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

Supervising Scientist Division

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GPO Box 461, Darwin NT 0801

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Authors of this report:

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

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

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Contents

Tables

Figures

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 channel 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 channel billabongs near 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, Jones et al 2009).

Full details of methods and procedures described in this protocol are contained in a corresponding 'Operational manual' which is the working document used by monitoring staff at the Jabiru Field Station to conduct environmental document procedures. The additional material that the Operational manual contains (that is not contained in this published document) includes:

- photographs and maps of the location of sample transects
- fish identification photographs and summary information on key references and supporting studies
- instructions on use of meters and other instrumentation
- data-sheet pro-forma for collection of field data
- data codes for fish and environmental variables
- worked examples of statistical procedures
- a list of all required reports.

Acknowledgments

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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 have provided most of this assistance.

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Advice and assistance with access from Parks Australia North and Energy Resources of Australia (ERA) are gratefully acknowledged.

Dr Keith McGuinness, Charles Darwin University, provided most of the advice for, and review of, development of the statistical model and the statistical robustness and power of the impact detection methods used in this protocol. David Jones provided critical review of the draft protocol.

Gunther Schmida took the fish photographs and Mick Alderson, Violet Lawson, Kate Boyd and Kate Duigan provided advice on the Aboriginal names of the fish.

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

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

Supervising Scientist Division

1 Introduction

1.1 Objective

Detection of any[1] adverse effects of operations at the Ranger uranium mine on downstream fish communities resident in the channel billabongs of Magela Creek.

1.2 Background

Fish and benthic macroinvertebrates are key components of the aquatic ecosystems of Magela Creek that are potentially at risk from contaminants from the Ranger uranium mine. In particular, if contaminated waters are present, these organisms will be continuously exposed via the large surface areas of their gills (Humphrey et al 1990). ***eriss*** has developed procedures for monitoring the health of these ecosystem components as part of the Supervising Scientist Division's integrated physio-chemical and biological monitoring program (Van Dam et al 2002, Jones et al 2009). This protocol describes one of these procedures, used for monitoring fish in channel billabongs.

Channel billabongs are very important habitats in the seasonally-flowing streams of the NT's Top End since these relatively deep, permanent waterbodies serve as refuge sites for fish in the dry season (Bishop & Forbes 1991). The permanent waterbodies also serve as major sites for harvesting fish for consumption by local residents, particularly the larger-growing species.

The monitoring procedure measures the abundances of individual species comprising the fish community structure. Whilst univariate metrics of community structure such as species richness (or number) and abundance are acquired, the focus is on the use of multivariate statistical procedures that quantify the extent of similarity of different communities from different samples or sites, by use of metrics that incorporate collective species abundance information. Monitoring whole communities has the potential advantage that species with a wider range of sensitivities to different stressors will be included in the assessment. At least 32 fish species have been recorded in Magela Creek downstream from Ranger Mine (Bishop et al 1990).

A non-destructive monitoring method is preferred for monitoring programs that require repetitive sampling in highly-valued conservation areas such as Kakadu National Park. The protocol described here uses visual observation as the method for characterising the status of fish communities. The effectiveness of this method in the Alligator Rivers Region (ARR) where high water clarity provides adequate visibility has been demonstrated by a significant

[1] Positive as well as negative impacts on fish are possible. In particular, inputs of low levels of solutes to receiving waters could induce non-natural enhanced (hormetic) effects in fish communities which could provide a trigger for management action.

body of research (Bishop et al 1986, Pidgeon & Humphrey 1992, Pidgeon & Boyden 1995). The monitoring is timed to coincide with periods of maximum visibility in the recessional flow period at the end of the wet season. The present design of the monitoring program was implemented in 1994, noting that fish community data and environmental data had previously been collected in Mudginberri Billabong from 1989 to 1993 (for methods used during this time see Pidgeon et al 1992).

1.3 Principle of the monitoring technique

The monitoring method is designed to detect effects of water quality, integrated over a wet season, on fish communities in a channel billabong downstream of the Ranger Mine. Data are obtained annually during the recessional flow period (ie early dry season) when fish movement between refuge billabongs is restricted by falling water levels. The abundances of different fish species are measured along the margins of two channel billabongs: one located downstream of the minesite ('exposed' site, Mudginberri) and one located on a separate catchment unaffected by mining activity ('control' site, Sandy).

