3.8	1.2	5.0	6.1	3.5	6.5	30.2	0.6	1.1	1.4
EC	**µS cm^{-1}**	1322.5	353		267.3	50	25.5	51.5	79.8	30	21	28.5	21	31	16.5
Temp	**°C**	27.94	27.20		27.02	26.08	27.38	23.21	24.69	21.95	22.93	26.71	25.06	21.90	21.95
pH	**Units**	6.73	7.08		6.32	6.42	6.11	6.31	7.04	5.83	5.94	6.50	6.05	6.28	5.54
DO	**mg L^{-1}**	94.45	68.53		38.15	27.98	22.25	33.93	73.05	2.5	21.35	40.95	8.75	13.2	58.55
DO%	**%**	172.75	139		104	109.25	144.25	175.25	123.5	145	125	126.5	88.5	112	202
Al_F	**µg L^{-1}**	26.4	3.6		5.1	17.1	15.2	141	1.8	11.2	14.7	595.5	12.3	3.3	13.9
Cu_F	**µg L^{-1}**	11.75	0.425		0.28	0.51	0.24	0.35	0.23	0.2	0.4	0.75	0.25	0.2	0.3
Fe_F	**µg L^{-1}**	10	150		150	175	180	515	300	140	70	280	120	80	70
Mn_F	**µg L^{-1}**	489.25	6.73		17.75	18.8	12.35	4.84	1.99	48.5	2.85	2.4	7.85	23.5	5.15
Pb_F	**µg L^{-1}**	9.94	<0.05		<0.05	<0.05	<0.05	0.15	<0.05	<0.05	<0.05	0.28	<0.05	<0.05	<0.05
U_F	**µg L^{-1}**	2940	3.658		0.506	0.585	0.043	0.023	0.006	0.009	0.025	0.035	0.011	0.006	0.016
Zn_F	**µg L^{-1}**	32	1		1.3	13.1	0.8	0.8	1	1.3	1	0.8	1.5	1.5	1
Ca_F	**mg L^{-1}**	28.4	3.6		2.3	1.0	0.7	2	3.7	1.3	0.3	1	0.9	1.7	0.6
K_F	**mg L^{-1}**														

Variables	Units	Waterbody												
		RP2	RP1	DJKB	CJBB	GTB	GULB	BARB	JBL	CORB	WINB	MALB	ANGB	BUBB	SDSB
Mg_F	mg L^{-1}	201	35.4		27.0	3.0	1.2	2.0	4.3	0.9	1	0.7	0.9	1.6	0.3
Na_F	mg L^{-1}														
SO4_F	mg L^{-1}	874.6	142.3		96.6	5.1	0.3	0.1	0.2	0.1	<0.1	0.1	0.1	<0.1	<0.1
Cl_F	mg L^{-1}														
OH	mg L^{-1}														
HCO3	mg L^{-1}														
CO3	mg L^{-1}														
Total Alkalinity	mg L^{-1}														
NO3_N	mg L^{-1}	1.775	<0.005		<0.005	0.028	<0.005	<0.005	0.011	<0.005	<0.005	<0.005	0.01	0.013	<0.005
NH3_N	mg L^{-1}	0.05	<0.005		<0.005	0.013	0.007	0.008	0.008	0.01	0.01	0.005	<0.005	0.005	<0.005
PO4_P	mg L^{-1}	<0.005	<0.005		<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
TOC	mg L^{-1}														
DOC	mg L^{-1}														
1996															
Turbidity	NTU														
EC	µS cm^{-1}		159.2	646	104	32.5	27.78	66.6	86.9	26.74			29.02	50.22	15.24
Temp	°C														
pH	Units		6.81	6.92	6.18	6.21	6.38	6.81	7.30	6.16			6.33	6.44	5.98
DO	mg L^{-1}														
DO%	%														
Al_F	µg L^{-1}				13**	58**	34**	140**		24**				20**	18**
Cu_F	µg L^{-1}				<0.5**	<0.5**	<0.5**	<0.5**		<0.5**				<0.5**	<0.5**
Fe_F	µg L^{-1}				0.68**	1.2**	1.3**	1.5**		1.8**				1.5**	0.18**
Mn_F	µg L^{-1}		12.19*		28**	13**	57**	49**		72**				27**	<2**
Pb_F	µg L^{-1}				<0.2**	<0.2**	<0.2**	<0.2**		<0.2**				<0.2**	<0.2**
U_F	µg L^{-1}		0.37*	2.6**	0.08**	0.17**	0.03	0.05		<0.02**				<0.02**	<0.02**

Variables	Units	Waterbody												
		RP2	RP1	DJKB	CJBB	GTB	GULB	BARB	JBL	CORB	WINB	MALB	ANGB	BUBB	SDSB
Zn_F	**µg L^{-1}**				<2**	<2**	<2**	<2**		<2**				<2**	<2**
Ca_F	**mg L^{-1}**			2.7**	1.2**	0.82**	0.68**	2.2**		1.6**				1.8**	0.46**
K_F	**mg L^{-1}**			1.2**	0.9**	0.32**	0.35**	0.98**		1.1**				0.82**	0.1**
Mg_F	**mg L^{-1}**		15.32*	37**	7.9**	1.6**	1**	2**	3.79723***	0.96**			0.978377***	1.7**	0.26**
Na_F	**mg L^{-1}**			4**	6**	2.9**	4**	11**		4.3**				3.4**	2.2**
SO4_F	**mg L^{-1}**		45.1*	92**	5.6**	1.2**	0.1**	0.2**		0.1**				0.1**	0.1**
Cl_F	**mg L^{-1}**			3.2**	4.1**	2.4**	3.8**	13**		3.4**				2.8**	2.9**
OH	**mg L^{-1}**														
HCO3	**mg L^{-1}**														
CO3	**mg L^{-1}**														
Total Alkalinity	**mg L^{-1}**				35**	9.2**	10**	22**		12**				15**	1.6**
NO3_N	**mg L^{-1}**			<0.1**	<0.01**	<0.01**	<0.01**	<0.01**		<0.01**				<0.01**	<0.01**
NH3_N	**mg L^{-1}**				<0.03**	<0.03**	<0.03**	<0.03**		<0.03**				<0.03**	<0.03**
PO4_P	**mg L^{-1}**				<0.002**	<0.002**		<0.002**		<0.002**					0.01**
TOC	**mg L^{-1}**				6.6**	4.7**	5.4**	4.4**		6.5**				4.5**	3.5**
DOC	**mg L^{-1}**				6.2**	4.3**	4.3**	3.8**		5.3**				3.6**	3.3**
1995															
Turbidity	**NTU**		2												
EC	**µS cm^{-1}**		200	830	120	50			45					41	28
Temp	**°C**		26.5	25.2	25.7	25.1			27					25.8	26.2
pH	**Units**		7.5	6.9	7.3	7.1			7.3					5.8	5.6
DO	**mg L^{-1}**		6.58	0.85	2.58	4.18			7.02					3.97	5.18
DO%	**%**		79.7	10	30.7	49.2			85.6					47.2	63
Al_F	**µg L^{-1}**			51**	21**	57**								67**	33**
Cu_F	**µg L^{-1}**		15.77*	<0.5**	<0.5**	<0.5**			<0.5**					<0.5**	<0.5**
Fe_F	**µg L^{-1}**			130**	1100**										840**

Variables	Units	Waterbody												
		RP2	RP1	DJKB	CJBB	GTB	GULB	BARB	JBL	CORB	WINB	MALB	ANGB	BUBB	SDSB
Mn_F	**µg L^{-1}**		1.33*	180**	31**	240**								36**	18**
Pb_F	**µg L^{-1}**		2*	<0.5**	<0.5**	<0.5**								<0.5**	<0.5**
U_F	**µg L^{-1}**		0.41*	<0.5**	<0.5**	<0.5**								<0.5**	<0.5**
Zn_F	**µg L^{-1}**		<2*	1.8**	<0.5**	1.2**								<0.5**	<0.5**
Ca_F	**mg L^{-1}**			230**	8.7**	1.3**								0.22**	0.59**
K_F	**mg L^{-1}**			2.5**	0.87**	2.1**								0.73**	<0.05**
Mg_F	**mg L^{-1}**		19.56*	120**	7.1**	2.7**			1.8495***					1.4**	0.38**
Na_F	**mg L^{-1}**		6.13*	15**	8.6**	6.1**								3.6**	2.1**
SO4_F	**mg L^{-1}**		70.87*	230**	8.7**	1.3**								0.22**	0.59**
Cl_F	**mg L^{-1}**			12**	5.4**	4.6**								3.2**	2.9**
OH	**mg L^{-1}**														
HCO3	**mg L^{-1}**														
CO3	**mg L^{-1}**														
Total Alkalinity	**mg L^{-1}**			260**	34**	25**								14**	1.9**
NO3_N	**mg L^{-1}**			0.03**	0.02**	0.01**								0.01**	0.02
NH3_N	**mg L^{-1}**			0.05**	0.2**	<0.03**								<0.03**	0.05**
PO4_P	**mg L^{-1}**														
TOC	**mg L^{-1}**			14**	8.1**	8.8**								1.6**	3.8**
DOC	**mg L^{-1}**			14**	7.6**	8.5**								5.2**	3.6**
1979															
Turbidity	**NTU**				5.6*	14.77*									
EC	**µS cm^{-1}**				38.67*	35*									
Temp	**°C**				25.67*	27.67*									
pH	**Units**				6.13*	6.77*									
DO	**mg L^{-1}**														
DO%	**%**														

Variables	Units	Waterbody												
		RP2	RP1	DJKB	CJBB	GTB	GULB	BARB	JBL	CORB	WINB	MALB	ANGB	BUBB	SDSB
Al_F	µg L^{-1}														
Cu_F	µg L^{-1}				<0.5*	0.8*									
Fe_F	µg L^{-1}				196.67*	323.33*									
Mn_F	µg L^{-1}				9.6*	1.77*									
Pb_F	µg L^{-1}				<0.5*	0.5*									
U_F	µg L^{-1}				0.3*	0.6*									
Zn_F	µg L^{-1}				2.8*	1.43*									
Ca_F	mg L^{-1}				0.74*	0.69*									
K_F	mg L^{-1}				1.14*	0.72*									
Mg_F	mg L^{-1}				0.97*	1.57*									
Na_F	mg L^{-1}				5.13*	3.17*									
SO4_F	mg L^{-1}				0.27*	0.43*									
Cl_F	mg L^{-1}				3.53*	3*									
OH	mg L^{-1}														
HCO3	mg L^{-1}				15.67*	12.23*									
CO3	mg L^{-1}														
Total Alkalinity	mg L^{-1}														
NO3_N	mg L^{-1}				0.015*	0.05*									
NH3_N	mg L^{-1}				0.035*	0.035*									
PO4_P	mg L^{-1}				<0.003*	0.003*									
TOC	mg L^{-1}														
DOC	mg L^{-1}														

Table A1.5. Antecedent water chemistry data for Georgetown Billabong. Blank cells = no data collected

Variables	Units	1979			1995			1996			2006			2009			2011			2013		
		Dry	Wet	*Mean*	Dry	Wet	*Mean*	Dry	Wet	*Mean*	Dry	Wet	*Mean*	Dry	Wet	*Mean*	Dry	Wet	*Mean*	Dry	Wet	*Mean*
EC	**µS cm^{-1}**	45	31	*38*	88.4	19.7	54.05	59.15	27	*43.08*	69	33.8	*51.4*	91.6	53.98	*72.79*	128.25	38.1	*83.18*	95.1	64	*79.55*
U	**µg L^{-1}**	0.45	0.2	*0.33*	0.9	0.25	0.57	0.51	0.35	*0.43*	0.37	0.64	*0.5*	0.46	0.3	*0.38*	0.38	0.4	*0.39*	0.46	0.23	*0.34*
Ca	**mg L^{-1}**	0.87	0.67	*0.77*				2.30		*2.3*	0.5	0.73	*0.61*	1.15	1.4	*1.28*	1.83	0.9	*1.36*	1.3	1.7	*1.5*
Cl	**mg L^{-1}**	8.4	1.8	*5.1*								2.4	*2.4*	3.7	3	*3.35*	6.88	1.6	*4.24*	5.2	2.7	*3.95*
Mg	**mg L^{-1}**	1.08	1.5	*1.29*	2.94	1.03	1.98	2.16	0.98	*1.57*	1.9	2.25	*2.08*	5.88	4.35	*5.11*	8.6	2.75	*5.68*	4.65	3.9	*4.28*
K	**mg L^{-1}**	1.65	0.42	*1.04*				2.10		*2.1*				1.4	0.7	*1.05*	4.4	0.4	*2.4*	2.9	0.5	*1.7*
Na	**mg L^{-1}**	5.05	1.5	*3.28*	5.75		5.75	6.10		*6.10*				5.5	3.5	*4.5*	8.43	1.95	*5.19*	8.2	2.6	*5.4*
SO_4	**mg L^{-1}**	2.85	0.5	*1.68*	6.4	1.12	3.76	1.14	1.88	*1.51*	1.3	4.65	*2.98*	6.68	9.8	*8.24*	20.5	4.8	*12.65*	8.2	10	*9.1*
Mn	**µg L^{-1}**	38.5	2.7	*20.6*	17.54	162.67	90.11	43.07	12.13	*27.6*	3.13	11.85	*7.49*	11.75	8.61	*10.18*	18.5	5.41	*11.95*	15.5	15.5	*15.5*

Antecedent data obtained by calculating the median of the median monthly data for each season for the relevant preceding wet/dry periods for each year sampling was done. Dates for each season considerted to be; Wet (Dec-31 May) and Dry (1 Jun- Nov). Exact wet season start also based on drop in EC levels seen in the billabong as the Magela Creek flow starts, so can vary with year (this date also used for Coonjimba, RP1 and Djalkmara billabongs as the start of the wet season for each year). Data sources ERA LIMS, NT Roads & Transport, and SSB

Table A1.6. Antecedent water chemistry data for Coonjimba Billlabong. Blank cells = no data collected

		1979			1995			1996			2006			2009			2011			2013		
		Dry	Wet	*Mean*	Dry	Wet	*Mean*	Dry	Wet	*Mean*	Dry	Wet	*Mean*	Dry	Wet	*Mean*	Dry	Wet	*Mean*	Dry	Wet	*Mean*
EC	**µS cm^{-1}**	86	28	*57*	97.5	110	*103.75*	103.2	90	*96.6*	361.65	233.2	*297.43*	323.45	372.18	*347.81*	332.75	200	*266.38*	233.5	514	*373.75*
U	**µg L^{-1}**	0.15	0.4	*0.28*	0.44	0.39	*0.41*	0.27	0.36	*0.31*	1.04	1.23	*1.13*	1.06	0.72	*0.89*	0.64	0.72	*0.68*	0.72	0.83	*0.78*
Ca	**mg L^{-1}**	0.27	0.76	*0.51*							3.2	2.4	*2.80*	3.95	4.38	*4.16*	3.55	2.4	*2.98*	1.83	7.8	*4.81*
Cl	**mg L^{-1}**	16.6	2.3	*9.45*													12	3	*7.5*	6.45	3.95	*5.2*
Mg	**mg L^{-1}**	0.31	0.95	*0.63*	6.42	7.47	*6.95*	8.24	6.75	*7.5*	39.2	23.4	*31.3*	33.35	41.38	*37.36*	31.83	17.85	*24.84*	18.75	41.55	*30.15*
K	**mg L^{-1}**	3.6	0.84	*2.22*		1.27	*1.27*										2.5	0.9	*1.7*	1.75	1.45	*1.6*
Na	**mg L^{-1}**	11.45	3	*7.23*													15.6	6.3	*10.95*	15.85	12.7	*14.28*
SO_4	**mg L^{-1}**	1.1	0.4	*0.75*	4.51	21.54	*13.02*	7.27	12.61	*9.94*	70.1	87.5	*78.8*	67.65	150.53	*109.09*	76.13	67.6	*71.86*	31.8	224.5	*128.15*
Mn	**µg L^{-1}**	36.1	6.8	*21.45*	104.81	10.64	*57.72*	129.49	54.12	*91.8*	31.25	9.9	*20.58*	97.75	63.25	*80.50*	40.38	14.5	*27.44*	43.75	474.5	*259.13*

Table A1.7. Antecedent water chemistry data for Retention Pond 1. Blank cells = no data collected

Variable	Units	1995			1996			2006			2009			2011			2013		
		Dry	Wet	*Mean*	Dry	Wet	*Mean*	Dry	Wet	*Mean*	Dry	Wet	*Mean*	Dry	Wet	*Mean*	Dry	Wet	*Mean*
EC	**$\mu S\ cm^{-1}$**	152	118.48	*135.24*	284	157.7	*220.85*	549	378.15	*463.58*	677.43	708.23	*692.83*	522.5	268.75	*395.63*	445	475	*460*
U	**$\mu g\ L^{-1}$**	0.48	0.5	*0.49*	0.62	0.64	*0.63*	10.03	4.61	*7.32*	9.66	3.99	*6.82*	6.77	3	*4.88*	6.35	3.85	*5.1*
Ca	**$mg\ L^{-1}$**		1.3	*1.3*		1.32	*1.32*	6	4	*5*	8.63	9.80	*9.21*	7.48	3.58	*5.53*	5.1	8.6	*6.85*
Cl	**$mg\ L^{-1}$**		3.19	*3.19*		3.02	*3.02*		2.8	*2.8*				4.35	1.85	*3.10*	4.55	3.4	*3.98*
Mg	**$mg\ L^{-1}$**	14.29	9.56	*11.92*	24.46	13.74	*19.1*	61.2	35.78	*48.49*	79.63	86.23	*82.93*	57.93	27.35	*42.64*	44.2	48.9	*46.55*
K	**$mg\ L^{-1}$**		1.23	*1.23*		1.36	*1.36*		1.95	*1.95*				2.85	1.05	*1.95*	2.5	2.3	*2.4*
Na	**$mg\ L^{-1}$**	5.26	4.79	*5.02*	8.65	4.5	*6.57*		8.2	*8.2*				8.9	5.70	*7.30*	12.35	9.8	*11.08*
SO_4	**$mg\ L^{-1}$**	27.9	33.58	*30.74*	89.94	46.33	*68.13*	223	133	*178*	304.5	321.25	*312.88*	201.25	100.28	*150.76*	151.5	221	*186.25*
Mn	**$\mu g\ L^{-1}$**	2.92	8.14	*5.53*	2.38	8.1	*5.24*	1.8	5	*3.4*	0.63	22.75	*11.69*	0.73	3.60	*2.16*	7.35	77.50	*42.43*

Table A1.8. Antecedent water chemistry data for Djalkmara Billabong. Blank cells = no data collected

Variable	Units	1995			1996		
		Dry	Wet	*Mean*	Dry	Wet	*Mean*
EC	**µS cm^{1}**	1100	830	965	970	340	655
U	**µg L^{-1}**	28.65	6.2	17.43	13.6	2	7.8
Ca	**mg L^{-1}**	12	7.45	9.73	13	2.6	7.8
Cl	**mg L^{-1}**	22.5	7.35	14.93	15.875	3.1	9.49
Mg	**mg L^{-1}**	150	103	126.5	145	14	79.5
K	**mg L^{-1}**	5.7	1.7	3.7	3.3	0.7	2
Na	**mg L^{-1}**	32	13	22.5	21.5	2.6	12.05
SO_4	**mg L^{-1}**	470	350	410	375	51	213
Mn	**µg L^{-1}**	104.5	1354	729.25	477.5	40	258.75

Table A1.9. Summary of sediment chemistry results (means) for the <63 µm fraction. Results displayed are for the 1M HCl digest. Blank cells indicate variable not measured for that site. See Table 1 for site codes.

Variable	Units	Waterbody												
		ANGB	BARB	BUBB	CJBB	CORB	GTB	GULB	JBL	MALB	RP1	RP2	SDSB	WINB
2013														
Al	**mg kg^{-1}**	4196	2756	3116	5940	3808	1993	4834	1304	2445	1497	4258	4154	2796
Ba	**mg kg^{-1}**	124.2	107.68	131.8	20.86	125.7	147.9	86.32	32.24	141.66	33.035	19.76	160.267	124
Ca	**mg kg^{-1}**	1299	772	1047.4	374.8	1324	1113.4	976.8	522.2	1162	326.6	2248	681.2	554.8
Cd	**mg kg^{-1}**	0.048	0.01	0.027	0.044	0.04	0.023	0.042	0.03	0.034	0.006	0.114	0.044	0.02
Cr	**mg kg^{-1}**	1.6	8.8	3	2.8	1	3	1.2	6	4.8	1.8	12	1.66667	1
Cu	**mg kg^{-1}**	9.415	5.06	8.12	8.39	10.38	15	8.19	9.9	11.69	5.52	139.9	9.75	6.575
Fe	**mg kg^{-1}**	1872	5830	5829	5592	4084	7705	2730	6222	4801	3612	5648	3622	2602
Mg	**mg kg^{-1}**	506.2	1203.2	416.8	1007.6	337.8	706.8	402	364.8	318.6	755.8	4398	194.4	417
Mn	**mg kg^{-1}**	27.34	30.76	80.78	29.44	50.84	76.34	61.8	40.3	82.06	22.94	990.6	41.8667	31.12
Ni	**mg kg^{-1}**	6.395	2	1.64	1.63	2.325	2.205	1.87	0.975	1.755	1.015	17.58	3.36	1.61
Pb204	**mg kg^{-1}**													
Pb206	**mg kg^{-1}**													
Pb207	**mg kg^{-1}**													
Pb208	**mg kg^{-1}**													
Pb	**mg kg^{-1}**	5.545	30.46	13.81	13.98	11.15	8.615	11.77	26.39	35.58	9.08	289	6.11	7.195
Rb	**mg kg^{-1}**	2.652	1.352	1.792	1.648	1.62	1.668	1.424	1.17	1.762	1.606	1.384	1.912	0.984
S	**mg kg^{-1}**	36	22	22.5	1250	44.5	33	79	42	26	1057.5	929	38.3333	31.5
U	**mg kg^{-1}**	1.636	2.172	3.927	9.946	1.987	16.742	2.57	1.99	2.16	33.72	3236	1.56267	2.861
Zn	**mg kg^{-1}**	1.01	1.36	1.36	5.26	1.98	1.46	3.74	9.03	3.08	1.81	390.28	1.02	1.13
2011														
Al	**mg kg^{-1}**	5092	4274	5424	5007	4928	4143	9574	2500	2492	1172	4170	3613	4520
Ba	**mg kg^{-1}**	171.4	147	160.8	53.11	142.4	106.67	149.4	68.1	151.62	13.842	21.18	137.71	118.12
Ca	**mg kg^{-1}**	1288.2	1014.4	1209.6	800.4	1486	586.6	234.4	1377.2	1314.4	569.6	3940	341.8	549.6

Variable	Units	Waterbody												
		ANGB	BARB	BUBB	CJBB	CORB	GTB	GULB	JBL	MALB	RP1	RP2	SDSB	WINB
Cd	mg kg^{-1}													
Cr	mg kg^{-1}													
Cu	mg kg^{-1}	13.26	7.41	11.64	10.88	11.49	14.44	9.81	10.53	14.36	7.51	99.8	13.1	7.83
Fe	mg kg^{-1}	2214	2978	3836	7568	4532	5392	4982	5854	4116	7049	4038	4742	2232
Mg	mg kg^{-1}	405.2	638.4	323.2	1749	258	673.6	157.2	698.8	313.6	1372.6	6000	153.2	425.6
Mn	mg kg^{-1}	31.87	40.58	88.06	54.12	73.22	50.65	57.12	32.824	75.9	22.6	558	44.73	34.412
Ni	mg kg^{-1}													
Pb204	mg kg^{-1}	0.1	0.33	0.19	0.21	0.21	0.095	0.16	0.36	0.52	0.15	0.27	0.115	0.08
Pb206	mg kg^{-1}	1.945	5.92	3.74	5.195	3.85	3.88	3.13	6.4	8.47	8.76	167.36	2.225	1.64
Pb207	mg kg^{-1}	1.54	5.36	3.03	3.38	3.29	1.665	2.57	5.79	8.4	2.975	23.14	1.875	1.33
Pb208	mg kg^{-1}	3.815	12.93	7.39	7.89	7.91	3.775	6.25	13.9	19.42	5.755	11.71	4.67	3.28
Pb	mg kg^{-1}	7.38	24.54	14.36	16.68	15.29	9.4	12.1	26.42	36.82	17.63	202.2	8.875	6.33
Rb	mg kg^{-1}	3.916	1.792	2.712	2.704	2.42	2.56	1.444	2.18	2.388	2.504	1.328	0.954	1.444
S	mg kg^{-1}													
U	mg kg^{-1}	2.145	2.058	4.948	19.98	2.28	19.591	3.24	2.42	2.406	74.68	4794.8	1.84	2.4744
Zn	mg kg^{-1}	1.37	1.88	1.5	16.34	4.54	3.79	1.16	7.6	1.9	2.6	117.42	1.55	1.1

Appendix 2 Relationships between EC and Mg in minesite waterbodies

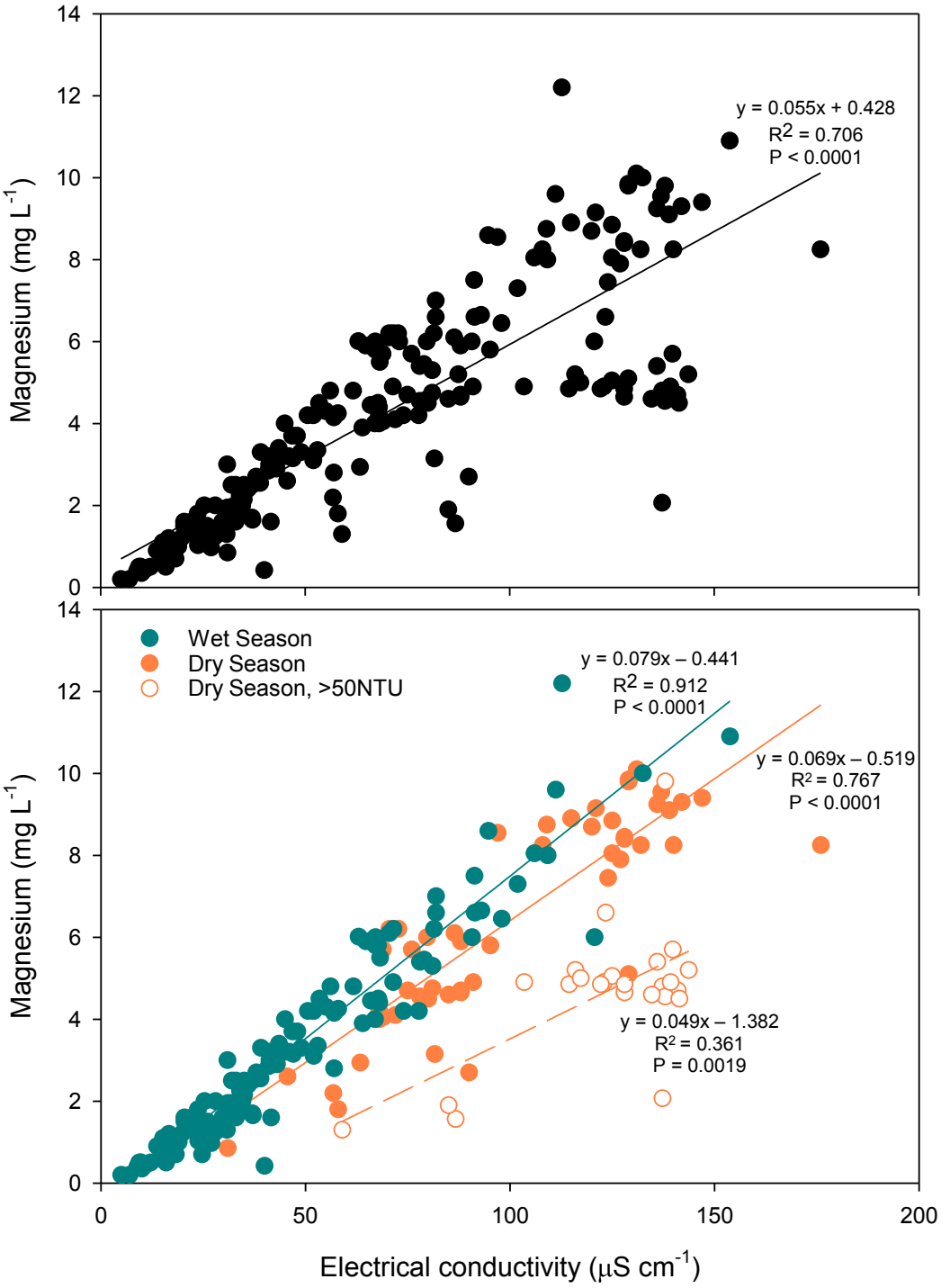


Figure A2.1 Georgetown Billabong magnesium/electrical conductivity relationship (top) all available data (bottom) seasonal data. This data is from the antecedent years only. Where turbidity measures were ≥ 50NTU the data was separated.

