Australian Government

Department of Sustainability, Environment,
Water, Population and Communities
Supervising Scientist

internal report 589

Environmental monitoring protocols to assess potential impacts from Ranger minesite on aquatic ecosystems: Fish community structure in shallow lowland billabongs

Supervising Scientist Division

June 2011

(Release status – Unrestricted)

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Supervising Scientist Division

Supervising Scientist Division
GPO Box 461, Darwin NT 0801

June 2011

Registry Files: SG2008/0243, SG2003/0015, SG2003/0016, SG2003/0017, SG2003/0018, SG2003/0019, SG2003/0020, SG2004/0152, SG2008/0241, JR-05-170, SG2001/0187

Project Number: MON-1992-001

Australian Government

Department of Sustainability, Environment, Water, Population and Communities
Supervising Scientist

How to cite this report:

Supervising Scientist Division 2011. Environmental monitoring protocols to assess potential impacts from Ranger minesite on aquatic ecosystems: Fish community structure in shallow lowland billabongs. Internal Report 589, June, Supervising Scientist, Darwin.

Location of final PDF file in SSIMS (SSDX Sharepoint)

Supervising Scientist Division > PublicationWork > Publications and Productions > Internal Reports (IRs) > Nos 500 to 599 > IR589 Protocols - shallow billabong fish

Location of all key data files for this report in SSDX Sharepoint:

Supervising Scientist Division > SSDX > Environmental Impact of Mining - Monitoring and Assessment > Fish > Ranger > Shallow Billabongs > Data (popnet data Access version 2000)

Authors of this report:

Supervising Scientist Division – GPO Box 461, Darwin NT 0801, Australia

Supervising Scientist
Department of Sustainability, Environment, Water, Population and Communities
GPO Box 461, Darwin NT 0801 Australia

e-mail: publications_ssd@environment.gov.au

Internet: www.environment.gov.au/ssd (www.environment.gov.au/ssd/publications)

Printed and bound in Darwin NT by Supervising Scientist Division

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Executive summary

The Supervising Scientist Division (SSD) operates an integrated chemical (including radiological), physical and biological monitoring program to ensure protection of the aquatic ecosystems of the ARR from the operation of uranium mines in the region. This stream monitoring program is an independent assurance program, unlike the compliance and check water chemistry monitoring programs of the mining company (Ranger Mine, Energy Resources of Australia Ltd) and the NT government regulator respectively (DoR, Department of Resources).

The techniques and 'indicators' used in the monitoring program satisfy two important needs of environmental protection: (i) the early detection of significant changes in measured indicators to avoid short or longer term ecologically important impacts; and (ii) assessing ecological or ecosystem-level effects by way of measured changes to surrogate indicators of biodiversity.

SSD has prepared protocols for the measurement programs required to implement each of these monitoring techniques. For each technique, two types of protocols have been prepared, high-level protocols and detailed operational manuals. This document is the high-level protocol, describing the science underpinning one of the ecosystem-level techniques, namely use fish community structure in shallow lowland billabong monitoring.

This protocol for the structure of fish community monitoring technique provides an overview of the monitoring principles and objectives, experimental and statistical design, test, data analysis and impact assessment procedures and reporting requirements.

Preamble

This document details the experimental design and data interpretation methods used to monitor fish community structure in shallow backflow billabongs around the Ranger Mine. The monitoring of fish in these billabong environments is a component of the multiple lines of evidence monitoring program implemented by the Supervising Scientist Division (Van Dam et al 2002).

Full details of the operational methods and procedures described in this protocol are contained in the companion 'Operational manual' which is the working document used by staff running the monitoring activity. The additional material provided in the operational manual includes:

- Photographs and maps of the location of sites and sample transects for current and historical sampling sites;
- Fish identification photographs and summary information from key references and supporting studies;
- Instructions on use of meters and other instrumentation;
- Data-sheet pro-forma for recording of field data;
- Data codes for fish and environmental variables;
- Worked examples of statistical procedures;
- Examples of all required reports.

Acknowledgments

The following ***eriss*** personnel have been involved in the development of this protocol since it was first conceived in 1992: Ben Bayliss, James Boyden, Ian Brown, Duncan Buckle, Chris Humphrey, Robert Luxon, Bob Pidgeon and Bruce Ryan.

Many volunteers have assisted with the fieldwork as data recorders, crocodile spotters and general field hands over this time. Volunteers working with Conservation Volunteers Australia provided much of this assistance, particularly in the early years of development of the methodology.

Traditional owners of the country containing the sampling sites (Gagadju and Mirrar) have generously allowed access to the sites and assisted in the fieldwork on many occasions. In recent years they have constituted the majority of the field team.

Advice and assistance with site access and information about local site conditions from Parks Operation and Tourism Branch and Energy Resources of Australia (ERA), operators of the Ranger uranium mine, are gratefully acknowledged. Dr Keith McGuinness, Charles Darwin University, provided most of the advice for development of the statistical models, as well as review, of the statistical robustness and power of the impact detection methods used in this protocol. Dr David Jones of ***eriss*** provided critical comments and valuable suggestions upon a draft of this report.

Contact officers

Dr Chris Humphrey
Environmental Research Institute of the
Supervising Scientist, PO Box 461, Darwin
NT 0801
08 89201160
Chris.Humphrey@environment.gov.au

Duncan Buckle
Environmental Research Institute of the
Supervising Scientist, PO Box 461, Darwin
NT 0801
08 89201393
Duncan.Buckle@environment.gov.au

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Environmental monitoring protocols to assess potential impacts from Ranger minesite on aquatic ecosystems: Fish community structure in shallow lowland billabongs

Supervising Scientist Division

1 Introduction

1.1 Objective

The objective is the detection of any[1] effects of mining at the Ranger uranium mine[2] on fish communities inhabiting shallow billabongs adjacent to Magela Creek.

1.2 Background

The lowland reaches of most streams in the Top End of the NT are bordered by numerous small shallow wetlands, most often at the confluence of small seasonal tributaries and the main stream channel. They are formed by the development of mainstream levees that have restricted outflow from the tributary streams. They are generally termed 'billabongs' in the NT, but are called 'lagoons' in Queensland (Herbert & Peeters 1995). Because water from the mainstream typically enters these billabongs at high flows and drains out again when flow recedes, they have also been termed 'back-flow billabongs' (Davy & Conway 1974, Bishop et al 1986). Lowland billabongs are depositional basins and those downstream of the Ranger uranium mine can potentially receive and accumulate mine-derived waste substances.

In the dry season, the billabongs are important sources of food, especially turtles and geese, for traditional owners of the area. During the wet season they provide habitat for fish recruitment (Bishop & Forbes 1991). Many fish utilise these lentic conditions and their dense aquatic vegetation for reproduction and feeding. Monitoring of fish communities in these billabongs provides the potential for detecting downstream impact from the minesite and for providing assurance that environmental health is being maintained.

Two of the billabongs (Georgetown and Coonjimba – see Figure 1 for location) are located immediately downstream of Ranger uranium mine and receive inputs of solutes contained in runoff water that leaves the site. Due to their close proximity to the minesite, any mine-related changes to fish communities in the catchment would be first expected to occur in these waterbodies.

Research aimed at developing techniques for detection of long-term effects on fish communities in lowland billabongs has been conducted by ***eriss*** (formerly Alligator Rivers

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[1] Enhanced (hormetic) effects may indicate the response of fish communities to low-level contaminant concentrations in receiving waters which could provide sufficient cause to trigger management action.

[2] While any impacts arising from mining would be most evident as a consequence of changes to receiving water quality, changes to the hydrology of Coonjimba Billabong as a result of the upstream impoundment RP1 should also be considered in assessing any mine-related impacts.

Region Research Institute) since 1980. Initially, fish communities were monitored using gill and seine nets for sampling. In the late 1980s these sampling methods could no longer be used due to increases in aquatic plant density (Bishop & Walden 1988, Boyden & Pidgeon 1994, Buckle et al 2004) occurring as a result of the removal, in the 1980s, of buffalo from Stage 1 of Kakadu National Park. The composition of fish communities was also altered by the vegetation changes. The higher densities of plants and alterations to plant community structure led to the exclusion of some larger-growing fish species. Early research into fish community monitoring by Bishop et al (1990) demonstrated considerable seasonal variation in fish community structure in shallow billabongs. Bishop et al (1995) noted that the highest species richness was found during the late wet–early dry season when major dispersal movements and migrations of fish had ceased, preventing rapid changes in the billabong fish communities. As a result, sampling of fish communities in shallow billabongs is conducted at the onset of the dry season when outflow from the billabongs has ceased or declined to a level that prevents significant movement of fish and before water quality deteriorates following cessation of creek flow (Humphrey et al 1990).

This protocol describes the monitoring technique currently used for quantitative sampling of fish communities in shallow billabongs. It involves use of a pop-net procedure (Serafy et al 1988) (described in detail below, section 3.8). The procedure has proved to be cost effective and, relative to other methods available, to provide adequate representation of fish community structure in waterbodies containing dense aquatic vegetation (Serafy et al 1988, Paradis et al 2008), such as shallow billabong margins. As with all fish sampling methods, the technique has its biases, and these are reported to be under-sampling of larger-growing species (Jacobsen & Kushlan 1987). The monitoring program commenced in 1994 with the sampling of ten shallow billabongs, including four directly-'exposed' billabongs (Georgetown, Coonjimba, Gulungul and Djalkmara) (Boyden & Pidgeon 1994, Pigeon et al 2000). This was reduced to nine billabongs after 1996 when Djalkmara was isolated from Magela Creek at the onset of mining of Ranger Pit 2 (Pidgeon et al 2000). In 2006, the monitoring design was further refined to include just six billabongs comprising three control-impact sitepairs, with sampling conducted once every two years (Buckle 2010, Humphrey & Buckle 2008).

1.3 Principle of the monitoring technique

This sampling procedure is designed to detect effects of water quality, integrated over a wet season, on fish communities in shallow billabongs downstream of the Ranger Mine. Sampling is conducted biennially (ie once every two years), during the recessional flow period (ie early dry season). It measures the abundance of different fish species in the shallow margins of six shallow billabongs.

The sampling method involves the need for personnel to enter the water to operate the traps. The presence of crocodiles has required the introduction of stringent safety procedures to minimise risk to operators, including use of exclosure barriers which, by necessity, have influenced aspects of the experimental design.

Three of the billabongs are located in Magela Creek downstream of the minesite (exposed sites, E) and three are located on catchments unaffected by mining activity (control sites, C); the locations of these billabongs are shown on Figure 1. Pop-net traps are used to capture fish from dense vegetation in 10 quadrats, paired within five crocodile exclosure areas, in each billabong. Trained fish observers identify and count the numbers of each fish species captured. Fish are released alive after counting. Sampling of the ten quadrat (replicate) samples ensures adequate representation of the array of species present (see section 3.8). Habitat data on water depth and vegetation biomass and composition are recorded for each

quadrat. Water physico-chemistry is measured at each crocodile exclosure area and a single water sample for measurement of chemical analytes is collected from each billabong.

The structure of fish communities – species and relative numbers – is compared between billabongs potentially exposed to contaminants from mine waters and control billabongs in different catchments, and with community structure values obtained in previous years.

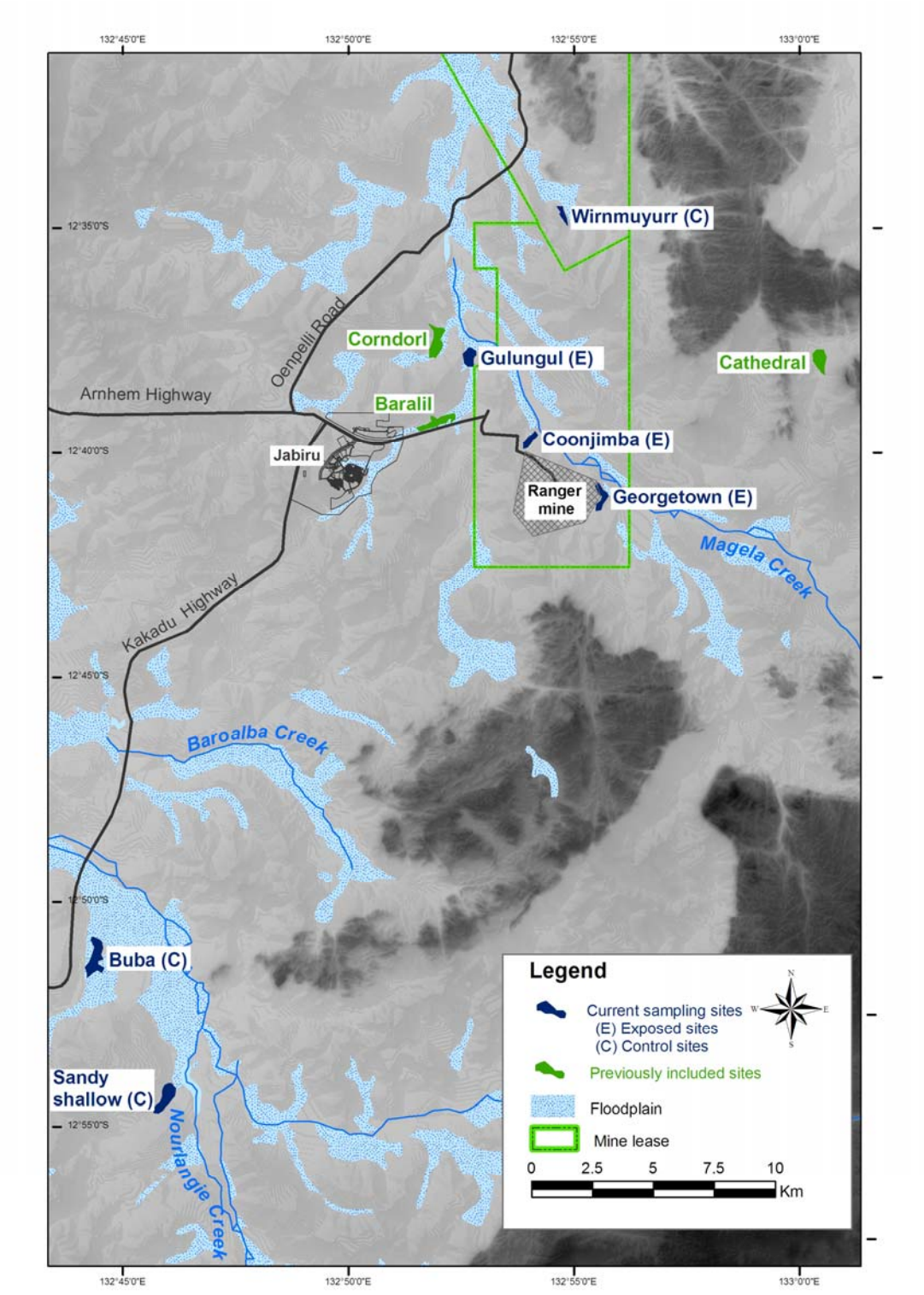


Figure 1 Location of shallow billabong fish community monitoring sites (E – exposed; C – control)

2 Experimental design

2.1 Statistical design

This monitoring technique uses three control-impact sitepairs that form a multiple Before-After-Control-Impact-Paired differences (BACIP) design which in its simple (single-pair) form was originally described by Stewart-Oaten et al (1986, 1992). The BACIP design uses a form of temporal replication. The *difference* between sampled responses at the *paired* sites (Control-minus-impact) at any one time is regarded as a replicate observation (Stewart-Oaten et al 1986, 1992). Where community data are derived, the mainstay of the present procedure, multivariate *dissimilarity* values are used as the measure of difference between the sites at each time of sampling (Faith et al 1991, 1995). These values reduce the differences between the two communities for many different species to a single value. A value of 0 indicates fish communities identical in structure, while a value of 100 (percent) indicates totally dissimilar communities, sharing no common taxa.