For safety reasons (presence of saltwater crocodiles) observations are made from a boat equipped with a transparent observation bow. The fish species and their abundances are recorded from 5 fixed transects located along the bank in each billabong. Two trained observers are used to make four counts in each of the five transects to minimise effects of observer bias and maximise the number of species detected.

The structures of the fish communities as measured by species and relative numbers are compared between the two billabongs, and with community structure values obtained for previous years.

2 Experimental design

Fish community structure data are collected at the same time of year from 5 transects from within each of two billabongs. At each transect, four replicate visual fish counts are made by two trained observers. The count data from the four observations at each transect are pooled (average taken) to best represent the sampled area (see section 3.6 for details). The two billabongs include one *exposed* site billabong on Magela Creek (Mudginberri Billabong), *directly* exposed to contaminants from the Ranger uranium mine and one *control* site located in Nourlangie Creek (Sandy Billabong). Figure 1 illustrates the experimental design used.

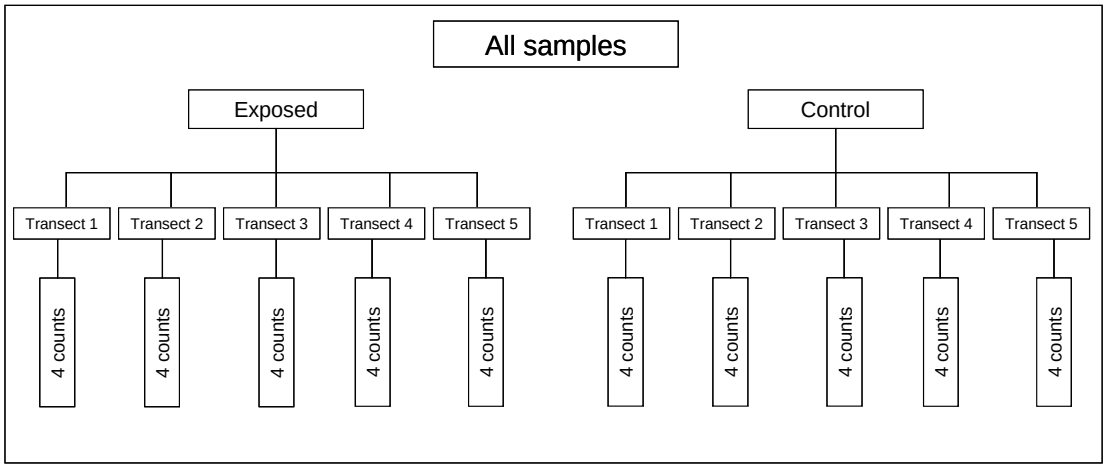

Figure 1 Channel billabong fish community monitoring design

2.1 Statistical design and analysis

This monitoring technique is based on principles of a Before-After-Control-Impact-Paired differences (BACIP) design described by Stewart-Oaten et al (1986, 1992). The BACIP design uses a form of temporal replication. The *difference* between sampled responses at the *sitepairs* (control-minus-exposed) at any one time is regarded as a replicate observation. 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 monitoring (Faith et al 1991, 1995); these values reduce the differences between the two communities over 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 may, for example, represent before versus after periods.

Specifically, replicate observations in the model are Bray-Curtis dissimilarity values derived for each of the randomly-paired, exposed-control transects (from section 2 and Figure 1); the replicate observations provide an estimate of error (or residual) variation. For the community data collected for the billabong 'sitepair', five dissimilarity values are derived, each representing one of the five possible randomly-paired exposed and control site transects. Mean dissimilarity values are calculated before and after (BA) an event or period of interest.

2.1.1 BACIP design with ANOVA

1 BACIP design and ANOVA model

The BA values are statistically tested (compared) using a nested two factor ANOVA model.

The model for the ANOVA is: BA + Year(BA).

In this model, BA is a fixed factor (or effect), testing for differences between 'before' and 'after' periods. 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).