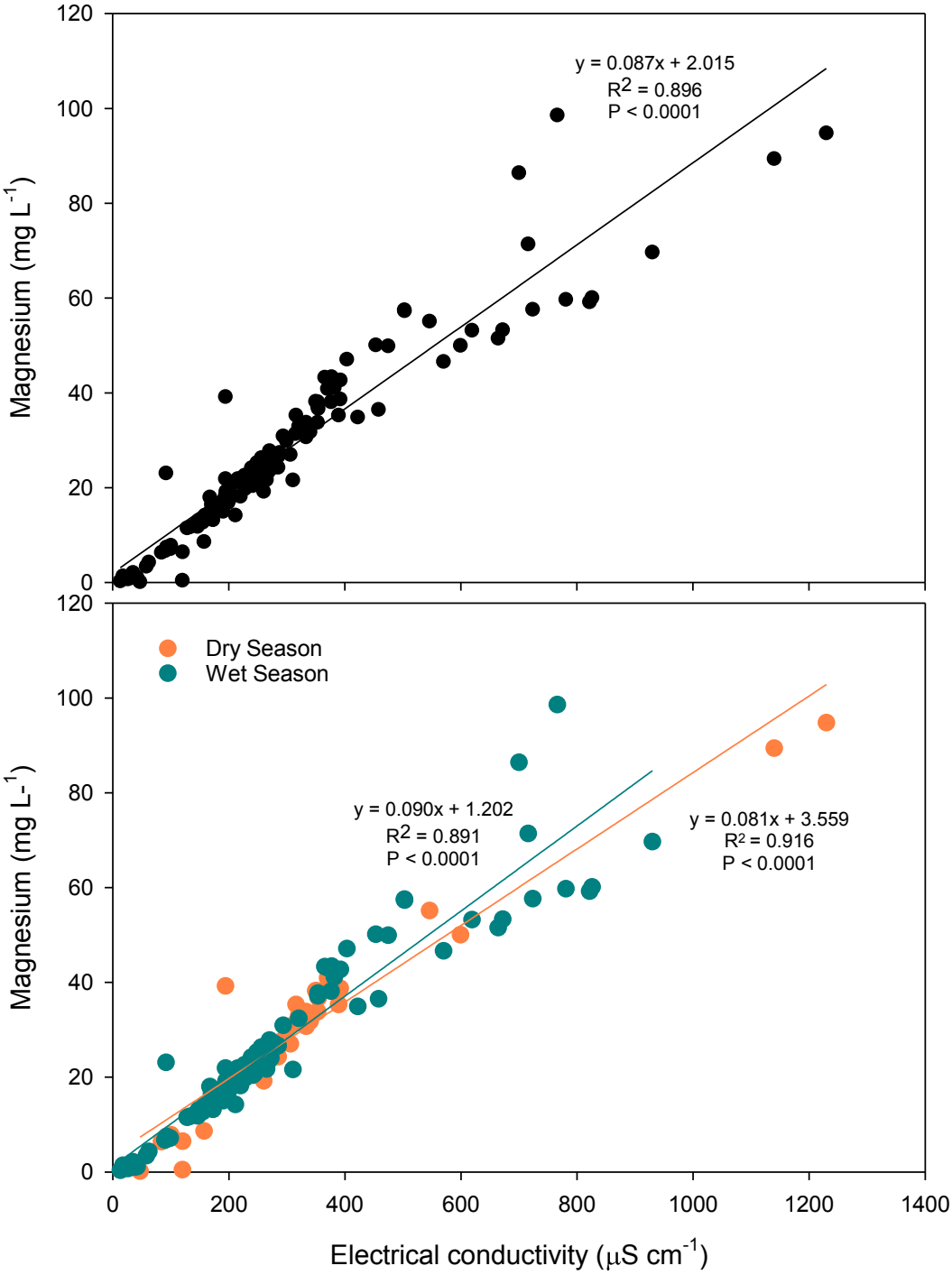


Figure A2.2 Coonjimba Billabong magnesium vs electrical conductivity relationship (top) all available data (bottom) seasonal data. This data is from the antecedent years only.

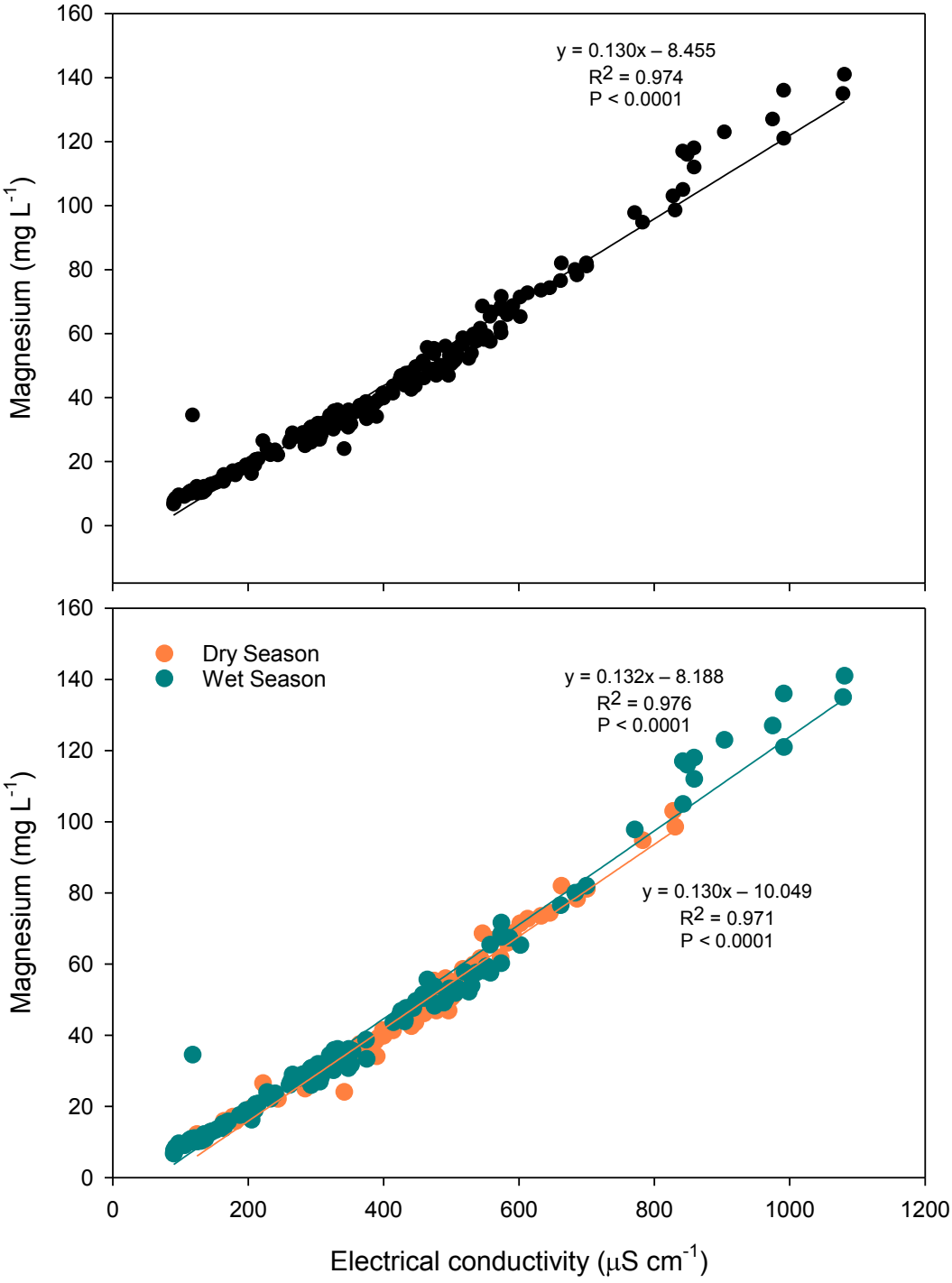


Figure A2.3 RP1 magnesium vs electrical conductivity relationship (top) all available data (bottom) seasonal data. This data is from the antecedent years only.

Appendix 3 Correlations amongst all environmental variables from the waterbodies for the sampling periods between 1995 and 2013

Spearman rank correlations (rho, correlation coefficient) for pairwise comparisons of all measured environmental variables. P <0.01 indicated in red font.

	Long	Rainfall	Area (sqm)	Catchment	Catchment Area	Depth Rank	Total Percentage Cover	Macrophyte taxa richness	FA Total	EBL Total	ENL Total	SEF Total	SNF Total	FF Total	Turb	EC	pH	Temp	DO	DO%	WQ associated EC	Al_F µg/L	Cu_F µg/L	Fe_F µg/L	Mn_F µg/L	Pb_F µg/L	U_F µg/L	Zn_F µg/L	Ca_F mg/L	K_F mg/L	Mg_F mg/L	Na_F mg/L	SO4_F mg/L	Cl_F mg/L	Bicarbonate Alkalinity	Total Alkalinity	Total N mg/L	NO3_N mg/L	NH3_N mg/L	PO4_P mg/L	Total P mg/L	TOC	DOC	Calculated Mg	Mg/Ca ratio
Lat	0.53	0.01	-0.54	-0.80	0.07	-0.06	0.04	0.18	0.28	0.30	-0.11	-0.02	-0.10	-0.13	-0.14	0.26	0.12	-0.10	-0.33	-0.33	0.28	-0.04	-0.17	0.10	0.20	-0.12	0.01	-0.01	0.16	0.44	0.22	0.53	0.03	0.47	0.28	0.34	0.33	-0.16	0.02	-0.10	0.30	0.37	0.12	0.25	0.07
Long		-0.02	-0.15	-0.80	-0.27	0.07	-0.14	-0.24	0.32	-0.01	-0.06	-0.28	-0.17	-0.39	-0.16	0.42	0.06	0.02	-0.26	-0.24	0.44	0.07	0.11	0.08	0.26	-0.03	0.57	0.06	0.11	0.28	0.51	0.46	0.56	0.16	0.42	0.41	0.02	0.07	0.03	-0.12	-0.05	0.49	0.32	0.43	0.19
Rainfall			-0.01	0.01	0.02	0.03	0.11	0.00	-0.06	0.02	0.17	-0.06	-0.03	0.01	-0.15	-0.12	-0.11	-0.05	0.28	0.23	-0.08	0.11	0.13	0.30	-0.08	0.03	0.12	0.00	-0.05	-0.10	-0.03	-0.02	-0.17	0.03	--	-0.18	--	0.18	-0.22	0.22	-0.23	-0.24	-0.32	-0.11	-0.56
Area (sqm)				0.12	-0.63	0.54	-0.34	-0.44	-0.27	-0.14	-0.11	-0.20	-0.04	-0.29	-0.29	0.23	0.02	0.40	0.56	0.59	0.20	-0.24	0.14	-0.06	-0.27	0.04	0.23	-0.06	0.18	-0.10	0.31	-0.08	0.35	-0.15	0.21	0.02	-0.29	0.26	0.06	0.10	-0.19	-0.33	-0.14	0.23	0.07
Catchment					0.30	-0.28	0.25	0.17	-0.23	-0.14	0.14	0.23	0.29	0.38	0.25	-0.57	-0.14	-0.20	0.10	0.08	-0.60	0.03	0.02	-0.13	-0.19	-0.02	-0.37	-0.07	-0.41	-0.51	-0.59	-0.71	-0.46	-0.46	-0.65	-0.57	-0.08	-0.05	-0.09	0.14	-0.17	-0.32	-0.11	-0.57	-0.14
Catchment Area						-0.68	0.42	0.49	0.03	0.32	0.13	0.18	0.23	0.43	0.36	-0.58	0.01	-0.48	-0.31	-0.32	-0.52	0.14	-0.01	-0.24	0.20	-0.04	-0.44	0.21	-0.42	-0.21	-0.54	-0.45	-0.52	-0.30	-0.43	-0.41	0.58	-0.06	0.02	-0.05	0.25	-0.03	-0.13	-0.57	-0.04
Depth Rank							-0.48	-0.37	-0.09	-0.04	-0.31	-0.18	-0.20	-0.25	-0.27	0.34	0.23	0.39	0.56	0.59	0.34	0.04	0.32	0.06	-0.27	0.25	0.39	0.00	0.30	0.07	0.28	0.23	0.38	0.20	0.51	0.06	-0.38	0.03	0.01	0.07	0.16	-0.35	-0.31	0.33	-0.03
Total Percentage Cover								0.21	0.13	-0.03	0.41	0.14	0.24	0.32	0.21	-0.50	0.01	-0.59	-0.29	-0.32	-0.50	-0.07	-0.25	-0.05	-0.05	-0.36	-0.45	0.07	-0.41	-0.31	-0.46	-0.46	-0.52	-0.30	-0.63	-0.49	0.20	-0.15	-0.31	-0.11	0.34	0.03	-0.03	-0.51	-0.02
Macrophyte taxa richness									0.16	0.16	-0.08	0.44	0.44	0.36	0.10	-0.40	-0.02	-0.32	-0.27	-0.29	-0.41	-0.06	-0.19	0.07	-0.13	-0.14	-0.55	-0.10	-0.30	-0.23	-0.44	-0.32	-0.55	-0.21	-0.48	-0.32	0.00	-0.28	-0.20	-0.09	-0.01	-0.03	-0.11	-0.40	0.00
FA Total										-0.03	-0.35	0.06	0.08	-0.03	-0.13	-0.08	0.21	-0.33	-0.37	-0.37	-0.06	-0.10	-0.20	0.15	-0.13	-0.25	-0.01	-0.06	-0.16	0.06	-0.05	0.20	-0.07	0.05	0.00	0.19	-0.27	-0.25	-0.21	-0.05	-0.08	0.28	0.11	-0.09	0.04
EBL Total											-0.17	-0.01	0.00	0.09	0.07	0.07	0.11	-0.14	-0.02	-0.02	0.07	-0.02	0.03	-0.19	0.07	0.03	-0.01	0.11	0.05	0.17	0.05	0.09	-0.02	0.08	0.26	0.12	0.52	0.14	0.08	0.14	0.35	-0.06	-0.19	0.08	-0.02
ENL Total												-0.12	-0.31	0.09	0.26	-0.05	-0.22	0.03	-0.14	-0.15	-0.06	0.20	0.06	0.05	0.29	0.12	0.00	0.13	0.03	0.02	-0.02	-0.08	-0.12	-0.18	0.19	-0.04	0.55	0.17	0.10	-0.13	0.17	-0.08	0.05	-0.05	0.12
SEF Total													0.02	0.16	-0.05	-0.24	-0.08	-0.16	-0.27	-0.28	-0.22	-0.09	-0.27	0.05	-0.15	-0.15	-0.35	-0.18	-0.16	-0.34	-0.28	-0.33	-0.28	-0.24	-0.33	-0.38	-0.14	-0.23	-0.13	0.12	0.00	-0.26	-0.26	-0.25	-0.17
SNF Total														0.02	-0.01	-0.45	0.02	-0.41	0.00	-0.02	-0.49	-0.17	-0.12	-0.12	-0.23	-0.30	-0.39	-0.12	-0.47	-0.42	-0.42	-0.47	-0.37	-0.18	-0.75	-0.44	0.03	-0.14	-0.23	0.00	-0.12	0.08	0.03	-0.44	-0.02
FF Total															0.34	-0.44	-0.05	-0.37	-0.09	-0.10	-0.39	0.00	-0.12	-0.09	-0.07	-0.06	-0.42	0.17	-0.21	-0.02	-0.52	-0.35	-0.46	-0.25	-0.35	-0.34	0.12	-0.15	-0.15	0.04	0.14	0.05	-0.02	-0.43	-0.28
Turb																-0.13	0.03	-0.09	-0.18	-0.18	-0.12	0.23	0.11	0.06	0.15	0.13	-0.10	0.14	-0.07	-0.08	-0.17	-0.15	-0.15	-0.15	-0.07	-0.07	0.45	0.16	0.01	0.03	0.12	0.40	0.25	-0.11	-0.08
EC																	0.25	0.58	0.06	0.09	0.97	-0.11	0.13	0.15	0.17	0.20	0.56	0.05	0.87	0.72	0.93	0.86	0.77	0.49	0.96	0.96	0.16	0.22	0.23	0.06	-0.16	0.30	0.27	1.00	0.22
pH																		-0.24	-0.01	0.05	0.31	-0.44	0.06	0.17	-0.32	-0.34	0.07	0.07	0.35	0.32	0.29	0.32	0.31	0.15	0.44	0.50	-0.04	-0.22	-0.19	0.07	-0.09	0.40	0.26	0.23	0.17
Temp																			0.37	0.39	0.57	0.25	0.28	0.30	0.16	0.57	0.48	-0.03	0.54	0.25	0.55	0.27	0.50	0.30	0.65	0.24	-0.06	0.40	0.37	0.05	-0.63	-0.27	0.04	0.58	0.11
DO																				0.99	0.09	0.04	0.26	-0.15	-0.29	0.20	0.17	0.00	0.15	0.13	0.11	0.00	0.09	0.06	0.20	-0.02	-0.28	0.13	-0.07	0.06	0.11	-0.44	-0.43	0.06	-0.17
DO%																					0.12	0.03	0.29	-0.15	-0.28	0.22	0.20	0.00	0.17	0.11	0.13	0.00	0.13	0.02	0.21	-0.01	-0.26	0.12	-0.05	0.05	0.14	-0.44	-0.40	0.09	-0.15
WQ associated EC																						-0.13	0.11	0.13	0.18	0.17	0.55	0.09	0.88	0.80	0.95	0.90	0.76	0.52	0.96	0.98	0.16	0.23	0.19	0.03	-0.16	0.37	0.32	0.97	0.21
Al_F µg/L																							0.47	0.00	0.49	0.78	0.41	0.15	-0.20	0.05	-0.17	0.01	-0.02	0.08	-0.04	-0.23	0.48	0.29	0.41	-0.05	0.08	-0.23	-0.17	-0.10	0.02
Cu_F µg/L																								-0.14	0.00	0.55	0.51	0.26	0.01	-0.05	0.10	0.00	0.23	-0.03	0.01	-0.02	0.24	0.17	0.16	-0.03	0.16	-0.26	-0.14	0.14	0.04
Fe_F µg/L																									-0.02	-0.07	0.09	-0.34	0.19	0.24	0.10	0.20	0.08	0.13	0.14	0.32	-0.11	-0.02	0.02	0.22	-0.81	0.53	0.27	0.15	-0.08
Mn_F µg/L																										0.41	0.35	0.25	0.06	0.39	0.16	0.38	0.17	0.32	0.25	0.22	0.48	0.38	0.46	-0.24	0.11	0.11	0.21	0.18	0.35
Pb_F µg/L																											0.46	0.33	0.12	0.15	0.11	0.19	0.20	0.29	0.13	-0.03	0.17	0.46	0.49	-0.16	0.01	-0.35	-0.20	0.21	0.09
U_F µg/L																												0.12	0.27	0.34	0.59	0.50	0.75	0.10	0.68	0.45	0.22	0.34	0.25	0.04	-0.20	0.12	0.18	0.58	0.24
Zn_F µg/L																													0.10	0.33	0.07	0.22	0.03	0.24	0.16	0.15	0.48	0.31	0.05	-0.31	0.36	0.02	-0.05	0.05	0.08
Ca_F mg/L																														0.73	0.80	0.80	0.54	0.46	0.91	0.86	0.08	0.21	0.18	0.05	-0.12	0.31	0.22	0.86	-0.04
K_F mg/L																															0.63	0.79	0.41	0.61	0.68	0.77	0.27	0.19	0.27	-0.15	-0.05	0.41	0.45	0.71	0.07
Mg_F mg/L																																0.82	0.79	0.42	0.90	0.92	0.16	0.28	0.17	0.00	-0.12	0.32	0.28	0.93	0.24
Na_F mg/L																																	0.52	0.71	0.93	0.88	0.13	0.26	0.26	-0.27	-0.01	0.29	0.34	0.86	0.06
SO4_F mg/L																																		0.17	0.70	0.67	0.23	0.23	0.28	0.14	-0.36	0.45	0.40	0.78	0.21
Cl_F mg/L																																			0.32	0.53	0.03	0.20	0.23	-0.23	-0.02	0.09	0.09	0.48	-0.12
Bicarbonate Alkalinity																																				1.00	0.14	0.46	0.38	-0.29	0.28	-0.16	-0.04	0.95	0.55
Total Alkalinity																																					0.14	0.20	0.28	-0.14	-0.20	0.40	0.48	0.95	0.09
Total N mg/L																																						0.65	0.44	0.19	0.53	0.33	0.17	0.17	0.26
NO3_N mg/L																																							0.42	-0.06	-0.20	-0.03	-0.09	0.24	0.07
NH3_N mg/L																																								0.22	-0.20	-0.13	0.06	0.22	0.17
PO4_P mg/L																																									-0.47	0.31	0.01	0.07	-0.39
Total P mg/L																																										-0.41	-0.52	-0.17	0.32
TOC																																											0.95	0.33	-0.03
DOC																																												0.29	0.31
Calculated Mg																																													0.22

Appendix 4 Results of Principal Components Analysis of environmental data from all waterbodies

Key influential variables for each axis (ie eigenvector coefficients > 0.25 highlighted in grey shading.

Table A4.1 Eigenvalues from PCA of all available environmental data for each waterbody from 1995, 1996, 2006, 2011 and 2013

PC	Eigenvalues	%Variation	Cum.%Variation
1	6	30.0	30.0
2	3.22	16.1	46.1
3	2.16	10.8	56.9
4	1.52	7.6	64.5
5	1.16	5.8	70.3

Table A4.2 Eigenvectors (Coefficients in the linear combinations of variables making up PCs) from PCA of all available environmental data for each waterbody from 1995, 1996, 2006, 2011 and 2013

Variable	PC1	PC2	PC3	PC4	PC5
Latitude	-0.207	0.426	-0.026	0.073	-0.181
Longitude	-0.270	0.358	-0.019	-0.076	-0.072
Rainfall	0.026	-0.002	0.105	-0.376	-0.301
Year	0.094	0.128	-0.319	-0.339	0.296
Area (sqm)	-0.185	-0.379	-0.237	0.042	-0.086
Catchment	0.276	-0.358	0.046	-0.080	0.137
Catchment Area	0.269	0.243	0.156	0.170	-0.071
Depth Rank	-0.219	-0.201	-0.370	0.128	-0.215
Total Percentage Cover	0.283	0.161	0.118	-0.241	0.196
Macrophyte taxa richness	0.252	0.187	-0.093	0.230	-0.004
FA Total	-0.046	0.345	-0.307	-0.062	0.302
EBL Total	0.068	0.263	-0.051	0.022	-0.281
ENL Total	0.047	-0.085	0.582	-0.231	-0.013
SEF Total	0.118	-0.051	-0.045	0.398	0.496
SNF Total	0.206	-0.015	-0.341	-0.331	-0.217
FF Total	0.102	0.142	0.179	0.374	-0.211
Log(EC)	-0.345	0.046	0.135	-0.037	0.290
pH	-0.304	-0.125	0.074	0.211	-0.105
Log(U_F)	-0.307	0.004	0.121	-0.216	0.065
Log(Mg_F)	-0.344	0.071	0.138	-0.098	0.245

Table A4.3 Eigenvalues from PCA of all available environmental data for each waterbody from 2006, 2011 and 2013

PC	Eigenvalues	%Variation	Cum.%Variation
1	8.11	25.4	25.4
2	4.4	13.8	39.1
3	3.26	10.2	49.3
4	2.61	8.1	57.4
5	2.51	7.8	65.3

Table A4.4 Eigenvectors (Coefficients in the linear combinations of variables making up PCs) from PCA of all available environmental data for each waterbody from 2006, 2011 and 2013

Variable	PC1	PC2	PC3	PC4	PC5
Latitude	-0.153	0.284	-0.219	0.073	-0.008
Longitude	-0.212	0.249	-0.164	-0.025	-0.201
Rainfall	0.028	0.095	0.383	0.159	-0.003
Area	-0.163	-0.345	0.075	0.081	-0.020
Catchment	0.217	-0.227	0.210	-0.124	0.070
Catchment area	0.210	0.196	-0.132	0.097	0.079
Depth Rank	-0.213	-0.221	-0.001	-0.026	0.178
Total Percent Cover	0.232	0.130	0.053	0.109	-0.178
Macrophyte taxa richness	0.206	0.082	-0.222	0.065	0.215
FA Total	-0.074	0.153	-0.285	-0.183	-0.020
EBL Total	0.087	0.108	-0.165	0.216	-0.070
ENL Total	0.047	0.169	0.399	0.158	0.016
SEF Total	0.022	-0.011	-0.154	0.320	0.144
SNF Total	0.181	-0.180	-0.058	-0.136	-0.328
FF Total	0.128	0.145	-0.092	0.053	0.353
Turb	0.051	0.116	0.014	-0.353	0.318
Log(EC)	-0.327	0.046	-0.008	0.076	0.054
pH	-0.241	-0.130	0.096	0.145	0.249
Temp	-0.112	-0.033	-0.297	-0.245	0.142
DO	-0.076	-0.360	0.049	-0.006	-0.012
Log(Al_F)	0.074	0.166	0.210	-0.310	0.228
Log(Cu_F)	0.023	-0.011	0.185	-0.408	0.195
Log(Fe_F)	-0.112	0.236	0.165	0.123	0.288
Log(Mn_F)	-0.011	0.346	0.173	-0.048	-0.117
Log(U_F)	-0.274	0.097	0.142	-0.231	-0.079
Log(Zn_F)	0.104	0.141	0.118	-0.086	-0.065
Log(Ca_F)	-0.227	-0.052	-0.019	0.234	0.307
Log(Mg_F)	-0.326	0.059	0.024	0.062	-0.029
Log(SO4_F)	-0.306	0.071	0.048	-0.121	-0.084
Log(NO3_N)	-0.012	0.089	0.279	0.230	-0.062
Log(NH3_N)	-0.012	0.136	-0.055	0.010	0.028
Log(Mg/Ca ratio)	-0.255	0.125	0.049	-0.113	-0.299

Table A4.5 Eigenvalues from PCA of water chemistry data (only) for each waterbody from 2006, 2011 and 2013

PC	Eigenvalues	%Variation	Cum.%Variation
1	5.32	31.3	31.3
2	2.72	16.0	47.3
3	1.99	11.7	59.0
4	1.62	9.5	68.5
5	1.48	8.7	77.2

Table A4.6 Eigenvectors (Coefficients in the linear combinations of variables making up PCs) from PCA of water chemistry data (only) for each waterbody from 2006, 2011 and 2013

Variable	PC1	PC2	PC3	PC4	PC5
Turb	0.052	-0.314	0.401	-0.104	0.234
Log(EC)	-0.421	0.013	-0.044	-0.037	0.065
pH	-0.294	0.157	0.147	-0.390	-0.077
Temp	-0.152	0.066	0.224	0.266	0.472
DO	-0.057	0.353	0.297	-0.169	-0.343
Log(Al_F)	0.105	-0.419	0.336	-0.217	-0.038
Log(Cu_F)	0.038	-0.221	0.531	0.026	-0.136
Log(Fe_F)	-0.137	-0.314	-0.102	-0.518	0.083
Log(Mn_F)	-0.048	-0.463	-0.298	0.110	0.058
Log(U_F)	-0.340	-0.217	0.158	0.170	-0.232
Log(Zn_F)	0.127	-0.260	-0.153	0.111	-0.222
Log(Ca_F)	-0.308	0.154	-0.046	-0.339	0.235
Log(Mg_F)	-0.427	-0.003	-0.057	0.029	-0.003
Log(SO4_F)	-0.397	-0.103	0.041	0.223	-0.047
Log(NO3_N)	-0.025	-0.162	-0.302	-0.288	-0.395
Log(NH3_N)	-0.013	-0.129	-0.186	-0.106	0.455
Log(Mg/Ca ratio)	-0.326	-0.135	-0.038	0.328	-0.202

Appendix 5 Macroinvertebrate data

Table A5.1 Summary of macroinvertebrate results (means) for 2006, 2011 and 2013. Data for 1995 and 1996 can be found in O'Connor et al (1995), O'Connor et al (1997). Data presented as mean abundance per replicate for each sampling year.