The dissimilarity data from a 'baseline' time series collected before an 'event' are compared with data obtained after suspected contamination by mine discharge or some other event or over a particular period of interest. The comparison of fish community data between operational and rehabilitation phases of mining, for example, may represent before versus after periods, respectively, of interest.

Fish community structure data are collected at the same time of year from 10 quadrats from within each of six billabongs. The 10 quadrats are divided into five pairs, each pair situated within crocodile exclusion areas (exclosure area). The count data from each of the pairs are pooled (average taken) to best represent the sampled area (see section 5.1.3 for details). The billabongs include three impact or exposed site billabongs on Magela Creek, directly exposed to contaminants from the Ranger uranium mine and three control sites on different stream catchments, two located in Nourlangie Creek and the other in a small tributary to the Magela floodplain (see Figure 1 for locations). Figure 2 illustrates the experimental design used for each site pair.

Replicate observations in the model are the Bray-Curtis dissimilarity values derived for each of the randomly-paired, exposed-control exclusion areas; the replicate observations provide an estimate of error (or residual) variation. For the community data collected for each billabong sitepair, five dissimilarity values are derived, each representing one of the five possible randomly-paired exposed and control site exclosure areas. Mean dissimilarity values are calculated for each sitepair, from before and after (BA) an event or period of interest. The BA values for the three sitepairs are statistically tested (compared) using a three factor ANOVA model.

The model for the ANOVA is:

Model: BA + Years(BA) + Sitepair + BA*Sitepair + Year*Sitepair

In this model, BA is a fixed factor (or effect), testing for differences between 'before' and 'after' periods. Sitepair is also a fixed factor, testing for differences amongst the sitepairs. However, Year(BA) is a *random* factor, nested in the BA factor because different years are sampled before and after the event (or between the periods of interest).

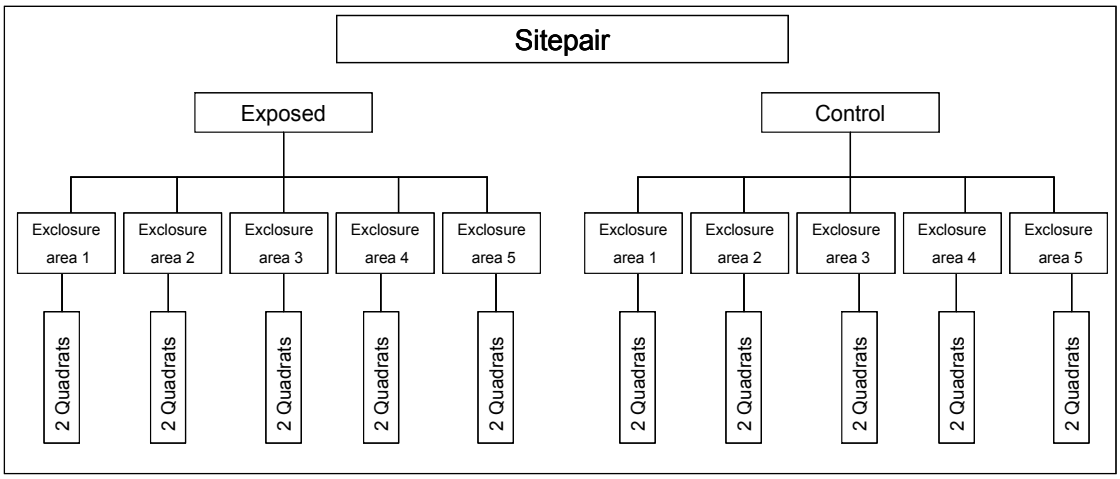


Figure 2 Shallow billabong fish community monitoring design for one of three control-impact sitepairs

Some comment is required on the choice of Years(BA) as a *random* factor since there is debate in the literature about whether 'time' in ANOVA designs should be assigned as 'fixed' or 'random' (see Quinn & Keough 2002). Although the years are a sequential (and not random) sample, the primary aim of this analysis is to answer the question: Does the magnitude of any change from before to after exceed that expected, given the natural variation from year to year? Treating Year(BA) as a random factor, addresses this question because years are not selected on the basis of particular characteristics they possess that may influence the response. While the assignment of time as a random factor would likely lead to a more conservative (less powerful) test (compared to fixed-factor designation), it is also worth noting that the BA test performed in this situation is comparable to the Student *t*-test method recommended by Stewart-Oaten et al (1986), authors of the original BACIP analysis method, and subsequently adopted routinely in numerous subsequent studies, where 'Seasons' (or time) are also treated as random.

Community summaries such as species richness and abundances are not used by the current implementation of this method. However, they could be included as response measures, using corresponding (univariate) difference values between the randomly-paired, exposed-control exclosure areas as input data to the ANOVA model.

The ANOVA table is described in Table 1.

Table 1 ANOVA table used for monitoring fish communities in shallow billabongs around Ranger Mine. BA and Sitepair are fixed factors, years(BA) is regarded as a random factor. (*b* = years before; a=years after; *sp* = number of sitepairs; *n* = number of replicates).

Source	df	F
BA	1	MS_{BA}/ $MS_{Years(BA)}$
Years(BA)	(*b*-1)+(*a*-1)	$MS_{Years(BA)}$/ $MS_{Year*Sitepair}$
Sitepair	(*sp*-1)	$MS_{sitepair}$/ $MS_{Year*Sitepair}$
BA*Sitepair	(*sp*-1)	$MS_{BA*Sitepair}$/ $MS_{Year*Sitepair}$
Year*Sitepair	*(sp-1)* ((*b*-1)+(*a*-1))	$MS_{Year*Sitepair}$/MS_{Error}
Error	*sp* (*b*+*a*)(*n*-1)	
Total	*spn*(*b*+*a*)-1	

Where significant differences are found to occur between a time-series of sitepair dissimilarity or difference values, further investigation is required to assess whether or not the change is associated with inputs from the mine. Water quality variables measured over the duration of the wet season in Georgetown and Coonjimba Billabongs, and in Ranger Retention Pond 1 upstream of Coonjimba (ERA water chemistry monitoring program) and at the time of fish sampling, are used to assist in determining the potential influence of mine waste-water inputs upon fish communities. Habitat variables measured at the time of fish sampling, together with wet season hydrological variables, are used as possible covariates to identify natural causes of any change in fish communities, thereby reducing the risk of Type I statistical error. Where natural covariates are identified as directly contributing to trends or change in fish communities over time, there is potential in future to incorporate these into the ANOVA model using ANCOVA analysis.

PERMANOVA (PERmutational Multivariate ANalysis Of Variance) (Anderson 2001, McArdle & Anderson 2001, Anderson et al 2008), an add-on function of PRIMER software (Clarke and Gorley 2001, Clarke and Gorley 2006), is an analysis method that has become available since the inception of the original BACIP design. PERMANOVA can use any distance measure appropriate to the data (including Bray-Curtis), and uses permutations to perform hypothesis tests which are largely, but not entirely free of distribution type. As such, and by adopting an approach to partitioning of variation like that employed in ANOVA, it can perform analyses of multivariate (or univariate) data in the same manner as the more complex experimental designs and models associated with BACIP and ANOVA that are only applicable to univariate data and that are used in the current protocol.

Using the current design configuration, PERMANOVA is employed: 1) to better interpret the Multi-Dimensional Scaling (MDS) ordination graphic (section 6.2.1) and 2) as a comparative analysis to the ANOVA design (see Appendix 1).

2.2 Hypothesis testing

The monitoring technique is designed to test the primary null hypothesis that there has been no change in fish community structure at the Magela Creek 'exposed' sites, relative to control sites:

a between two time periods of interest, eg before and after a possible impact event, or

b between the current wet season and all previous wet seasons, or

c before and after mine rehabilitation etc.

Specifically, the null hypothesis of primary interest is:

1 **H_0: Mean dissimilarity before event (or period of interest) equals mean dissimilarity after event (or period of interest).**

If the test for BA effect is significant, then the null hypothesis (1) is rejected. That is, the mean dissimilarity after the event differs (is either smaller or larger) from that before the event.

Interpretation of the BA effect may be confounded if the BA*Sitepair interaction is significant. The BA*Sitepair interaction tests that the mean dissimilarity from before to after event, or period of interest, does not differ among the three sitepairs. Specifically, the null hypothesis is:

2 **H_0: The change, in mean dissimilarity, from before to after, does not differ among sitepairs.**

If the test for the BA*Sitepair interaction is significant, then the null hypothesis (2) that the change (from before to after) is the same at all sitepairs is rejected. A rejected null hypothesis indicates that one or more of the sitepairs differ amongst or between the BA periods. In this case further investigation using pairwise comparison (ie Tukey's) is required to determine the differences between the sitepairs.

The second factor in the model, Year(BA), can be used to determine whether, within the Before and After periods, any set of dissimilarity values for a year are significantly different, even if a test of the primary hypothesis shows that the mean dissimilarity values before and after the event (or period of interest) are not significantly different. The null hypothesis is:

3 **H_0: Mean dissimilarity amongst years in the before and/or after event (or period of interest) does not differ.**

If the test is significant, then the null hypothesis (3) is rejected. The mean dissimilarity amongst years differs within either the before and after period.

Interpretation of the BA effect may be confounded if the Year(BA)*Sitepair interaction is significant. The Year(BA)*Sitepair interaction tests that the mean dissimilarity amongst years within either the before and after period does not differ among the three sitepairs. Specifically, the null hypothesis is:

4 **H_0: The change, in mean dissimilarity, among years, within either the before and after period, does not differ among sitepairs.**

If the test is significant, then the null hypothesis (4) is rejected: mean dissimilarity amongst years differs amongst sitepairs within either the before and after period. Interpretation of either H_0 3 or 4 requires further analysis using pairwise comparisons to determine if significant differences occur only in the After period for all, or one, sitepair, (variability) that could indicate impact.

The third factor in the model, Sitepair, (null hypothesis 5) can be used to determine whether dissimilarity or difference values across all years are significantly different amongst sitepairs. The null hypothesis is:

5 **H_0: The mean dissimilarity, across years, does not differ among sitepairs**

If the test is significant, then the null hypothesis (5) is rejected: mean dissimilarity amongst sitepairs differs. Further testing using pairwise comparisons is used to determine which sitepair is significantly different.

Possible causes of any observed changes or trend are assessed and/or investigated further to ensure accurate inference about mining impact.

3 Sampling procedures

3.1 Occupational health and safety

eriss has established project and field safety approvals processes, guidelines and procedures that must be followed prior to and during all field work. This includes completion of a risk-based field safety analysis (FSA) of the required works. All participants are made aware of potential dangers and the procedures implemented to minimise risks by communicating and understanding the FSA before field work commences. Special arrangements are in place to facilitate communication of this information to indigenous personnel engaged as day labour.

The main health and safety risks in this project are heat stress, dehydration, back strain, mosquitoes and crocodile attack. Due to the potential for crocodile attack, entry into the water should only occur within the safety of crocodile exclusion nets (section 3.7). Details on mitigation measures for the identified health and safety risks for this work are discussed in the operational manual for this protocol.

3.2 Consultations required for site access

Sampling is conducted within Kakadu National Park (KNP) and the Ranger uranium mine lease. The Aboriginal people within KNP maintain strong cultural ties with their lands and take responsibility in its management. The following stakeholders need to be consulted, via SSD's community liaison officer, to ensure necessary protocols are followed, prior to sampling:

- Local traditional owners should be consulted directly through their community organisations for approval to access their land and to conduct the sampling. The Northern Land Council should be advised of intended activities. This should be carried out well in advance of sampling.
- Approval from Energy Resources of Australia Pty Ltd (ERA), environmental management branch, should be obtained prior to accessing, the Ranger and Jabiluka mineral leases ERA security should be notified prior to start of work in these areas.
- Parks Operation and Tourism Branch project officer and local district offices should be notified to ensure that park management activities do not conflict with sampling dates (eg feral animal control, fire management).

Work on vertebrate animals such as fish that involves their handling in any way requires approval of the Charles Darwin University Animal Ethics Committee (AEC). Hence, approval must be obtained in writing at least 3 months in advance of intended fish sampling activities (see operational manual for further details). Approval is usually granted for a two-year period. On approval, a progress report is required after the first year, then a final report after the second year.

Formal approval to use non-recreational fishing methods is required from the Northern Territory Government, Department of Resources – Fisheries Licencing. A special permit to collect fish and aquatic life should be held by the project leader and other ***eriss*** staff members regularly involved in this work. The Director of Fisheries issues permits for a one-year term.

3.3 Timing of sampling

For community-based monitoring in the ARR, sampling is conducted annually, during post wet season recessional flows, unless circumstances dictate otherwise. At this time species richness and abundances are highest and the monitoring is most likely to integrate, the effects of wet season inputs of mine runoff waters (Humphrey et al 1990). Access to sites is also practicable.