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 in analyses of the data gathered in the current protocol. However, they could be included as response measures, using corresponding (univariate) difference values between the randomly-paired, exposed-control transect areas as input data to the ANOVA model.

The ANOVA results table is described in Table 1.

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

Source	df	F
BA	1	$MS_{BA}/ MS_{Years(BA)}$
Years(BA)	$(b - 1) + (a - 1)$	$MS_{Years(BA)}/MS_{Error}$
Error	$(b + a)(n - 1)$	
Total	$n (b + a) - 1$	

Where significant differences are found to occur between a time-series of sitepair dissimilarity 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 Magela and Gulungul creeks upstream (Environmental Research Institute of the Supervising Scientist and Energy Resources of Australia water chemistry monitoring programs) and in each billabong at the time of fish monitoring, 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 monitoring, 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.

2 Hypotheses

The monitoring technique based upon the ANOVA model is designed to evaluate the primary null hypothesis that there has been no change in fish community structure at the Magela Creek exposed site, relative to the Nourlangie Creek control site:

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 the period of interest) equals mean dissimilarity after event (or the 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.

The second factor in the model, Year(BA), can be used to determine whether, within the Before and/or After periods, any set of dissimilarity values are significantly different amongst years, 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:

(2) 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 (2) is rejected. The mean dissimilarity amongst years differs within either the before or after period.

Interpretation of the Year(BA) factor requires further analysis using Tukey's pairwise comparison, to determine if significant differences occur only in the 'After' period (variability) that could indicate impact.

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

2.1.2 BACI design and PERMANOVA model

PERMANOVA (PERmutational Multivariate ANalysis Of Variance) (Anderson 2001, McArdle & Anderson 2001, Anderson et al 2008), an add-on function of PRIMER multivariate software (Clarke & Gorley 2001, Clarke & 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.

Unlike the BACIP approach where sitepair dissimilarities are employed to meet data assumptions of independence of temporal replicates, PERMANOVA is not so constrained. It uses data from the individual sites and as such offers increased partitioning of data variation (and hence increased factors). Its ability to use the complete multivariate dissimilarity 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 Appendix 1 for further information).

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).

2.1.3 Trend analysis and accounting for temporal variation in the BACIP design

Temporal variation in fish responses associated with longer-term climate events (eg interannual, including decadal-scale, cycles in rainfall), or unpredictable and stochastic events, must be considered in the monitoring design and associated analyses to determine whether or not trends over time are occurring. A trend over time that is unrelated to mining could result in problems for detection of an impact based on a comparison of the dissimilarities between two time periods (before versus after). Aside from the fact that trends, natural or mine-related, would indicate that the annual replicate observations (dissimilarities) are not statistically independent (hence violating an important ANOVA assumption), a significant difference in the dissimilarity value between a before and after period could mistakenly be interpreted as a mine-related change unless such temporal variation is explained and accounted for by way of suitable natural covariates.

A significant trend in the monitoring response data over time, either before or after putative impact, may be evident from one or both of the following observations:

a. Most obviously, from a plot of the annual dissimilarities against time (years); and/or
b. When testing for ANOVA assumptions (section 5.2.2), lack of independence (autocorrelation) of the temporal replicates (ie the annual dissimilarity values) is observed.

1 Trend analysis using regression

The existence of a trend in the data arising from triggering one or both of (a) and (b) above can be tested for using regression analysis. For this, the dependent variable, sitepair dissimilarity, is regressed with time and against a null hypothesis, in the case of simple linear regression, that the slope of the line, ß = 0 (ie no significant relationship). Quadratic (unimodal), or higher order polynomials for increasingly cyclic relationships (such as peaks and troughs that can occur over decadal periods of rainfall variation), may also be fitted and tested for, against null hypotheses that the quadratic, cubic etc terms are also = 0.