	Angbangbang Billabong			Baralil Billabong		
	2006	2011	2013	2006	2011	2013
Hydridae	0	0	0.04	0.04	0	0
Turbellaria	0.03	0.22	0.12	0	0	0
Temnocephalidae	0	0	0	0	0	0
Nematoda	5.35	17.82	20.74	0.25	8.95	7.56
Gordioidea	0	0	0	0.04	0	0
Hyriidae	0	0	0	0	0	0
Viviparidae	0	0	0	0	0	0
Thiaridae	0	0	0	0	0	0
Bithyniidae	9.47	3.13	0.48	9.05	1.74	0.71
Lymnaeidae	0	0	0	0.57	0.30	0.00
Ancylidae	0	0.07	0	0.37	0.40	0.16
Planorbidae	2.69	0.45	1.08	4.54	2.38	5.77
Oligochaeta	4.15	23.06	18.04	1.19	10.12	18.47
Glossiphoniidae	0.16	0.00	0.09	0.12	0	0
Hirudinidae	0	0.07	0	0	0.00	0
Ornithobdellidae	0	0	0	0.16	0.00	0
Conchostraca (imm.)	0.06	0.00	0.50	0.04	0.27	0
Cyclestheriidae	0	0.33	0.66	0	0	0.00
Caridea (imm./damaged)	0	0	0	0.61	0.07	0
Atyidae	0.95	0.04	0.00	14.41	10.05	0.78
Palaemonidae	0.06	0.01	0.00	3.03	0.09	0.02
Parathelphusidae	0	0	0	0	0	0
Parastacidae	0	0	0	0	0	0
Oribatida	4.65	12.86	15.11	4.42	17.22	27.43
Acarina	6.68	0.84	1.23	0.61	0.44	1.09
Symphypleona	0.22	0	0.04	0.04	0.03	0.16
Ephemeroptera	0	0.07	0.12	0	0	0
Baetidae	5.29	0.70	1.16	2.01	0.87	0.55
Caenidae	7.31	8.67	0.73	7.61	7.34	6.08
Leptophlebiidae	0	0	0	0	0	0
Zygoptera	1.74	1.42	1.00	0.61	1.47	0.55
Coenagrionidae	5.38	0.55	0.37	4.30	0.57	0.32
Protoneuridae	0	0	0	0	0	0
Isostictidae	0	0	0	0	0	0
Lestidae	0	0	0	0	0	0
Anisoptera	2.41	1.82	0.73	1.31	0.80	0.70
Aeshnidae	0.06	0	0.00	0.12	0.00	0

	Angbangbang Billabong			Baralil Billabong		
	2006	2011	2013	2006	2011	2013
Gomphidae	0.03	0	0	0	0	0
Lindeniidae	0.03	0	0	0.04	0	0
Corduliidae	0	0	0	0	0.00	0
Libellulidae	4.75	0.10	0.21	1.27	0.24	0.36
Veliidae	0.95	0.15	0.08	1.47	0.13	0.16
Gerridae	0.09	0.00	0.00	0.65	0.00	0
Hebridae	0.03	0.40	0	0.16	1.31	0.08
Hydrometridae	0.13	0	0	0.41	0.07	0
Mesoveliidae	0.13	0	0	2.09	0.60	0.08
Saldidae	0	0	0	0	0	0
Nepidae	0.13	0.00	0.00	0.12	0.00	0.00
Belostomatidae	0.51	0.01	0.01	2.62	0.14	0.02
Corixidae	3.36	0.47	0.42	0.37	0.13	0.08
Naucoridae	6.24	0.03	0.02	2.01	0.14	0.09
Notonectidae	0.13	0.00	0.00	0.41	0.10	0.08
Pleidae	4.97	0.22	0.54	1.92	1.11	0.55
Sisyridae	0	0.04	0.04	0	0.00	0
Carabidae (A)	0	0	0	0	0	0
Dytiscidae (L)	0.28	0.01	0.01	0.65	0.01	0.09
Dytiscidae (A)	5.06	0.00	0.00	4.63	0.27	0.01
Gyrinidae (L)	0	0	0	0	0	0
Gyrinidae (A)	0	0	0	0	0.00	0
Haliplidae (L)	0.03	0.00	0	0	0	0
Haliplidae (A)	0	0	0	0	0	0
Hygrobiidae (A)	0	0	0	0	0	0
Noteridae (L)	0	0.00	0	0	0	0
Noteridae (A)	0.38	0.00	0.00	0.04	0	0.00
Hydrophilidae (L)	0.13	0.01	0.04	0.86	0.30	0.00
Hydrophilidae (A)	1.52	0.01	0	6.02	0.57	0.00
Hydraenidae (A)	0	0	0	0.12	0	0
Staphylinidae (L)	0	0	0	0	0	0
Staphylinidae (A)	0	0	0	0	0	0
Scirtidae (L)	0	0	0	0	0	0
Scirtidae (A)	0	0	0	0	0	0
Elmidae (L)	0	0	0	0	0	0
Elmidae (A)	0	0	0	0	0	0
Limnichidae (A)	0	0	0	0.16	0	0
Chrysomelidae (L)	0	0	0	0	0	0
Chrysomelidae (A)	0	0	0	0	0	0
Brentidae (L)	0	0	0	0	0	0.00
Curculionidae (L)	0	0	0	0	0	0

	Angbangbang Billabong			Baralil Billabong		
	2006	2011	2013	2006	2011	2013
Curculionidae (A)	0.25	0.07	0.01	0.33	0	0.00
Cecidomyiidae(L)	0	0	0	0	0	0
Chaoboridae (L)	0	0	0	0	0	0
Chaoboridae (P)	0	0	0	0	0	0
Culicidae (L)	0.60	0.41	0.04	0.74	1.04	0
Culicidae (P)	0.03	0	0	0	0.00	0
Chironomidae (L)	8.36	14.39	27.60	9.54	19.80	21.59
Chironomidae (P)	0.16	0.58	1.08	0.37	0.57	0.39
Ceratopogonidae (L)	2.47	6.96	4.43	1.80	3.55	4.05
Ceratopogonidae (P)	0.09	0.37	0.00	0.20	0.20	0
Psychodidae (L)	0	0	0	0.04	0	0
Tipulidae (L)	0	0	0	0.16	0.13	0
Tipulidae (P)	0	0	0	0	0	0
Tabanidae (L)	0	0	0	0.16	0.07	0.00
Empididae	0	0	0	0	0	0
Stratiomyidae (L)	0	0	0	0	0	0
Dolichopodidae	0	0	0	0	0	0
Sciomyzidae (L)	0	0	0	0	0	0
Ephydridae (L)	0	0	0	0	0	0
Muscidae (L)	0	0	0	0	0	0
Trichoptera (L)	0.38	0	0.62	0.08	0	0
Trichoptera (P)	0	0	0	0	0	0
Ecnomidae (L)	0.44	0.15	0	0.37	0.61	0
Hydroptilidae (L)	0.79	2.19	1.54	0.53	4.26	0.47
Hydroptilidae (P)	0.06	0.04	0.04	0	0.17	0.08
Leptoceridae (L)	0.47	1.09	0.89	2.09	1.01	0.55
Leptoceridae (P)	0	0	0	0.04	0	0.00
Philopotamidae (L)	0	0	0	0	0	0
Polycentropodidae (L)	0	0	0	0	0	0.16
Crambidae (L)	0.35	0.15	0.13	2.05	0.34	0.78
Crambidae (P)	0	0	0.00	0	0.00	0.00

	Buba Billabong			Coonjimba Billabong		
	2006	2011	2013	2006	2011	2013
Hydridae	0	0	0	0	0.47	0.91
Turbellaria	0	0	0.16	0	0.49	0
Temnocephalidae	0	0	0	0	0	0
Nematoda	3.07	22.65	29.83	2.96	14.10	0.07
Gordioidea	0	0	0	0	0	0
Hyriidae	0	0	0	0	0	0
Viviparidae	0.11	0	0	0	0	0
Thiaridae	0	0	0	0	0	0
Bithyniidae	4.07	1.30	0.22	0	0.63	0.28
Lymnaeidae	1.41	0.43	0.60	0	0	0
Ancylidae	0.30	0.21	0	0.32	0.30	0
Planorbidae	7.70	4.59	3.71	1.93	9.13	0.00
Oligochaeta	2.58	15.08	24.54	2.01	12.04	32.55
Glossiphoniidae	0.95	0.05	0.17	0	0.06	0
Hirudinidae	0.03	0	0.00	0	0	0
Ornithobdellidae	0.11	0.00	0	0	0	0
Conchostraca (imm.)	0.33	0	0	0.12	0.24	0
Cyclestheriidae	0	0.00	0.06	0	0.68	0.00
Caridea (imm./damaged)	0.14	0	0	0	0	0
Atyidae	0.76	0.19	0.00	0.43	5.36	0
Palaemonidae	0.24	0.00	0	0.08	0.01	0.00
Parathelphusidae	0	0	0	0	0	0
Parastacidae	0	0	0	0	0	0
Oribatida	3.17	19.75	16.03	5.01	0.60	12.64
Acarina	17.61	2.09	8.56	2.05	0.75	0.21
Symphypleona	0	0.05	0	0.04	0.06	0.28
Ephemeroptera	0	0	0.11	0	0	0
Baetidae	2.60	0.24	0.98	7.81	3.95	5.31
Caenidae	15.38	6.63	0.28	4.54	2.07	0.63
Leptophlebiidae	0	0	0	0	0	0
Zygoptera	0.46	0.64	0.22	1.54	3.00	2.30
Coenagrionidae	3.28	0.27	0.07	12.59	1.88	1.47
Protoneuridae	0	0	0	0	0	0.14
Isostictidae	0	0	0	0	0	0
Lestidae	0	0	0	0	0	0
Anisoptera	2.39	0.89	0	2.01	0.74	0.91
Aeshnidae	0.14	0.00	0.00	0.08	0.02	0.01
Gomphidae	0.03	0	0	0.12	0	0
Lindeniidae	0	0.00	0	0.12	0.01	0
Corduliidae	0	0	0	0	0.06	0
Libellulidae	5.13	0.04	0.37	1.78	0.89	0.98

	Buba Billabong			Coonjimba Billabong		
	2006	2011	2013	2006	2011	2013
Veliidae	1.03	0.51	0	0.95	0.23	0
Gerridae	0.16	0.00	0	0.55	0.18	0
Hebridae	0	0	0	0	0.15	0
Hydrometridae	0.57	0.03	0	0.39	0	0
Mesoveliidae	0.65	0.14	0	0.24	0.38	0.42
Saldidae	0	0	0	0	0	0
Nepidae	0.14	0	0	0.12	0.12	0
Belostomatidae	0.73	0.03	0.00	1.62	0.19	0.01
Corixidae	0.38	0.56	0.06	0.08	6.17	0.42
Naucoridae	3.96	0.01	0.11	0.43	0.03	0.02
Notonectidae	0.11	0.00	0.00	0.04	0	0
Pleidae	2.85	0.35	0.66	0.28	0.93	0.21
Sisyridae	0	0	0	0	0.15	0
Carabidae (A)	0	0	0	0	0	0
Dytiscidae (L)	0.46	0.06	0.01	1.30	0.07	0.02
Dytiscidae (A)	1.98	0.01	0.01	4.78	0.60	0.01
Gyrinidae (L)	0	0	0	0	0	0
Gyrinidae (A)	0	0.00	0	0	0.06	0
Haliplidae (L)	0.03	0	0.06	0	0	0
Haliplidae (A)	0	0	0.00	0	0	0.00
Hygrobiidae (A)	0	0	0	0	0	0
Noteridae (L)	0.11	0	0.00	0	0	0
Noteridae (A)	0.14	0	0	0.67	0.12	0
Hydrophilidae (L)	0.73	0.09	0.06	1.34	0.44	0.15
Hydrophilidae (A)	3.09	0.17	0.06	11.25	2.67	0.18
Hydraenidae (A)	0.03	0	0	0.04	0	0
Staphylinidae (L)	0	0	0	0.08	0	0
Staphylinidae (A)	0	0	0	0.08	0	0
Scirtidae (L)	0	0	0	0.67	0.07	0
Scirtidae (A)	0	0	0	0	0	0
Elmidae (L)	0	0	0	0	0	0
Elmidae (A)	0	0	0	0	0	0
Limnichidae (A)	0.03	0	0.00	0.12	0.00	0
Chrysomelidae (L)	0	0	0	0	0.00	0
Chrysomelidae (A)	0	0	0	0	0	0
Brentidae (L)	0	0	0.00	0	0.11	0.14
Curculionidae (L)	0	0	0	0.04	0	0
Curculionidae (A)	1.17	0.05	0.00	1.18	0.05	0
Cecidomyiidae(L)	0	0	0	0	0	0
Chaoboridae (L)	0	0	0	0.04	0	0.00
Chaoboridae (P)	0	0	0	0	0	0

	Buba Billabong			Coonjimba Billabong		
	2006	2011	2013	2006	2011	2013
Culicidae (L)	0.33	0.48	0.06	0.36	0	0
Culicidae (P)	0	0	0	0	0	0
Chironomidae (L)	4.99	16.66	9.38	15.90	16.60	37.79
Chironomidae (P)	0.14	0.91	0.16	0.51	1.07	0.42
Ceratopogonidae (L)	1.11	2.44	2.56	6.39	2.73	0.63
Ceratopogonidae (P)	0.19	0.13	0.00	0.24	0.64	0
Psychodidae (L)	0	0	0	0	0	0
Tipulidae (L)	0.08	0.00	0	0.12	0.00	0.00
Tipulidae (P)	0	0	0	0	0	0
Tabanidae (L)	0.08	0.16	0.00	0.28	0.00	0.14
Empididae	0	0	0.00	0	0	0
Stratiomyidae (L)	0	0	0	0	0	0
Dolichopodidae	0	0	0	0	0	0
Sciomyzidae (L)	0	0.00	0	0	0	0
Ephydridae (L)	0	0.00	0	0	0	0
Muscidae (L)	0	0	0	0	0	0
Trichoptera (L)	0	0	0	0	0.05	0
Trichoptera (P)	0	0.05	0	0	0	0
Ecnomidae (L)	0.43	0.09	0	0.83	0.74	0
Hydroptilidae (L)	0.57	1.10	0.16	0.39	0.78	0.14
Hydroptilidae (P)	0.43	0.21	0	0	0.30	0
Leptoceridae (L)	1.17	0.51	0.11	2.45	5.92	0.14
Leptoceridae (P)	0	0	0	0.12	0	0
Philopotamidae (L)	0	0	0	0	0	0
Polycentropodidae (L)	0	0	0	0.08	0	0
Crambidae (L)	0.16	0.11	0.60	0.51	0.88	0.45
Crambidae (P)	0	0	0	0	0.06	0.01

	Corndorl Billabong			Georgetown Billabong		
	2006	2011	2013	2006	2011	2013
Hydridae	0	0.05	0	0	0	0.63
Turbellaria	0	0.52	0.05	0	0	0
Temnocephalidae	0	0.10	0	0	0	0
Nematoda	4.00	15.32	14.98	6.14	4.19	10.03
Gordioidea	0	0	0	0	0	0
Hyriidae	0	0	0	0	0	0
Viviparidae	0	0	0	0	0	0
Thiaridae	0	0	0	0	0	0
Bithyniidae	8.49	0.57	0.16	9.48	1.13	0.18
Lymnaeidae	0.03	0.00	0.05	0.08	0	0.06
Ancylidae	0.03	0	0.05	0.31	0.42	0.17
Planorbidae	8.93	2.30	6.51	0.25	0.22	1.16
Oligochaeta	7.36	23.49	26.60	9.39	14.57	14.10
Glossiphoniidae	1.06	0.00	0	0.45	0	0
Hirudinidae	0.26	0.00	0	0	0	0
Ornithobdellidae	0.16	0.00	0	0	0	0
Conchostraca (imm.)	0.67	0.63	0.42	0.20	0	1.49
Cyclestheriidae	0	0.16	0.47	0	0	0.12
Caridea (imm./damaged)	0	0.05	0	0.20	0.23	0.23
Atyidae	0.58	0.11	0.00	4.77	5.37	0.48
Palaemonidae	0.16	0.01	0.01	0.36	0.36	0.01
Parathelphusidae	0	0	0	0	0	0
Parastacidae	0	0	0	0	0	0
Oribatida	8.36	16.89	20.97	13.71	4.53	27.80
Acarina	4.48	1.75	5.26	0.73	1.11	0.63
Symphypleona	0.06	0	0	0.03	0	0
Ephemeroptera	0	0.68	0.05	0	0	0.23
Baetidae	1.47	0.34	0.60	2.08	1.83	1.43
Caenidae	13.83	10.78	0.88	6.87	7.14	2.47
Leptophlebiidae	0	0.03	0	0	0	0
Zygoptera	0.61	1.12	0.88	0.90	1.65	0.92
Coenagrionidae	5.12	0.34	0.37	10.04	2.38	0.57
Protoneuridae	0	0	0.00	0	0	0
Isostictidae	0	0	0	0	0	0
Lestidae	0	0	0	0	0	0
Anisoptera	1.73	1.59	0.42	0.84	1.32	0.57
Aeshnidae	0	0.01	0.00	0	0	0.00
Gomphidae	0	0.05	0	0.31	0	0
Lindeniidae	0	0	0	0	0.00	0.00
Corduliidae	0	0	0	0	0	0
Libellulidae	3.36	0.31	0.12	2.55	0.11	0.15

	Corndorl Billabong			Georgetown Billabong		
	2006	2011	2013	2006	2011	2013
Veliidae	0.67	0.31	0.00	0.06	0.08	0
Gerridae	0.06	0.01	0	0.03	0.08	0
Hebridae	0.06	0.57	0.19	0	0.06	0.00
Hydrometridae	0.03	0.05	0	0	0	0
Mesoveliidae	0.26	0.31	0.09	0.42	1.02	0.00
Saldidae	0	0	0	0	0	0
Nepidae	0	0	0	0.17	0	0
Belostomatidae	1.15	0.01	0.03	1.57	0.27	0.07
Corixidae	0.13	0.24	0.00	0.11	0	0.75
Naucoridae	3.01	0.17	0.01	0.90	0.73	0.02
Notonectidae	0.16	0.09	0.00	0.08	0.15	0.06
Pleidae	2.69	1.64	1.63	0.62	0.33	0.40
Sisyridae	0.03	0.21	0	0	0	0
Carabidae (A)	0	0	0	0	0	0
Dytiscidae (L)	0.64	0.11	0.61	1.01	0.01	0.01
Dytiscidae (A)	0.67	0.00	0.01	0.39	0.36	0.01
Gyrinidae (L)	0	0	0	0	0	0
Gyrinidae (A)	0	0	0	0	0	0
Haliplidae (L)	0	0	0	0.06	0	0.00
Haliplidae (A)	0.06	0	0	0.03	0	0.00
Hygrobiidae (A)	0	0	0	0	0	0
Noteridae (L)	0.10	0	0.05	0	0	0.00
Noteridae (A)	0.16	0	0	0.06	0.00	0.00
Hydrophilidae (L)	0.96	0.06	0.00	0.64	1.11	0.13
Hydrophilidae (A)	3.68	0.16	0.05	1.96	0.31	0.01
Hydraenidae (A)	0.03	0	0	0	0	0
Staphylinidae (L)	0	0	0	0	0	0
Staphylinidae (A)	0	0	0	0	0	0
Scirtidae (L)	0	0	0	0	0	0
Scirtidae (A)	0	0	0	0	0	0
Elmidae (L)	0	0	0	0	0	0
Elmidae (A)	0	0	0	0	0	0
Limnichidae (A)	0	0.10	0	0.08	0	0
Chrysomelidae (L)	0	0	0	0	0	0
Chrysomelidae (A)	0	0	0	0	0	0
Brentidae (L)	0	0.00	0	0	0	0
Curculionidae (L)	0	0	0	0.03	0	0
Curculionidae (A)	1.54	0.00	0.05	0.90	0	0.00
Cecidomyiidae(L)	0	0	0	0	0	0
Chaoboridae (L)	0.13	0	0.05	0	0.05	0.00
Chaoboridae (P)	0	0	0	0	0	0

	Corndorl Billabong			Georgetown Billabong		
	2006	2011	2013	2006	2011	2013
Culicidae (L)	0.51	0.13	0.00	0.20	0.56	0.06
Culicidae (P)	0.06	0.00	0	0	0	0.06
Chironomidae (L)	7.59	11.75	12.37	11.86	27.12	23.45
Chironomidae (P)	0.10	0.60	0.14	0.22	1.52	1.66
Ceratopogonidae (L)	2.53	4.31	4.84	3.06	14.93	7.45
Ceratopogonidae (P)	0.26	0.13	0.23	0.20	0.39	0
Psychodidae (L)	0	0	0	0	0	0
Tipulidae (L)	0.03	0.00	0	0	0	0
Tipulidae (P)	0	0	0	0	0	0
Tabanidae (L)	0.48	0.11	0.00	0.03	0.00	0.06
Empididae	0	0	0.00	0	0	0
Stratiomyidae (L)	0	0	0	0	0	0
Dolichopodidae	0	0	0	0	0	0
Sciomyzidae (L)	0	0	0	0	0	0
Ephydridae (L)	0	0.00	0	0	0	0
Muscidae (L)	0	0	0	0	0	0
Trichoptera (L)	0.10	0.05	0	0.34	0	0
Trichoptera (P)	0	0	0	0	0	0
Ecnomidae (L)	0.03	0.11	0	0.98	1.23	0.06
Hydroptilidae (L)	0.74	0.70	0.19	1.74	0.80	1.49
Hydroptilidae (P)	0	0.10	0	0.11	0	0.17
Leptoceridae (L)	0.48	0.50	0.33	1.68	1.05	0.29
Leptoceridae (P)	0	0	0	0.03	0	0.00
Philopotamidae (L)	0	0	0	0	0	0
Polycentropodidae (L)	0	0	0	0	0	0
Crambidae (L)	0.10	0.22	0.29	0.76	1.26	0.35
Crambidae (P)	0	0.00	0	0	0.00	0

	Gulungul Billabong			Jabiru Lake		
	GUL2006	GUL2011	GUL2013	JBL2006	JBL2011	JBL2013
Hydridae	0.36	0	0.14	0.11	0	0.26
Turbellaria	0.09	0	0	0	0	0
Temnocephalidae	0	0	0	0	0	0
Nematoda	2.91	16.18	17.31	0.87	14.54	6.69
Gordioidea	0	0	0	0	0	0
Hyriidae	0	0	0	0	0.00	0
Viviparidae	0	0	0	0.02	0	0
Thiaridae	0	0	0	0	0.07	0
Bithyniidae	9.65	1.58	0.15	7.25	3.76	2.01
Lymnaeidae	0	0	0	1.62	0.21	0.01
Ancylidae	0.36	0	0	0.02	0.07	0
Planorbidae	8.78	0.21	1.36	2.01	1.54	0.47
Oligochaeta	5.05	23.18	22.83	1.76	14.47	12.74
Glossiphoniidae	0.23	0	0.07	0.05	0.00	0.07
Hirudinidae	0.09	0	0	0.02	0	0
Ornithobdellidae	0.23	0.01	0	0	0	0
Conchostraca (imm.)	0	0	0	5.38	2.37	0.06
Cyclestheriidae	0	0.00	0.08	0	0.63	1.30
Caridea (imm./damaged)	0	0.10	0	0	0	0
Atyidae	0.27	0.56	0.08	0	0.07	0.00
Palaemonidae	0.50	0.00	0.16	0.16	0.02	0.00
Parathelphusidae	0	0	0	0	0	0
Parastacidae	0	0	0	0	0	0
Oribatida	2.82	11.99	13.56	8.87	24.90	32.17
Acarina	2.09	1.13	0.82	21.45	1.32	2.47
Symphypleona	0.05	0	0.07	0.02	0	0
Ephemeroptera	0	0.30	0.89	0	0	0.32
Baetidae	3.69	0.17	0.34	1.92	1.18	2.08
Caenidae	12.20	7.79	5.45	3.92	2.78	5.72
Leptophlebiidae	0	0	0	0	0	0
Zygoptera	0.41	0.59	1.02	0.41	1.39	0.71
Coenagrionidae	5.23	0.20	0.43	3.21	1.11	0.66
Protoneuridae	0	0	0	0	0	0
Isostictidae	0	0	0	0	0	0
Lestidae	0.14	0	0	0	0	0
Anisoptera	1.55	1.38	0.27	1.76	2.57	0.58
Aeshnidae	0.05	0.00	0	0	0.07	0.00
Gomphidae	0	0	0	0.32	0	0
Lindeniidae	0	0	0.00	0.16	0.07	0
Corduliidae	0	0	0	0.02	0	0
Libellulidae	2.46	0.18	0.89	2.90	0.49	0.53

	Gulungul Billabong			Jabiru Lake		
	GUL2006	**GUL2011**	**GUL2013**	**JBL2006**	**JBL2011**	**JBL2013**
Veliidae	0.86	0	0	0.55	0.90	0.39
Gerridae	0.36	0.00	0	0.50	0.42	0.13
Hebridae	0.09	0.30	0	0.36	1.53	1.30
Hydrometridae	0.59	0	0	0	0	0
Mesoveliidae	0.59	0.00	0	0.11	0.49	0.06
Saldidae	0	0	0	0	0	0
Nepidae	0.09	0.00	0	0.14	0.00	0.00
Belostomatidae	3.00	0.01	0.02	0.57	0.00	0.08
Corixidae	0.32	0.20	0.07	2.87	2.86	1.37
Naucoridae	3.10	0.18	0.15	0.27	0.00	0.00
Notonectidae	0.09	0.11	0.00	0.30	0.21	0.07
Pleidae	0.50	0.06	0.28	1.62	0.90	0.52
Sisyridae	0	0.05	0	0	0.00	0
Carabidae (A)	0	0	0	0	0	0
Dytiscidae (L)	1.09	0.00	0.07	0.16	0.14	0.00
Dytiscidae (A)	4.42	0.01	0.15	8.03	0.28	0.01
Gyrinidae (L)	0	0	0	0	0	0
Gyrinidae (A)	0.05	0.01	0	0	0	0
Haliplidae (L)	0	0	0	0.05	0	0.00
Haliplidae (A)	0	0	0	0.11	0	0.00
Hygrobiidae (A)	0	0	0	0	0	0
Noteridae (L)	0.05	0	0	0	0	0
Noteridae (A)	0.27	0	0.00	0.05	0	0.00
Hydrophilidae (L)	1.09	0.07	0.00	1.05	0.01	0.20
Hydrophilidae (A)	3.28	0.14	0	1.98	0.29	0.03
Hydraenidae (A)	0	0	0	0	0.00	0
Staphylinidae (L)	0	0	0	0	0	0
Staphylinidae (A)	0	0	0	0	0	0
Scirtidae (L)	0	0	0	0	0	0
Scirtidae (A)	0	0	0	0	0	0
Elmidae (L)	0	0	0	0	0	0
Elmidae (A)	0	0	0	0	0	0
Limnichidae (A)	0.05	0	0	0.02	0	0
Chrysomelidae (L)	0	0	0	0	0	0
Chrysomelidae (A)	0	0	0	0	0.00	0
Brentidae (L)	0	0.00	0	0	0.00	0
Curculionidae (L)	0.05	0	0.00	0	0	0
Curculionidae (A)	0.77	0.00	0.01	0.02	0	0.00
Cecidomyiidae(L)	0	0	0	0	0	0
Chaoboridae (L)	0	0	0.00	0.02	0	0
Chaoboridae (P)	0	0	0	0	0	0

	Gulungul Billabong			Jabiru Lake		
	GUL2006	**GUL2011**	**GUL2013**	**JBL2006**	**JBL2011**	**JBL2013**
Culicidae (L)	1.55	0.06	0.00	0.43	0.49	0.13
Culicidae (P)	0.18	0.00	0.00	0.02	0.00	0
Chironomidae (L)	11.97	23.23	26.03	9.53	9.18	15.47
Chironomidae (P)	0.59	0.89	0.48	0.11	0.42	0.13
Ceratopogonidae (L)	2.55	5.67	3.00	3.92	5.70	8.32
Ceratopogonidae (P)	0.18	0.20	0.07	0.50	0.00	0.00
Psychodidae (L)	0	0	0	0	0	0
Tipulidae (L)	0	0.00	0	0	0.00	0
Tipulidae (P)	0	0.00	0	0	0	0
Tabanidae (L)	0.23	0.10	0	0.09	0.00	0.00
Empididae	0	0	0	0	0	0.06
Stratiomyidae (L)	0	0	0	0.57	0.98	0.52
Dolichopodidae	0.05	0	0	0	0	0
Sciomyzidae (L)	0	0	0	0.02	0.14	0
Ephydridae (L)	0	0	0	0	0	0
Muscidae (L)	0	0	0	0.02	0	0
Trichoptera (L)	0.09	0	0	0.05	0	0.32
Trichoptera (P)	0	0	0	0	0	0
Ecnomidae (L)	0.36	0.83	0	0.57	0.01	0.00
Hydroptilidae (L)	0.86	1.28	0.89	0.32	0.63	1.82
Hydroptilidae (P)	0.09	0.10	0.07	0.02	0.07	0.06
Leptoceridae (L)	0.41	0.79	2.59	0.59	0.56	0.00
Leptoceridae (P)	0	0	0	0	0	0.00
Philopotamidae (L)	0	0	0	0	0	0
Polycentropodidae (L)	0	0	0	0	0	0
Crambidae (L)	0.96	0.13	0.22	0.21	0.15	0.08
Crambidae (P)	0	0	0.00	0	0.00	0.00

	Malabanbandju Billabong			Retention Pond 1		
	2006	2011	2013	2006	2011	2013
Hydridae	0	0.13	0.09	0.04	0.05	0
Turbellaria	0	0.23	0	0	0	0
Temnocephalidae	0	0.05	0.09	0	0	0
Nematoda	2.41	20.89	3.44	1.78	13.30	8.18
Gordioidea	0	0	0	0	0	0
Hyriidae	0	0.87	0.00	0	0	0
Viviparidae	0.22	0	0.00	0	0	0
Thiaridae	0	0	0	0	0	0
Bithyniidae	1.41	0.12	0.12	0	0	0.13
Lymnaeidae	0.03	0	0.00	0	0.04	0
Ancylidae	0.08	0	0.00	0.19	0.21	0.27
Planorbidae	1.36	2.13	7.93	1.70	0.12	1.20
Oligochaeta	3.54	13.33	6.46	2.50	10.24	33.36
Glossiphoniidae	7.56	0	0.01	0	0	0
Hirudinidae	0	0	0	0	0	0
Ornithobdellidae	0	0	0	0	0	0
Conchostraca (imm.)	0.94	0	1.21	6.47	0.00	0
Cyclestheriidae	0	0.20	0.55	0	0.48	2.42
Caridea (imm./damaged)	1.11	0	0	0	0	0
Atyidae	3.54	4.00	0.12	0.87	0.37	0.13
Palaemonidae	0.42	0.10	0.09	0.42	0	0
Parathelphusidae	0	0	0	0	0	0
Parastacidae	0.06	0	0	0	0	0
Oribatida	4.10	11.27	2.15	4.99	14.26	5.47
Acarina	5.95	3.56	1.29	6.58	0.08	0.53
Symphypleona	0.06	0.13	0.09	0	0	0.76
Ephemeroptera	0	0	0.60	0	0	0
Baetidae	1.55	3.30	1.56	2.08	1.40	3.87
Caenidae	14.32	12.05	1.46	1.59	4.07	0.49
Leptophlebiidae	0	0	0	0	0	0
Zygoptera	0.61	0.34	0.34	1.21	0.63	3.42
Coenagrionidae	5.68	0.62	0.22	10.97	1.42	0.96
Protoneuridae	0	0.00	0	0	0	0.27
Isostictidae	0	0	0	0	0	0
Lestidae	0	0	0	0	0	0
Anisoptera	1.00	0.32	0	4.31	1.57	1.11
Aeshnidae	0	0	0	0.26	0.10	0
Gomphidae	0.14	0.03	0	0.42	0	0
Lindeniidae	0.03	0	0.01	0.45	0.00	0
Corduliidae	0	0	0	0.23	0	0
Libellulidae	2.24	0.26	0.11	5.07	0.08	0.68

	Malabanbandju Billabong			Retention Pond 1		
	2006	2011	2013	2006	2011	2013
Veliidae	0.28	0.27	0.09	0.11	0.25	0
Gerridae	0.08	0.00	0	0.04	0.45	0
Hebridae	0.08	0	0	0.04	0.24	0
Hydrometridae	0.03	0	0	0.04	0	0
Mesoveliidae	0.33	0.52	0	0.38	0.35	0.13
Saldidae	0	0	0	0	0	0
Nepidae	0.36	0.13	0.00	0.49	0.05	0
Belostomatidae	1.47	0.05	0.01	1.32	0.01	0.02
Corixidae	1.38	1.29	0.95	3.18	0	0.80
Naucoridae	4.04	0.08	0.02	0.15	0	0
Notonectidae	0.25	0.13	0.00	0.64	1.02	0
Pleidae	0.80	0	2.16	4.50	0.31	0.40
Sisyridae	0	0	0.00	0	0	0
Carabidae (A)	0	0	0	0	0	0
Dytiscidae (L)	1.16	0.00	0.26	0.64	0	0.54
Dytiscidae (A)	3.30	0.04	0.03	3.78	0.13	0.27
Gyrinidae (L)	0	0	0	0	0	0
Gyrinidae (A)	0	0	0	0	0	0
Haliplidae (L)	0.03	0.00	0.09	0	0	0
Haliplidae (A)	0.19	0	0.10	0.04	0	0
Hygrobiidae (A)	0	0.00	0	0	0.00	0
Noteridae (L)	0.03	0	0	0	0	0
Noteridae (A)	0.06	0	0	0	0	0.00
Hydrophilidae (L)	0.86	0.18	0.00	1.89	0.62	0.14
Hydrophilidae (A)	2.19	0.06	0.00	4.54	0.63	0.43
Hydraenidae (A)	0	0	0	0.04	0	0
Staphylinidae (L)	0	0	0	0	0	0
Staphylinidae (A)	0	0	0	0	0	0
Scirtidae (L)	0	0	0	0.04	0	0
Scirtidae (A)	0	0	0	0	0	0
Elmidae (L)	0	0.10	0	0	0	0
Elmidae (A)	0	0	0	0	0	0
Limnichidae (A)	0	0	0	0.04	0	0
Chrysomelidae (L)	0	0.10	0	0	0	0
Chrysomelidae (A)	0	0	0	0	0	0.01
Brentidae (L)	0	0.00	0	0	0	0
Curculionidae (L)	0.03	0	0	0	0	0
Curculionidae (A)	0.28	0.16	0	0.38	0	0
Cecidomyiidae(L)	0	0	0	0	0	0
Chaoboridae (L)	0	0	0.09	0.08	0	0.09
Chaoboridae (P)	0	0	0	0	0	0