A gauge height reading of 11.6 m or less at the Magela Creek Gauging station GS8210067 (near the upstream (control) water quality monitoring site) or a stage height reading less than 1.65 m (equivalent flow of around 173 577 ML) at gauging station G8210009 (near the Magela downstream monitoring point), indicates conditions suitable for sampling and conditions that are dry enough to enable overland access to the sampling sites. The gauge board located at GS8210067 is easily accessed in the field, whilst stage height data from G8210009 is accessed online.

In most years, significant rainfall finishes by early April, resulting in commencement of sampling in mid May. When rainfall ceases earlier or later than early April, the timing of sampling is adjusted accordingly.

3.4 Sampling schedule

The total sampling time over all billabongs should be minimised as far as possible to reduce temporal changes that may occur over the sampling period. Billabongs dry up quickly so a prolonged sampling period may result in natural changes in fish communities during the sampling period, thereby potentially confounding mining impact assessment.

It is not practical to implement a random sampling strategy for sites due to physical and biological constraints. The occurrence of sites in different catchments and the travel distances between sites necessitates a pre-determined visitation schedule, which is generally the same from year-to-year.

3.5 Sampling sites

Six sites are sampled in the current monitoring design (Figure 1; Table 2) which is a reduction from a total of nine sites that were sampled up until 2005. The original nine sites were selected to provide three different treatment categories (directly exposed, indirectly exposed and control), while the six current sites form three control–(directly) exposed sitepairs.

All sites are shallow depositional basins, mostly less than 2 m deep, but some have pockets of deeper water up to 5 m deep. The shallower waters around the margins dry out in the dry season. All sites are densely vegetated. Shallower margins are dominated by sedges (*Eleocharis* spp), and spiny mud grass (*Pseudoraphis spinescens*) while deeper water is dominated by water lilies (*Nymphoides* spp and *Nymphaea* spp). *Utricularia* sp, *Chara* sp, *Najas* sp and *Ceratophyllum demersum* are the dominant submerged macrophytes.

The high mobility of fish communities along stream systems in this region (Bishop et al 1995) makes upstream – downstream comparisons of doubtful value for detecting long-term effects due to upstream sites not being truly independent of downstream sites (viz fish movements) and thereby not constituting a true control. The use of control sites on separate catchments circumvents this problem.

3.5.1 Exposed sites

Sites potentially *directly exposed* to contaminants from Ranger uranium mine in the Magela Creek catchment include Georgetown, Coonjimba, and Gulungul Billabongs. Georgetown and Coonjimba are adjacent to the minesite. Georgetown receives potentially contaminated surface water via Corridor Creek, which drains the southern edge of the mine area. Coonjimba billabong receives overflow water from the RP1 pond that collects seepage and surface flow from areas of clean catchment and from waste rock and tailings dam wall areas. Spray irrigation of pond water also occurs in the catchment areas for Georgetown and Coonjimba Billabongs. Gulungul Billabong lies at the confluence of Gulungul Creek and Magela Creek. It is potentially influenced by surface runoff and seepage from the western margins of the minesite and by possible contaminants from Jabiru township via Baralil Creek.

Table 2 Location of shallow lowland billabongs and treatment designation for monitoring of fish communities

Billabong	Treatment designation	Catchment	Longitude Latitude	Australian Grid reference (WGS 84) 1:50 000
Georgetown	Exposed to Ranger Mine	Magela	S12°40.471 E132°55.872	2-75-300 m E 85-97-600 m N Mount Brockman (5472 1)
Coonjimba	Exposed to Ranger Mine	Magela	S12°39.741 E132°54.303	2-72-400 m E 85-97-600 m N Mount Brockman (5472 1)
Gulungul	Exposed to Ranger Mine	Magela	S12°37.822 E132°53.112	2-70-200 m E 86-02-800 m N Mount Brockman (5472 1)
Wirnmuyurr	Control	Magela	S12°34.586 E132°55.086	2-73-800 m E 86-08-700 m N
Sandy Shallow	Control	Nourlangie	S12°54.04 E132°46.4	2-58-500 m E 85-72-700 m E Nourlangie Creek (5427 2)
Buba	Control	Nourlangie	S12°50.93 E132°45.12	2-55-500 m E 85-78-400 m E Mount Cahill (5427 3)

3.5.2 Control sites

Buba and Sandy (Shallow) Billabongs are located in the Nourlangie Creek catchment of the South Alligator River system. There is no mining activity or urban development in the Nourlangie catchment. Wirnmuyurr Billabong is located on Wirnmuyurr Creek, a tributary to the floodplain reaches of Magela Creek well downstream and to the north of the Ranger minesite. Wirnmuyurr Creek drains an undisturbed catchment. Whilst there is the possibility of inputs from Ranger Mine affecting fish migration and recruitment into Wirnmuyurr Billabong, the volume of water on the Magela floodplain provides a large area for dilution of any mine inputs. Furthermore, historical minesite input into Magela Creek, measured by concentrations of U and Mg, are orders of magnitude lower than those known to adversely affect larval fishes (Hogan et al 2005, Cheng et al 2010, van Dam et al 2010).

3.6 Sampling transects

At each of the control and exposed sites (billabongs), sampling is conducted each year along the same shoreline 'transects' (Table 3). These transects were originally selected to provide areas that are suitable for the pop-net trapping method with adequate vehicle access for equipment and low gradient shallow waters (0.3–1.0 m deep) harbouring a variety of aquatic plants.

The structure of the aquatic plant community varies amongst sites and along transects and may vary from year to year. To maintain sample integrity and safety of personnel, the location of the transects may need slight alteration as the billabong edge habitat changes over time. Billabong size and extent of available habitat may vary as a consequence of trees, submerged logs or steep gradients which make sampling difficult and unsafe. As a consequence, transects will vary in length and may need to be divided into two sections to accommodate these obstacles and conditions. Table 3 provides details on the location of each transect at each site.

Table 3 Location of shoreline transects on shallow billabong sample sites

Billabong	Number and location (Latitude/Longitude) of transects
Georgetown	One continuous transect starting from Gauge board on the western bank at the northern end of the billabong. Transect length = 620 m. Start: S12°40.471 E132°55.872, Finish: S12°40.722 E132°55.841
Coonjimba	One continuous transect located on eastern side of billabong. Transect length = 300 m Start: S12°39.741 E132°54.303, Finish: S12°39.618 E132°54.311
Gulungul	Two transect sections either side of outflow: Transect length section A = 600 m of south eastern shore line. Start: S12°37.822 E132°53.112, Finish: S12°37.684 E132°53.015 Transect length section B = 200 m of Northern shore line. At least two exclosures (this section has not been accessible after cyclone Monica, April 2006). Start: S12°37.772 E132°52.986, Finish: S12°37.868 E132°52.975
Wirnmuyurr	One continuous transect located on Western Side. Transect length = 400 m Start: S12°34.586 E132°55.086, Finish: S12°34.738 E132°55.154
Sandy Shallow	One continuous transect located on northern shoreline. Transect length = 380m Start: S12°54.040 E 132°46.400, Finish: S 12°54.150 E 132°46.250
Buba	Two transect sections: Transect length section A = 120 m on western side at northern end of billabong. Start: S12°50.930 E132°45.120 Finish: 12°51.020 E132°45.170 Transect length section B = 160 m on western side at southern end of billabong. Start: S12°51.100 E132°45.320, Finish: S12°51.220 E132°45.380

3.7 Exclosure areas

Exclosure areas are formed by the setting of crocodile exclusion nets (heavy gauge (12 mm) cargo netting (mesh 200 mm)) to provide safe operation of sample quadrats (section 3.8). Exclosure areas were introduced in 2001 due to the increasing presence of, and hence risk from, crocodiles at the sampling sites and so the need for protection. It is not practical to set any more than five crocodile exclusion areas as deployment is time consuming, labour intensive and additional nets would disturb the sampling area, possibly limiting the quality of results.

The five exclosure areas are randomly located along the transect at each sample site. Each area requires approximately 25 m of shoreline to optimise the independent placement, and operation, of two quadrats (see section 3.8). The random position of the exclosure area along the transect is determined each year by dividing the billabong transect into 25 m blocks beginning at a randomly-selected starting distance within the first 25 m (0 to 25 m at the downstream end of the billabong). Five positions are then randomly selected using the random function in Excel by consecutively numbering the possible starting points; the positions mark the downstream starting point for each exclosure.

3.8 Sample quadrat (pop-net trap)

Sampling quadrats are defined by the 2 m x 2 m square frame of the pop-net traps (open-ended enclosures of 2 mm netting that extend from the bottom to the surface of the water body), modified from the design described by Serafy et al (1988). Ten pop-net traps are sampled from billabong margins (0.5–1.0 m depth) at each site. Method investigation during 1992 (unpublished data and illustrated in Buckle et al 2004, p12) indicated that ten pop-net traps are adequate to reflect and distinguish fish community structure differences amongst billabongs. Since the introduction of crocodiles exclusion nets in 2001, two sample quadrats

have been deployed within each of the five exclosure areas (section 3.7). To maximise independence of each sample quadrat by sampling undisturbed area, their placement is allocated according to operational criteria.

3.9 Fish sampling

Pop-net traps (section 3.8) are deployed on the billabong substrate overnight to allow animals to recolonise the vegetation after initial disturbance when the trap is set. The trap is triggered remotely which allows the top floating frame to rise ('pops') to the surface, trapping the fauna within the pop-net trap. The plants are then removed by hand and the fish are subsequently removed using a 2 mm mesh seine net.

(a) Deploying a crocodile exclusion net using an Argo amphibious vehicle

(b) Pop-net trap being checked after assembly on shore

Figure 3 Crocodile exclusion net and pop-net trap used to sample fish in shallow billabongs

Seining is repeated until three consecutive effective seines retrieve zero fish. If three consecutive zero fish seines cannot be achieved and a single species of fish continues to intermittently appear, sampling that pop-net trap can cease.

Any fish found caught within the removed pop-net trap or the removed aquatic vegetation are added to the respective pop-net fish tally for that species.

The identity and number of each fish species and a total combined weight of all fish within the pop-net trap is recorded. Most fish species are very small and in large numbers making it impractical to weight individual fish species.

Fish of uncertain identity are transported live to the ***eriss*** laboratories for detailed identification (see section 3.11.2), or grown out if juveniles.

On completion of each pop-net trap the fish are released immediately into a relatively undisturbed area of the billabong.

3.10 Measurement of environmental variables

Change in fish community structure may be associated with changes in water quality or habitat structure among sites and years that may arise from either natural environmental perturbations or through human-induced change. For example, macrophyte vegetation can be influenced by feral animals, fire regimes and wet season water levels and their trends, and so structure of the vegetation may change from year to year.

Consequently, at each pop-net trap and at the time of fish sampling, variables relating to water chemistry, water depth and the structure of the habitat created by vegetation, are measured. Rainfall and hydrology data from the just-completed wet season are also obtained. These may serve as potential environmental correlates of fish community structure data for that year.

3.10.1 Water chemistry

Water quality monitoring to characterise in situ water quality condition in each of the fish traps is completed in the morning of fish sampling, just after pop-net traps have been triggered and before the disturbance of fish collection procedures.

A water sample for later laboratory determination of ions, metals, nutrients and organic carbon is collected from two locations from each billabong, in areas best representing the location of the 10 pop-nets.

Measured variables are described in Table 4.

3.10.2 Habitat structure

Assessment of habitat structure variables in each pop-net trap is undertaken after traps have been triggered and prior to fish removal. Measured variables are outlined below and in Table 4.

Surface vegetation cover

Surface vegetation and open water (proportion of the water's surface not covered by any vegetation) are characterised within an area encompassing a 5-metre radius from the centre of the pop-net trap. Estimates are made by using a visual percentage water cover technique. Total values for each pop-net trap should equal 100%.

Water depth and distance to shore

Measurements of water depth and distance to shore are taken at each pop-net soon after the aquatic plant surface area assessments are completed (Table 4, see also operational manual for further details).

Vegetation biomass and composition

Aquatic vegetation is physically removed from each pop-net so it can be weighed. Once the vegetation is weighed, the aquatic plants are sorted into species and the percentage volume of each plant species is estimated. The percentages are then standardised to weight and the pop-net volume estimated to provide plant density in kg/m^3.

3.10.3 Rainfall and hydrology

Water level in each billabong is recorded from an in-situ depth gauge.

Discharge data for Magela and Nourlangie Creeks are available from the NT Department of Natural Resources, Environment, the Arts and Sport (NRETAS). Daily creek discharge (ML) and stage height information for Magela Creek are sourced from gauging station G8210009. Discharge data from G8210009 are used to determine start and cease of flow which is used as an indicator for length of dry and wet seasons for both streams. Up to the 2005–06 wet season, Nourlangie Creek hydrology data were sourced from gauging station G8200112. Due to the decommissioning of this station in the dry season of 2006, the duration of dry (no creek flow) and wet season (creek flow) can no longer be derived specifically for Nourlangie Creek. However, data can be derived for Nourlangie Creek annual flow using a regression formula to Magela G8210009, details for which are outlined in the operational manual.

Table 4 Site physico-chemistry and habitat description measured for shallow billabong fish monitoring

Category	Feature/analyte	Units
In situ water physico-chemistry[A]	Temperature	^{0}C
	Dissolved oxygen	mg/L & % saturation
	pH	Units
	Electrical conductivity	µS/cm
	Turbidity	NTU
Laboratory-measured water physico-chemistry[B]	Ca^{2+}, Mg^{2+}, SO_4^{2-},DOC	mg/L (filtered)
	TP, TN, TOC	mg/L (unfiltered)
	Al, Cu, Fe, Mn, Pb, U, Zn	µg/L (filtered)
Rainfall and hydrology[C]	Length of wet season	Days
	Length of previous dry season	Days
	Annual flow in Magela or Nourlangie Creeks	ML (Megalitres)
Habitat structure	Billabong relative depth (single in situ gauge board)	cm
	Distance of inner trap from shore[D]	m
	Shore side trap depth[D]	cm
	Outer side trap depth[D]	cm
	Outer side + 2m depth[D]	cm
	Surface area of each aquatic plant species[D]	%
	Density of each aquatic plant species[D]	kg/m^3

Superscripts A= Water quality variables measured in the field at each enclosure area, B = Samples collected from two locations at each billabong and sent to a NATA accredited laboratory, required filtration is done in the field, C = Data collated after the wet season form gauging station G8210009, D = measurements taken at each pop-net trap on the day of fish sampling.