Keough and Mapstone (1995) advise that data exhibiting trends may be used in analysis for impact detection by comparing trends in data between impact and control locations before and after putative impact. However, methods that account for temporal variability, preferably using natural covariates that have a strong causal relationship with the response variable, or are useful surrogates for other explanatory variables, are preferred over approaches that use time per se as the independent variable. In this way, known (natural) explanatory covariates may be discounted in a causal manner where a significant result is observed. Two approaches, Time-series intervention analysis or Analysis of covariance are potentially suited to monitoring data that exhibit temporal trends. Time-series analysis requires at least 50 data observations (eg Rasmussen et al 1993). However, since the present monitoring technique has acquired only 17 observation years using the sitepair design, this method of data analysis cannot be used.

2 ANalysis of COVAriance (ANCOVA)

Where natural covariates are identified as directly contributing to trends or changes in fish communities over time and a regression relationship between fish response (eg dissimilarity) and the environmental variables is established, there is potential to incorporate the covariates into ANalysis of COVAriance (ANCOVA) to test for changes between two time series of results. ANCOVA is a merger of ANOVA and regression, and tests whether, in the case of putative mining impact, associated changes in water quality (for example) alter the sitepair dissimilarity after removing the variance for which the quantitative predictors (covariates) account. In simple terms, ANCOVA determines whether a (biological) response-environment relationship that is deemed to be uninfluenced by mining has changed over time, which could infer a mine-related impact.

The main null hypothesis in ANCOVA is that there is no difference in sampled means of *Y* (dissimilarity) when these are adjusted for a common mean of *X* (covariate) and a common regression line.

The BIOENV routine from the PRIMER multivariate analysis software (Clarke & Gorley 2001, Clarke & Gorley 2006) is used to determine suites of environmental variables that best correlate to the fish assemblage patterns (based upon the dissimilarity matrix derived for the biological data). This procedure, together with Pearson correlation, is used to assist in identifying potential environmental covariates that may be used in regression and subsequent ANCOVA.

3 Patterns in dominant fish species

In the event a significant trend in time in the sitepair dissimilarity value is observed, and whether or not a covariate of useful explanatory value can be found, the dissimilarity is 'decomposed' to determine which of the constituent fish species are most influential in contributing to the trend in the dissimilarity. This is achieved using the SIMilarity PERcentages (SIMPER) multivariate routine from the PRIMER (v6) software package which examines the taxa contributing most to the differences between control and exposed locations (Clarke & Gorley 2001, Clarke & Gorley 2006). Abundance data for the influential fish species are plotted for the control and exposed locations over time and trends in these species,

in turn, tested for. When trends are seen, identification of significant explanatory covariates is sought, and suitable regression relationships between fish abundance and the covariate(s) are derived. The ANCOVA method from step 2 above may be applied to the fish abundance and covariate data to test for impact.

2.1.4 Multivariate analyses to assist in impact assessment

Inferences about mining impact are not solely reliant upon formal statistical testing techniques that underpin the approaches described above. Fish community data are also displayed according to the underlying Bray-Curtis dissimilarity matrix by Multi-Dimensional Scaling (MDS) ordination (Clarke & Gorley 2001, Clarke & Gorley 2006). The resulting ordination graphic depicts the patterns in fish community structure for each billabong transect over time and the relationship of these transect communities to each other. Points on the MDS with greatest separation represent transects with greatest differences in fish community structure. While the visual arrangement of transect communities shown in the MDS may be formally tested (using PERMANOVA, see section 2.1.2 above), the graphical representation of the sample points alone may assist in determining the significance of year to year changes in the structure of fish communities from the control and exposed billabongs.

The multivariate SIMPER and BIOENV routines described above can also assist in identifying possible causal mechanisms by providing a greater understanding of the fish species and environmental (including habitat) factors contributing to the separation of billabongs.

The suite of multivariate analyses described in this section is not simply an add-on to the formal statistical tests for impact detection described above in 2.1.3. Rather, the two approaches are run side-by-side and each informs the other to increase the power of the approach for not only detecting impact, but also assigning a cause.

3 Monitoring procedures

3.1 Occupational health and safety

eriss has established project and field safety approval 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, mosquito borne disease and crocodile attack. Due to the potential for crocodile attack, all monitoring and observations are conducted from within boats, and the smaller visual boat is only used in close proximity to the two accompanying larger and more stable boats that are used by the data recorder and designated crocodile spotter respectively. Details on how to mitigate the identified health and safety risks for this project are discussed in the Operational manual for this protocol.