	Malabanbandju Billabong			Retention Pond 1		
	2006	**2011**	**2013**	**2006**	**2011**	**2013**
Culicidae (L)	0.44	0.86	0.18	0.15	0.18	0.13
Culicidae (P)	0	0.00	0.00	0	0.00	0
Chironomidae (L)	13.38	15.78	39.95	18.34	32.86	27.75
Chironomidae (P)	0.53	0.76	0.95	0.83	0.94	0.53
Ceratopogonidae (L)	1.02	2.18	1.29	2.61	11.12	1.38
Ceratopogonidae (P)	0.03	0.37	0.00	0.04	0.35	0
Psychodidae (L)	0	0	0	0	0	0
Tipulidae (L)	0	0	0	0	0	0
Tipulidae (P)	0	0	0	0	0	0
Tabanidae (L)	0.03	0	0	0.08	0.00	0
Empididae	0	0	0	0	0	0
Stratiomyidae (L)	0	0	0	0	0.05	0
Dolichopodidae	0	0	0	0	0	0
Sciomyzidae (L)	0	0	0	0	0	0
Ephydridae (L)	0	0	0	0	0	0
Muscidae (L)	0	0	0	0	0	0
Trichoptera (L)	0.75	0	0	0	0	0.13
Trichoptera (P)	0	0	0	0	0	0
Ecnomidae (L)	2.27	0.10	0.27	1.21	0.77	0.13
Hydroptilidae (L)	3.16	2.30	23.94	0.23	0.54	2.49
Hydroptilidae (P)	0.14	0.10	0.52	0	0	0
Leptoceridae (L)	2.35	0.44	0.95	1.51	0.67	0.09
Leptoceridae (P)	0.08	0.00	0	0.04	0	0
Philopotamidae (L)	0	0	0	0	0	0
Polycentropodidae (L)	0	0	0	0	0	0.40
Crambidae (L)	0.25	0.05	0.22	0.49	0.02	0.86
Crambidae (P)	0	0	0	0	0	0.01

	Retention Pond 2			Sandy Shallow Billabong		
	2006	2011	2013	2006	2011	2013
Hydridae	0	0	0.11	0	0	0
Turbellaria	0	0	0	0	0	0
Temnocephalidae	0	0	0	0	0	0
Nematoda	0	15.79	0.16	3.64	13.87	8.81
Gordioidea	0	0	0	0	0	0
Hyriidae	0	0	0	0	0	0
Viviparidae	0	0	0	0	0	0
Thiaridae	0	0	0	0	0	0
Bithyniidae	0	0	0	0	0	0.10
Lymnaeidae	0	0	0	0	0	0
Ancylidae	0	0	0	0	0	0
Planorbidae	0.23	0	0	1.19	0.25	0.31
Oligochaeta	0	16.73	2.61	4.78	20.66	7.85
Glossiphoniidae	0	0	0	0	0	0.00
Hirudinidae	0	0	0	0.06	0	0
Ornithobdellidae	0	0	0	0	0	0
Conchostraca (imm.)	0	0	7.02	0.17	0	0.03
Cyclestheriidae	0	0	0	0	0	1.72
Caridea (imm./damaged)	0	0	0	0.03	0.06	0
Atyidae	0	0	0	0.40	0.21	0.00
Palaemonidae	0	0.01	0.16	0.54	0.00	0.04
Parathelphusidae	0	0	0	0	0	0
Parastacidae	0	0	0	0	0	0
Oribatida	0.23	0	0	5.75	17.97	22.55
Acarina	3.03	0.16	0	13.08	1.34	1.27
Symphypleona	0	0.14	0	0.20	0.06	0
Ephemeroptera	0	0	0	0	0.06	0.14
Baetidae	0	1.16	2.67	3.92	0.66	0.52
Caenidae	0	0	0	5.46	3.31	1.79
Leptophlebiidae	0	0	0	0	0	0
Zygoptera	0.47	0.55	1.45	1.05	0.78	1.00
Coenagrionidae	1.17	0.39	1.29	6.80	0.30	0.83
Protoneuridae	0	0	0	0	0	0
Isostictidae	0	0	0	0	0	0
Lestidae	0	0	0	0.03	0	0
Anisoptera	8.16	1.18	1.27	3.24	1.72	1.17
Aeshnidae	0	0	0.00	0	0.00	0
Gomphidae	0	0.10	0.00	0.06	0	0
Lindeniidae	0	0	0.00	0.06	0	0
Corduliidae	0	0	0	0	0	0
Libellulidae	0	0	0.04	3.38	0.28	0.19

	Retention Pond 2			Sandy Shallow Billabong		
	2006	2011	2013	2006	2011	2013
Veliidae	0.93	0.29	0	1.34	0.16	0.14
Gerridae	3.96	0.15	0.00	0.23	0.00	0.00
Hebridae	0	0	0	0.14	0.50	0.21
Hydrometridae	0.93	0	0.00	0.23	0	0
Mesoveliidae	3.26	1.99	0.00	0.54	0	0.00
Saldidae	0	0	0	0	0	0
Nepidae	1.63	0	0.09	0.23	0.00	0
Belostomatidae	1.17	0.10	0.01	1.05	0.01	0.04
Corixidae	1.63	0.26	0.65	4.52	1.28	0.07
Naucoridae	4.20	0.16	0	2.19	0.04	0.04
Notonectidae	1.40	0.01	0	0.14	0.06	0.04
Pleidae	1.17	0.42	0	2.50	0.35	0.76
Sisyridae	0	0	0	0.06	0.00	0.00
Carabidae (A)	0	0	0	0	0	0
Dytiscidae (L)	2.33	0	0.27	0.20	0.00	0.07
Dytiscidae (A)	45.69	0.80	0.00	5.94	0.04	0.00
Gyrinidae (L)	0.70	0	0	0	0	0
Gyrinidae (A)	0.47	0.07	0	0	0	0
Haliplidae (L)	0	0	0	0	0	0
Haliplidae (A)	0	0	0	0.03	0	0
Hygrobiidae (A)	0	0	0	0.03	0	0
Noteridae (L)	0	0	0	0.03	0	0
Noteridae (A)	0	0.01	0	0.23	0	0.00
Hydrophilidae (L)	0	0	0	0.34	0.01	0.00
Hydrophilidae (A)	1.86	1.60	0.00	2.87	0.15	0.00
Hydraenidae (A)	0	0.25	0	0.03	0	0
Staphylinidae (L)	0	0	0	0	0	0
Staphylinidae (A)	0	0	0	0.06	0	0
Scirtidae (L)	0	0	0	0	0	0
Scirtidae (A)	0	0	0	0	0	0
Elmidae (L)	0	0	0	0	0	0
Elmidae (A)	0	0	0	0	0	0
Limnichidae (A)	0.47	0.01	0.00	0.26	0	0
Chrysomelidae (L)	0	0	0	0	0	0.00
Chrysomelidae (A)	0	0	0	0	0	0.00
Brentidae (L)	0	0	0	0	0.00	0
Curculionidae (L)	0	0	0	0.06	0	0
Curculionidae (A)	0	0	0	0.34	0.00	0.04
Cecidomyiidae(L)	0	0	0	0	0	0
Chaoboridae (L)	0	0	0	0.03	0	0.00
Chaoboridae (P)	0	0	0	0	0	0.00

	Retention Pond 2			Sandy Shallow Billabong		
	2006	2011	2013	2006	2011	2013
Culicidae (L)	0	0	0	1.59	0.04	0.00
Culicidae (P)	0	0	0	0.37	0	0.03
Chironomidae (L)	12.82	53.76	81.54	12.66	24.69	33.15
Chironomidae (P)	0	2.15	0.41	0.71	0.91	0.79
Ceratopogonidae (L)	0.47	1.21	0.16	4.07	7.59	11.22
Ceratopogonidae (P)	0	0	0.00	0.20	0.00	0.31
Psychodidae (L)	0	0	0	0	0	0
Tipulidae (L)	0	0	0	0	0	0.00
Tipulidae (P)	0	0	0	0	0	0
Tabanidae (L)	0	0.49	0.01	0	0	0.00
Empididae	0	0	0	0	0	0
Stratiomyidae (L)	0	0	0	0	0	0
Dolichopodidae	0	0	0	0	0	0
Sciomyzidae (L)	0	0	0	0	0.06	0
Ephydridae (L)	0	0	0.08	0	0	0
Muscidae (L)	0	0	0	0	0	0
Trichoptera (L)	0	0	0	0.43	0.06	0.41
Trichoptera (P)	0	0	0	0	0	0
Ecnomidae (L)	0	0.07	0	0.46	0.08	0.00
Hydroptilidae (L)	0	0	0	0.65	1.37	3.65
Hydroptilidae (P)	0.23	0	0	0.09	0.06	0.17
Leptoceridae (L)	1.17	0	0	0.97	0.37	0.07
Leptoceridae (P)	0	0	0	0	0.00	0
Philopotamidae (L)	0	0	0	0	0	0
Polycentropodidae (L)	0	0	0	0	0	0
Crambidae (L)	0.23	0	0	0.37	0.60	0.42
Crambidae (P)	0	0	0	0	0.00	0.00

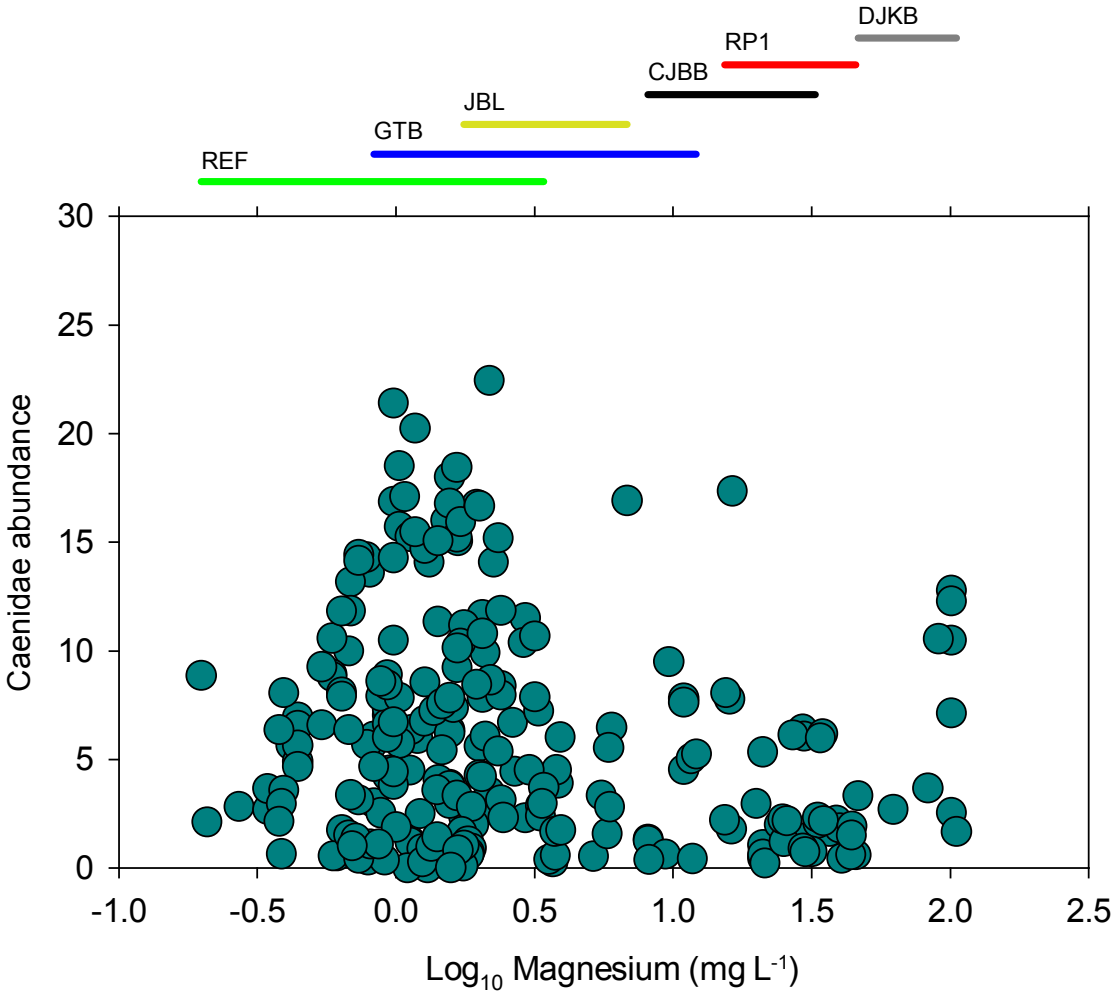
	Wirnmuyurr Billabong		
	2006	2011	2013
Hydridae	0	0	0
Turbellaria	0	0	0.09
Temnocephalidae	0.11	0	0
Nematoda	4.43	20.06	31.01
Gordioidea	0	0	0
Hyriidae	0	0	0
Viviparidae	0	0	0
Thiaridae	0	0	0
Bithyniidae	0	0	0.05
Lymnaeidae	0	0	0
Ancylidae	0.05	0	0
Planorbidae	0.53	0.09	0.36
Oligochaeta	5.55	15.83	17.27
Glossiphoniidae	0.05	0	0.09
Hirudinidae	0.05	0	0
Ornithobdellidae	0	0	0
Conchostraca (imm.)	0	0	0.36
Cyclestheriidae	0	0.13	0.14
Caridea (imm./damaged)	0	0	0
Atyidae	0	0.09	0.00
Palaemonidae	0.11	0.03	0.01
Parathelphusidae	0	0	0
Parastacidae	0.16	0	0
Oribatida	3.52	13.40	15.39
Acarina	4.59	0.63	2.15
Symphypleona	0.16	0	0.09
Ephemeroptera	0	0	0
Baetidae	7.10	0.46	0.99
Caenidae	10.94	2.93	0.14
Leptophlebiidae	0	0	0
Zygoptera	0.16	0.67	0.98
Coenagrionidae	6.24	0.18	0.59
Protoneuridae	0	0	0
Isostictidae	0	0	0
Lestidae	0.11	0	0
Anisoptera	1.76	0.54	0.31
Aeshnidae	0	0.00	0.00
Gomphidae	0	0	0
Lindeniidae	1.01	0	0.00
Corduliidae	0	0	0
Libellulidae	4.86	0.04	0.17

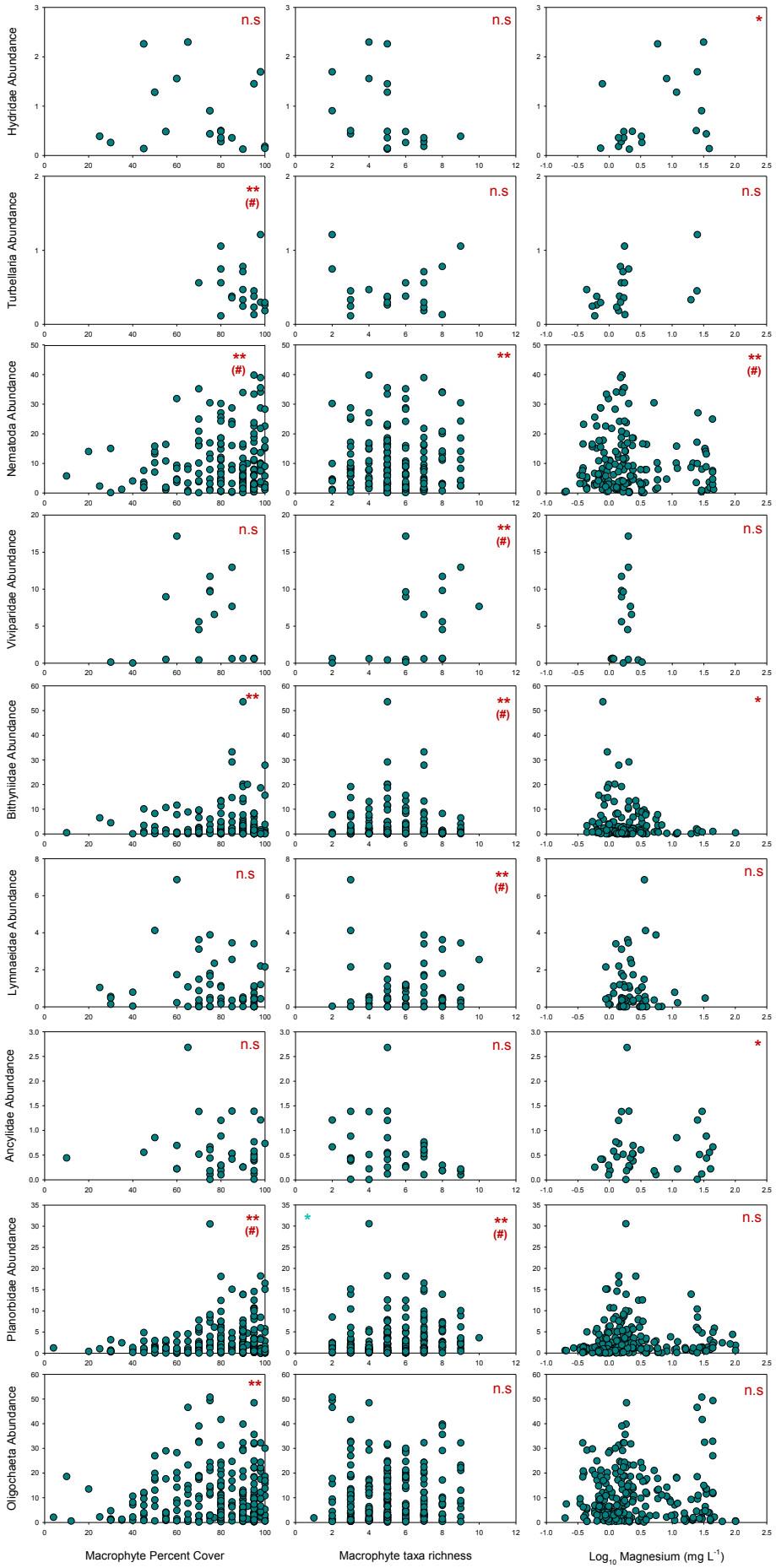
	Wirnmuyurr Billabong		
	2006	2011	2013
Veliidae	0.43	0.04	0.13
Gerridae	0.37	0.00	0.00
Hebridae	0.05	0.17	0.04
Hydrometridae	0.59	0	0
Mesoveliidae	1.23	0.25	0.09
Saldidae	0	0	0
Nepidae	0.21	0	0
Belostomatidae	3.36	0.05	0.03
Corixidae	0.05	0.00	0.37
Naucoridae	4.70	0.05	0.07
Notonectidae	0	0.00	0.00
Pleidae	0.96	0.38	0.76
Sisyridae	0	0.04	0
Carabidae (A)	0	0	0
Dytiscidae (L)	0.48	0.04	0.09
Dytiscidae (A)	5.02	0.04	0.00
Gyrinidae (L)	0	0	0
Gyrinidae (A)	0	0	0
Haliplidae (L)	0	0	0
Haliplidae (A)	0	0	0
Hygrobiidae (A)	0	0	0
Noteridae (L)	1.87	0	0.13
Noteridae (A)	1.01	0.00	0.00
Hydrophilidae (L)	0.91	0.00	0.00
Hydrophilidae (A)	1.33	0.00	0.00
Hydraenidae (A)	0	0	0
Staphylinidae (L)	0	0	0
Staphylinidae (A)	0	0	0
Scirtidae (L)	0	0	0
Scirtidae (A)	0	0	0
Elmidae (L)	0	0	0
Elmidae (A)	0	0	0
Limnichidae (A)	0	0	0
Chrysomelidae (L)	0	0	0
Chrysomelidae (A)	0	0	0
Brentidae (L)	0	0.00	0
Curculionidae (L)	0	0	0
Curculionidae (A)	0.80	0.00	0.00
Cecidomyiidae(L)	0	0.04	0
Chaoboridae (L)	0	0	0
Chaoboridae (P)	0	0	0

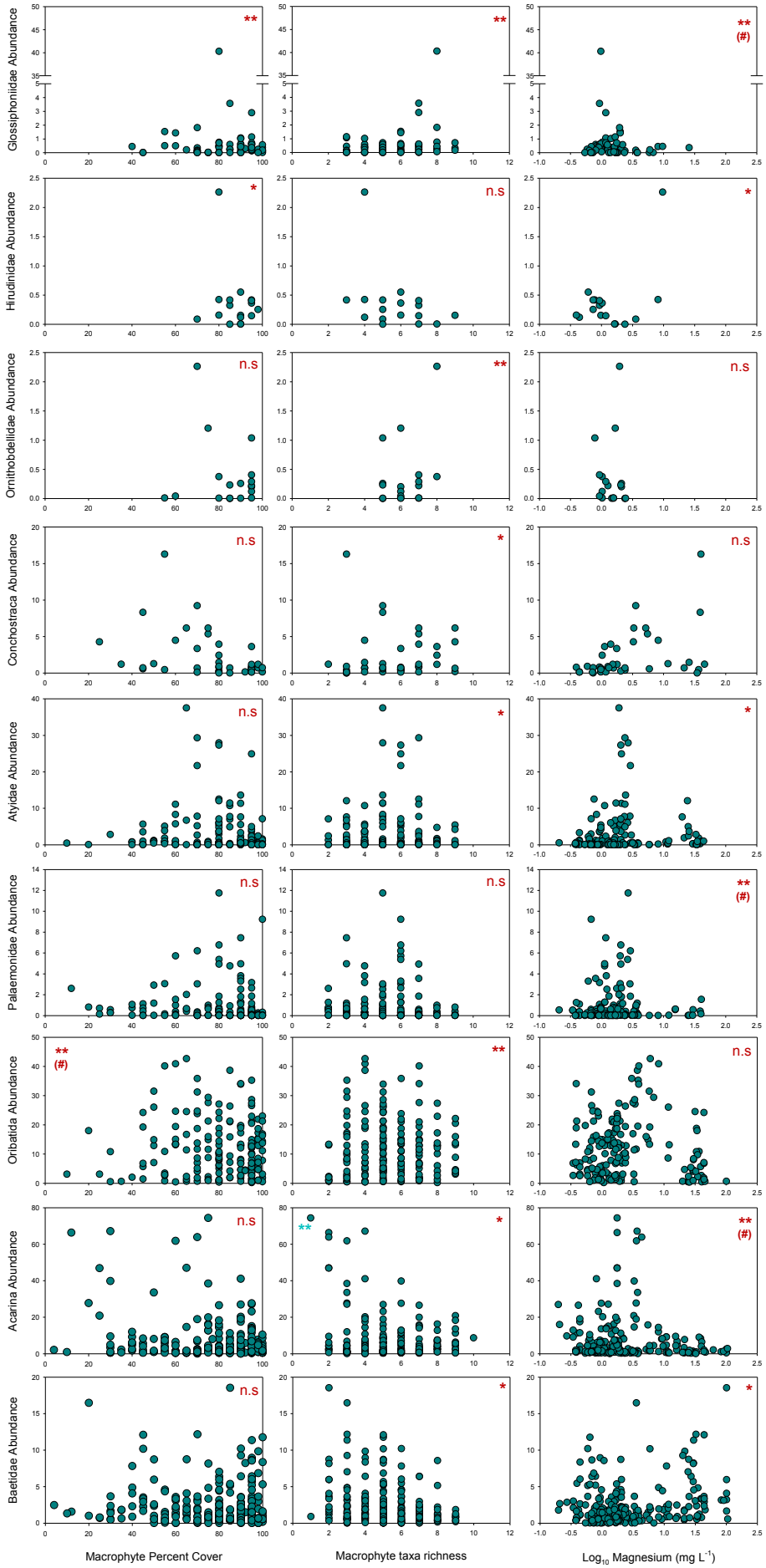
	Wirnmuyurr Billabong		
	2006	**2011**	**2013**
Culicidae (L)	1.81	0.04	0.27
Culicidae (P)	0.11	0	0.00
Chironomidae (L)	13.23	30.65	20.76
Chironomidae (P)	0.59	1.09	0.36
Ceratopogonidae (L)	5.76	9.92	4.43
Ceratopogonidae (P)	0.27	0.17	0.09
Psychodidae (L)	0	0	0.04
Tipulidae (L)	0	0	0
Tipulidae (P)	0	0	0
Tabanidae (L)	0.48	0.00	0
Empididae	0	0	0
Stratiomyidae (L)	0	0	0
Dolichopodidae	0	0	0
Sciomyzidae (L)	0.05	0	0
Ephydridae (L)	0	0	0
Muscidae (L)	0	0	0
Trichoptera (L)	0.27	0	0
Trichoptera (P)	0	0	0
Ecnomidae (L)	0.05	0.08	0.00
Hydroptilidae (L)	1.49	0.67	0.72
Hydroptilidae (P)	0.11	0.08	0.13
Leptoceridae (L)	0.27	0.63	1.07
Leptoceridae (P)	0	0.00	0
Philopotamidae (L)	0	0	0
Polycentropodidae (L)	0	0	0
Crambidae (L)	0.64	0.46	0.19
Crambidae (P)	0	0.00	0.00

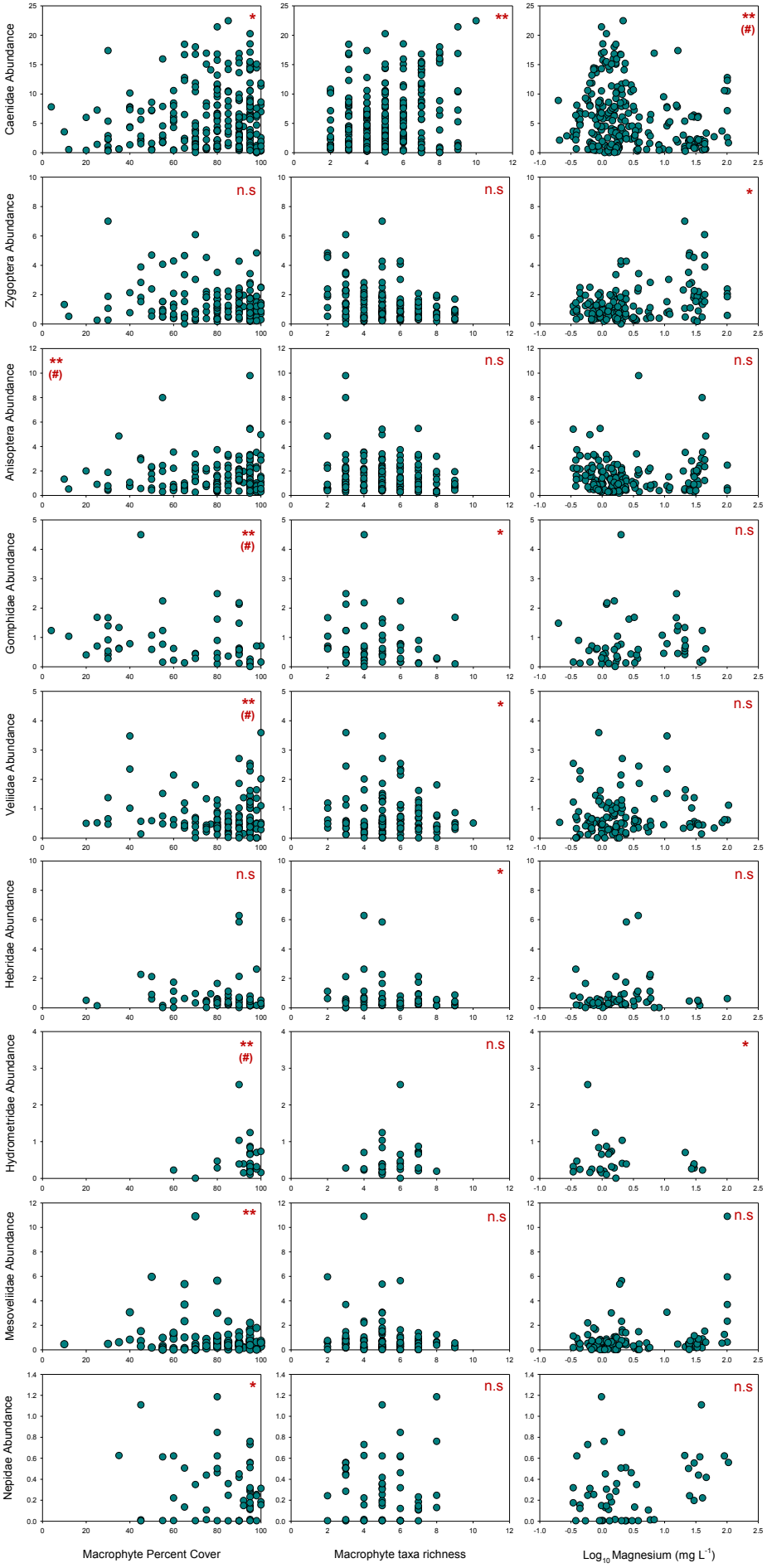
Appendix 6 Correlations between taxa and key environmental variables

Highly correlated macroinvertebrate taxa with either macrophyte percent cover, macrophyte taxa richness or natural log of magnesium concentration. Taxa abundance values are the relative percent of the total abundance for each sample. Symbols on graphs ** Highly significant ($p<0.005$), * Significant ($P<0.05$) and n.s. not significant # significance after Bonferroni correction ($p<0.05$). * Significant 90%ile quantile regression




Hydridae Abundance
Turbellaria Abundance
Nematoda Abundance
Viviparidae Abundance
Bithyniidae Abundance
Lymnaeidae Abundance
Ancylidae Abundance
Planorbidae Abundance
Oligochaeta Abundance
n.s
**
(#)
*
Macrophyte Percent Cover
Macrophyte taxa richness
Log10 Magnesium (mg L-1)