3.11 Quality Assurance (QA)/Quality Control (QC) procedures

3.11.1 Training

Sampling is conducted, or supervised, by trained personnel. Personnel with limited experience are accompanied by trained staff until deemed competent. Experienced operators must be present at all times throughout the sampling operation to minimise the influence of bias.

3.11.2 Fish identification

Quality control and assurance of fish identifications is ensured by the most senior fish identifier being abreast with fish nomenclature and current identification keys. The senior fish identifier regularly checks other operators' identifications, particularly species that are typically problematic.

To ensure the accuracy of field identifications, a selection of specimens, with emphasis on problematic species, are preserved for later identification in the laboratory using current taxonomic keys.

Identification of live or preserved specimens can be confirmed by a fish taxonomist or other known fish specialist when there is uncertainty. Specimens confirmed by a taxonomist are retained for voucher and training purposes.

3.12 Observer bias

Techniques for long-term monitoring that involve numerous observers, as staff recruit and leave, are prone to observer bias, particularly visual estimates. For correct interpretation of

results it is essential that the influence of observer bias be well documented so that differences among observers can be distinguished from real spatial or temporal patterns (Thompson & Mapstone 1997). In this monitoring technique, observer biases are minimised by thorough training (section 3.11.1), complemented by the following recalibration procedures.

Prior to sampling each year, trained staff discuss the sampling procedures to ensure the procedures are well understood among observers. The most senior trained staff person (usually project manager) regularly checks on all aspects of the operation throughout the program. This includes habitat assessments and correct assignment of difficult-to-identify fish species, especially small juvenile specimens.

Observers recording % surface cover and density of vegetation recalibrate each other by joint assessments of 10 pop-net traps (one billabong) at the start of each year.

4 Data storage, entry and QA/QC

Further details on the storage and entry of data, and the data QA/QC methods described below are available in the corresponding operational manual.

4.1 Data storage

Original datasheets and relevant printed photographs are archived on SEWPAC/SSD registry files.

Fish observations and habitat data are stored electronically in a relational Access database located within the ***eriss*** computer network on SSIMS Sharepoint.

> **\\Environmental Impact of Mining - Monitoring and Assessment\Fish\Ranger\Shallow Billabongs\Data\popnetdata, access version2000.mdb**

Water chemistry data recorded for the billabongs are currently located in an Excel spreadsheet in the ***eriss*** computer network on SSIMS Sharepoint.

> **\\Environmental Impact of Mining - Monitoring and Assessment\Fish\Water chem and Hydrology data\Popnet - channels water chem data.xls**

Hydrology data obtained for Magela and Nourlangie Creeks (pre 2006) are currently located in an Excel spreadsheet in the ***eriss*** computer network on SSIMS Sharepoint.

> **\\Environmental Impact of Mining - Monitoring and Assessment\Fish\Water chem and Hydrology data\Monthly flow magela – Nourlangie.xls**

Backup, CD/external hard drive, copies are made annually and stored in the Darwin office.

4.2 Data entry and QA/QC

Field data QA/QC checks must be performed prior to data being entered into the database.

Data entered onto databases are verified by an independent person.

A commercial laboratory conducts water chemistry analysis. The results of these analyses are scrutinised before being entered into the Excel database.

Water chemistry QA/QC involves the following:

- Check field blank samples for contamination;
- Compare replicate sample results checking for any discrepancy that would indicate contamination (<20%)
- Check results for unusually high measurements.
- For further information refer to the Surface water chemistry interpretation and reporting operational manual.

5 Data analysis

5.1 Data preparation

For greater detail, including that of the worked example, of the statistical methods described below, refer to the corresponding operational manual.

5.1.1 Rejection of data

Data are only rejected if they are not representative of the experimental design requirements. For example, if sampling equipment failed and gave rise to an anomalous sample, the associated data should be rejected. An example could be a pop-net trap found to have a very large tear in the side netting (allowing fish to escape) – those data would not be representative.

5.1.2 Standardising fish species used in analysis

Fish previously thought to be the same species may now be more readily distinguished as separate species. As a result, data pertaining to some congeners need to be pooled to ensure data are standardised over the time period. Data for the following fish species should be pooled as follows:

Oxyeleotris selheimi and *O. lineolata* should be combined as *Oxyeleotris* spp

Porochilus obbesi and *P. rendahli* should be combined as *Porochilus* spp

5.1.3 Pooling of quadrats (pop-nets) in exclosure areas

The allocation of two quadrats to each exclusion area limits the independence of samples as they are likely to represent similar habitat because of their close proximity to one another. To this end, the values from quadrats within each exclosure area are averaged, for each year sampled and for each billabong. This results in five representative exclosure area values for each billabong and for each year.

5.1.4 Billabong sitepairs

For the comparison of exposed and control sites, each of the three exposed Magela billabongs is paired off with one of the two Nourlangie Creek and Wirnmuyurr Creek control billabongs. The most appropriate pairings were assigned primarily on criteria relating to depth (presence/absence of permanent water) and to a lesser extent general similarity in plant community structure (which may change over time) and longitudinal positioning of the billabong in the catchment. The current site pairings are:

- Georgetown vs Sandy shallow
- Gulungul vs Wirnmuyurr
- Coonjimba vs Buba

5.1.5 Random pairing of pooled exclosure areas

For each year of sampling and each billabong sitepair configuration (from section 5.1.4) data for each of the five exclosure areas (from section 5.1.3) at the control billabong are randomly paired (using a random-without-replacement method) with those at a corresponding exclosure area from the exposed billabong. For each of these five replicate control-exposed pairs within each billabong sitepair, a Bray-Curtis dissimilarity value is calculated. The replicate exclosure pairs for each year remain the same for all ensuing analyses relevant to that year of study.

5.2 Impact detection

Fish community data are analysed for detection of mining impact using a BACIP (using ANOVA) design described in section 2. There are two scenarios in need of consideration when dealing with mining impact: (1) analysis of the year of interest data ('after' impact) with past years' data ('before' impact); and, (2) analysis of two or more 'after' years data with those from previous 'before' years. Either of the two scenarios represents an unbalanced design which is the more common scenario in impact detection generally.

ANOVA analysis using dissimilarity values is conducted in parallel with regression analysis (section 5.2.3) to determine whether or not a trend over time has occurred. A natural trend over time could cause problems for detection of an impact based on a comparison of the dissimilarities between two time periods because the annual replicate observations (dissimilarities) are not independent.

5.2.1 Testing of ANOVA assumptions

ANOVA analyses are conducted on the dissimilarity values arising from the observations from each year. Five values for each year and sitepair are derived from the randomly-paired exclosure areas between the control and exposed billabong (5.1.5). It is important to check that the full dataset to be analysed (dissimilarity values) conforms to underlying assumptions of ANOVA, including normality, homogeneity of variance and independence (eg Stewart-Oaten et al 1986, Sokal & Rohlf 1995).

Assumptions of **normality** and **equal variances** are checked graphically, as recommended by McGuinness (2002). For this, plots of the *residuals* or *errors* (ie the dissimilarity between an observation and the mean for the group) are examined. A worked example of this procedure is provided in the Operational manual using Minitab software. Both assumptions are invariably satisfied for the shallow billabong fish community data collected since 1994. ANOVA is considered to be robust to these assumptions, particularly with large datasets. Therefore, even if the assumptions are not met for a particular test, absolute compliance with these assumptions is not an essential requirement.

If the residuals are arranged in time order of data collection, they should succeed each other in a random sequence. In this case, the datasets from each year meet the assumption of **independence**. Departure from independence would be indicated in the event of an extended sequence of positive residual values followed by an equally long sequence of negative values, ie *positive autocorrelation*, or regular periodicity of positive and negative values, ie *negative autocorrelation*.

The plot of residuals versus observation order (in Minitab) may be used as an initial screening assessment for lack of independence, with formal testing conducted using the von Neumann test as detailed in Sokal & Rohlf (1995, 394–396). An example of the use of the latter test is provided in the Operational manual where it is shown that dissimilarity data (from 1994 to 2009) are positively serially correlated. This result indicates the need to identify the

environmental or biological factors that result in non-independent data. This variable(s), if identified, can then be used as a covariate in the ANOVA analysis explained below (section 5.2.2).

Checks of the data to ensure compliance with the assumptions of the ANOVA method should be made after additional data is added.

5.2.2 BACIP (ANOVA) analysis

Analysis for potential or suspected minesite perturbation is conducted using the randomly-paired (section 5.1.5) multivariate dissimilarity values, based upon log [x+1] transformation of the raw data (Figure 4). The dissimilarity values for the two time periods of interest are formally analysed using a General Linear Model (GLM) (see section 2 for ANOVA model) in Minitab. Log [x+1] is the preferred transformation of the data used to derive the dissimilarity values because it down-weights the abundant species, allowing not only the mid-range but also the rare species to exert some influence on the calculation of the dissimilarity value (Clarke & Warwick 2001).

Analyses of the sitepair difference data for species richness or total abundance can be performed in a similar manner, however has not been used in this protocol.

Application of the BACIP test is compromised by the absence of data for some sitepairs in a number of years. Consequently, data for some earlier years need to be omitted from analyses using the designated model.

By way of example, data for the three sitepairs have been analysed for seven years 'before' (1998, 2000 and 2001 combined, 2002, 2003, 2004, 2005, 2007) with the most recent year 'after' (2009), to examine whether a potential impact has occurred in the 'after' period (Figure 4). In the absence of an observed impact from the mine that is likely to influence fish communities, this before/after analysis is performed using the most recent wet season data (year of interest) to determine if fish communities differ from previous years. Note that years 1994, 1995 and 1996 were omitted from the analysis due to data not being available for the Gulungul-Wirnmuyurr sitepair. Dissimilarity data for 2000 and 2001 were combined to provide a complete dataset for 2000/01 across the three sitepairs. This has been required to ensure a balanced design and to maximise the time series of data available for analysis. Details of how to set up the model and run the analysis in Minitab are provided in the Operational manual.

As illustrated in Table 5, the results of the three-factor ANOVA based upon the replicate billabong-sitepair fish community dissimilarities showed no significant difference from Before to After impact, across or within sitepairs' (BA source, $p = 0.474$; BA*Sitepair $p = 0.057$). However the marginal non-significance (p value) for the BA*Sitepair interaction indicates the dissimilarities from before to after may not be consistent amongst sitepairs. Interpretation from Figure 4 suggests the Coonjimba-Buba pairing differs from the other two site pairs by having reduced dissimilarities in the after period relative to before (considering years 1994–1996 have been removed from the analysis). The Sitepair*year(BA) interaction is significant in the same analysis ($p = 0.001$), this simply indicates that dissimilarity values for the different sitepairs – regardless of their status (Before, After) – show (natural) differences through time. The dissimilarity plot shown in Figure 4 corroborates these results and is most noticeable for the Coonjimba-Buba sitepair.

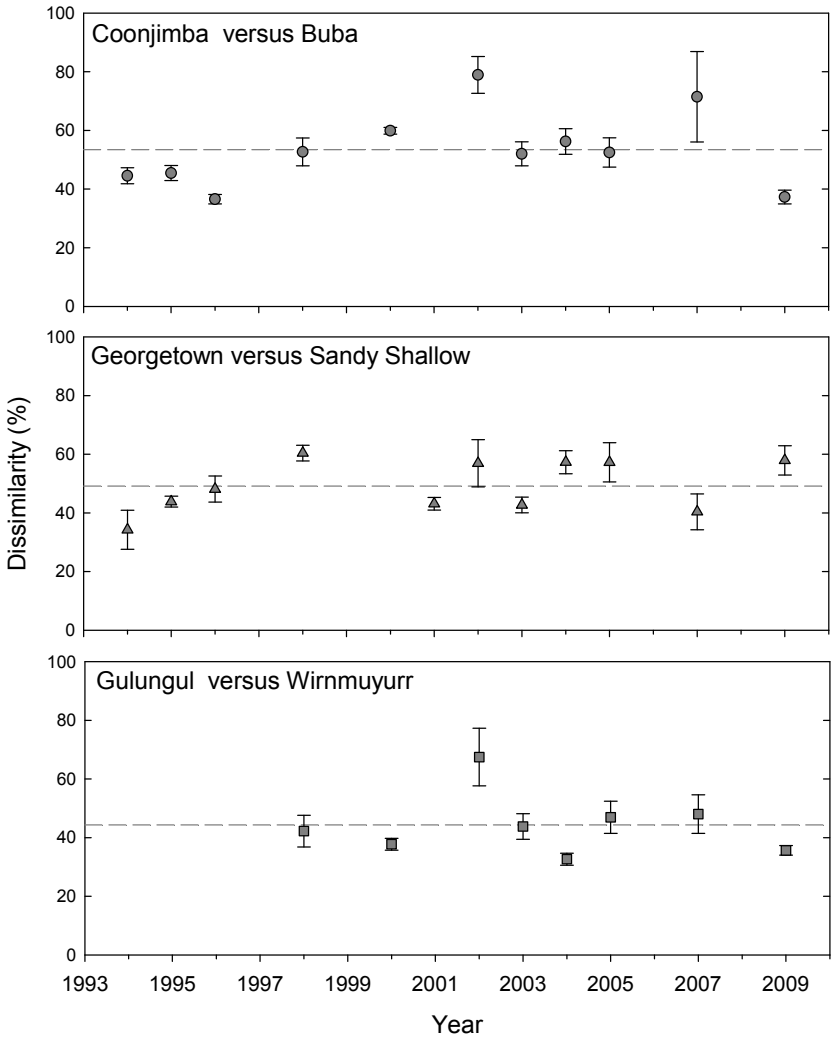


Figure 4 Paired control-exposed site dissimilarity values (using the Bray-Curtis measure) calculated for community structure of fish in 'exposed' Magela and 'control' Nourlangie and Magela Billabongs in the vicinity of the Ranger uranium mine over time. Values are means (± standard error) of the 5 possible (randomly-selected) pairwise comparisons of average trap exclosure data between the pairwise billabong comparisons, Coonjimba-Buba, Gulungul-Wirnmuyurr and Georgetown-Sandy shallow Billabongs.

The large variation in dissimilarity values (Figure 4), which appears to be caused by natural changes in habitat rather than any mine influence (Buckle & Humphrey 2008, see also sections 6.2.1.2 and 6.2.2 below), makes the formal ANOVA testing for a BA factor limited in its ability to distinguish mine-related change in the absence of a covariate that may be incorporated to account for the natural variation. This is particularly true for any analysis examining the 'year of interest' when only one year of data after a potential impact is available and when, in that year, such habitat-related variation is particularly evident.