3.2 Consultations required for site access

The monitoring is conducted within Kakadu National Park (KNP) which is jointly managed by Parks Operations and Tourism and the local traditional owners who maintain strong cultural ties with their lands. The following stakeholders need to be consulted, via SSD's community liaison officer, to ensure necessary protocols are followed, prior to the start of work:

- Local traditional owners should be consulted directly through their community organisations for approval to access their land and to conduct the monitoring. The

Northern Land Council should be advised of intended activities well in advance of starting the field work.

- The Parks Operation and Tourism Branch project officer and local district offices should also be notified in advance to ensure that park management activities do not conflict with monitoring dates (eg feral animal control, fire management).

3.3 Timing of monitoring

The fish community monitoring is conducted annually, during post wet season recessional flows. At this time, species richness and abundances are highest and the monitoring is most likely to capture the integrated effects of inputs over the wet season of mine runoff waters (Humphrey et al 1990). This is also the earliest that access to the sites can be achieved.

A gauge height reading of 11.8 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 1.77 m (equivalent flow of around 370000ML per day) at gauging station G8210009 (near the Magela downstream monitoring point), indicates conditions suitable for monitoring (no significant movement of the dominant fish species) and conditions that are dry enough to enable overland access to the monitoring 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 monitoring in mid May. However, when rainfall ceases earlier or later than early April, the timing of monitoring is adjusted accordingly, with particular emphasis on ensuring no migrations of the dominant fish species are occurring during the period that the observations are being made. When rainfall ceases early it is best to allow the stream height to fall further (no less than 11.4 m at the upstream gaugeboard) to ensure any follow-up rains that could trigger fish migrations, do not occur during the monitoring period. A late end to the wet season has a reduced risk of follow-up rains and sampling should occur as early as possible before water temperatures fall (see operational manual for further details).

3.4 Monitoring sites

The high mobility of fish along stream systems in this region (Bishop et al 1995) makes the use of upstream-downstream comparisons of doubtful value for detecting effects since the upstream sites are not truly independent of downstream sites. The use of control sites on separate catchments circumvents this problem.

The potentially-impacted site is Mudginberri Billabong, the only large channel billabong occurring in the lowland sand channels of Magela Creek downstream of the Ranger uranium mine. The control site is Sandy Billabong, a channel billabong on Nourlangie Creek, which has no mining in its catchment (Figure 2). Nourlangie Creek is a major tributary of the South Alligator River system and has no direct connection with the East Alligator River system which contains the Magela Creek catchment. Other potentially suitable sites on Nourlangie Creek exist but they are located in culturally sensitive areas and access has not been possible. As a consequence of this and resource constraints, no spatial replication of either Impact or Control treatment currently exists in the monitoring design.