Glossiphoniidae Abundance
Hirudinidae Abundance
Ornithobdellidae Abundance
Conchostraca Abundance
Atyidae Abundance
Palaemonidae Abundance
Oribatida Abundance
Acarina Abundance
Baetidae Abundance
Macrophyte Percent Cover
Macrophyte taxa richness
Log10 Magnesium (mg L-1)


Caenidae Abundance
Zygoptera Abundance
Anisoptera Abundance
Gomphidae Abundance
Veliidae Abundance
Hebridae Abundance
Hydrometridae Abundance
Mesoveliidae Abundance
Nepidae Abundance
Macrophyte Percent Cover
Macrophyte taxa richness
Log10 Magnesium (mg L-1)
*
**
**
(#)
n.s
n.s
*
**
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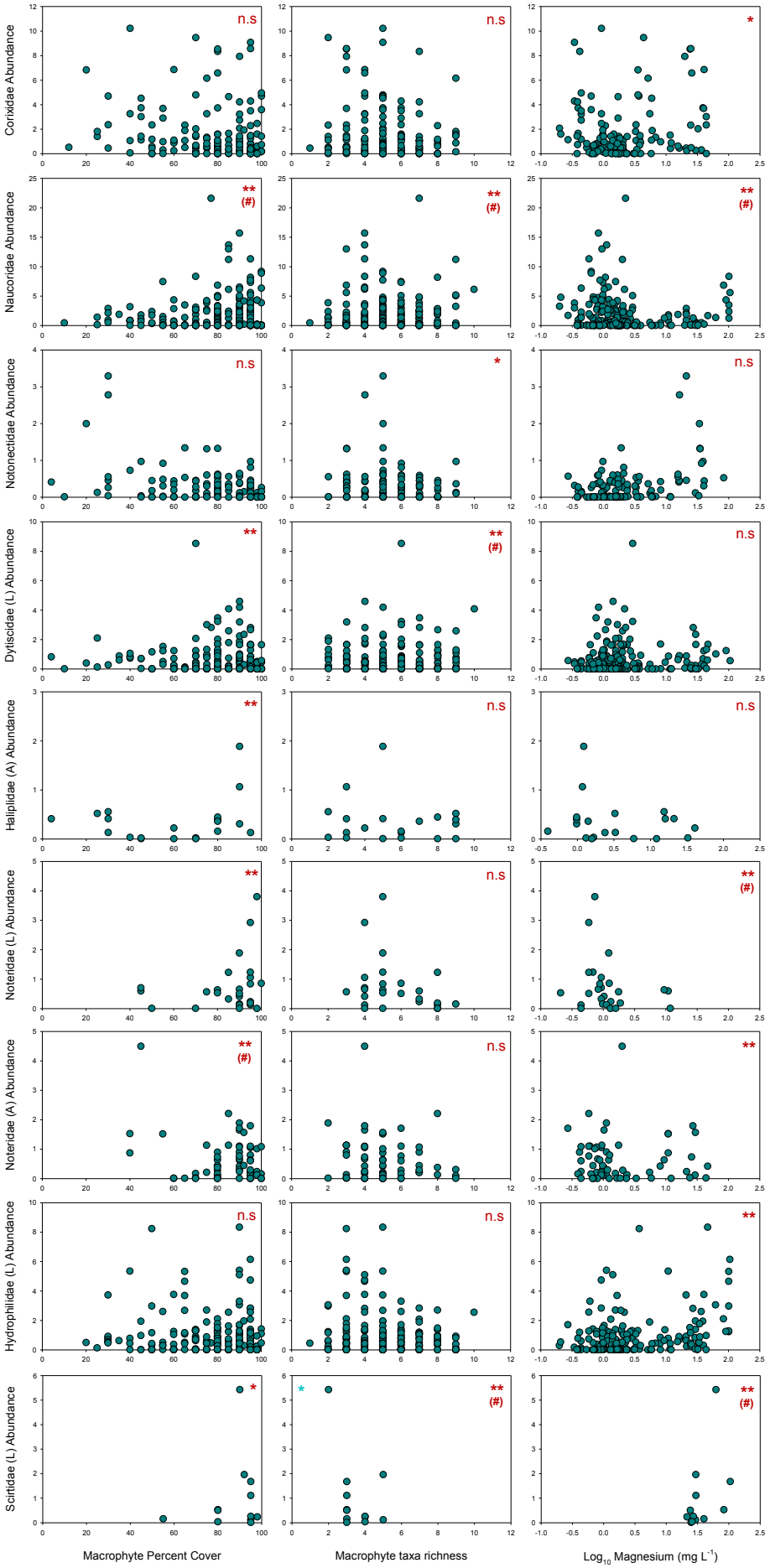
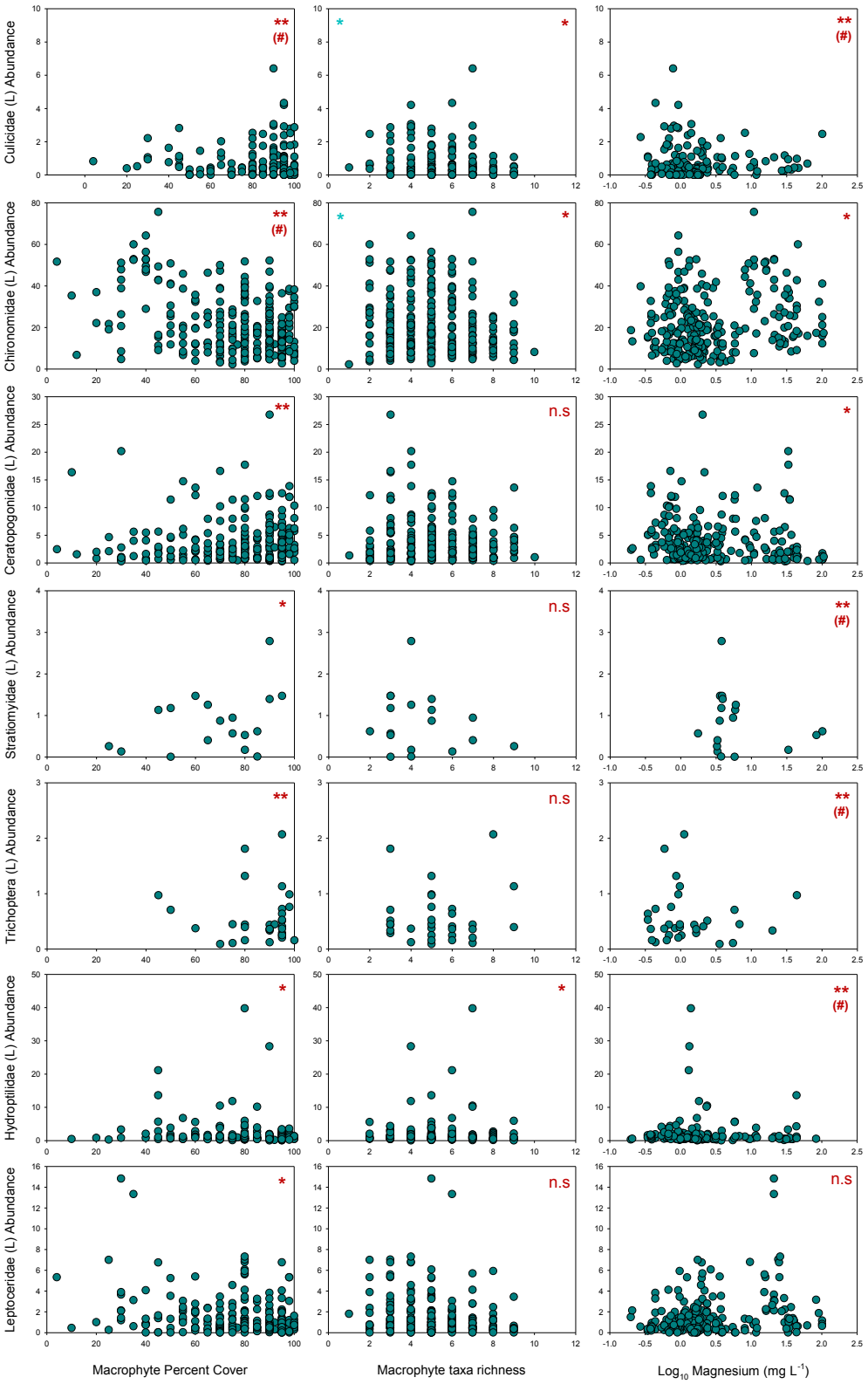

Corixidae Abundance
Naucoridae Abundance
Notonectidae Abundance
Dytiscidae (L) Abundance
Haliplidae (A) Abundance
Noteridae (L) Abundance
Noteridae (A) Abundance
Hydrophilidae (L) Abundance
Scirtidae (L) Abundance
n.s
*
**
(#)
Macrophyte Percent Cover
Macrophyte taxa richness
Log10 Magnesium (mg L-1)


Culicidae (L) Abundance
Chironomidae (L) Abundance
Ceratopogonidae (L) Abundance
Stratiomyidae (L) Abundance
Trichoptera (L) Abundance
Hydroptilidae (L) Abundance
Leptoceridae (L) Abundance
**
(#)
*
n.s
Macrophyte Percent Cover
Macrophyte taxa richness
Log10 Magnesium (mg L-1)

Appendix 7 Assessment of other possible causes of biological changes in GTB

Other factors have potential to cause the biological changes observed in GTB in 2011, including differences in sample processing methods since 1995 and habitat changes (viz aquatic plant communities). These are assessed in turn in this Appendix.

A7.1 Differences in sample processing methods since 1995[9]

For ARR waterbodies sampled in the present study, different sample processing methods have been applied over time. In 1995, 1996 and 2006, live macroinvertebrates were extracted from samples by eye in the field – so-termed live-sorting. In 2009, 2011 and 2013, the processing method differed; samples were preserved in the field and later subsampled and sorted in the laboratory under a stereo microscope, i.e. laboratory-processed samples. This change in methodology has potential to confound results observed to date, with a key question to address: are the changes in GTB macroinvertebrate communities observed after 2006 (particularly for 2011) an artefact of the method change?

The most effective way to assess potential confounding of this type is to compare analyses of community structure datasets derived (i) from the samples first live-sorted in field, then (ii) from the same sample residues preserved and later subsampled and sorted in the laboratory. Some limited local datasets were available for this from specific investigations conducted in GTB and/or Gulungul Billabong (GUL) in 1996 and 2013 (18 samples). A much larger dataset, however, was also available from the results of an extensive, Australia-wide AUSRIVAS bioassessment study, acquired from the mid to late 1990s (80 reference-site samples).

As part of the Commonwealth's National River Health Program, the Monitoring River Health Initiative (MRHI) was established in the mid 1990s, to develop a bioassessment approach in Australia using riverine macroinvertebrate communities. The MRHI was the precursor to development of regional AUSRIVAS predictive models for bioassessment of Australia's rivers. ***eriss*** and a collaborator conducted a QA/QC study for the MRHI in the late 1990s with results reported in (Humphrey et al 2000). A key objective of the QA/QC was to assess the performance of operators sorting macroinvertebrate samples either live in the field or from preserved subsamples using a microscope in the laboratory. ***eriss*** staff received a large number of preserved macroinvertebrate samples, representing a variety of different riverine habitats, from different State and Territory agencies. Most agencies (QLD, NSW, VIC, TAS & WA) adopted live-sorting as the sample processing protocol, and assessment of operator performance involved:

1 further processing in the laboratory of the preserved residues from field live-sorted (LS) samples using microscopic examination of residue subsamples; and

2 comparison of community structure data from live-sorted samples and their associated laboratory-processed component. The latter component was termed the 'whole sample

[9] Modified excerpt from:

Humphrey C & Chandler L 2015. Developing water quality closure criteria for Ranger billabongs using macroinvertebrate community data. In ***eriss*** *research summary 2013-2014*. Supervising Scientist Report 209, Supervising Scientist, Darwin NT, 166-180.

estimate' (WSE), i.e. results that would have arisen had the same (live-sorted) sample been sub-sampled and processed in the laboratory.

A key aspect of the comparative assessment in (ii) above, was whether operators were missing taxa when live-sorting, as a consequence of the cryptic and small size of specimens. The most significant findings from the QA/QC were: (a) small and/or cryptic taxa were commonly overlooked during live-sorting (chironomid pupae, ceratopogonids, empidids, elmid larvae, hydroptilids, oligochaetes, Acarina), while conversely (b) some taxa were better represented in live-sort data, including large or mobile, but less abundant taxa (odonates, shrimps and adult beetles) (Humphrey et al 2000).

While the MRHI QA/QC study determined that sample processing biases resulted in (subtly) different community structure data for the two sample processing methods, the important aspect to address is whether group comparisons (in this instance GTB versus reference waterbodies) give similar or different results when either live-sorted or laboratory processed data are analysed. If GTB versus reference waterbody comparisons, by way of number of taxa, ANOSIM R or Bray-Curtis similarity, are very similar for live-sorted or associated laboratory processed data, this indicates sample processing method is *not* a confounding factor in the interpretation of results from the full dataset (1995 to 2013) used in the present study.

To mimic the GTB versus reference waterbody comparison that is conducted separately for each year of the present study, the LS and associated WSE community structure data from local and MRHI datasets were assessed:

1 ANOSIM R and Bray-Curtis similarity were calculated between different sample groups from the larger sample pool using both the LS and the associated WSE data, i.e. LS-to-LS and WSE-to-WSE comparisons. LS and equivalent WSE results from the various group comparisons were then compared.
2 Number of taxa was derived for LS and WSE components of each MRHI and local (ARR) sample. A mean LS/WSE number of taxa ratio was derived for sample groups of interest.

For ANOSIM R and Bray-Curtis similarity response measures, two types of group to group comparison by processing method (i.e. LS-LS and WSE-WSE) were applied: (a) all possible pairwise combinations of MRHI state to state (e.g. QLD vs VIC, QLD vs WA, NSW vs TAS, etc), and (b) habitat from one state compared to the same habitat from all other states combined (e.g. QLD macrophyte vs macrophyte from all other states, NSW riffle vs riffle from all other states, etc). The habitat comparison described in (b) was most relevant to the present study where replicates from one billabong (GTB) are compared to replicates from all reference billabongs combined because the sample numbers were similar (typically 4-6 'replicates' from one state versus ~20 or more replicates from all others states) and the local (ARR) macrophyte habitat dataset described above (GTB vs GUL) could be directly assessed in the same analysis. State to state and within-habitat relationships between ANOSIM R values calculated on live-sort or whole-sample-estimate data are shown in Figure A7.1.1.

A median LS/WSE number of taxa ratio was also derived for each state and local dataset. These results are shown in Figure A7.1.2.

Despite taxa biases and ensuing differences in macroinvertebrate community structure data represented in the two sample processing methods, pairwise ANOSIMs from the same method (LS-LS and WSE-WSE) yield very similar results (Figure A7.1.1). Indeed, the

plotted relationships for state-to-state and within-habitat comparison are almost a 1:1 relationship. Macrophyte habitat data for the comparison of five GTB with five GUL replicates processed using both methods are plotted in the within-habitat relationship (Figure A7.1.1b). The ANOSIM R values for the GTB-GUL processing method comparisons are also very similar (R values of 0.52 and 0.55 for LS and WSE methods respectively).

Processing method comparisons using the Bray-Curtis similarity measure were also conducted. The results are not provided here but these showed a slightly higher correlation between LS and WSE groupwise comparison, i.e. very high concordance between the results for each sample processing method.

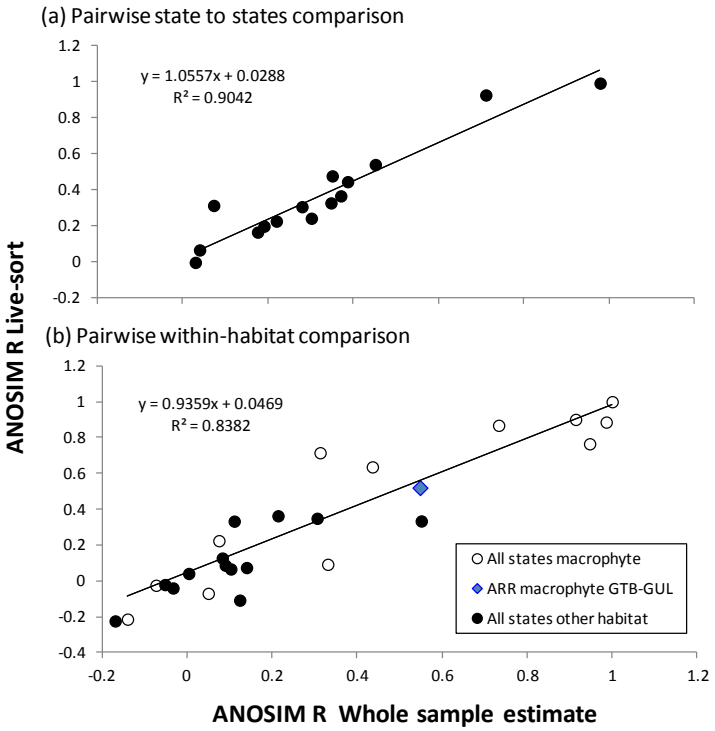


Figure A7.1.1 (a) State to state and (b) within-habitat relationships between ANOSIM R values calculated on live-sort or whole-sample-estimate data.

Number of taxa comparisons between the two sample processing methods (Figure A7.1.2) showed a LS/WSE ratio of close to 1 for all state and local groups considered. This is despite the fact that taxa represented in LS and WSE components were not always the same.

These collective results show that groupwise comparisons of (i) macroinvertebrate community structure and (ii) number of taxa, are preserved, whichever of the two sample processing methods is used. Thus, there is no evidence that the GTB-reference waterbody ANOSIM, similarity and number of taxa values are confounded by the change in sample processing method that occurred after 2006.

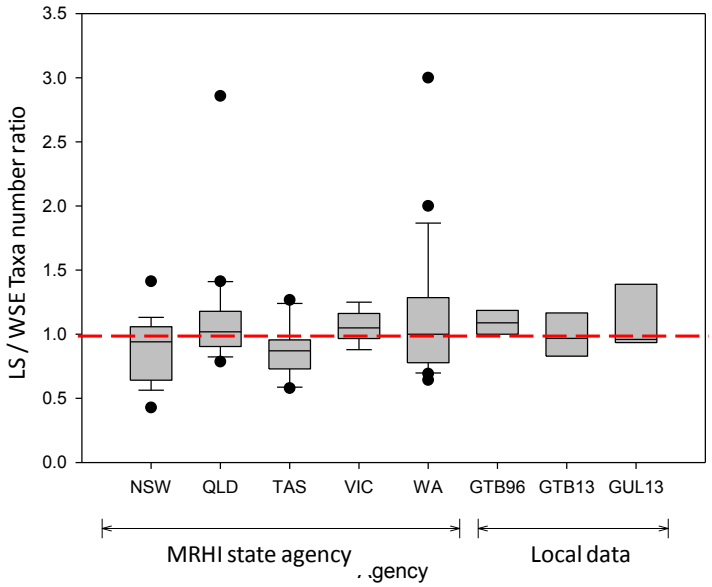


Figure A7.1.2 Boxplot showing comparison of LS-WSE (live-sort/whole sample estimate ratio) for the different MRHI state agencies and within GTB 1996 (10 samples), GTB 2013 (5) and Gulungul 2013 (5) samples.

Box plots show median, 25th and 75th percentile, range within these inter-quartiles and outliers (asterisk).

A final assessment was made of variability and separation in ordination space between live-sorted and laboratory processed samples. The LS and associated WSE datasets used above and representative of different habitats were ordinated using MDS, with results shown in Figure A7.1.3. For each habitat type, there is high overlap in LS and corresponding WSE samples with group separation (LS vs WSE) for each habitat very small (ANOSIM R values <0.15). Variabiity in ordination space was measured using PERMDISP (see section 2.5.3.1 of the main report) with results shown in Figure A7.1.4. Variability for all habitats is consistently greater in the live-sorted components of samples. The results indicate that ordinations combining LS and WSE-based samples will maintain fidelity of community structure position in ordination space whichever component (LS or WSE) is used. 'Spread' of data points in ordination space will be greater amongst LS samples than WSE-based samples.

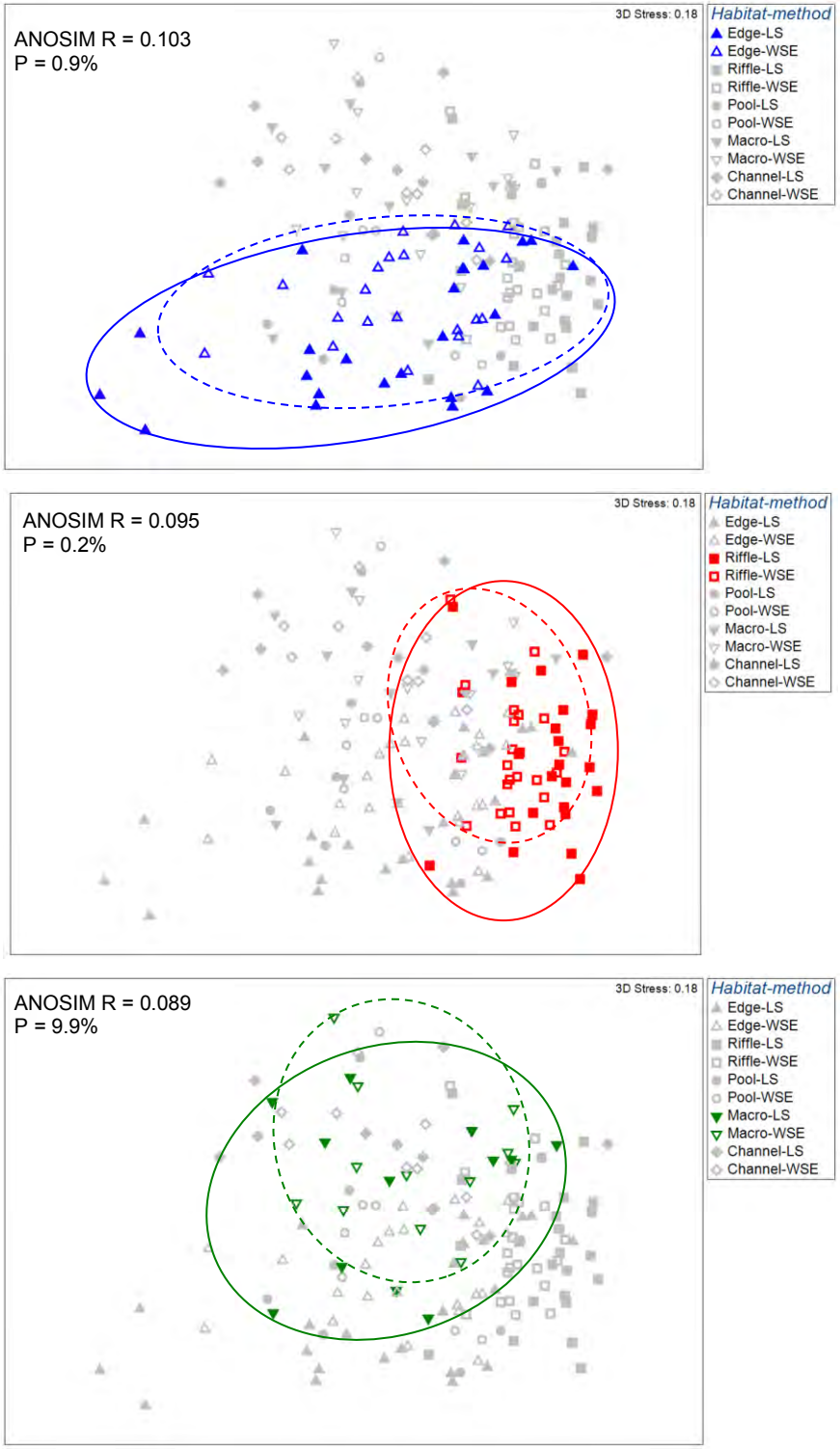


Figure A7.1.3 MDS ordination showing separation of agency data processed using different methods, by habitat Edge (top), Riffle (middle) and Macrophyte (bottom). LS = live-sorted samples, WSE = whole sample estimate based on laboratory subsampling and sorting

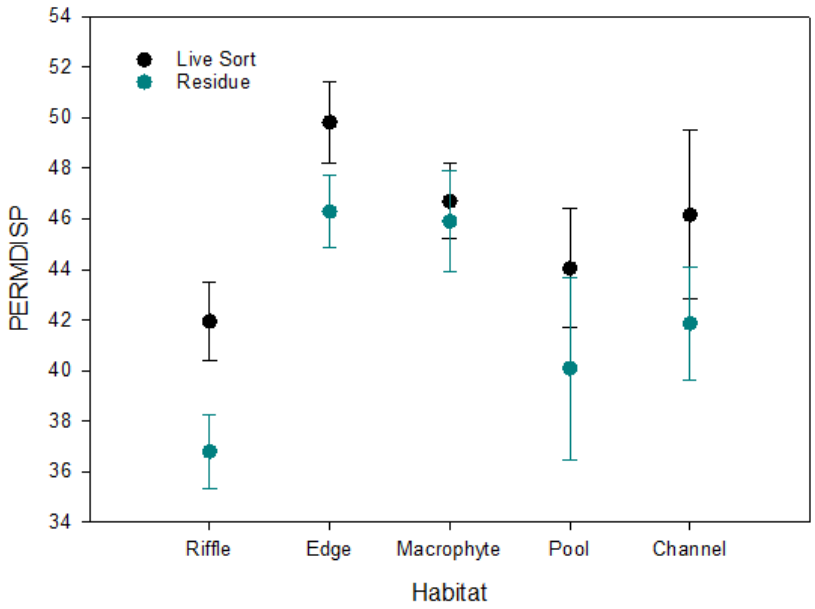


Figure46. Variability in community structure, as depicted using the mean distance from the centroid with PERMDISP, between Live sort and WSE ("Residue") components of agency samples for different habitat.

A7.1.1 References

Humphrey CL, Storey AW & Thurtell L 2000. AUSRIVAS: operator sample processing errors and temporal variability – implications for model sensitivity. In *Assessing the Biological Quality of Freshwaters. RIVPACS and Similar Techniques*, eds Wright JF, Sutcliffe DW & Furse MT, Freshwater Biological Association, Ambleside, UK, 143-163.

A7.2 Analysis of aquatic vegetation community data from shallow waterbodies of the Alligator Rivers Region[10]

A7.2.1 Background

Development of closure criteria for aquatic ecosystems associated with the rehabilitation of Ranger Uranium Mine has, to date, focused primarily on key water quality variables. As the mine moves towards rehabilitation, a wider range of ecological information will be required to ensure that site conditions, biological communities and key ecological processes meet the Environmental Requirements.

A review of aquatic ecosystem literature for the Alligator Rivers Region highlighted a knowledge gap associated with aquatic vegetation in sandy creek channels and shallow billabongs of the lowlands. While aquatic vegetation data are collected as the 'habitat' component of current monitoring projects for macroinvertebrate and fish communities, these data have not been analysed and evaluated for their own value, both in terms of responses of aquatic vegetation to natural and mine-related stressors, and in the context of ecosystem establishment and rehabilitation targets.

A recurring theme in discussions around aquatic vegetation is that rehabilitation targets will need to capture natural variation within vegetation communities. Rather than relying solely on (early) baseline vegetation data, which may have changed over time, targets will need to reflect natural changes associated with contemporary reference or analogue conditions.

This project aimed to evaluate the natural variation of aquatic plant communities in shallow waterbodies around Ranger by analysing existing data collected by ***eriss*** between 1993 and 2014. The objective of the study was to characterise aquatic vegetation communities, determine the level of spatial and temporal community change and seek possible environmental determinants of the patterns observed. Such knowledge may inform establishment methods and targets for rehabilitation of aquatic systems both on- (within the Ranger Project Area) and off-site.

The first task associated with the project was to evaluate and amalgamate relevant datasets in a form that could address the key research needs. Having undertaken this, the following research questions could then be posed:

- Do the aquatic vegetation communities of shallow, lowland mine disturbed and reference waterbodies differ?
- Have shifts in vegetation community composition occurred over time?
- For any spatial or temporal differences or shifts in composition observed, were there specific taxa distinguishing these changes or differences?

A7.2.2 Methods

A7.2.2.1 Data preparation

Aquatic vegetation data were compiled from two key ***eriss*** programmes:

- billabong macroinvertebrate monitoring
- shallow lowland billabong fish monitoring.

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[10] Modified excerpt from: Supervising Scientist (2015). Annual Report 2014–15. Commonwealth of Australia 2015. Section 4.3.

These programmes examined 15 shallow waterbodies in total. Of these, five were considered to be mine-disturbed, either through changes in water quality and/or catchment alteration (RP1 and RP2, and Georgetown, Coonjimba and Djalkmara billabongs). The remaining waterbodies were all considered to be reference sites for the purposes of this study (Gulungul, Corndorl, Baralil and Wirnmuyurr billabongs and Jabiru Lake (Magela Creek catchment); Buba, Sandy Shallow, Angbangbang and Malabanbandju billabongs (Nourlangie Creek catchment); and Cathedral Billabong (East Alligator River catchment)).

Aquatic vegetation communities were assessed by visual estimation methods (percent cover). Genus-level assessments were undertaken, except for *Najas*, *Nitella*, *Chara* and *Utricularia*, which are submerged fine-feathery taxa that in some early years were not distinguished in the field. These taxa were combined under a 'submerged feathery' category. To account for different methods of vegetation assessment, presence/absence data were used for all analyses, removing bias in percent estimation assessments amongst years and between (macroinvertebrate and fish) programmes. Further validity in combining aquatic vegetation datasets from macroinvertebrate and fish monitoring studies is provided in section A6.2.3.1 below.

A7.2.2.2 Data analyses

Community composition data from replicate locations within the different waterbodies have been collated and analysed using community summaries and multivariate statistical techniques in PRIMER. Community summaries were based on number of taxa (mainly genus). Multivariate techniques included:

1. Hierarchical cluster analysis, where samples of similar assemblages are grouped, with the groups forming clusters at lower levels of similarity. A group average linkage was used to derive the resultant dendrogram;
2. Multi-Dimensional Scaling (MDS) ordination, depicted as two-dimensional plots based on the sample by sample Bray-Curtis similarity matrices;
3. Analysis of Similarity (ANOSIM), examining the degree and significance of separation of *a priori* groups in ordination space. (ANOSIM was effectively an analogue of the univariate ANOVA based upon rank similarities between samples in the underlying Bray-Curtis similarity matrices.); and
4. SIMPER, examining taxa that were contributing to the differences in groups identified from ANOSIM analyses.