Covariate(s) that could be used in the ANOVA model to potentially explain the variations in dissimilarity are currently being sought. Where a natural covariate is identified for any trends or changes in fish community structure over time, they will be incorporated into the ANOVA design (section 2) and the data then analysed using Analysis of Covariance (ANCOVA). Until such covariate(s) are identified, impact detection relies on detailed interpretation of the sitepair dissimilarity values and other community indices (species richness and total abundance) using a combination of complementary analysis techniques. These additional methods for data analysis are described below (Section 6.2).

Table 5 ANOVA results for shallow billabong fish community dissimilarity values using three billabong sitepairs: Coonjimba vs. Buba; Georgetown vs. Sandy shallow; and Gulungul vs. Wirnmuyurr. Years before include 1998, 2000–01, 2003–2007; years after are 2009.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
BA	1	466.3	466.3	466.3	0.58	0.474
Year(BA)	6	4797.4	4797.4	799.6	2.15	0.122
Sitepair	2	3390.9	1984.0	992.0	2.67	0.110
BA*Sitepair	2	2733.6	2733.6	1366.8	3.68	0.057
Sitepair*Year(BA)	12	4461.5	4461.5	371.8	2.22	0.016
Error	96	16087.1	16087.1	167.6		
Total	119	31936.9				

5.2.3 Regression analysis

Regression analysis is performed on the dissimilarity values over time. The purpose is to determine whether any trends over time are evident, which may or may not be associated with a mine-related impact. Under natural conditions, regional congruence in waterbody behaviour in response to natural environmental drivers would be expected, so that no trends in the sitepair dissimilarities would be evident (which would indicate a progressive divergence in the structure of the communities from the two sites over time).

For the dataset from 1994 to 2009, no trends in dissimilarity have been identified for any of the sitepairs.

6 Impact assessment

6.1 Background

The possibility of an impact resulting from mine-derived contamination of creek waters downstream of Ranger may be inferred from a statistically significant change or trend in the multivariate dissimilarity value associated with control and exposed sites between any two time periods. The assessment of possible mine-related change and significance of any possible impact is based on comparison of the fish community results with other environmental information, especially water chemistry, hydrology and habitat data.

At the time of publication of this protocol, no impact arising from mining at Ranger has been identified from fish community analyses conducted since 1994 when the current BACIP design was instigated. However, the sitepair dissimilarity data are highly variable over time (Figure 4) as a result of changes through time in natural aquatic vegetation in each billabong (Buckle & Humphrey 2008, and see below sections 6.2.1.2 and 6.2.2). Such variation in the data limits the capacity to detect potential impacts from mining if the Impact detection analysis above (section 5.2.2) does not include a covariate that accounts for the influence of aquatic vegetation changes. Furthermore, due to an absence of pre-mining fish community data for the 'impacted' waterbodies, there are no true 'before' data represented in the BACIP design. This limits the capacity of the impact detection design to detect changes that may have occurred prior to 1994.

To ensure correct inference of mining impact (as distinct from natural environmental changes), the monitoring of fish communities in shallow billabongs forms part of the 'weight-of-evidence' approach intrinsic to the integrated Ranger stream monitoring and assessment

program (van Dam et al 2002, Jones et al 2009). Conclusions about an off-site impact of mining operations at Ranger uranium mine need to be based on multiple lines of evidence provided by other components of the Ranger stream monitoring and assessment program (laboratory and field ecotoxicology, water chemistry including measurement of radionuclides, bioaccumulation, macroinvertebrate communities and fish communities in channel billabongs).

6.2 Assessment of impact

The absence of true pre-mining fish community data and the limitations for detecting mining impact due to the variation observed in sitepair dissimilarity values has required further exploratory analysis. The complementary statistical techniques, in addition to the primary impact detection analysis, are used to provide supportive interpretation of observed changes, if any, in fish community data. These are particularly important to identify the causes of variations (including significant changes or trends) in sitepair dissimilarity values that are evident from the time series data (Figure 4) and which cannot at this stage be accounted for by specific covariates in the ANOVA model (see section 5.2.2). Identifying likely causes for these variations is necessary so that correct inferences can be made.

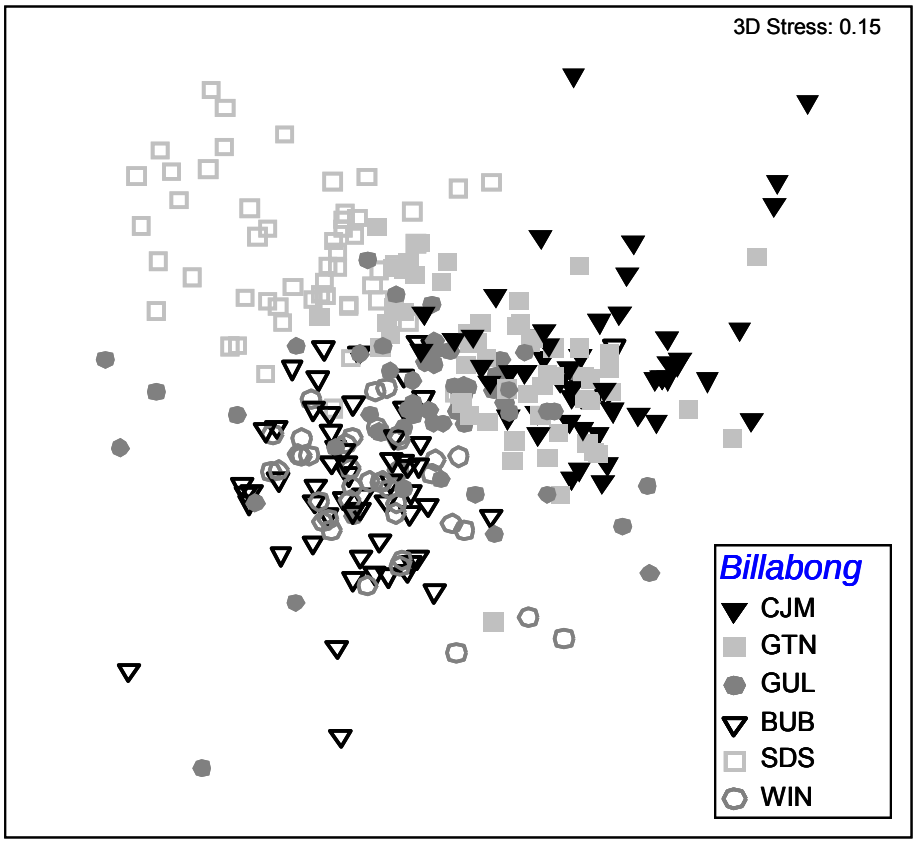
At the time of writing this protocol, aquatic vegetation weight (kg per m^3) appeared to be most closely correlated to variations in the sitepair dissimilarity value (Buckle & Humphrey 2008; see sections 6.2.1.2. and 6.2.2). This observation highlights the importance of recording at the time of sample collection the key environmental conditions that could influence fish communities, in order to draw correct inference.

Complementary techniques used for analysis of fish community data have been divided into those for 'Fish community structure' and 'Fish community summaries'. Not all of the data analysis techniques described below are required for the routine biennial (once every two years) assessments of fish communities in the billabongs. Further, all or some of the analyses may be used on either the complete dataset or subsets of the data, depending upon the question to be answered.

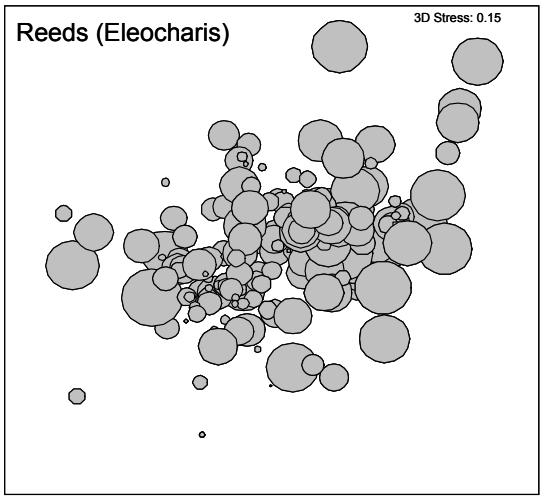
6.2.1 Fish community structure

An ordination of the complete fish community dataset (all sites data available from 1994-2009), is displayed in Figure 5A using the Multi-Dimensional Scaling (MDS) method (Clarke and Gorley 2001, Clarke and Gorley 2006). Data points represent the structure of fish communities from replicate exclusion areas (see section 5.1.3) in each billabong and year. In particular, the graphic depicts the patterns in fish community structure for each billabong and year and the similarity of the communities to each other based upon the underlying Bray-Curtis dissimilarity matrix. Points on the MDS with greatest separation represent exclosures with greatest differences in fish community structure.

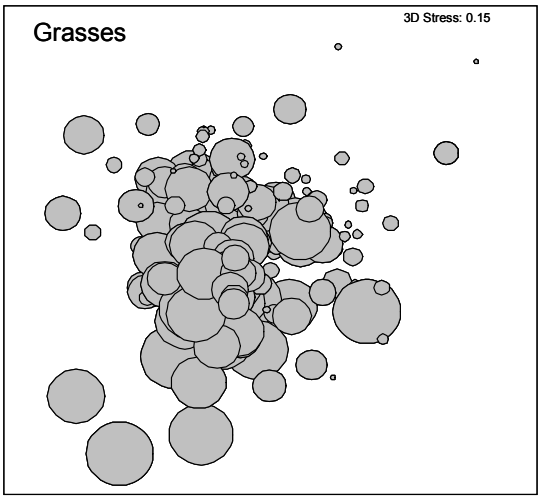
While it is evident from Figure 5A that there are not substantial differences in fish communities amongst billabongs (sites largely overlap in ordination space), each billabongs is defined by a region of ordination space, indicating that there are slight differences in fish community structure amongst waterbodies. This is best displayed by Buba, Coonjimba and Sandy shallow Billabongs (Figure 5A). Furthermore, the billabongs in close proximity to the Ranger Mine, Coonjimba and Georgetown Billabongs, are represented by points tending to the right hand side of this ordination, which if local provenance or catchment issues, and/or additional natural environmental factors, were not considered (see below), could suggest subtle mine influence.



(a) MDS ordination of fish community structure using Log(X+1) transformation from six shallow billabongs including three control (Buba (BUB), Sandy Shallow (SDS) and Wirnmuyurr (WIN)) and three exposed billabongs (Coonjimba (CJM), Georgetown (GTN), and Gulungul (GUL)).



(b) Eleocharis species weight (kg/m³)



(c) Emergent grass species % surface cover

Figure 5 Axis 1 and 3 of a three dimensional MDS ordination based upon fish community structure data from three control and three exposed sites. (a) depicts fish community structure data, (b) depicts *Eleocharis* species weights superimposed over fish community values as bubble plots, and (c) depicts emergent grass species percentage cover superimposed over fish community values as bubble plots. For bubble plots, circle size denotes relative magnitude of plant weight (5b) or surface cover (5c).

Further investigation of the separation of fish community structure between sites was conducted using the exploratory technique of PERMANOVA (introduced in Section 2.1). Three factors were included in the analysis: i) Exposure (Fixed Factor, control or impact), ii)

Billabong (Random Factor – nested within Exposure), and iii) Years (Random Factor). Results of the analysis are shown in Table 6. All factors and the Years* Billabong (Exposure) interaction are significant, indicating that fish communities differ amongst billabongs, years and across exposure types, and that differences amongst years and billabongs are not consistent. The non significant Years*Exposure interaction indicates differences amongst years and between exposure type are consistent. These results show that significant differences occur amongst the fish communities within one, or both, exposure types (Further analysis shows significant differences occur within both exposure types.) and that the variation from year to year is not consistent amongst billabongs.

Whilst natural catchment differences between most of the control and exposed billabongs, and the close geographical proximity of the exposed billabongs to one another, may explain the significant 'exposure' effect, lack of pre-mining baseline data means that this interpretation cannot be accepted without some further investigation.

Table 6 Results from a three-factor PERMANOVA (main factors and interactions) for shallow billabong fish community dissimilarity values using the three exposed (Coonjimba, Georgetown and Gulungul) and three control billabongs (Sandy shallow, Wirnmuyurr and Buba) for all years available (1994 to 2009)

Factors	Test significance
Years	0.0001
Exposure	0.0188
Billabong (exposure)	0.0001
Years* exposure	0.6728
Years* billabong (exposure)	0.0001

An understanding of the fish species and environmental (including habitat) factors contributing to the separation of billabongs by exposure, can assist in identifying causal mechanisms. These analyses can be conducted using the SIMilarity PERcentages (SIMPER) and the linking of multivariate biotic patterns to suites of environmental variables (BIOENV) routines of the PRIMER multivariate software (Clarke & Gorley 2001, Clarke & Gorley 2006) respectively, as follows.

6.2.1.1 Influential fish species

SIMPER (from PRIMER) was used to identify fish species contributing to the dissimilarities between control-exposed sitepairs, and amongst years. By way of example, the average dissimilarity between Coonjimba (exposed) and Buba (control) Billabongs from 1994 to 2009 was 54%, 69% of which was influenced by the abundances of five fish species. These are in order of importance: *Porochilus* spp, *Denariusa bandata*, *Melanotaenia splendida inornata*, *Ambassis agrammus* and *Oxyeleotris* spp. The fish species that most influence the sitepair dissimilarity may be investigated further to determine if patterns observed in their abundances are related to habitat or contaminants present in mine-derived waters. Whilst detailed analysis of fish species abundances has not been completed to date, the patterns of occurrence of the influential species identified above appear to be most related to differences in the aquatic plant communities of the billabongs. Buba Billabong is typically dominated by emergent grasses, providing habitat favoured by *Porochilus* spp and *D. bandata.* Coonjimba Billabong is dominated by the reed, *Eleocharis*, which, at intermediate densities, provides more open water habitat preferred by *M. splendida inornata*, compared to the aquatic grasses, whilst possibly still providing suitable ambush hunting conditions for the predatory sleepy cod

species (*Oxyeleotris* spp). *A. agrammus* favours either habitat, providing plant densities are not too high.

6.2.1.2 Influential environmental variables

Environmental variables were analysed to determine which, if any, correlate with multivariate differences in the fish communities. Natural (non-mine-related) variable(s) identified in this analysis that can account for fish community differences may potentially serve as covariate(s) for analyses conducted for impact detection (see section 5.2.2), thereby enabling greatly improved inference about the effects of mining. Mine-related variables correlated with differences in fish communities, after taking into account natural covariates of the fish patterns, may then be regarded as potential causative factors.