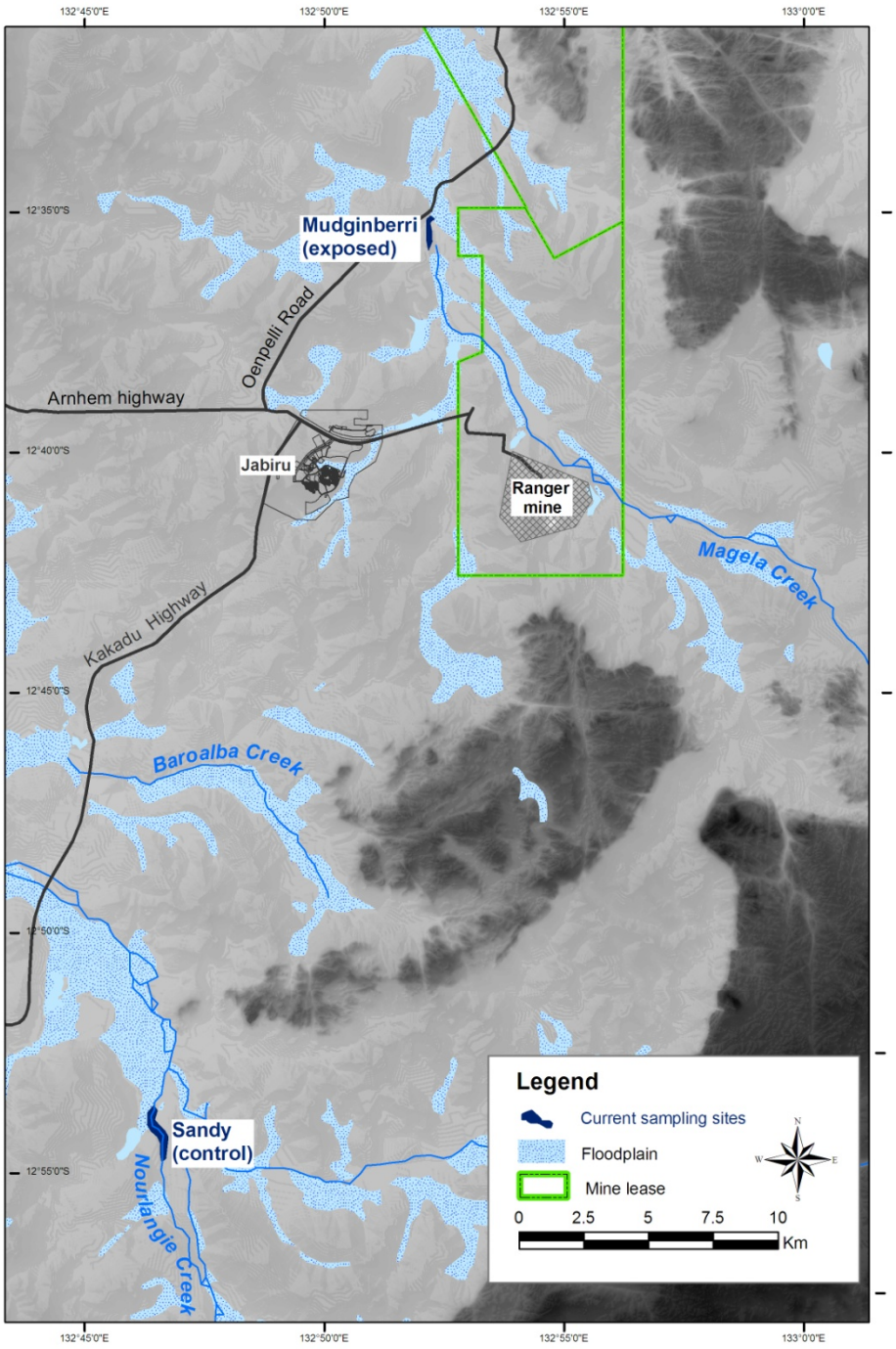

Figure 2 Location of channel billabongs used for monitoring fish communities, using visual observation, in relation to Ranger uranium mine

Mudginberri Billabong is located downstream of the Jabiru township. Contaminants from Jabiru can enter Baralil Creek from surface runoff from the town and from secondary treated sewage water that is disposed of by land application. Baralil Creek then enters Gulungul Creek downstream of Ranger which in turn enters Magela Creek upstream of Mudginberri. Contaminants from these other non-mining sources have the potential to confound interpretation of effects associated with mining operations on fish communities, and this program cannot differentiate these two possible sources of impacts in isolation of associated water chemistry data obtained from strategic sites along Magela and Gulungul Creeks.

3.4.1 Mudginberri Billabong (exposed site)

The impact site, Mudginberri Billabong, is located about 12 km downstream of Ranger Mine. It is a large waterhole 1.4 km long with an average width of about 80 m. The late-dry-season depth at the centre ranges from 1 to 5 m along its length. Much of the billabong margins are lined with the shrub *Pandanus aquaticus* and the riparian zone is otherwise dominated by large trees (*Melaleuca argentia*, *M. leucadendra* and *Syzygium* spp) that can shade the banks and shallow billabong margins, influencing the light climate and aquatic vegetation of this zone. Roots and fallen branches from these trees provide structural complexity to the fish habitat that can influence distribution and abundance of different fish species.