A priori groups used for these analyses included:

i. Reference versus mine-disturbed sites; and

ii. Three year-class intervals determined on the basis of (i) a relatively abrupt change in water chemistry observed in RP1, Coonjimba and Georgetown billabongs[11] (i.e. between interval 1993–2000 and interval 2001–2011), together with (ii) examination of ordination plots for the vegetation data for each billabong separately (results not shown here), which showed the last three years (i.e. interval

[11] Humphrey C & Chandler L 2015 Developing water quality closure criteria for Ranger billabongs using macroinvertebrate community data In: ***eriss*** *research summary 2013-2014*. Supervising Scientist Report 209, Department of the Environment, Darwin NT,

2012–2014) in most waterbodies grouping and separating out together in ordination space.

A7.2.3 Results and discussion

A7.2.3.1 Spatial and temporal patterns in aquatic plant communties

The median number of taxa per reference waterbody was consistently higher than that in mine-disturbed billabongs (Figure A6.2.1). There also appeared to be a trend of increasing number of taxa for both reference and mine-disturbed sites over time.

A hierarchical cluster analysis was run to examine any patterns of grouping in the data. Significant clusters, as determined by the SIMPROF permutation test, indicated that the highly modified artificial waterbodies, Jabiru Lake and Retention Pond 2 (RP2), were significantly separated from all other sites, while mine disturbed sites were generally separated from reference sites (results not shown here). RP2 and Jabiru Lake data were subsequently removed from the analyses due to a tendency to greatly skew the data.

The MDS ordination for all remaining waterbodies is shown in Figure A6.2.2. Amongst reference sites and confirming the cluster analysis, there was interspersion of billabongs occurring in Magela and other (including Nourlangie Creek) catchments (Table A6.3.1), indicating that any separation of mine-disturbed and reference waterbodies evident in Figure A6.2.2 was not due to catchment. Reference sites were also interspersed according to macroinvertebrate and fish sampling programmes indicating, similarly, no method artefact amongst the reference billabongs (Table A6.3.1). In all other respects, the ordination was consistent with the cluster analysis. While some separation of the data was evident between mine-disturbed and reference waterbodies (Figure A6.3.2), ANOSIM indicated that such separation was minor (Table A6.3.1).

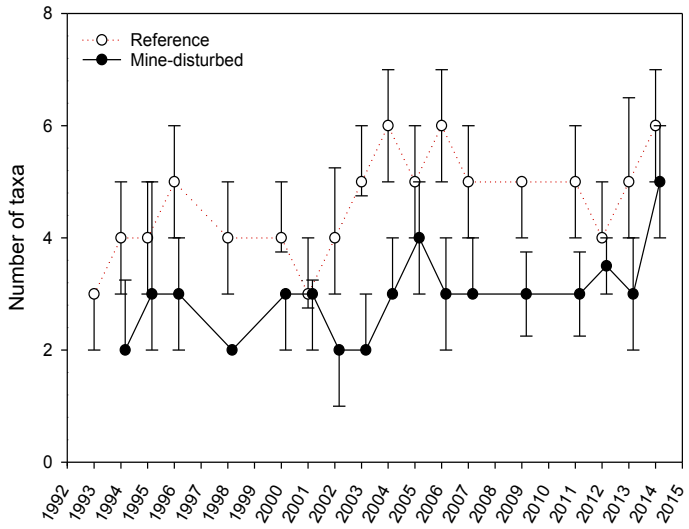

Figure A7.2.1 The median number of taxa for each year of sampling per reference and mine-disturbed waterbody. Error bars are 25th and 75th percentiles. No mine-disturbed sites are represented in 1993. Data from years 2006, 2011 and 2013 were derived from macroinvertebrate sampling, years 1993, 1994, 1998, 2000-2005, 2007, 2009, 2012 and 2014 from fish sampling, and 1995 and 1996 from both macroinvertebrate and fish sampling.

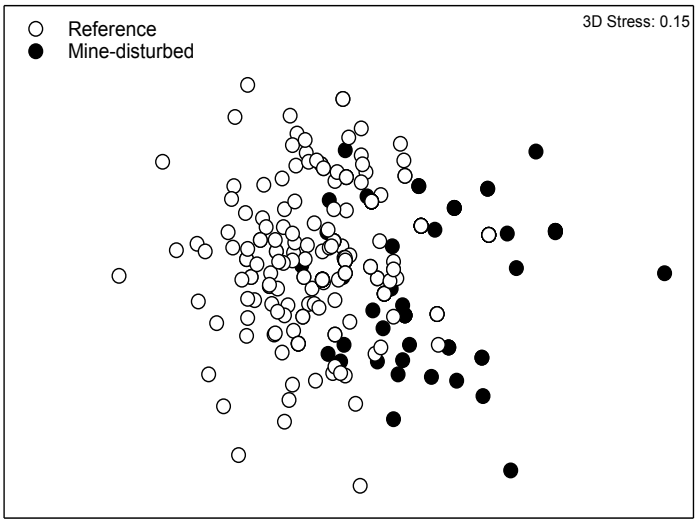

Figure A7.2.2 Ordination plot of plant composition (showing axis 1 and 2) for all years.

Compositional shifts over time were examined from ordinations derived separately for reference and mine disturbed waterbodies (Figure A6.2.3). For both waterbody types, all pairwise combinations of year groups were barely separable in ordination space (Table A6.3.1; R-statistic <0.25), indicating negligible changes in plant composition over time, even though mine-disturbed sites did show a slightly stronger separation between early years and latter years than reference sites for the same time comparison.

Table A7.2.1 ANOSIM results

Groups	R-Statistic[1]	P
Magela vs other reference billabongs		
Global Test	0.102	0.0001
Macroinvertebrate vs fish reference data		
Global Test	0.154	0.0001
Mine-disturbed versus reference waterbodies		
Global Test	0.209	0.0001
Year intervals in mine-disturbed waterbodies		
Global Test	0.082	0.011
1993-2000 vs 2001-2011	0.057	0.014
1993-2000 vs 2012-2014	0.208	0.006
2001-2011 vs 2012-2014	0.013	0.411
Year intervals in reference billabongs		
Global Test	0.042	0.048
1993-2000 vs 2001-2011	0.057	0.001
1993-2000 vs 2012-2014	0.079	0.092
2001-2011 vs 2012-2014	-0.024	0.647

1 The degree of separation between groups is denoted by the R-statistic, where R-statistic > 0.75 = groups well separated, R-statistic >0.5 = groups overlapping but clearly different, and R-statistic <0.25 = groups barely separable. A significance level of <5% =- significant effect/difference.

A7.2.3.2 Plant taxa distinguishing the observed spatial and temporal differences

SIMPER analysis was applied to the main (combined waterbody) ordination (Figure A7.2.2), as well as the separate mine-disturbed and reference waterbody ordinations (Figure A7.2.3a). *Caldesia*, submerged feathery species and *Nymphoides* had greater occurrences in reference billabongs while the emergent *Eleocharis* occurred more often in mine-disturbed waterbodies. The primary plant taxa contributing to the difference between time periods and waterbody type were submerged feathery species and *Eleocharis*.

These two taxa categories are presented as bubble plots over the MDS ordinations in Figure A7.2.3(b) and Figure A7.2.3(c), respectively. The size of the bubbles in these plots is related to the number of occurrences at each site over time. For both mine-disturbed and reference waterbodies, submerged feathery genera tended to be absent from earlier years (1993–2000), with generally greater frequency of occurrences in the reference billabongs, as noted above. *Eleocharis*, conversely, has tended to be more prevalent in the mine-disturbed sites (as noted) with consistent very low frequency of occurrence in just a few reference billabongs, including Corndorl, Sandy and Malabanbandju, over time.

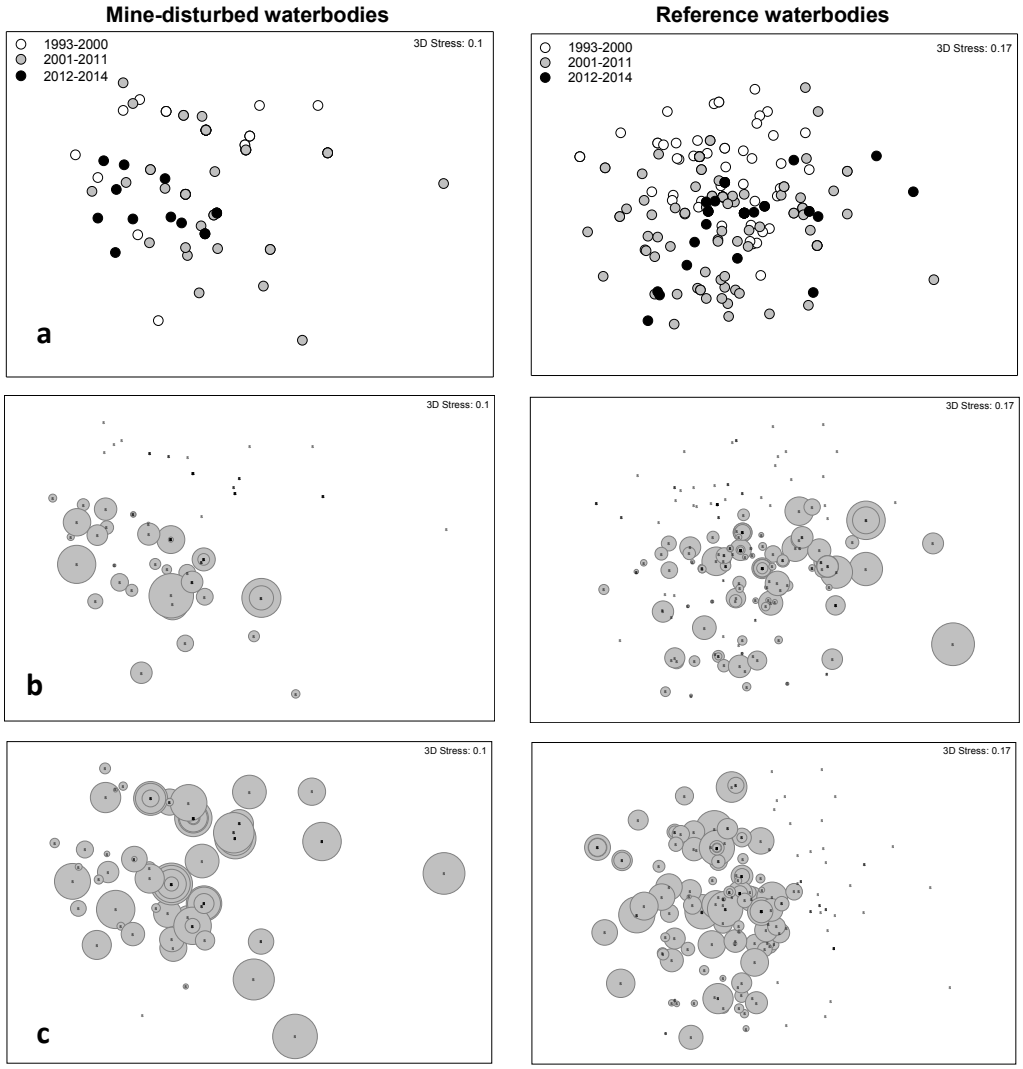

Figure A7.2.3 (a) Ordination plots showing axis 1 and 2 for mine-disturbed sites only (left) and reference sites only (right). Sites are classified by year groups. Bubble plots superimposed on the mine-disturbed and reference ordinations of influential species identified by SIMPER (b) Utricularia and (c) Eleocharis.

A7.2.4 Conclusions and further work

Initial analyses of aquatic vegetation data from shallow waterbodies indicate only a small difference in community composition between reference and mine-disturbed waterbodies, and only slight shifts in composition in both waterbody types since 1993 to 2014. Submerged feathery species and emergent *Eleocharis* appeared to be significant indicators of aquatic vegetation change between mine-disturbed and reference billabongs and for submerged feathery species, also over time.

Future analyses will seek relationships between key environmental variables and the multivariate patterns observed. The small changes noted in plant communities of mine-disturbed waterbodies over time are consistent with those similarly observed in reference billabongs, suggesting little influence of mine-related water quality. Supporting this, the magnitude of differences in number of taxa between the two waterbody types has been similar over the study period, a period that has included significant changes in water quality in the mine-disturbed sites (i.e. post-2000) (Figure A7.2.1). This could indicate that natural factors such as differences in waterbody morphometry or catchment size are more important determinants of plant community composition. Understanding possible causal mechanisms will be enhanced through further time series analyses, comparing the current dataset with species lists compiled during the 1980s.[12]

Incorporation into the statistical analyses of additional aquatic vegetation data collected by Energy Resources Australia Ltd (ERA) from smaller on-site (constructed) waterbodies will also potentially inform (i) water quality tolerances of aquatic vegetation and (ii) decisions on whether on-site waterbodies generally, natural and constructed, have potential to host the broader suite of aquatic plant communities found in similar shallow billabongs of Kakadu National Park.

[12] Finlayson C, Thompson K, von Oertzen I & Cowie I 1994. Vegetation communities of five Magela Creek billabongs, Alligator Rivers Region, Northern Territory. Technical memorandum 46, Supervising Scientist for the Alligator Rivers Region, AGPS, Canberra.

A7.3 Changes in aquatic vegetation amongst waterbodies over time[13]

Macroinvertebrate communities may respond to changes in habitat and for GTB and similar ARR lentic waterbodies, aquatic plant communities constitute the major habitat for residency. Assessment of aquatic vegetation composition and relative abundance has accompanied billabong macroinvertebrate sampling for every year of the present study, using the same methodology first developed in 1995. Changes in these plant communities were examined to assess the extent, if any, to which these changes may also account for macroinvertebrate responses in GTB over time.

Since 1995 and at each of the five locations within each of the 14 waterbodies sampled, macrophyte composition and relative abundance data have been collected. The visual-assessment methods are described in O'Connor et al (1995). In summary, a visual assessment of the total percentage cover of submerged and emergent macrophytes in the sampling location is made, as well as the percentage abundance of individual macrophyte taxa (usually genus-level) present. Typically, percentage abundance of the different taxa were grouped according to structurally-similar plant forms, after the schema of Sainty and Jacobs (2003), i.e. 'floating attached', 'submerged not feathery', 'submerged and emergent feathery', 'free floating', 'emergent narrow leaf' and 'emergent broad leaf' forms. GTB plant communities were compared to the same (up to) 9 reference waterbodies as the macroinvertebrate analyses described above. Assessment of aquatic vegetation changes in GTB relative to reference waterbodies entailed comparative plots of relative abundance of structurally-similar plant forms, number of taxa and mean (GTB-reference) similarity for community structure data. The number of taxa and similarity plots were prepared in the same manner as applied to macroinvertebrate data (i.e. Figures A7.3.2 & A7.3.3 respectively); number of taxa and similarity were plotted against antecedent (to sampling) Mg concentration. Of particular interest in the plots was whether changes in GTB plant communities, relative to reference waterbodies, were most evident in 2011, coincident with macroinvertebrate impacts and highest (on record) $MgSO_4$ contamination.

The analyses showed that, relative to reference waterbodies in 2011, GTB submerged vegetation was in low densities (Figure A7.3.1) and the average replicate number of taxa was also low (Figure A7.3.2). GTB aquatic plant community structure also had low resemblance to that of reference waterbodies in 2011 (Figure A7.3.3). However, these same characteristics were also evident for GTB in 1996 and 2006 (Figures A7.3.1, A7.3.2 & A7.3.3), years when GTB macroinvertebrate communities were similar to reference. This provides evidence that key aquatic plant habitat in GTB was not responsible alone, at least, for the relatively low macroinvertebrate diversity observed in 2011.

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[13] Modified excerpt from:

Humphrey C & Chandler L 2015. Developing water quality closure criteria for Ranger billabongs using macroinvertebrate community data. In ***eriss*** *research summary 2013-2014*. Supervising Scientist Report 209, Supervising Scientist, Darwin NT, 166-180.

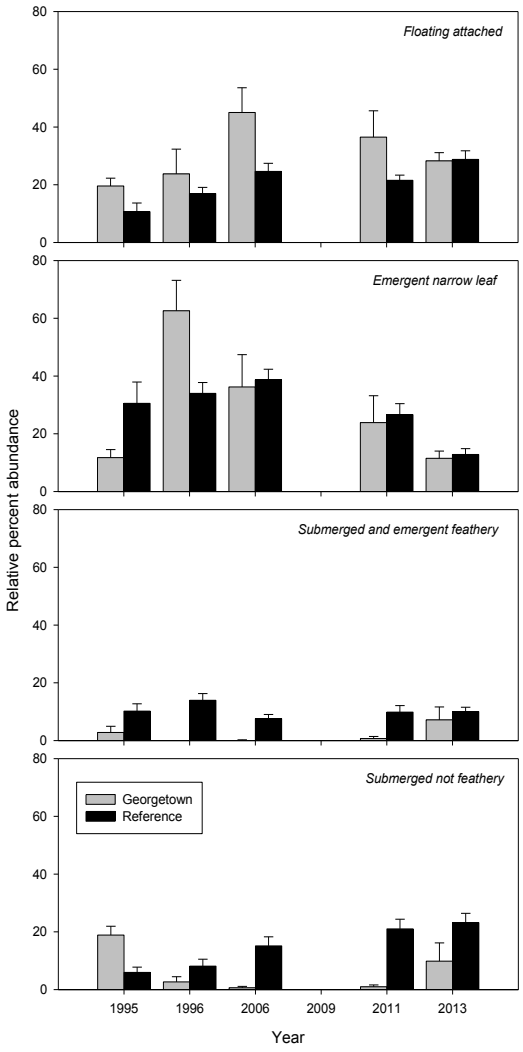


Figure A7.3.1 Mean (±SE) relative percent abundance of aquatic plants grouped by life-form in GTB and reference waterbodies for different years of sampling.

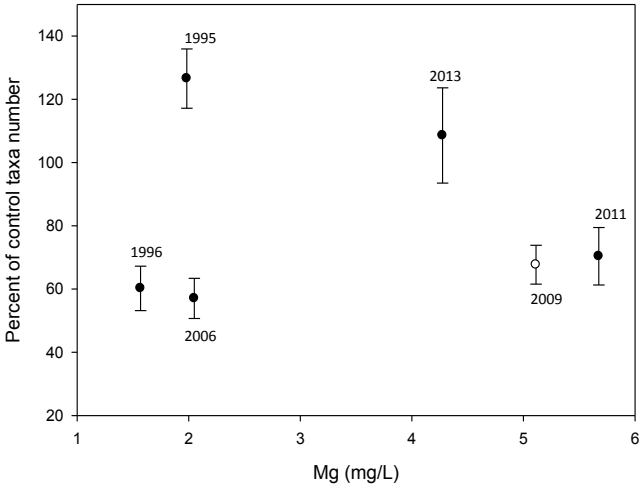


Figure A7.3.2 Mean (±SE) aquatic plant number of taxa (as % of mean reference waterbody number of taxa) in GTB in relation to year of sampling and median antecedent wet and dry season Mg concentration.

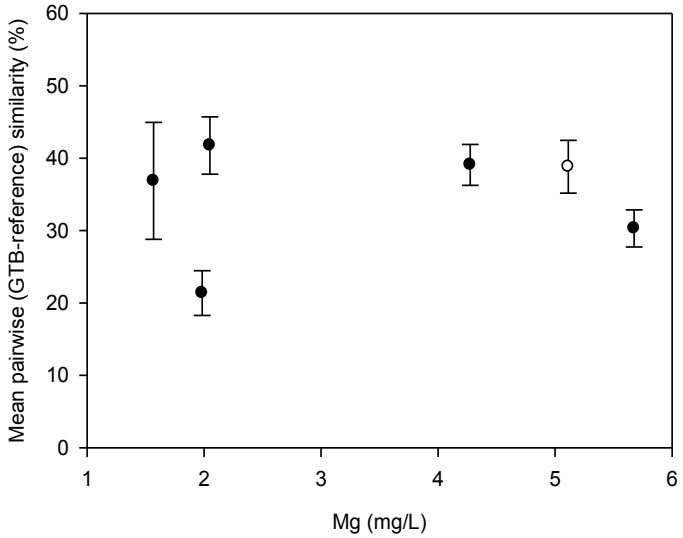


Figure A7.3.3 Mean (±SE) replicate pairwise Bray-Curtis similarity for GTB vs reference waterbody aquatic plant comparison, in relation to year and median antecedent wet and dry season Mg concentration.

A7.3.1 References

O'Connor R, Humphrey CL, Dostine PL, Lynch CM & Spiers AG 1995. A survey of aquatic macroinvertebrates in lentic waterbodies of Magela and Nourlangie Creek catchments, Alligator Rivers Region, NT. December 1995, Supervising Scientist, Canberra. Internal Report.

Sainty GR & Jacobs SWL 2003. *Waterplants in Australia.* 3rd edn, Sainty and Associates Pty Ltd, Potts Point, NSW.

A7.4 Satellite Derived Fire History Mapping for the Ranger Project Area

Table A7.4.1 Meta-data for Kakadu National Park Satellite Derived Fire History Mapping

Name:	Kakadu National Park Satellite Derived Fire History Mapping
Creator:	Darwin Centre for Bushfire Research, Charles Darwin University (formerly known as Bushfires NT Research)
Data type:	Raster
Projection:	Albers equal area projection, GDA94
Pixel size:	30 m, resampled for all image sources.
Image data sources:	Landsat 2 (1980-1981) Landsat 4 (1982-83) Landsat 5 (1984-1998; 2003-2012) Landsat 7 (1999-2002) Landsat 8 (2013-16)
Late Dry Season Supplementary image sources:	NOAA AVHRR (1990-99) MODIS 250 m (2000-16)
Methods:	Up until October 2008 satellite imagery was expensive, three or more Landsat satellite image dates were used annually: 1. Early in the dry season, approximately early June; 2. At the end of the early dry season (late July/early August); 3. As late in the season as possible before the onset of wet season cloud. Since October 2008, all possible image dates (at 16 day intervals) are used. Fire mapping after the last Landsat image acquisition were supplemented by MODIS derived mapping from NAFI, 2000-16, and AVHRR derived mapping from Landgate, WA from 1990 to 1999. No supplementary data were available prior to 1990.

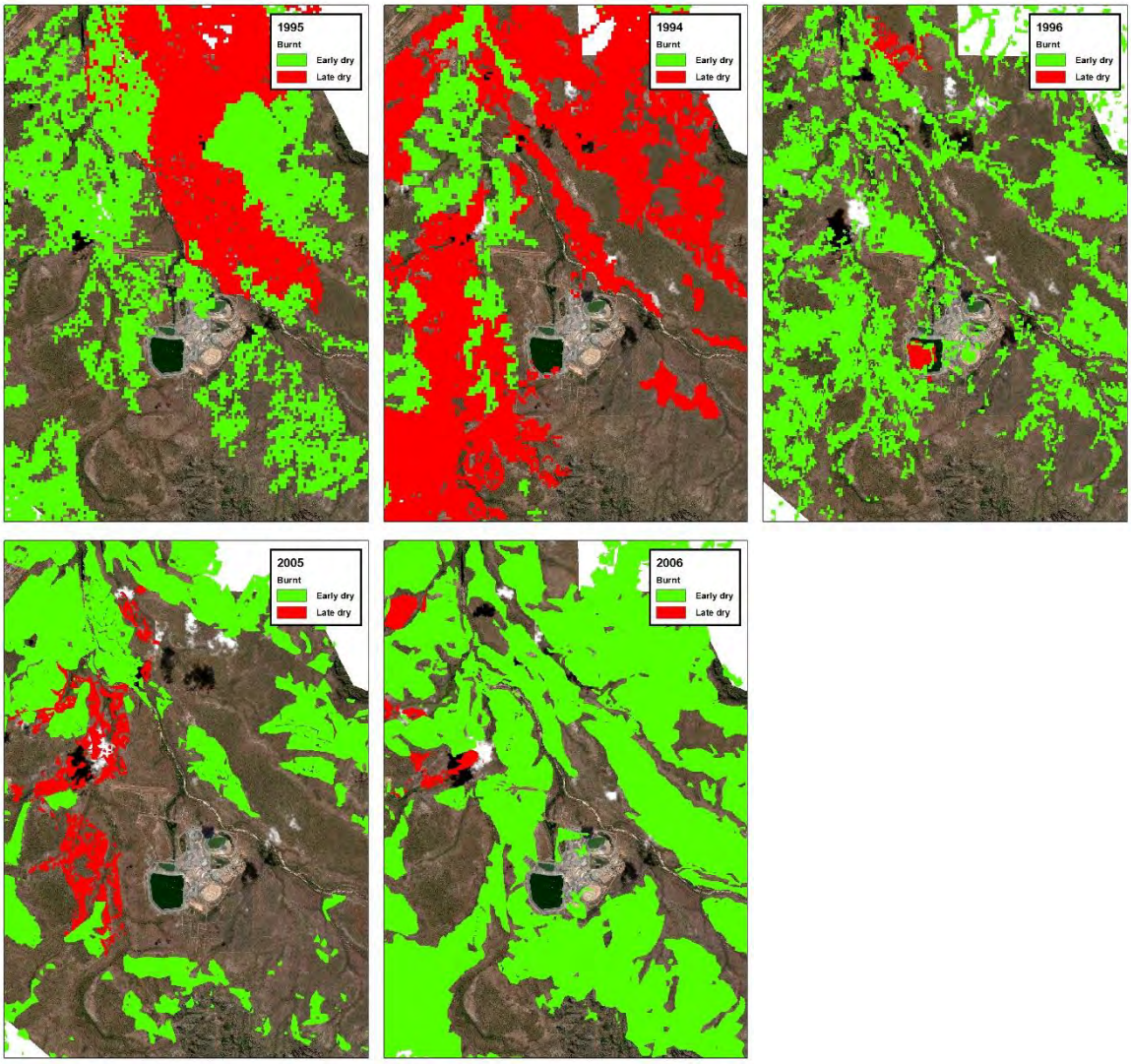


Figure A7.4.1 Fire intensity mapping for the Ranger minesite and adjacent landscape by way of early dry season (early June) and late (intense) dry season burning for the years of macroinvertebrate sampling and the year antecedent to sampling. Showing years 1994,1995,1996, 2005 and 2006

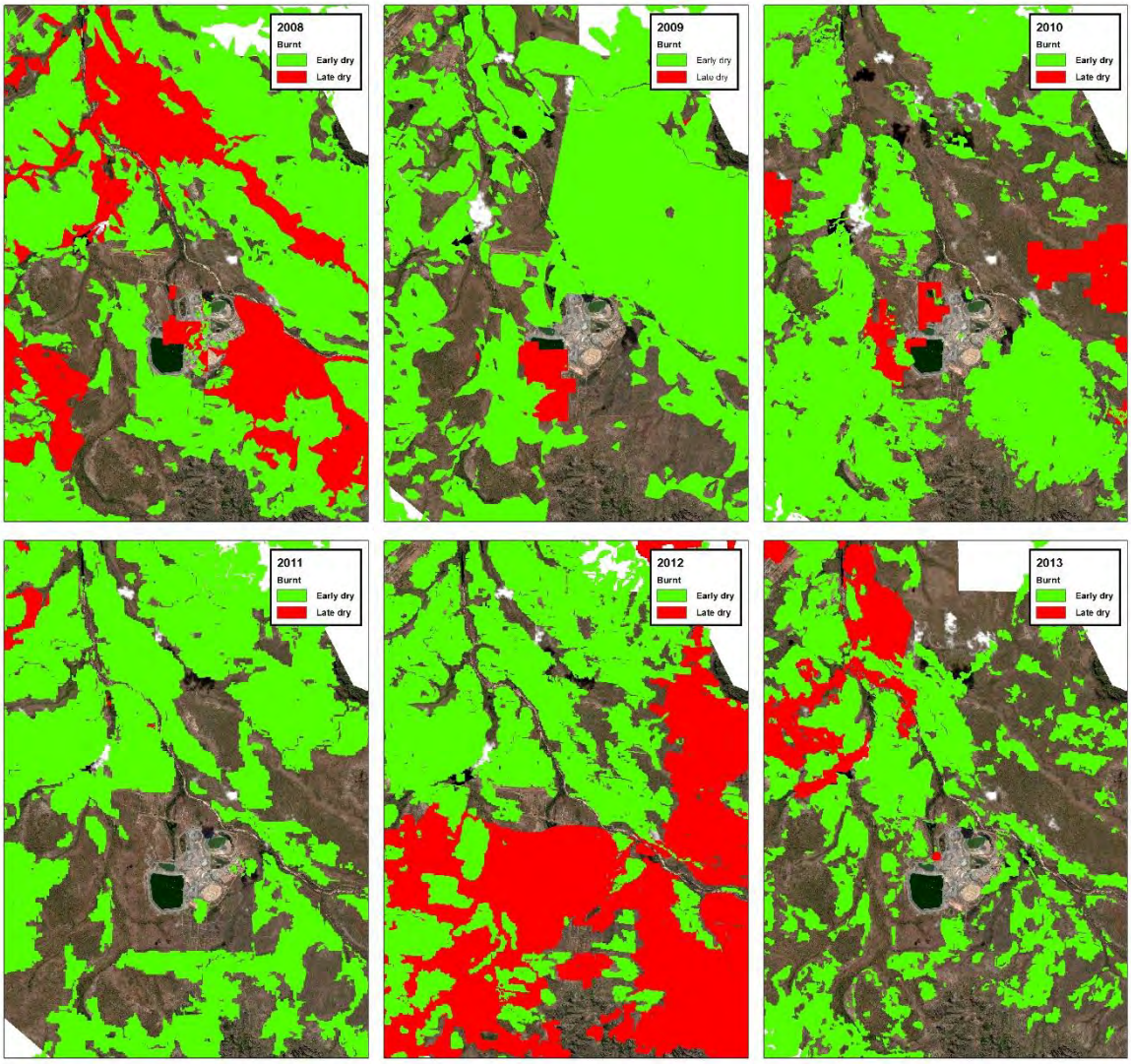
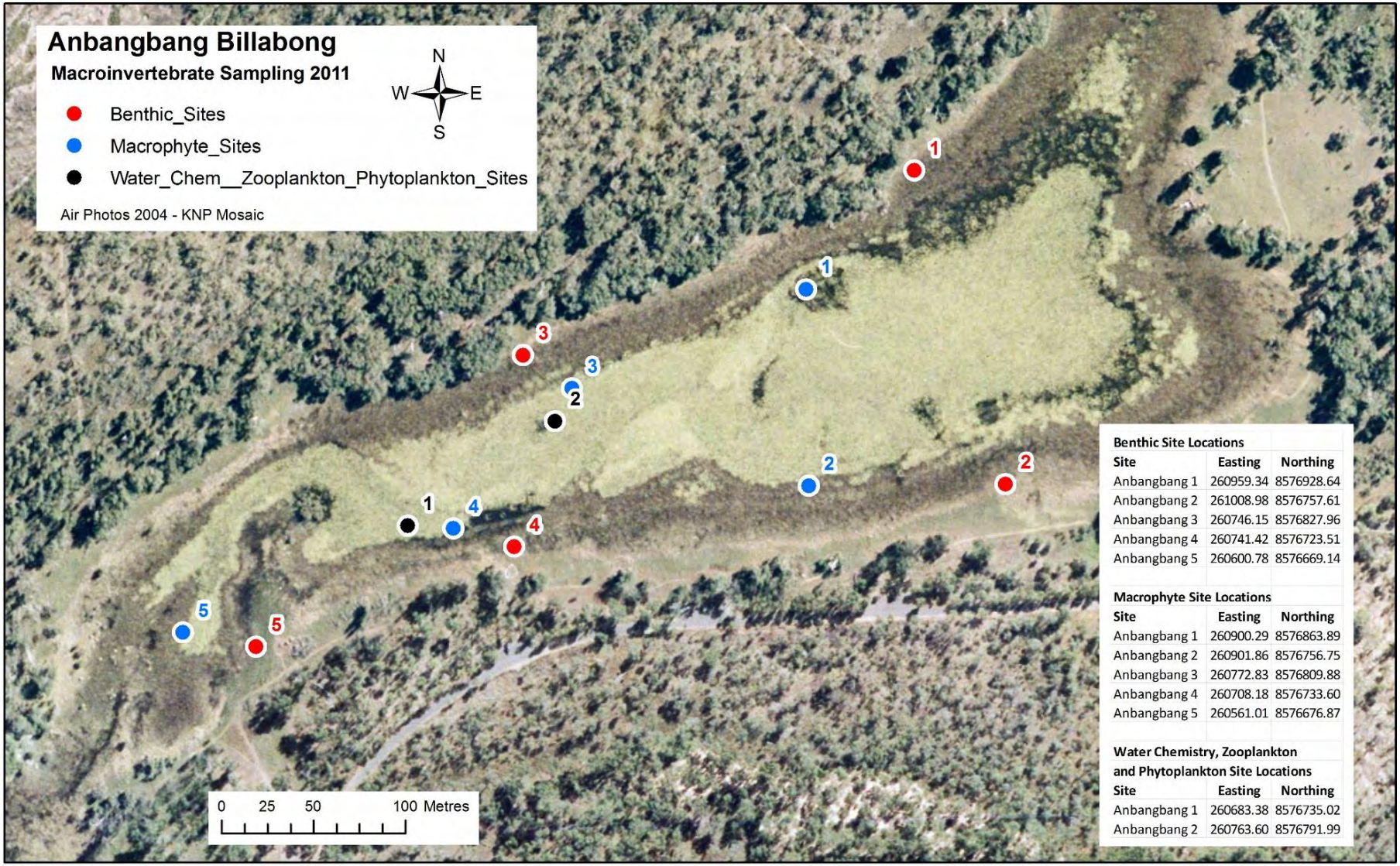