The BIO-ENV procedure from PRIMER was used to determine how well the patterns of an environmental variable, or groups of variables, correlate with the fish community patterns. The procedure uses Spearman rank correlation to compare the multivariate patterns in environmental data with those of fish community data. Missing SSD water chemistry data (metals and ions) for years 1998, 2000 and 2001 were replaced for Georgetown and Coonjimba Billabongs with data collected by ERA. This approach has been used to maximise the time series data available for the two billabongs most influenced by mining (Coonjimba and Georgetown).

Because there are no data available for the other billabongs over the three years 1998, 2000 and 2001, the overall average value for the data available (1994–2009) for each billabong was used. Since water quality data for the other billabongs indicate they are in reference condition with very low solute values compared with much higher values of some mine-related variables for Coonjimba and Georgetown, use of an average value will not reduce the value and validity of the analysis. Importantly, a comparison of this approach with removing years 1998, 2000 and 2001 from the analysis, indicates very little difference in the results (see operational manual).

Variables included in the analysis were: (i) habitat structure (trap volume (derived from depth of trap) and major aquatic vegetation categories (% surface coverage and weight in the trap (kg/m^3), see operational manual for further details); (ii) hydrological variables (total wet season creek discharge, length of dry and wet seasons); and (iii) water chemistry (Electrical Conductivity (EC), Magnesium (Mg), Calcium (Ca) and Uranium (U) as indicators of minesite contamination) with highly co-correlated variables (≥ 0.95) removed (for example Magnesium (Mg) is strongly correlated with SO_4 so SO_4 was removed from the dataset).

Inclusion of the above-listed environmental variables in the BIOENV analysis explained only a small proportion of the variation in fish community differences. For example, analysis of data from 1994 to 2009 using the six billabongs that comprise the three sitepairs showed that the best Spearman rank correlation obtained was 0.406 with the five variables: Surface % of open water, total creek discharge, surface % of waterlilies, weight of *Eleocharis* (Figure 5b) and surface % of total grasses (Figure 5c). (Figures 5b and c are bubble plots (from PRIMER) which superimpose the numeric value of *Eleocharis* and the % of total grasses respectively on the fish community MDS ordination as circles of proportional magnitude.) The results indicate that hydrology and aquatic plant habitat conditions have the greatest influence on fish community structure.

Further analysis, using the same variables, was conducted for Coonjimba Billabong alone (the exposed site most affected by the Ranger minesite) to determine if changes in water quality due to minesite runoff have influenced fish community structure. In this analysis, the best Spearman rank correlation was 0.650, with the same five variables: total creek discharge,

surface % cover of lilies, surface % cover of open water, length of the dry season and total aquatic vegetation weight (kg/m^3). These results provide further support that habitat and wet season flow conditions are the most influential variables on billabong fish communities with mine-derived water quality variables not appearing to be a factor affecting fish communities.

Of the water quality variables included in the BIOENV analysis, uranium concentration in the water appeared most often and occurred amongst the top 5 strings of significant variables for both analyses conducted (for all sites and Coonjimba Billabong only), however, it had a low individual Spearman correlation.

The lack of influence of water quality in explaining differences in fish communities amongst sites is perhaps not surprising given that the concentrations of uranium (the only variable identified as a possible correlate of fish community structure by BIOENV) recorded from Coonjimba Billabong (and all other billabongs) are typically well below the toxicologically-derived trigger value of 6 µg/L (for 99% ecosystem protection) for Magela Creek waters (Hogan et al 2005). The highest concentrations recorded in shallow billabongs during the fish monitoring period to date are in Coonjimba Billabong. Whilst spot measured concentrations have occasionally exceeded the trigger value, values are mostly well below (Figure 6). In addition, not only is the sensitivity of fish to uranium two orders of magnitude greater than the derived trigger value (Cheng et al 2010, Holdway 1992 and Markich & Camilleri 1997), but the concentrations of dissolved organic carbon (DOC) are higher in billabongs than in the Magela Creek water that was used as the diluent to test species sensitivities. The toxicity of U is reduced by complexation with DOC so this provides further protection in the billabong environment (see Houston et al 2009).

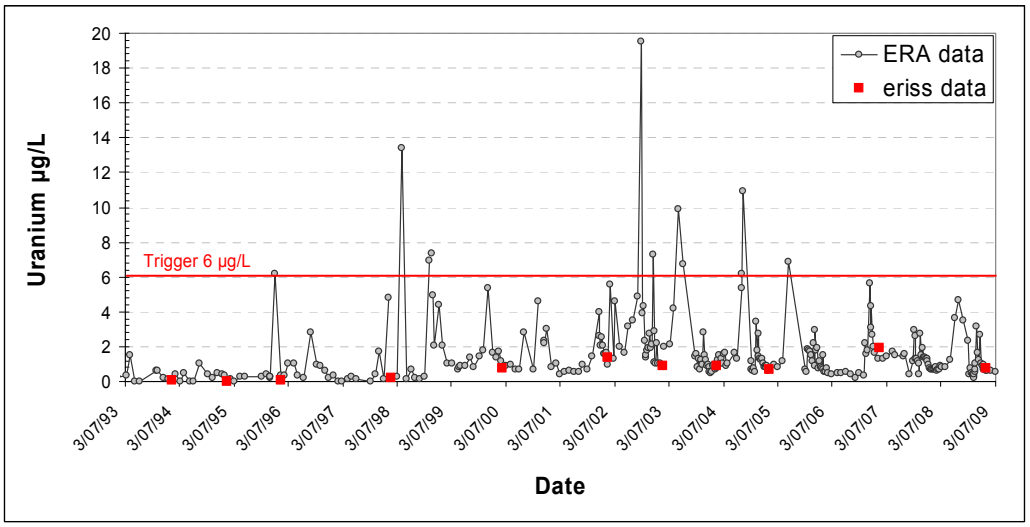


Figure 6 Concentrations of uranium in Coonjimba Billabong collected over the fish monitoring period. Samples have been collected by ERA as part of its routine water quality monitoring program.

Whilst magnesium (Mg) was not identified as a correlate of billabong fish community structure, recent research into the toxicity of Mg indicates Coonjimba Billabong has concentrations around the estimated IC50[3] for the local gudgeon *Mogurnda mogurnda* in low ionic conditions (Mg:Ca ratios >9:1) (van Dam et al 2010). Furthermore, the Mg:Ca ratios in Coonjimba Billabong have altered over time, from < 9:1 prior to 2000 to > 9:1 after 2000; such a change could infer higher toxicity of Mg to aquatic organisms (van Dam et al 2010).

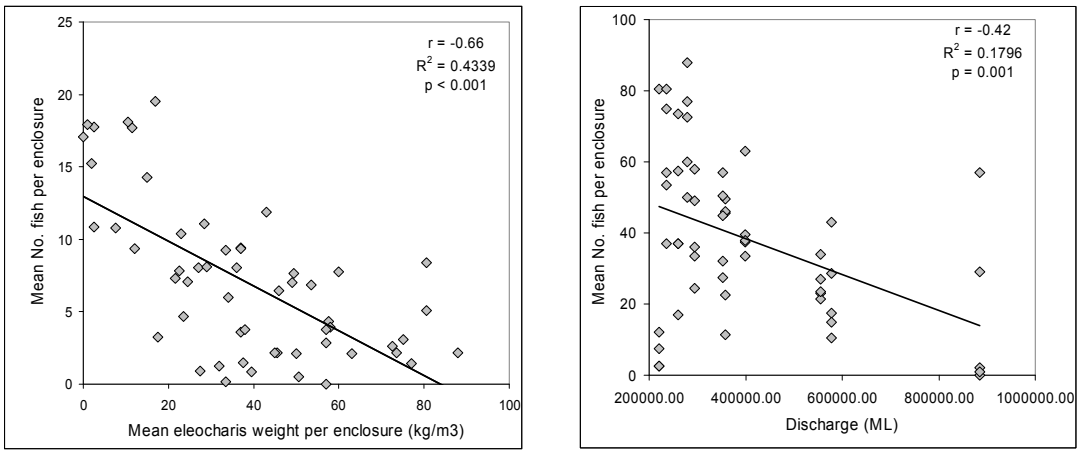
[3] IC50 – Median Inhibition Concentration, ie concentration that reduces the effect by 50%.

To this end, possible relationships between Mg concentrations in shallow billabongs and the corresponding fish communities will continue to be sought in future.

6.2.2 Fish community summaries

Analyses of fish species richness (number) and relative abundance for each crocodile exclusion area (average exclusion area value see 5.1.3) have been used to complement the analysis of fish community structure based upon dissimilarity values. Patterns observed in the community summaries of each billabong, including their relationships with habitat variables, may help explain changes observed in the paired site dissimilarity value. Indeed, as both summaries have some influence in the calculation of the Bray-Curtis dissimilarity value, relationships between the various indicators would not be unexpected.

Environmental variables (from section 6.2.1.2 above) have been analysed using descriptive (graphical) and statistical (correlation) analysis techniques to determine which, if any, correlate with fish community summaries. The analyses showed that of the environmental variables measured, aquatic vegetation weights have the greatest influence on community summaries (Buckle & Humphrey 2008). The effect of aquatic vegetation is best observed in Coonjimba Billabong where fish species richness and total abundance both decline with increasing weight of *Eleocharis* ($r = -0.36$, $p = 0.01$; $r = -0.65$, $p < 0.001$ respectively, Figure 7). Excessive plant densities are unfavourable for fish communities as fish movement, and hence residency, is physically restrained and prevented.



(a) (b)

Figure 7 Regression relationship between average fish abundance and (a) average weight of *Eleocharis* sp (kg/m³) per trap exclosure in Coonjimba Billabong (b) Discharge (ML) in Magela Creek (1994 to 2009)

Total fish abundance in Coonjimba Billabong also appears to decline with greater wet season (Magela) creek discharges (Figure 6b). However, this correlation appears to simply reflect the fact that increased duration of the dry season (negatively correlated with wet season discharge) results in a reduced biomass of *Eleocharis* ($r = -0.42$, $p = 0.001$) due to the reduced inundation period. Thus hydrological factors themselves influence the density of *Eleocharis*. The influence of *Eleocharis* and hydrology (direct or indirect) on fish community summaries provides supportive evidence to the BIOENV analysis above (section 6.2.1.2).

In contrast to habitat variables, mine-related water quality indicators (EC, U, SO_4, Mg and Ca) appear to be unrelated to either fish species richness or total abundance. The lack of influence of water quality on fish community summaries provides supportive evidence to the BIOENV analysis above (section 6.2.1.2).

7 Reporting

7.1 Overview

Different reporting mechanisms are required for different forums and stakeholder groups. Summarised below is a (more or less) chronological sequence of corporate and other reporting from the commencement of the calendar year – noting that the fish community assessment work is currently done once every two years:

- Reporting to Traditional Owners
- Supervising Scientist Annual Report (statutory requirement)
- Updating of the Internet monitoring pages following analysis of the collected data
- Review of ***eriss*** science program outputs by the Alligator Rivers Region Technical Committee (ARRTC)
- Report of SSD wet season monitoring program results to the Alligator Rivers Region Advisory Committee (ARRAC)
- Annual Research Summary (Supervising Scientist Report)
- Additional summary reports for stakeholders as required

7.2 Reporting results to Traditional Owners and Aboriginal residents

There are two components involved in communicating the work and outcomes of the monitoring program (including the fish community assessment) to Aboriginal people:

1 Informing people of what tasks are to be undertaken, when, by whom and why

2 Providing feedback to people on the results of the work and providing assurance that the environment and their lifestyle have been protected.

Communication occurs through a variety of mechanisms including:

- Involvement of Aboriginal people in the actual monitoring program, especially through employment.
- Regular updates and reports of monitoring results presented by the Community Liaison Officer at meetings and associations. Larger meetings or Open days may also be planned for this purpose. Monitoring staff (and more senior Darwin based staff) are available to people (particularly Traditional Owners and Aboriginal residents) to answer questions or provide additional information as requested. Information is provided on what programs are to be undertaken and their timetable. Feedback is also sought on any key questions and needs.
- Illustrated report of monitoring results for Traditional Owners and Aboriginal residents.

7.3 Supervising Scientist Annual Report

This statutory report is tabled in Parliament in the latter part of each year. A summary of the fish community monitoring results (which may be an abbreviated version of the summary reports described in section 7.4 below) is included in the Report.

7.4 Internet

Once the fish community monitoring data have been analysed and reported in the Supervising Scientist Annual Report the text and figures are adjusted appropriately for presentation on the SSD website:

http://www.environment.gov.au/ssd/monitoring/magela-bio.html

Papers and reports produced to address the other (listed in Section 7.1 above) communication requirements for the monitoring and science programs of SSD are also posted to the SSD website as these become available.

7.5 Alligator Rivers Region Technical Committee and Annual Research Summary (Supervising Scientist Report)

A verbal summary of results-to-date is reported to the first meeting of ARRTC that occurs in the mid to late wet season (typically February–April period). A full summary report of work conducted in the wet season prior is provided to the Alligator Rivers Region Technical Committee (ARRTC) for their late dry season review meeting. This summary provides the basis for reporting in the ***eriss*** Annual Research Summary (a Supervising Scientist Report), compiled late in the calendar year, together with results from other stream monitoring programs.

The Annual Research Summary is circulated to a wide audience, including the key stakeholders, Energy Resources Australia, the Northern Land Council and the NT Department of Regional Development, Primary Industry, Fisheries and Resources. A full list of recipients is available from the SSD Publications Section.

The technical reports should contain the following information, and adhere to the required layout proforma:

1 Brief description and background of the monitoring program.

2 Details of the just-completed wet season, noting any specific or unusual issues of relevance. This includes water flow timing and period of controlled or accidental discharge events, and may include unusual weather or hydrological events, etc.

3 Brief description of methods with reference to the protocols. Any variations from the accepted operational protocols and reasons for the variations should be reported.

4 Current wet season's results and comparisons to past wet seasons' trends and findings. This would include summary statistics for the data collected in the current season, BACIP (ANOVA) analysis of fish community dissimilarity values, and the relationship, if any, of these biological data to environmental conditions and variables.