3.4.2 Sandy Billabong (control site)

The control site, Sandy Billabong, is larger than Mudginberri Billabong. It is 3.5 km long but is structurally divided into two different basins: a wide shallow basin in the upstream two thirds which is joined, via a deep narrow channel, to a basin in the downstream one third that is very similar in dimensions (1.2 km long and 50 m wide) and riparian vegetation to Mudginberri Billabong. For this reason, the downstream section was selected as the control for Mudginberri.

3.5 Monitoring transects

Five 50 metre long transects have been established along the littoral margins of each of the two billabongs. The same transects are sampled each year (Table 2). The longitude and latitude values in Table 2 denote the downstream starting point for each transect.

Table 2 Map references for Mudginberri and Sandy billabongs and the monitoring transects within them

Mudginberri Billabong Australian Grid Reference (WGS 84) Mount Brockman. Sheet: 5472 1. Scale: 1 : 50 000 E 2 69 276 N 86 07 000	
Transect monitoring sites	Longitude, Latitude
MUD 1	S 12°35.564, E132°52.533
MUD 2	S 12°35.465, E 132°52.588
MUD 3	S 12°35.379, E 132°52.585
MUD 4	S 12°35.210, E 132°52.536
MUD 5	S 12°35.083, E 132°52.567
Sandy Billabong Australian Grid Reference (WGS 84) Nourlangie Creek Sheet: 5472 2. Scale: 1 : 50 000 E 2 58 850 N 85 72 900	
Transect monitoring sites	Longitude, Latitude
SDC 1	S 12°53.960, E 132°46.632
SDC 2	S 12°53.869, E 132°46.616
SDC 3	S 12°53.810, E 132°46.638
SDC 4	S 12°53.795, E 132°46.658
SDC 5	S 12°53.674, E 132°46.668

3.6 Fish observations and counting

Fish counts in each transect are made visually by an observer looking underwater through a clear acrylic dome located on the bow of a small customised boat. The boat-to-dome fit is designed to allow for visual observations of fish when a person lies prone in the bottom of the

boat and covered by a dark shroud (Figure 3). The boat is manoeuvred by a designated paddler, slowly and quietly along the transect, adjacent to the bank where possible, and in and around emergent obstacles (mostly tree trunks and branches) that block access along the bank.

Each transect is counted four times to obtain an adequate inventory of species present (Buckle 2010). Each count, along the 50 m transect, is standardised by time and must be completed within 15–25 minutes to ensure adequate, but not excessive, observation time. The 10 minute variation in time allows for differing degrees of complexity amongst transects (amount and formation of vegetation lining the banks) that limits manoeuvrability of the visual boat. The four successive counts made at each transect are done alternately by two trained observers to limit observer bias (see section 3.9) (total of two counts per observer). The observer verbally dictates the count to a data recorder located on a separate boat adjacent to the observation boat. The data recorder uses fish codes and pre-formatted datasheets (which includes the most commonly observed species codes) that ensure quick and effective data recording. This enables any uncertain fish identity and/or count to be confirmed with the observer *at the time*. (Such confirmation is not possible via audio recording, an alternative approach that could be employed).

Fish are identified to species level wherever this is possible. However, there are some species that are impossible to routinely distinguish, using visual characteristics, from other species in that genus. In these cases identification is only to genus level. Fish in this category are identified in Table 3.

Table 3 Fish Genus that are not possible to routinely distinguish to species when using the visual census monitoring technique in channel billabongs

Common name	Genus	Species
Glassfish	*Ambassis*	*A. agrammus, A. mulleri & A. macleayi*
Goby	*Glossogobius*	*G. giuris, G. aureus & G.* sp (Munro's goby)
Sleepy cod	*Oxyeleotris*	*O. lineolata & O. selheimi*
Eel-tailed catfish	*Neosilurus & Porochilus*	*N. hyrtlii, N. ater* (juveniles)[1], *P. rendahli, & P. obbesi*
Fork-tailed catfish[2]	*Neoarius*	*N. leptaspis, N. berneyi, N. graeffei & N. midgleyi*

1 Larger *Neosilurus ater* are readily distinguished from other large eel-tailed catfish

2 Uncommon in monitoring transect areas and Identification issues only occur in Sandy Billabong, as only one species (*N. leptaspis*) occurs in Mudginberri

Figure 3 Visual observation boat in operation at Mudginberri Billabong. The visual observer is prone on the bottom of the boat beneath the black shroud, with the boat being manoeuvred along a transect by the boat paddler. Both the data recorder and crocodile spotter are located in the closest of the larger two boats (labelled wetland research) which is attached to a 50 m transect line (the secondary larger boat is tied using quick release knots for rapid deployment if required).