Figure A7.4.2 Fire intensity mapping for the Ranger minesite and adjacent landscape by way of early dry season (early June) and late (intense) dry season burning for the years of macroinvertebrate sampling and the year antecedent to sampling. Showing years 2008,2009,2010,2011,2012 and 2013

Appendix 8 Site Maps



Benthic Site Locations

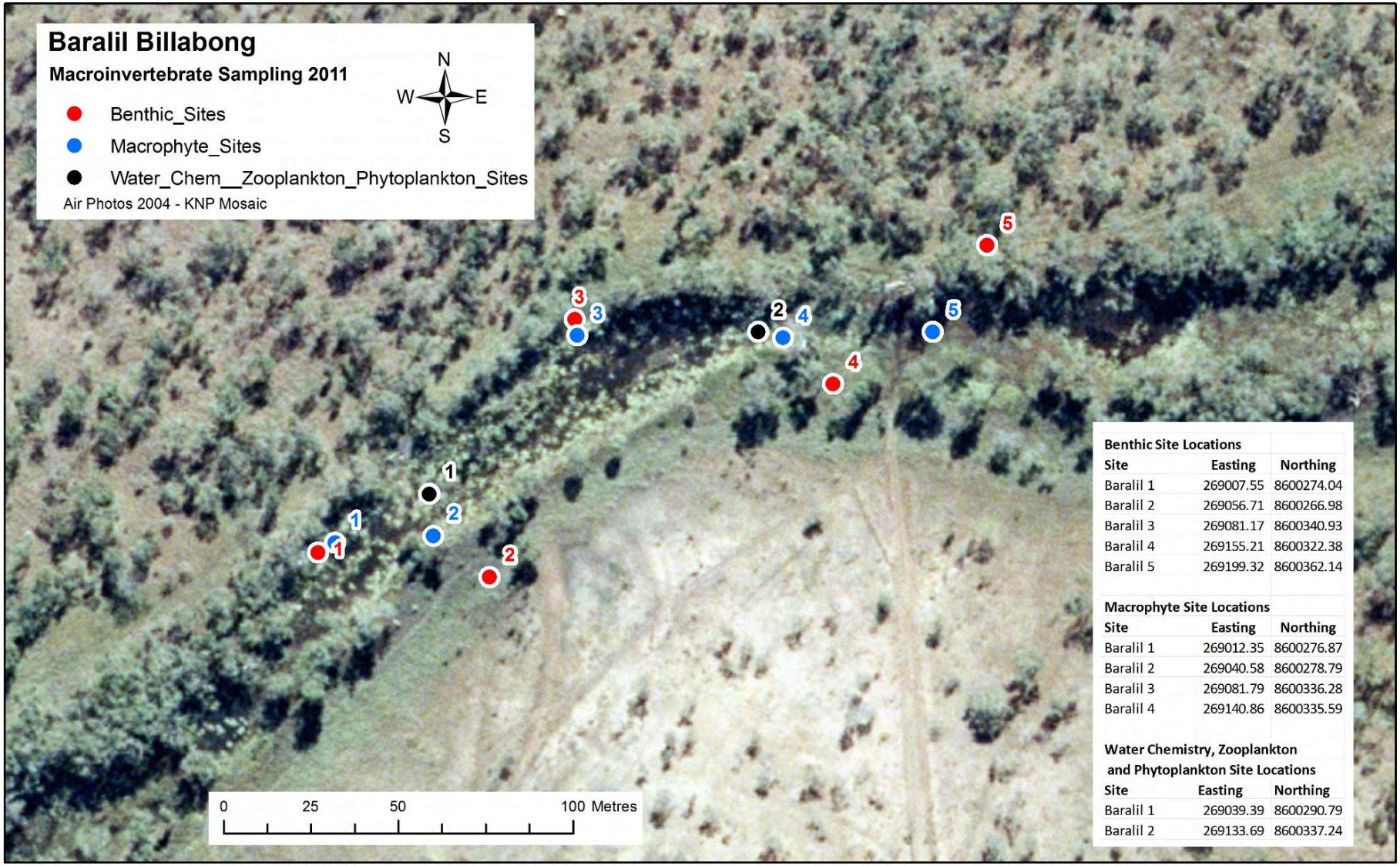
Site	Easting	Northing
Anbangbang 1	260959.34	8576928.64
Anbangbang 2	261008.98	8576757.61
Anbangbang 3	260746.15	8576827.96
Anbangbang 4	260741.42	8576723.51
Anbangbang 5	260600.78	8576669.14

Macrophyte Site Locations

Site	Easting	Northing
Anbangbang 1	260900.29	8576863.89
Anbangbang 2	260901.86	8576756.75
Anbangbang 3	260772.83	8576809.88
Anbangbang 4	260708.18	8576733.60
Anbangbang 5	260561.01	8576676.87

Water Chemistry, Zooplankton and Phytoplankton Site Locations

Site	Easting	Northing
Anbangbang 1	260683.38	8576735.02
Anbangbang 2	260763.60	8576791.99



Benthic Site Locations

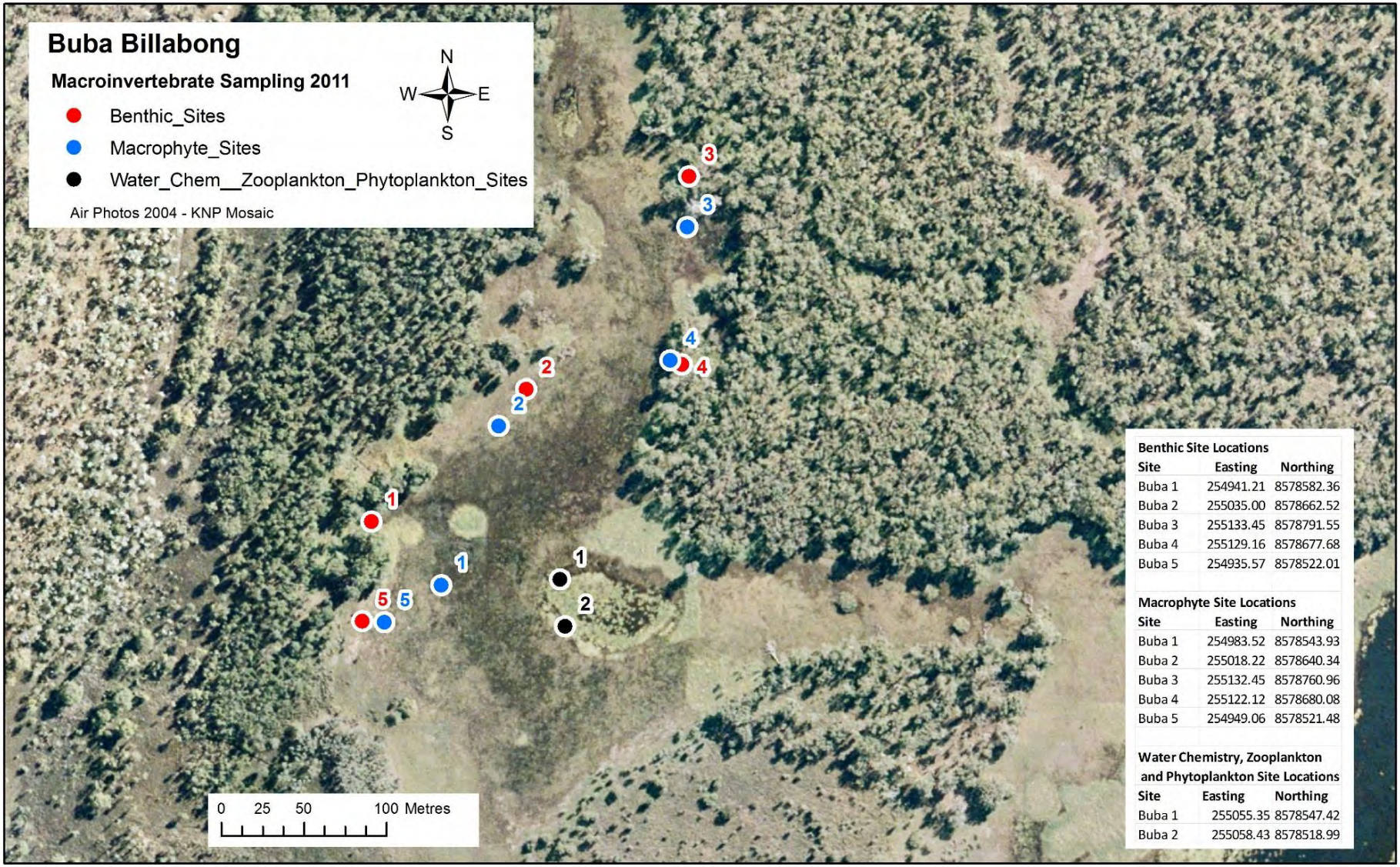
Site	Easting	Northing
Baralil 1	269007.55	8600274.04
Baralil 2	269056.71	8600266.98
Baralil 3	269081.17	8600340.93
Baralil 4	269155.21	8600322.38
Baralil 5	269199.32	8600362.14

Macrophyte Site Locations

Site	Easting	Northing
Baralil 1	269012.35	8600276.87
Baralil 2	269040.58	8600278.79
Baralil 3	269081.79	8600336.28
Baralil 4	269140.86	8600335.59

Water Chemistry, Zooplankton and Phytoplankton Site Locations

Site	Easting	Northing
Baralil 1	269039.39	8600290.79
Baralil 2	269133.69	8600337.24



Benthic Site Locations

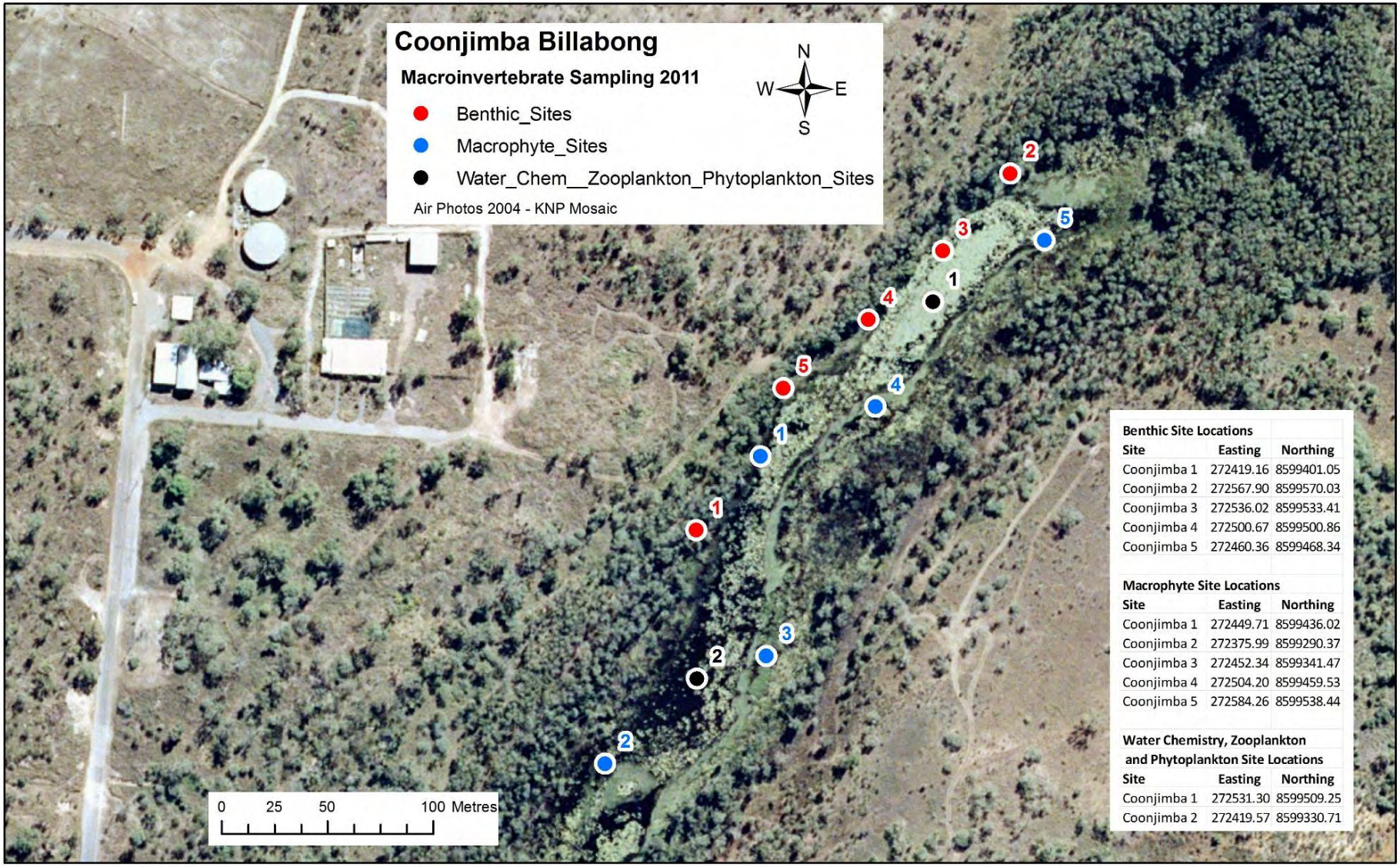
Site	Easting	Northing
Buba 1	254941.21	8578582.36
Buba 2	255035.00	8578662.52
Buba 3	255133.45	8578791.55
Buba 4	255129.16	8578677.68
Buba 5	254935.57	8578522.01

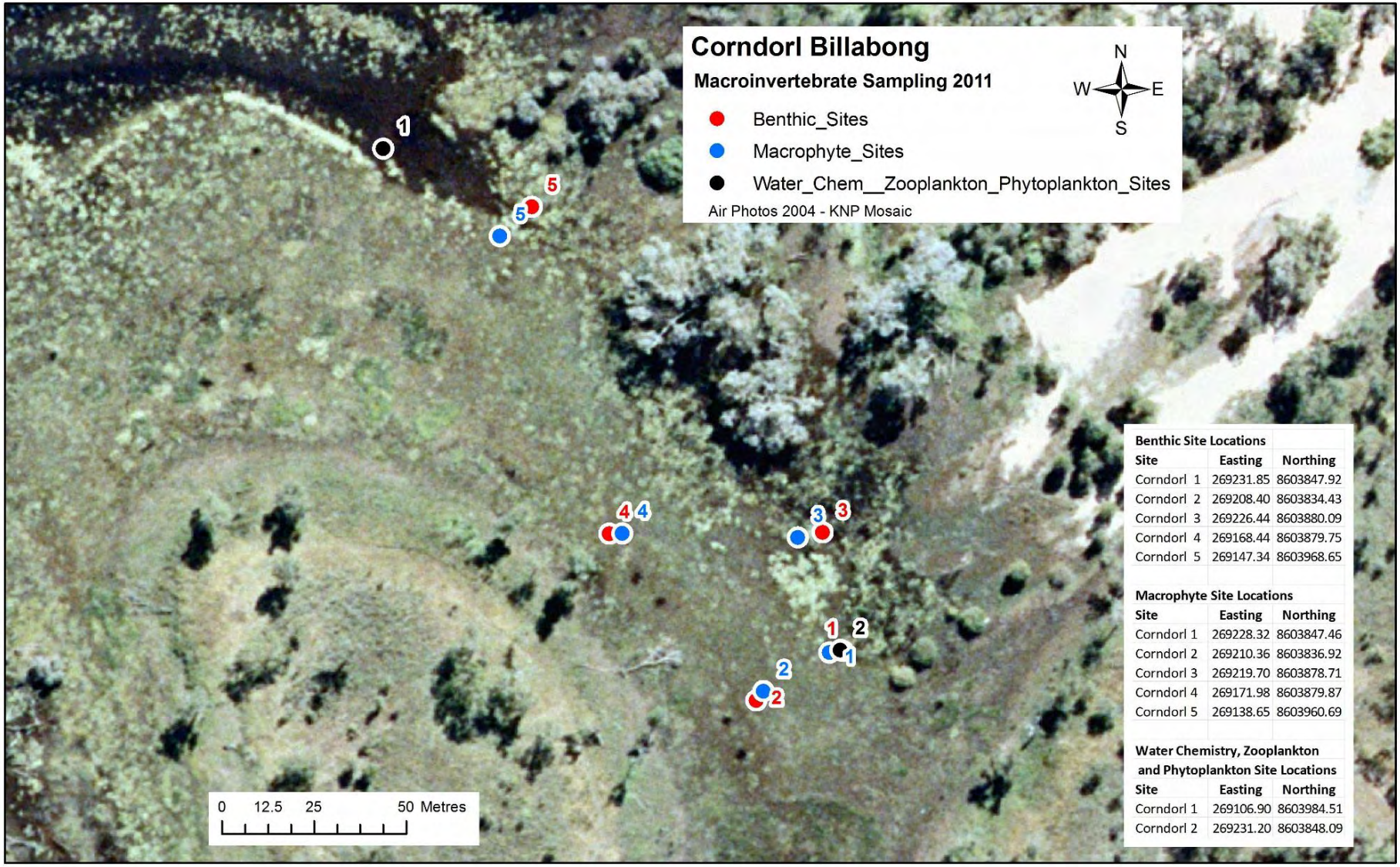
Macrophyte Site Locations

Site	Easting	Northing
Buba 1	254983.52	8578543.93
Buba 2	255018.22	8578640.34
Buba 3	255132.45	8578760.96
Buba 4	255122.12	8578680.08
Buba 5	254949.06	8578521.48

Water Chemistry, Zooplankton and Phytoplankton Site Locations

Site	Easting	Northing
Buba 1	255055.35	8578547.42
Buba 2	255058.43	8578518.99

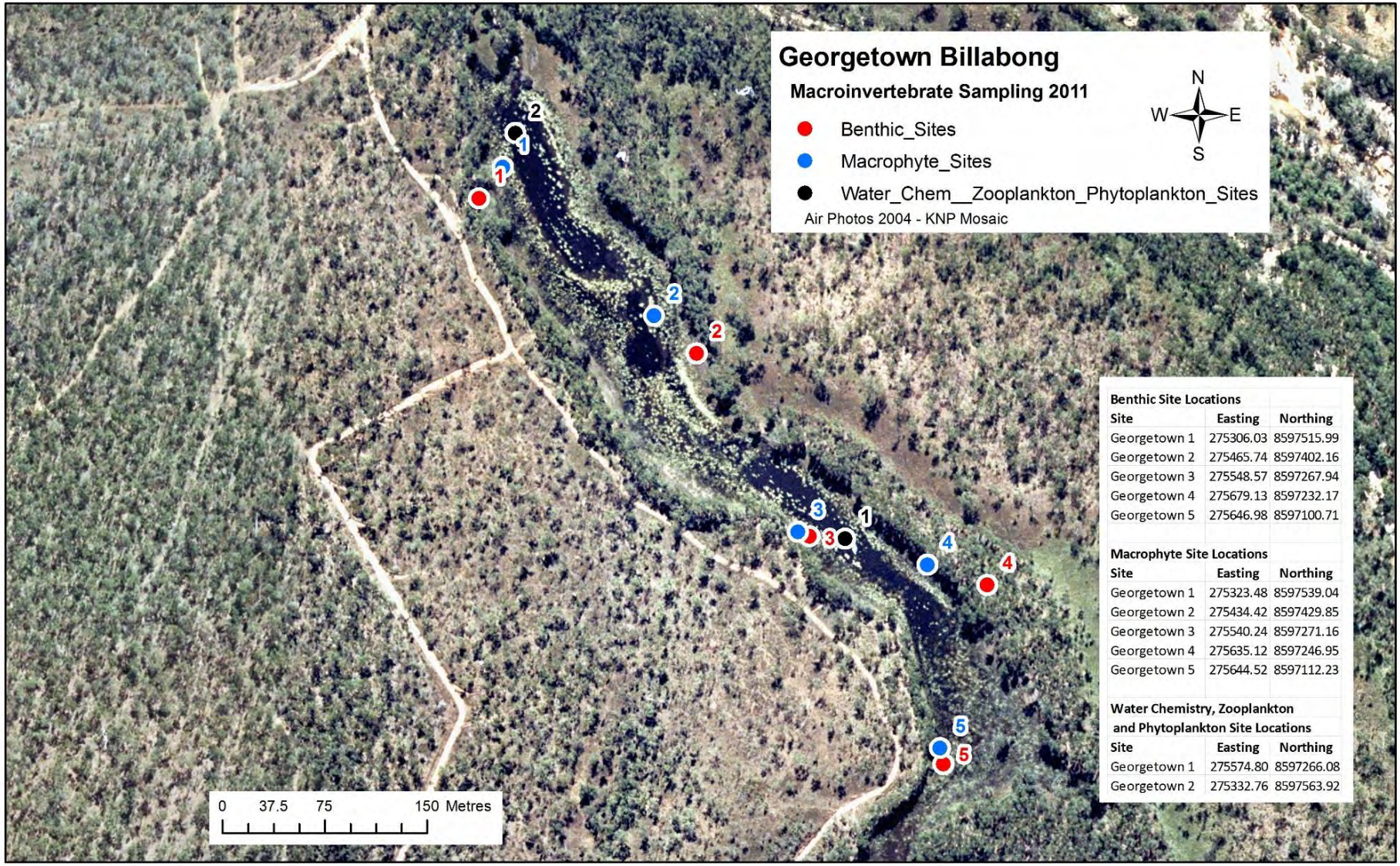

Coonjimba Billabong
Macroinvertebrate Sampling 2011
Benthic_Sites
Macrophyte_Sites
Water_Chem__Zooplankton_Phytoplankton_Sites
Air Photos 2004 - KNP Mosaic
N
W
E
S
0 25 50 100 Metres
Benthic Site Locations
Site Easting Northing
Coonjimba 1 272419.16 8599401.05
Coonjimba 2 272567.90 8599570.03
Coonjimba 3 272536.02 8599533.41
Coonjimba 4 272500.67 8599500.86
Coonjimba 5 272460.36 8599468.34
Macrophyte Site Locations
Site Easting Northing
Coonjimba 1 272449.71 8599436.02
Coonjimba 2 272375.99 8599290.37
Coonjimba 3 272452.34 8599341.47
Coonjimba 4 272504.20 8599459.53
Coonjimba 5 272584.26 8599538.44
Water Chemistry, Zooplankton and Phytoplankton Site Locations
Site Easting Northing
Coonjimba 1 272531.30 8599509.25
Coonjimba 2 272419.57 8599330.71



Benthic Site Locations		
Site	Easting	Northing
Corndorl 1	269231.85	8603847.92
Corndorl 2	269208.40	8603834.43
Corndorl 3	269226.44	8603880.09
Corndorl 4	269168.44	8603879.75
Corndorl 5	269147.34	8603968.65

Macrophyte Site Locations		
Site	Easting	Northing
Corndorl 1	269228.32	8603847.46
Corndorl 2	269210.36	8603836.92
Corndorl 3	269219.70	8603878.71
Corndorl 4	269171.98	8603879.87
Corndorl 5	269138.65	8603960.69

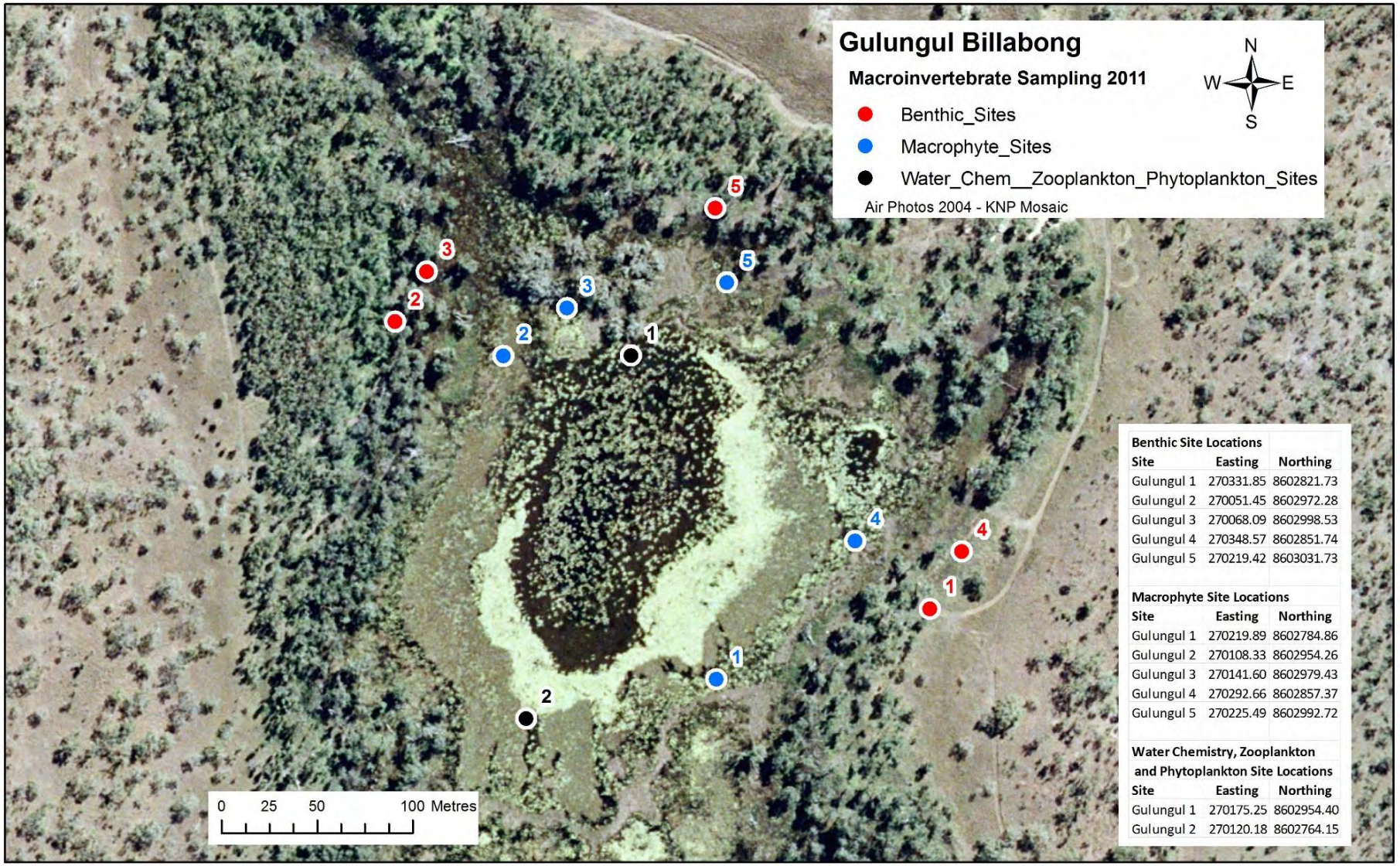
Water Chemistry, Zooplankton and Phytoplankton Site Locations		
Site	Easting	Northing
Corndorl 1	269106.90	8603984.51
Corndorl 2	269231.20	8603848.09