5 Evaluation of results in the context of any impact being detected.

6 Recommendations based on conclusions drawn from the evaluation.

7.6 Summary report for stakeholders

Consistent with the reporting to ARRTC and with similar timing, two reports and presentations are provided each calendar year to the Alligator Rivers Region Advisory Committee ARRAC, representing a wide range of stakeholders for the ARR (not necessarily with technical backgrounds). The reports contain a summary of major results and conclusions,

and should be in a more plain-english form to those reports described in sections 7.2 and 7.4 above, given the broader range of stakeholder participation in ARRAC.

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Appendix 1 Comparison of Analysis Of Variance (ANOVA) with PERmutational MANOVA

A1 Background

PERMANOVA (PERmutational Multivariate ANalysis Of Variance) (Anderson et al 2008), an add-on function of PRIMER software (Clarke and Gorley 2001, Clarke and Gorley 2006), represents an alternative and potentially superior data analysis method for impact assessment using the current channel billabong fish community dataset. The method of non-parametric multivariate analysis of variance was introduced by McArdle & Anderson (2001) and its application to community data by Anderson (2001). There are two main technical advantages of the PERMANOVA method that may ultimately prove superior to the ANOVA method that is currently used for impact detection:

1. Whereas ANOVA or the multivariate (and computationally difficult) equivalent, MANOVA, assume normal distributions and, implicitly, Euclidean distance (MANOVA), PERMANOVA can use any distance measure appropriate to the data (including Bray-Curtis), and uses permutations to perform hypothesis tests which are largely, but not entirely, free of distribution type. As such, and by adopting an approach to partitioning of variation like that employed in ANOVA, it can perform analyses of multivariate (or univariate) data in the same manner as the more complex experimental designs and models associated with BACIP and ANOVA that are restricted to univariate data such as used in the current protocol.
2. Unlike the BACIP approach used in the present protocol where sitepair dissimilarities are employed to meet data assumptions of independence of temporal replicates, PERMANOVA is not so constrained and offers increased partitioning of data variation (and hence increased factors) by way of its ability to use the complete multivariate dissimilarity matrix. Use of the complete data matrix enables PERMANOVA to better detect changes in direction in multivariate space that might otherwise be missed when using the simple sitepair dissimilarity metric data (See Figure A1 for hypothetical illustration of how the sitepair dissimilarity value might be similar for the before and after periods yet mask a real change that occurs in multivariate direction). (Note that this is not an issue when using univariate data as the difference can only be positive or negative.)

This appendix compares the results from the BACIP ANOVA analysis (section 5.2.2 from the main report) to results from PERMANOVA analysis on the equivalent dataset. This comparison has been carried out for two reasons:

1. To provide complementary results to the BACIP ANOVA conducted in section 5.2.2 (particularly in the context of the greater information available from PERMANOVA, viz advantage item 2 above); and
2. To compare and assess any differences in results between the two analysis approaches on the same monitoring dataset.

At this stage, the PERMANOVA method is not afforded more prominence in this protocol because it is relatively new (when compared with BACIP and associated ANOVA methods) and as such has not been assessed with the same statistical scrutiny as the BACIP ANOVA.

Because of this, the univariate ANOVA analysis technique using sitepair dissimilarity remains the formal statistical testing procedure used to analyse billabong fish data.

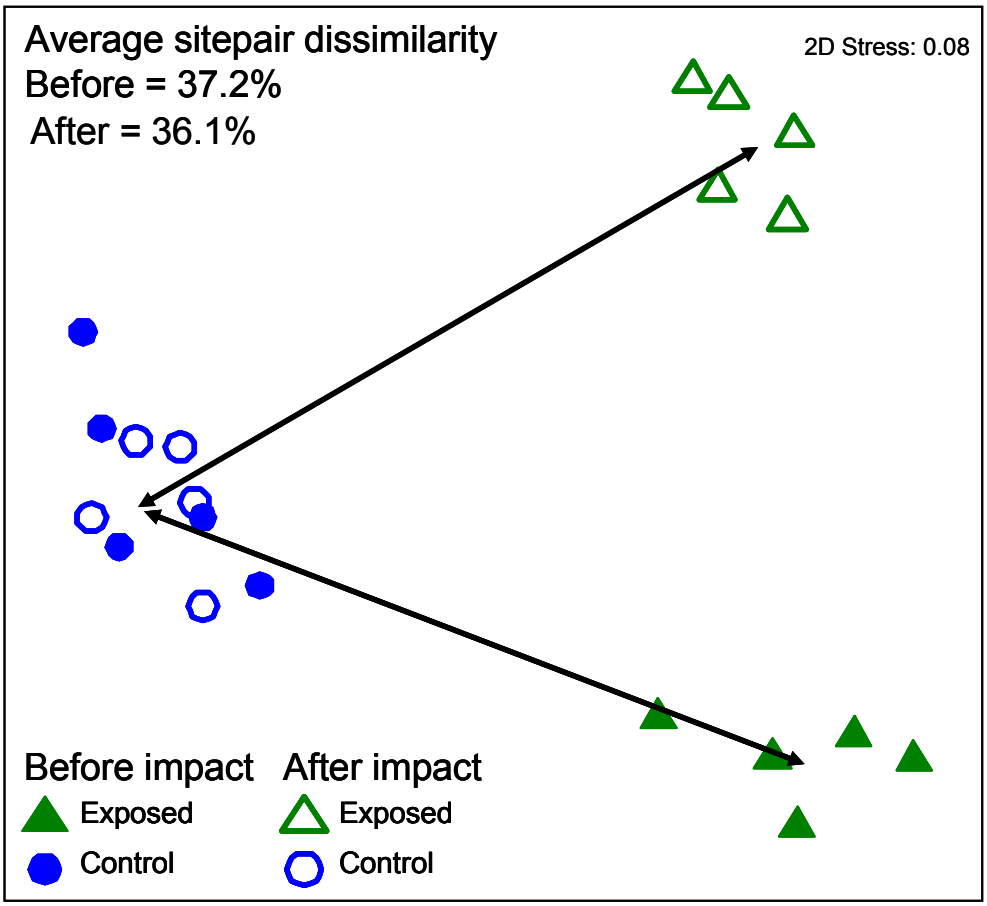

Figure A1 Hypothetical scenario showing that an analysis using a control-Impact sitepair dissimilarity value will not detect all changes that occur in multivariate direction. In this case, while a change has occurred at the exposed site after impact, the sitepair dissimilarity remains similar for the before and after periods.

A2 Comparison of PERMANOVA and BACIP ANOVA analysis approaches

While PERMANOVA and the BACIP ANOVA use the same original dataset, the actual analysis is undertaken on different manipulations of the same dataset, thus:

- PERMANOVA uses the complete dissimilarity matrix which enables the analysis to detect not only changes across billabongs, but also within each billabong.
- BACIP ANOVA uses control-impact sitepair dissimilarity values for each year in order to eliminate or reduce spatial and temporal variability. (As such, the approach assumes that, in the absence of human disturbance, natural variation between the billabong pair will be consistent from year to year.) This approach is an established design for impact detection, particularly with paired upstream and downstream comparisons (ANZECC & ARCANZ 2000). However, when using multivariate data, the BACIP sitepair approach reduces the multi-dimensional data to just one dimension (a metric scale from 0-100). As illustrated above (Figure A1), this results in a potential loss of information relating to *direction* of

Given the different analysis approaches, the hypotheses being tested with each analysis are somewhat different. Because the BACIP sitepair dissimilarity aims to eliminate or reduce temporal and spatial variation, interpretation is based upon the changes between sitepairs (dissimilarity value) for each year. PERMANOVA retains the spatial and temporal variation but partitions these sources of variation by additional and different factors, thus interpretation is based upon changes amongst years for each billabong.

To this end, the factors used to analyse the data differ between the two models and are outlined in Table A1. While both models include the 'Before vs After' (BA) and 'Years' factors, no other factors are shared. The BACIP ANOVA uses a fixed 'sitepair' factor to partition the replicate control-impact dissimilarity values within each of the three sitepairs. PERMANOVA, based on the complete dissimilarity matrix, requires two factors to partition the equivalent data by exposures and billabongs. To do this, PERMANOVA includes a 'fixed' exposure factor to partition the control and impact billabongs and a 'random' billabong factor (nested within exposure) to partition the three billabongs within each exposure. For PERMANOVA, the replicates are values (from the complete dissimilarity matrix) that represent the fish community structure within each billabong, enabling analysis of the before and after periods within each billabong.

Table A1 Description of factors used for PERMANOVA and BACIP ANOVA analysis with factor designation included (nested, and fixed or random)

Factors	Nested in	Fixed or Random	Analysis that includes the factor
Before vs. After (BA)		Fixed	PERMANOVA, ANOVA
Years	BA	Random	PERMANOVA, ANOVA
Sitepair		Fixed	ANOVA
Exposure		Fixed	PERMANOVA
Billabong	Exposure	Random	PERMANOVA

Furthermore, because the two analysis models use different datasets (and hence different factors) the approach to interpreting the two sets of results also differs. The interpretation of results arising from each model is detailed in Table A2. In summary, impact detection viz the BACIP ANOVA model is assessed using the 'BA' factor if the 'sitepair*BA' factor is not significant (ie that all three sitepairs show a consistent change from the before to after period). However, in this monitoring design the sitepair*BA interaction is likely to be the most important because it will indicate if a sitepair is responding differently to the other sitepairs (ie a sitepair that is closer to the minesite is more likely to show mine related change). In this situation further investigation of any aberrant sitepair would be required. In PERMANOVA, the BA*Exposure interaction (not available in ANOVA) is the important source of variation to interpret for impact detection after ensuring the BA*Billabong(Exposure) is not significant (ie that all billabongs within each exposure type show a consistent change from the before to after period). In this monitoring design, equivalent to the BACIP ANOVA model, the BA*Billabong(Exposure) interaction is likely to be the most important because it will indicate if billabongs within exposure types are responding differently to the other sitepairs (ie an

exposed billabong that is closer to the minesite is more likely to show mine related change). For PERMANOVA, the BA factor is not used for impact detection, but can be used to determine the nature of any natural change from before to after when the previous interactions are not significant (ie the BA change is consistent between exposures and amongst billabongs within exposures).

Inferences made about mining impact differ between the PERMANOVA and the BACIP ANOVA methods due to the different allocation of fixed versus random factors. Because the sitepair factor in the BACIP ANOVA is 'fixed', interpretation of the results is specific for the sitepairs identified. In PERMANOVA, the billabong factor is assigned 'random', and while in the present study random assignment does not change the exposed-reference billabong allocation (ie same billabongs assigned), the results may differ because of the underlying recognition, and hence, treatment of data, that inference is being made about fish communities generally (as opposed to specifically selected billabongs) from similar types of billabongs from across the region.

Analysis using the BACIP ANOVA on sitepair dissimilarity values is constrained in the number of years of data that are available for analysis (see section 5.2.2 in main report). The constraints arise because sitepair dissimilarities require, and are based upon, data that are gathered *concurrently* for each of the sitepairs. This has not been possible over the monitoring period. Furthermore, the ANOVA model employed (using Minitab) is sensitive to missing years of data. PERMANOVA is not constrained by the sitepair configuration (due to the analysis partitioning data for each billabong), and can be applied to unbalanced datasets resulting from missing data (see Anderson et al 2008, PERMANOVA unbalanced designs). As a consequence, PERMANOVA is capable of analysing the complete dataset. However, for this comparison, both analysis approaches have used the reduced dataset described in section 5.2.2 of the main report. The years included in the before period are 1998, 2000 & 2001 (combined), 2003-2007 with year 2009 as the after period.

The analysis methods for the BACIP ANOVA are described in section 2 of the main report. For PERMANOVA, the analysis has followed the BACIP ANOVA data preparation procedures with the exception of the sitepair stages. The default settings in the PERMANOVA analysis package (Anderson et al 2008) have been used with the following exceptions:

1 The number of permutations was increased to 9999.

2 An unrestricted model was selected in order to match the approach used by Minitab's General Linear Model (GLM, required for unbalanced designs) ANOVA. Selecting an unrestricted model in PERMANOVA is achieved by un-selecting the 'Fixed effects sum to zero'. See below (section A4) for a brief discussion on the different models.

Table A2 interpretation of each factor and interaction for the PERMANOVA and BACIP ANOVA analyses on fish community structure data in shallow billabongs. Important factors/interactions for interpreting impact detection are identified.

Factors	Relevant method	Interpretation
BA	PERMANOVA	A significant result Indicates change from the before to after periods across all billabongs and exposures. While this factor is not directly interpreted for impact detection, it can be interpreted for non significant BA*Exposure and BA*Billabong(Exposure) interactions to indicate if change from before to after has occurred.
	ANOVA	**Key factor for impact detection**, A significant result indicates change from the before to after period in the magnitude of control-impact dissimilarity across all sitepairs. A non-significant result for this factor needs to be interpreted carefully because a significant BA*sitepair interaction may indicate that the three sitepairs are not consistent within or between the before and after periods. A significant BA*sitepair interaction requires further investigation to understand the nature of the differences amongst sitepairs.
Years(BA)	PERMANOVA	A significant result indicates if years differ *within* the before or after period across all billabongs and exposures. This factor is not important for impact detection.
	ANOVA	A significant result indicates the magnitude in dissimilarity across all sitepairs differs amongst years. This factor is not important for impact detection.
Sitepair	ANOVA	A significant result indicates that the magnitude of control-impact dissimilarity differs amongst sitepairs. This factor is not important for impact detection.
Exposure	PERMANOVA	A significant result indicates differences between the two exposure types (across all billabongs within each exposure type). May indicate mining impact, though care in the interpretation is needed because any differences could be due to natural variation between catchments. Consideration of the Billabong(exposure) factor is needed to determine if natural variation between billabongs occurs (indicated if this factor is significant). Exploration of correlates of fish community structure using BIOENV analysis may be required to aid interpretation (ie natural habitat variation versus mine-related water quality).
Billabong(Exposure)	PERMANOVA	A significant result Indicates billabongs differ within either or both exposure type. Pairwise comparisons are required to explore a significant result to test whether differences occur amongst billabongs within control or exposed designations, or both. Differences amongst billabongs within both exposure types most likely indicate natural differences amongst billabongs.
BA*Sitepair	ANOVA	**Key interaction for impact detection**. A significant result indicates that the BA result above is not consistent amongst the three sitepairs which Indicates one or more of the sitepairs differs between, or within, the before and after periods. For a significant result, further investigation is required to understand the nature of the differences amongst sitepairs. For example, it is possible that one sitepair has changed significantly from the before to after period, but not the other two (eg one exposed site situated closer to the minesite is impacted). In this case the sitepair of interest would require further investigation to determine the cause of such changes.
Sitepair*Years(BA)	ANOVA	A significant result indicates that the magnitude of control-impact dissimilarity is not consistent amongst sitepairs over time. This interaction is not important for impact detection.
BA*Exposure	PERMANOVA	**Key interaction for impact detection**. A significant result indicates that across billabongs within each exposure type the change from before to after periods is not consistent between the control and exposed billabongs (suggesting minesite influence). A non-significant result for this factor needs to be interpreted carefully because a significant BA*Billabong(Exposure) interaction may indicate billabongs within either, or both, exposure type are not consistent within or between the before and after periods. A significant BA*Billabong(Exposure) interaction requires further investigation to understand the nature of the differences.
BA*Billabong (Exposure)	PERMANOVA	**Key interaction for impact detection**. A significant result indicates that billabongs within either, or both, exposures are not consistent within or between the before and after periods. When significant, this interaction is the most important for impact detection because, for example, it might reflect that one exposed billabong (closest to the minesite) is responding differently to all other billabongs. A significant result for this interaction requires further investigation to determine the nature of the inconsistencies and whether or not they indicate mine impact. Pairwise comparisons can be used to explore a significant result.
Year(BA)*Exposure	PERMANOVA	A significant result indicates that across billabongs within each exposure type the changes amongst Years(BA) are not consistent between the two exposures. This interaction is not important for impact detection.
Year(BA)*Billabong (Exposure)	PERMANOVA	A significant result indicates that the variation amongst Years(BA) is not consistent amongst billabongs within either, or both, exposure type. This interaction can be useful for impact detection if, for example, variation over time was only detected amongst exposed billabongs. Pairwise comparisons can be used to explore a significant result.

A3 Comparison of PERMANOVA and BACIP ANOVA results

The complete results for PERMANOVA are shown in Table A3. The complete results for the BACIP ANOVA analysis are available in section 5.2.2 (Table 5) of the main report. For both PERMANOVA and the BACIP ANOVA analyses, a summary interpretation is provided in Table A4.

From Tables 5 (main report) and A3, the PERMANOVA analysis has a greater total degrees of freedom (237) than the BACIP ANOVA (119) so, theoretically, PERMANOVA should provide greater statistical power due to the inclusion of more data.

Table A3 PERMANOVA results for shallow billabong fish community dissimilarity values using three exposed billabongs (Coonjimba, Georgetown & Gulungul) and three control billabongs (Buba, Sandy shallow & Wirnmuyurr). Years Before include 1998, 2000 & 2001 (combined), 2003-2007 and years After are 2009.

Source	df	SS	MS	Pseudo-F[1]	P(perm)[2]	Unique perms[3]
BA	1	2263.50	2263.50	0.61	0.9254	9904
Year(BA)	6	31554.00	5259.00	2.78	0.0051	9925
Exposure	1	29196.00	29196.00	2.13	0.0317	9906
Billabong(Exposure)	4	51146.00	12787.00	7.32	0.0008	9941
BA*Exposure	1	304.34	304.34	0.63	0.9137	9912
BA*Billabong(Exposure)	4	6987.80	1747.00	0.89	0.6136	9919
Exposure*Year(BA)	6	11350.00	1891.70	0.95	0.5565	9879
Year(BA)*Billabong(Exposure)	24	47844.00	1993.50	3.75	0.0001	9772
Res	190	100880.00	530.92			
Total	237	311840.00				

Superscripts 1 = Pseudo-F is the permutation equivalent to a standard F value, 2 = P(perm) is the permutation equivalent to a standard p (significance) value, 3 = Unique perms are the number of unique permutations used to determine P(perm).

A3.1 Impact detection

The results of the PERMANOVA analysis showed that exposed and control billabong fish communities are consistently similar between the 'before' and 'after' periods (BA*Exposure, $p = 0.9137$) and that billabongs within the two exposures are also consistent from before to after (BA*Billabong(Exposure), $p = 0.6136$) (Table A3). Because both these interactions are non-significant, the BA factor ($p = 0.9254$), also non-significant, indicates that no change was detected from the before to after period. Collectively, these results indicate that control and exposed billabong fish communities (or any billabong within an exposure type) have not changed from the before to after period and hence there is no indication of a mine-site influence at the exposed sites in the after period.

The results for the BACIP ANOVA indicate that no change in the difference (dissimilarity) between control-impact fish communities has occurred from the before and after periods across sitepairs (BA, $p = 0.474$) and this is consistent amongst the three billabong sitepairs (BA*sitepair, $p = 0.057$) (Table A4), though the marginal non-significance suggests some differences amongst the three sitepairs between before to after periods (See section 5.2.2 in main report for further discussion on this.). In this analysis, interpretation is not available for each control or impact billabong that makes up the sitepair and as a result, information on which billabong is changing over time is not available.

To this end, the inclusion of temporal and spatial variation in the PERMANOVA analysis (using the multivariate dissimilarity matrix) enables greater interpretation of the data and hence greater assurance that fish communities at the exposed billabongs have not been influenced by minesite activity (non-significant BA*Exposure and BA*Billabong(Exposure) interactions).

A3.2 Temporal variation

For PERMANOVA, variation over time is detected across all billabongs in the before or after period, or in both periods (Year(BA), P = 0.0051). Because there is no replication in the after period, this variation is within the before period. Furthermore, the variation over time is not consistent amongst billabongs within either exposure type (Year(BA)*Billabong(Exposure), p = 0.0001) (Table A3). Pairwise comparisons (using the pairwise comparison option in the PERMANOVA software) amongst years within all billabongs confirm (results not shown here) that years differ over time within billabongs of *both* exposure condition, indicating this is natural variation.

Despite the BACIP design approach (aims to remove temporal variation), temporal variation is detected by the BACIP ANOVA (sitepair*years(BA), p = 0.016) (Table A4) which appears to be related to the varying response of fish community to changes in aquatic plant type and density over time (see sections 6.2.1.2 and 6.2.2 in main report for further discussion on this). Because the variation is associated with the change in magnitude of the dissimilarity between control and exposed sitepairs, it is extremely difficult, or may not be possible, to identify a suitable covariate that can account for such natural variation. In the absence of a covariate that accounts for this natural variation, the BACIP ANOVA is limited in its ability to detect change that could be due to mining impact.

Whilst the dissimilarity data matrix used by PERMANOVA includes the natural variation in fish communities associated with changes in aquatic vegetation type and density, it is not restricted by the sitepair approach that is used by the BACIP ANOVA method. PERMANOVA allows fish communities to be compared within each billabong (or within each habitat type) and in doing so, the magnitude in change between two billabong fish communities (or the magnitude of change in habitat type and density) can be better explained. As a result, PERMANOVA is more suited to distinguishing potential mining impact within each exposed billabong from natural variation inherent at that site. Furthermore, there is enhanced potential to determine a suitable covariate to account (and hence model) natural variation when using the raw individual billabong data (ie viz BIOENV analysis).

A3.3 Spatial variation

Not surprisingly, the BACIP ANOVA successfully removes spatial variation in billabong fish communities by its control-impact sitepair approach.

The PERMANOVA analysis shows significant differences between control and exposed sites (Exposure p = 0.0317) and significant differences amongst some billabongs within either exposure type (Billabong(Exposure), p = 0.0008). Further analysis using pairwise comparisons (results not shown here) shows that across all years significant differences occur between billabongs within both exposure conditions. These results suggest that billabong fish communities are naturally different from one another. To this end, the significant difference between exposures could be simply due to natural catchment differences, given that exposed sites are located on Magela Creek in close proximity to one another. Exploratory analysis using BIOENV indicates mine-related water quality is not a driving influence over fish community structure (see section 6.2.1.2 of main report).

Table A4 Comparison of results from a four-factor PERMANOVA with the three factor ANOVA for shallow billabong fish community dissimilarity values using three billabong sitepairs: Coonjimba (CJM) vs. Buba (BUB); Georgetown (GTN) vs. Sandy swamp (SDS); and Gulungul (GUL) vs. Wirnmuyurr (WIN). N/A indicates test not available.

Factors	PERMANOVA	BACIP ANOVA	Interpretation
BA	0.9254	0.474	PERMANOVA; across both exposure types and billabongs, no change in fish communities between the before and after periods.
			ANOVA; across the three billabong sitepairs no change in the magnitude of fish community dissimilarity (control-impact) between the before and after periods. The result for this factor needs to be interpreted carefully, however, because of the low p value for BA*sitepair (p = 0.057) interaction, which indicates the three sitepairs are not consistent within or between the before and after periods (see BA*sitepair interaction below) .
Years(BA)	0.0051	0.122	PERMANOVA; across exposures and billabongs, fish communities show differences amongst years in the before period (no replication in the after period). The Year(BA)*Billabong(Exposure) interaction (below) indicates this variation is present amongst billabongs of both exposure types, suggesting natural variation over time.
			ANOVA; across all sitepairs, no differences in the magnitude of fish community dissimilarity (control-impact) amongst years in the before period (no replication in the after period), though this is not consistent amongst sitepairs – see Sitepair*Years(BA) interaction
Sitepair	N/A	0.110	Magnitude of dissimilarity is similar amongst sitepairs for all years.
Exposure	0.0317	N/A	Control and exposed sites differ, but within each exposure type billabongs also differ (significant Billabong(Exposure) interaction), suggesting natural catchment differences due to the close proximity of the exposed billabongs to one another. Exploratory analysis using BIOENV indicates mine-related water quality is not the driving influence over fish communities.
Billabong(Exposure)	0.0008	N/A	Billabong fish communities differ within both exposure types, confirmed by pairwise comparisons.
BA*Sitepair	N/A	0.057	The BA result above is consistent amongst the three sitepairs, though the marginal non-significance suggests some differences amongst the three sitepairs within or between before and after periods (see section 5.2.2 in main report for further discussion on this.) Exploratory analyses indicate fish communities are influenced by changes in aquatic plant type and density over time resulting in large variations in the sitepair dissimilarity values.
Sitepair*Years(BA)	N/A	0.016	The magnitude of fish community dissimilarity (control-impact) is not consistent within the before or after periods amongst the three sitepairs.
BA*Exposure	0.9137	N/A	The BA result is consistent between control and exposed billabong types. Given the BA response is consistent for billabongs within each exposure (see BA*Billabong(Exposure) below), ie the change from before to after is consistent for the control and exposed billabongs, and hence no minesite influence is evident.
BA*Billabong(Exposure)	0.6136	N/A	The BA result is consistent amongst billabongs within each of the two exposure types (ie no change from the before to after period for any billabong within either exposure). This result enables confident interpretation of the BA*Exposure interaction.
Year(BA)*Exposure	0.5565	N/A	The Years(BA) result is consistent between the two control and exposed billabong types. However the Year(BA)*Billabong(Exposure) interaction indicates annual variation is not consistent amongst billabongs within either exposure type.
Year(BA)*Billabong(Exposure)	0.0001	N/A	The years(BA) result is not consistent amongst billabongs within either exposure type. Pairwise comparison confirms fish communities vary over time in all billabongs, thus variation is considered natural.

A4 PERMANOVA and Minitab program functionality differences

There are two noticeable programming advantages to PERMANOVA over Minitab in this comparison. These are:

1. PERMANOVA allows pairwise comparison (eg between billabongs or years) with random factors (after a suitable warning). This is useful when exploring significant differences in factors that are considered random, but in reality are not truly random (ie the factors years and billabongs – same billabongs used over time after the initial selection). Minitab will not conduct pairwise comparisons on random factors.
2. PERMANOVA allows the choice of analysis model type (restricted or unrestricted models), but defaults to a restricted model to overcome the intrinsic over-parameterisation of the ANOVA model (see Anderson et al 2008, p. 45). The GLM ANOVA required in Minitab uses an unrestricted model. The choice of model (restricted versus unrestricted) appears to be still debated amongst statisticians (Quinn & Keough 2002, box 9.7 p. 233; Anderson et al 2008) and is not discussed further here. However, use of either of the two models does not influence the interpretation of results for impact detection when applied to the current dataset[4]. For other comparisons, however, differences can occur and this requires further consideration/advice to determine the most appropriate model.

A4.1 Conclusions

Interpretation of the PERMANOVA results support the ANOVA results in showing that no change in fish community structure has occurred from the before to after period. However, in this respect, PERMANOVA utilises the temporal and spatial variation (using the multivariate matrix) which enables further partitioning within the dataset and hence greater interpretation and greater assurance that fish communities are not influenced by minesite activity. In particular:

1. Its ability to enable interpretation of changes from before to after for *each* billabong fish community - rather than providing a result for possible change in the magnitude in dissimilarity between control-impact sitepair but without the ability to determine whether the change is occurring at either, or both, billabongs.
2. Its potential to detect changes in fish community structure that might occur in different directions in multivariate space but pass unnoticed in the one-dimensional sitepair dissimilarity data (see Figure A1).

The BACIP approach is an established design for impact detection, particularly with paired up and downstream comparisons and the graphical presentation of sitepair dissimilarity values is succinct, providing a control-chart approach to detecting changes in community structure between two sites. The analysis (using fewer factors) is also simpler to interpret. However, in the monitoring of fish communities in shallow billabongs, the influence of different aquatic plant types and densities upon fish communities results in temporal variation in the control-

[4] Comparison of restricted versus unrestricted models with the lowland billabong fish community data has been conducted. Results for the multivariate dissimilarity data analysed using PERMANOVA and using restricted and unrestricted models, were similar. However, analysis of the BACIP sitepair dissimilarity data (analysed using PERMANOVA) using both models gave different results for the Year(BA) factor (a significant result for the restricted model and a non significant result for the unrestricted model).

impact sitepair dissimilarity values that limits the BACIP ANOVA's ability to detect minesite influence (in the absence of a covariate to account for this variation).

From a programming perspective, the PERMANOVA package provides benefits over the Minitab program in relation to:

1. The analysis of unbalanced datasets (replicates missing from some years and/or billabongs): PERMANOVA is capable of analysing the complete dataset from 1994 to 2009.
2. Minitab does not allow pairwise comparisons on random factors. Pairwise comparisons on random factors need to be done with caution, as comparisons between terms which are truly randomised (ie sites randomly selected each sampling time) are meaningless.
3. The ability to select the analysis model type (restricted or unrestricted model).

A5 References

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