Benthic Site Locations		
Site	Easting	Northing
Georgetown 1	275306.03	8597515.99
Georgetown 2	275465.74	8597402.16
Georgetown 3	275548.57	8597267.94
Georgetown 4	275679.13	8597232.17
Georgetown 5	275646.98	8597100.71

Macrophyte Site Locations		
Site	Easting	Northing
Georgetown 1	275323.48	8597539.04
Georgetown 2	275434.42	8597429.85
Georgetown 3	275540.24	8597271.16
Georgetown 4	275635.12	8597246.95
Georgetown 5	275644.52	8597112.23

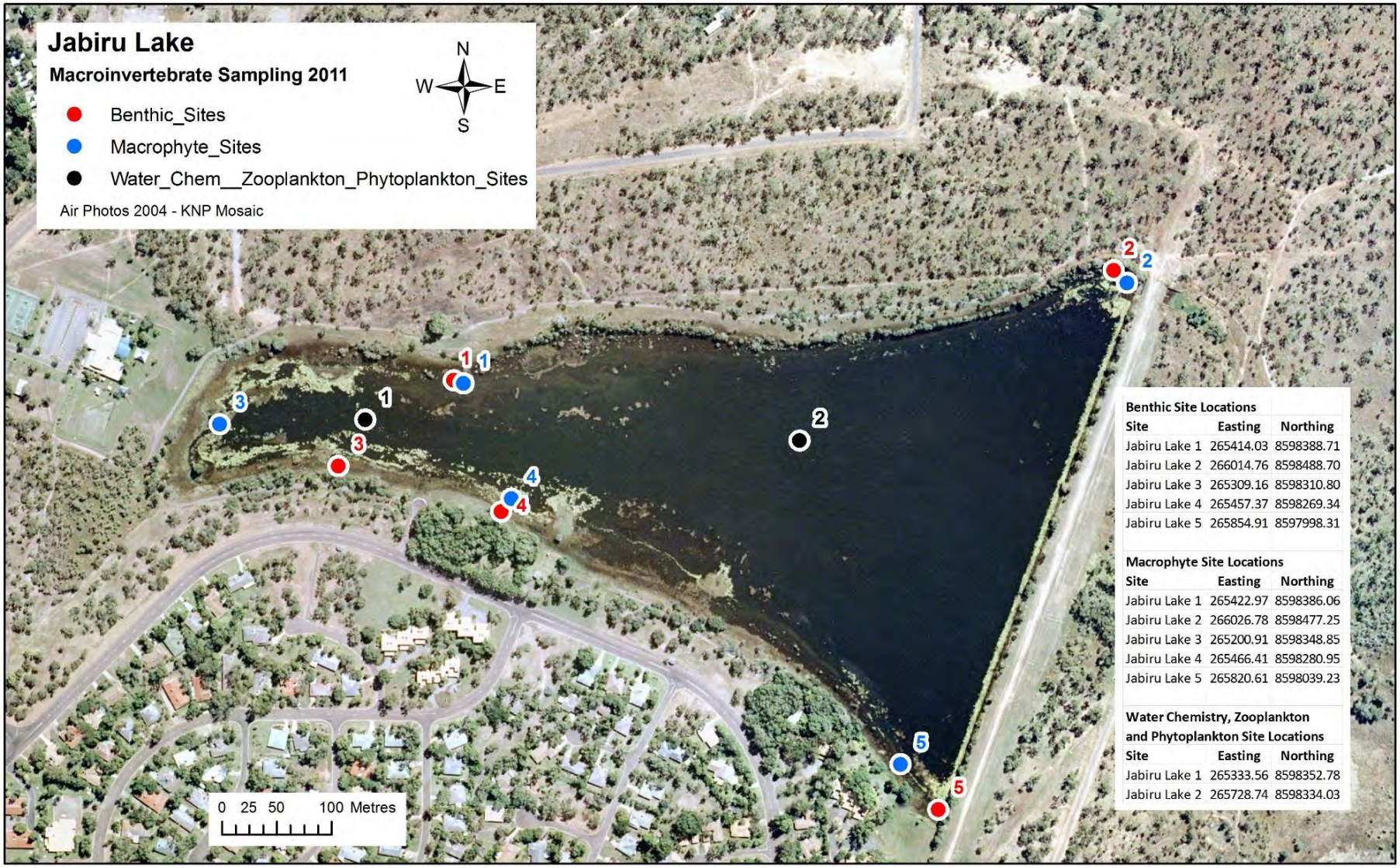
Water Chemistry, Zooplankton and Phytoplankton Site Locations		
Site	Easting	Northing
Georgetown 1	275574.80	8597266.08
Georgetown 2	275332.76	8597563.92



Benthic Site Locations		
Site	Easting	Northing
Gulungul 1	270331.85	8602821.73
Gulungul 2	270051.45	8602972.28
Gulungul 3	270068.09	8602998.53
Gulungul 4	270348.57	8602851.74
Gulungul 5	270219.42	8603031.73

Macrophyte Site Locations		
Site	Easting	Northing
Gulungul 1	270219.89	8602784.86
Gulungul 2	270108.33	8602954.26
Gulungul 3	270141.60	8602979.43
Gulungul 4	270292.66	8602857.37
Gulungul 5	270225.49	8602992.72

Water Chemistry, Zooplankton and Phytoplankton Site Locations		
Site	Easting	Northing
Gulungul 1	270175.25	8602954.40
Gulungul 2	270120.18	8602764.15



Benthic Site Locations

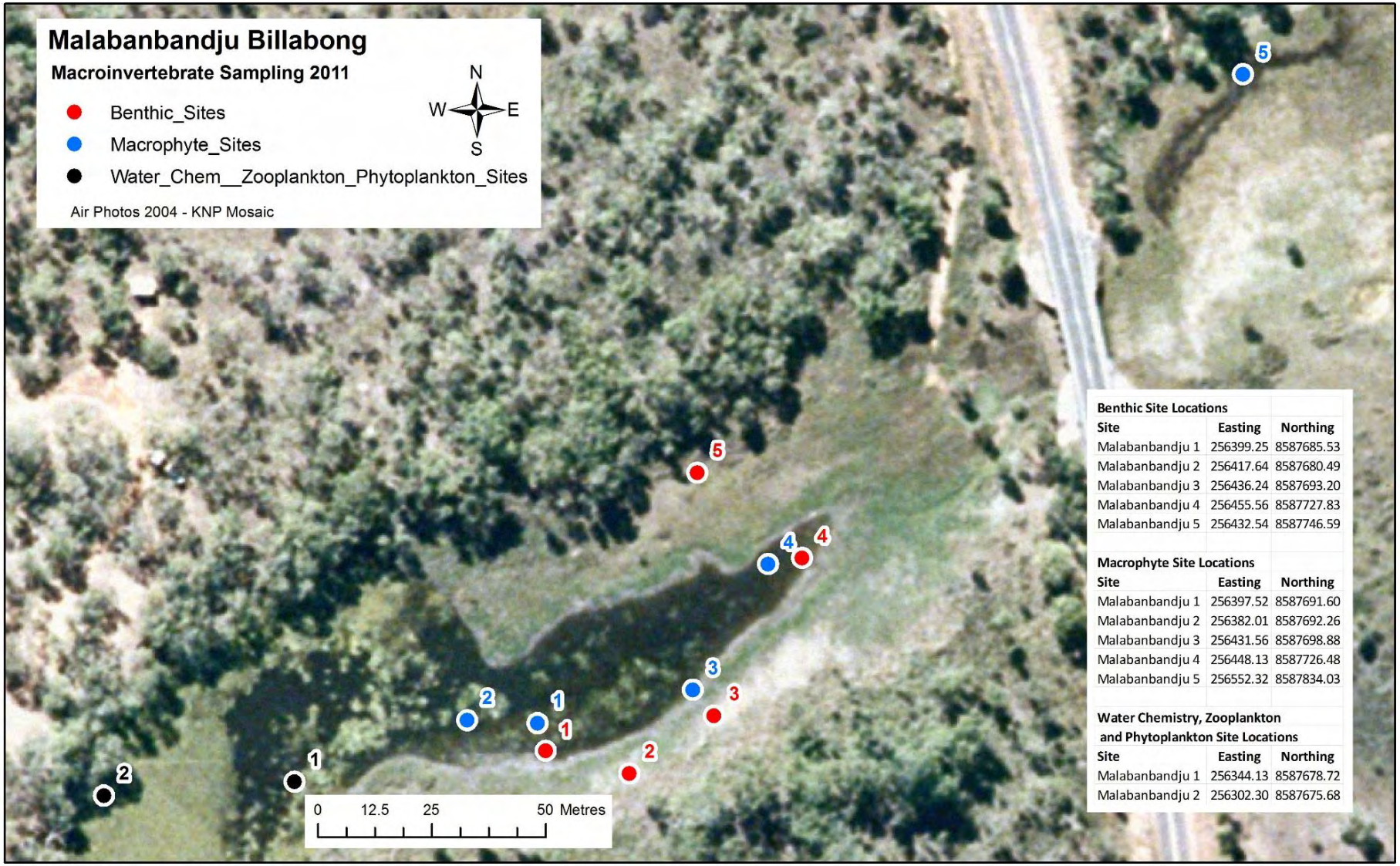
Site	Easting	Northing
Jabiru Lake 1	265414.03	8598388.71
Jabiru Lake 2	266014.76	8598488.70
Jabiru Lake 3	265309.16	8598310.80
Jabiru Lake 4	265457.37	8598269.34
Jabiru Lake 5	265854.91	8597998.31

Macrophyte Site Locations

Site	Easting	Northing
Jabiru Lake 1	265422.97	8598386.06
Jabiru Lake 2	266026.78	8598477.25
Jabiru Lake 3	265200.91	8598348.85
Jabiru Lake 4	265466.41	8598280.95
Jabiru Lake 5	265820.61	8598039.23

Water Chemistry, Zooplankton and Phytoplankton Site Locations

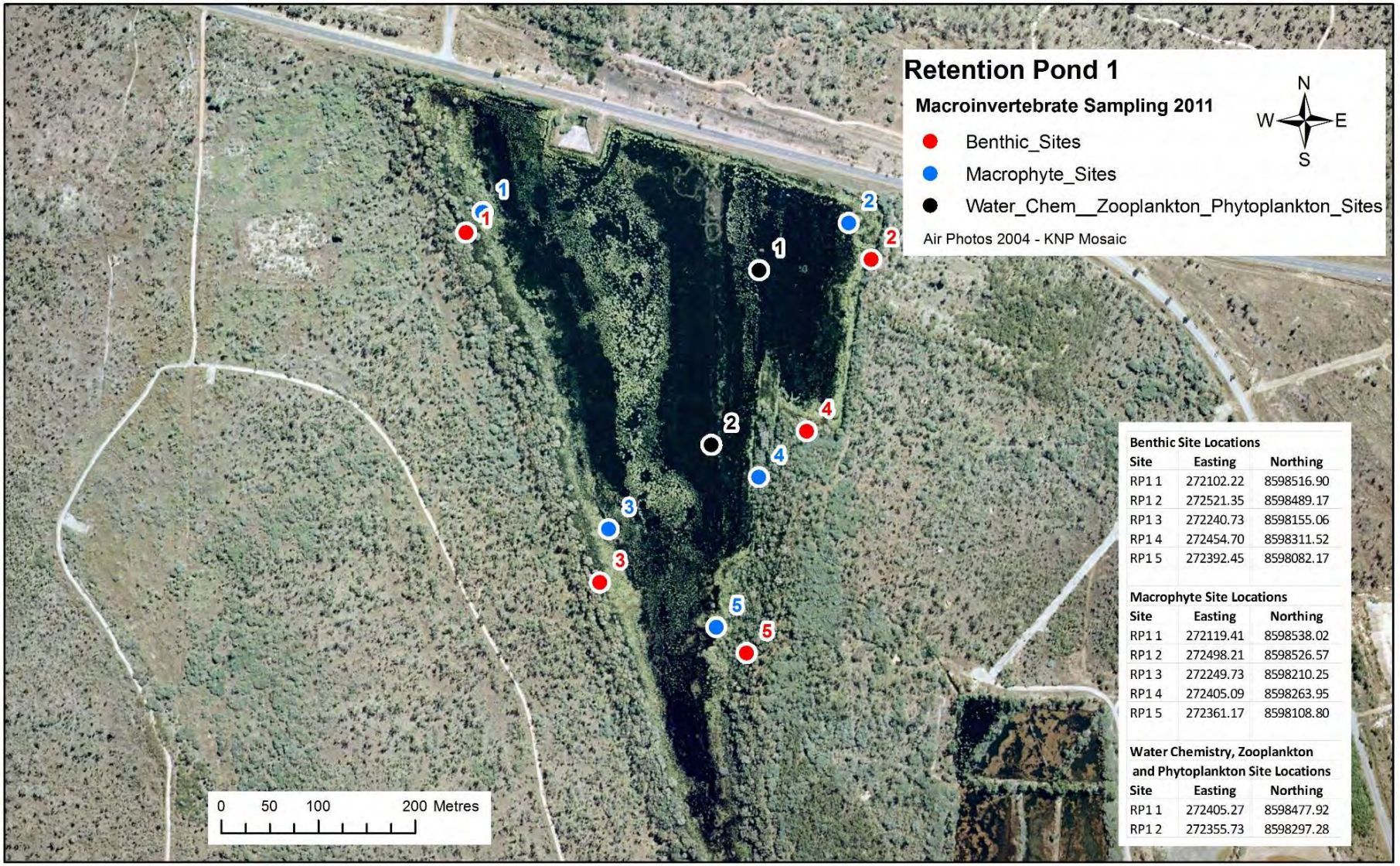
Site	Easting	Northing
Jabiru Lake 1	265333.56	8598352.78
Jabiru Lake 2	265728.74	8598334.03



Benthic Site Locations		
Site	Easting	Northing
Malabanbandju 1	256399.25	8587685.53
Malabanbandju 2	256417.64	8587680.49
Malabanbandju 3	256436.24	8587693.20
Malabanbandju 4	256455.56	8587727.83
Malabanbandju 5	256432.54	8587746.59

Macrophyte Site Locations		
Site	Easting	Northing
Malabanbandju 1	256397.52	8587691.60
Malabanbandju 2	256382.01	8587692.26
Malabanbandju 3	256431.56	8587698.88
Malabanbandju 4	256448.13	8587726.48
Malabanbandju 5	256552.32	8587834.03

Water Chemistry, Zooplankton and Phytoplankton Site Locations		
Site	Easting	Northing
Malabanbandju 1	256344.13	8587678.72
Malabanbandju 2	256302.30	8587675.68



Benthic Site Locations

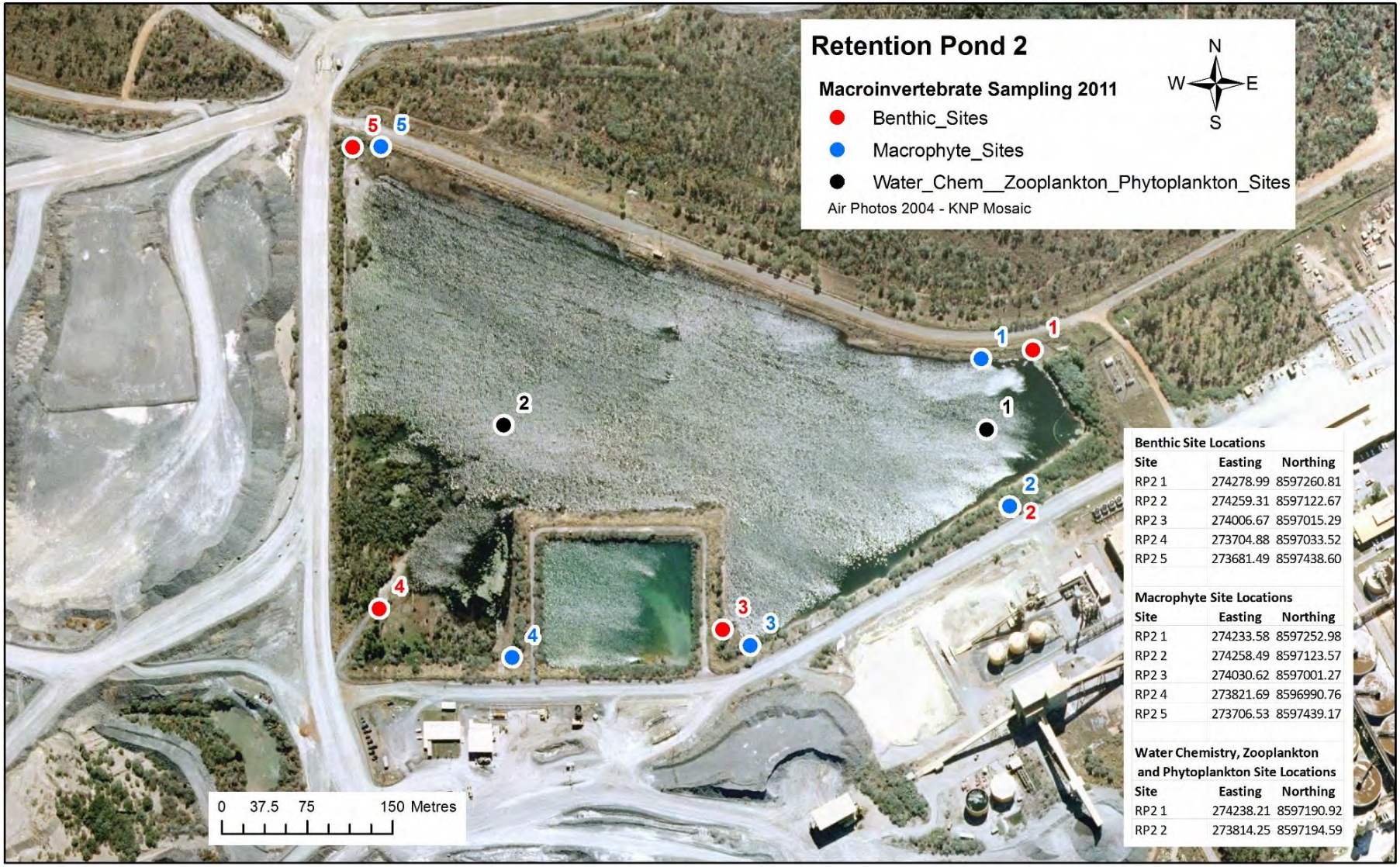
Site	Easting	Northing
RP1 1	272102.22	8598516.90
RP1 2	272521.35	8598489.17
RP1 3	272240.73	8598155.06
RP1 4	272454.70	8598311.52
RP1 5	272392.45	8598082.17

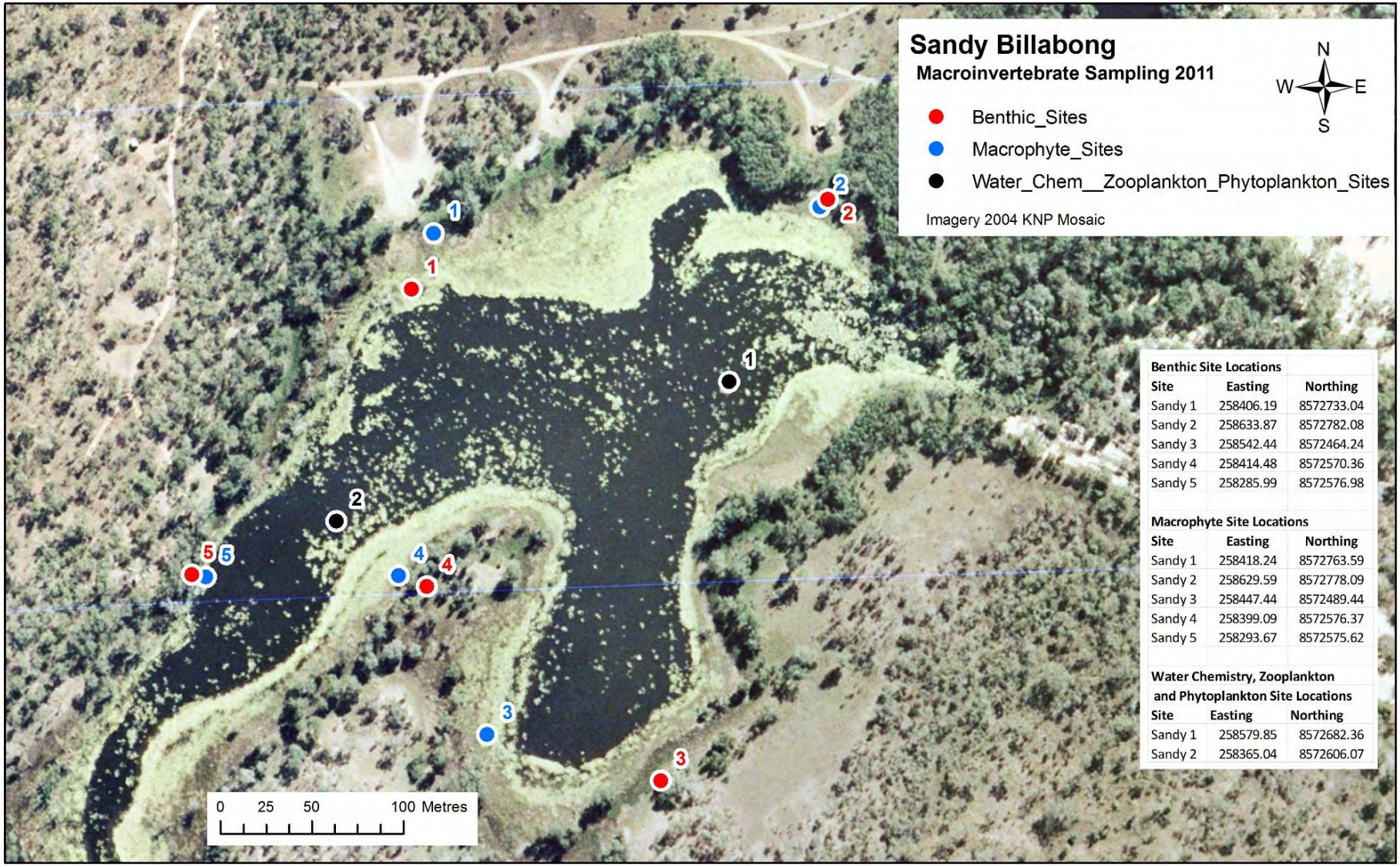
Macrophyte Site Locations

Site	Easting	Northing
RP1 1	272119.41	8598538.02
RP1 2	272498.21	8598526.57
RP1 3	272249.73	8598210.25
RP1 4	272405.09	8598263.95
RP1 5	272361.17	8598108.80

Water Chemistry, Zooplankton and Phytoplankton Site Locations

Site	Easting	Northing
RP1 1	272405.27	8598477.92
RP1 2	272355.73	8598297.28

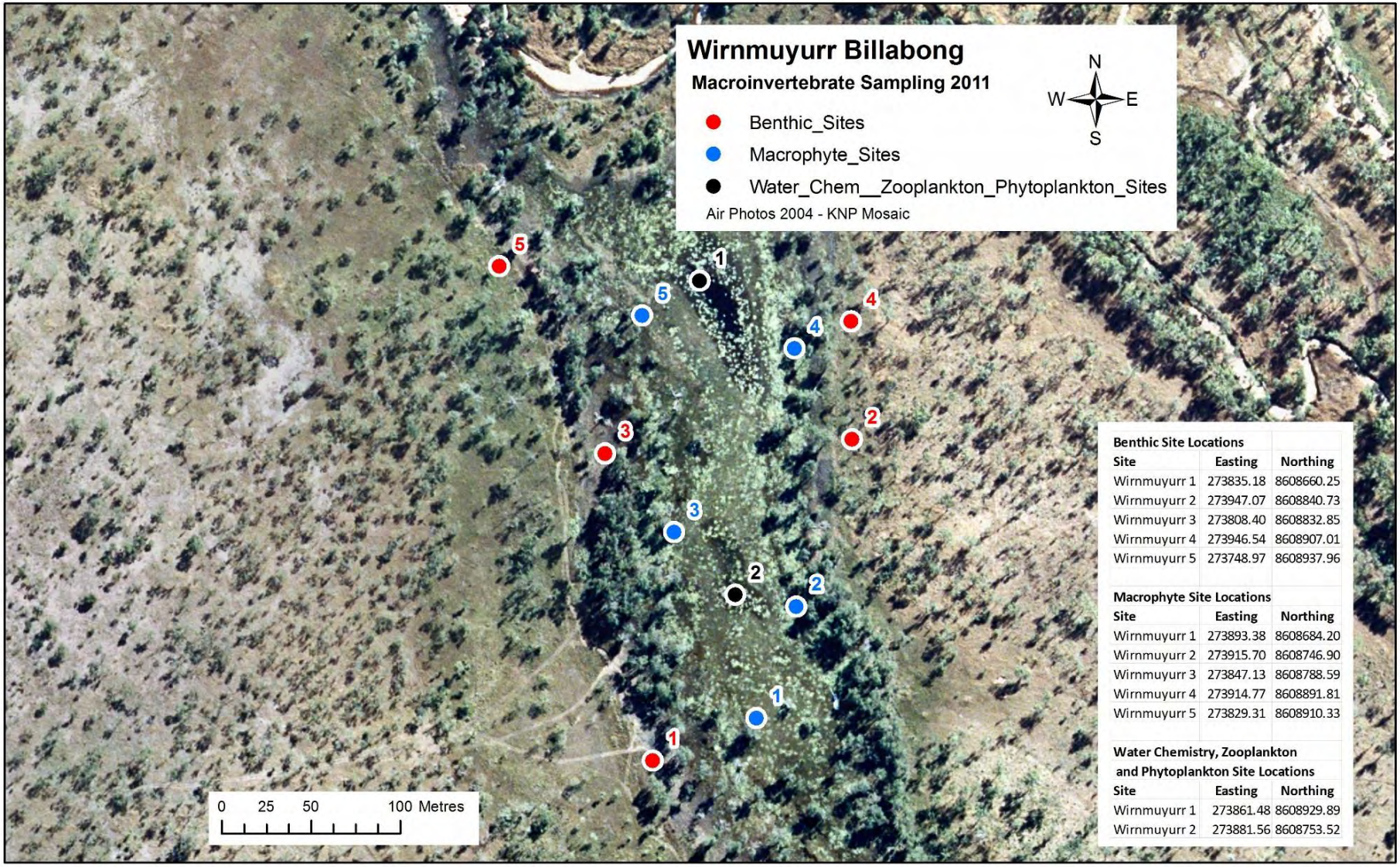

Retention Pond 2
Macroinvertebrate Sampling 2011
Benthic_Sites
Macrophyte_Sites
Water_Chem__Zooplankton_Phytoplankton_Sites
Air Photos 2004 - KNP Mosaic
N
W
E
S
0 37.5 75 150 Metres
Benthic Site Locations
Site Easting Northing
RP2 1 274278.99 8597260.81
RP2 2 274259.31 8597122.67
RP2 3 274006.67 8597015.29
RP2 4 273704.88 8597033.52
RP2 5 273681.49 8597438.60
Macrophyte Site Locations
Site Easting Northing
RP2 1 274233.58 8597252.98
RP2 2 274258.49 8597123.57
RP2 3 274030.62 8597001.27
RP2 4 273821.69 8596990.76
RP2 5 273706.53 8597439.17
Water Chemistry, Zooplankton and Phytoplankton Site Locations
Site Easting Northing
RP2 1 274238.21 8597190.92
RP2 2 273814.25 8597194.59



Benthic Site Locations		
Site	Easting	Northing
Sandy 1	258406.19	8572733.04
Sandy 2	258633.87	8572782.08
Sandy 3	258542.44	8572464.24
Sandy 4	258414.48	8572570.36
Sandy 5	258285.99	8572576.98

Macrophyte Site Locations		
Site	Easting	Northing
Sandy 1	258418.24	8572763.59
Sandy 2	258629.59	8572778.09
Sandy 3	258447.44	8572489.44
Sandy 4	258399.09	8572576.37
Sandy 5	258293.67	8572575.62

Water Chemistry, Zooplankton and Phytoplankton Site Locations		
Site	Easting	Northing
Sandy 1	258579.85	8572682.36
Sandy 2	258365.04	8572606.07



Benthic Site Locations		
Site	Easting	Northing
Wirnmuyurr 1	273835.18	8608660.25
Wirnmuyurr 2	273947.07	8608840.73
Wirnmuyurr 3	273808.40	8608832.85
Wirnmuyurr 4	273946.54	8608907.01
Wirnmuyurr 5	273748.97	8608937.96

Macrophyte Site Locations		
Site	Easting	Northing
Wirnmuyurr 1	273893.38	8608684.20
Wirnmuyurr 2	273915.70	8608746.90
Wirnmuyurr 3	273847.13	8608788.59
Wirnmuyurr 4	273914.77	8608891.81
Wirnmuyurr 5	273829.31	8608910.33

Water Chemistry, Zooplankton and Phytoplankton Site Locations		
Site	Easting	Northing
Wirnmuyurr 1	273861.48	8608929.89
Wirnmuyurr 2	273881.56	8608753.52

Appendix 9 Site photos

1979 Dry Season (Photo: R. Marchant)

1980 Wet Season (Photo: R. Marchant)

2009 Dry season -August (Photo: D. Buckle)

2009 April (Photo: D. Buckle)

Plate 1 Georgetown Billabong – showing changes over time

Angbangbang Billabong – June 2011

Baralil Billabong – June 2011

Buba Billabong – May 2006 (Photo: J Hanley)

Coonjimba Billabong – June 2011

Plate 2 Angbangbang, Baralil Buba and Coonjimba billabongs. Dates indicated

Corndorl Billabong – May 2006 (Photo: J. Hanley)

Gulungul Billabong – June 2011

Jabiru Lake – June 2013

Malabanbandju Billabong – June 2011

Plate 3 Corndorl and Gulungul billabongs, Jabiru lake and Malabanbandju Billabong. Dates indicated

Retention Pond 1 – July 2010 (Photo: K. Tayler)

Retention Pond 2 – June 2011

Sandy Shallow Billabong – June 2011

Wirnmuyurr Billabong – June 2013

Plate 4 Retention Ponds 1 and 2, Sandy Shallow and Wirnmuyurr billabongs. Dates indicated