Counting should be done between 0830 h and 1600 h to ensure adequate incident lighting. Sustained periods of heavy cloud cover affect the underwater light climate, and counts should not be conducted during these periods. The angle of the sun influences the counting efficiency. Observations are best conducted with sunlight penetrating the water column, rather than in areas shaded by riparian vegetation. Thus, transects on the east bank are counted in the afternoon and those on the west bank in the morning.

3.7 Environmental variables affecting fish

Change in fish community structure may be associated with changes in water quality or with changes in habitat structure between sites and amongst years. These changes may be caused by natural environmental perturbations or by human-induced change. For example, riparian vegetation which is considered to strongly influence fish habitat structure, can be influenced by altered fire regime, invasion by environmental weeds, large floods, cyclones or foraging by feral animals. As a result of these processes the structure of the riparian habitat available for fish may change from year to year.

For each transect and at the time of fish counting, variables relating to water chemistry, water depth and the structure of the riparian habitat, are measured. Rainfall and stream flow data from the immediate past wet season are also obtained. These may be found to be environmental correlates of any changes to fish community structure data for that year.

3.7.1 Water chemistry

Water quality monitoring (temperature, EC, pH, turbidity, dissolved oxygen) to characterise in situ water quality condition at each of the billabong transects is done during the counting procedure.

Two replicate water samples for later laboratory determination of major ions, metals, nutrients and organic carbon are taken once from a single location within each billabong, in an area adjacent to the five transects. Measured variables are described in Table 4.

3.7.2 Water clarity

Secchi depth is measured prior to any fish counting to ensure adequate visibility (>1.7 m). Measurements are also taken to correspond with each of the transect counts.

3.7.3 Habitat structure

Riparian and aquatic habitat structure found along each transect is measured at five locations, 10 m apart, along the transect. The habitat variables that are measured are described in Table 4.

3.7.4 Rainfall and hydrology

Water level in each billabong is recorded at the time of the survey 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, duration of dry and wet season 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 channel 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[A]	**Depths** – Water depth at 0.5, 1.0, 2.0, 3.0 and 4.0 meters perpendicular to the bank, measured using a depth pole.	m
	Submerged logs and branches – The presence or absence of logs and branches are recorded 0 m, 0.5 m, 1.0 m, 1.5 m and 2.0 m perpendicular to the bank. The depth pole can be used as an aid in determining log or branch presence within 0.5 m either side of each point. A total score, out of 25 for logs and 25 for branches, is recorded for the combined habitat replicates from each fish transect.	0 = absent 1 = present
	Submerged plant cover – Percentage cover for Salvinia, macrophytes (other than Salvinia), pandanus roots, and other roots estimated within a quadrat 4 m wide X 2 m deep adjacent to the bank	%
	Riparian vegetation cover – Percentage cover above the water of riparian vegetation above 2 m high and percentage cover of vegetation below 2 m are estimated within a quadrat 4 m wide along the bank by 2 m perpendicular from the bank.	%
	Distance of: 1) The distance that overhanging vegetation extends out over the water, 2) The distance that dense submerged structure extends perpendicular from the bank (ie pandanus edge) and 3) the distance to dense submerged structure perpendicular from the bank (ie area of open water behind structure)	M

A= measurements taken at each transect, on the day of fish monitoring or shortly thereafter. B = Field-filtered (and unfiltered) samples collected from one location at each billabong and sent to a NATA accredited laboratory, C = Data collated after the wet season form gauging station G8210009.

3.8 Field QA/QC

3.8.1 Training

The counting process 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 to minimise the influence of bias. Details on training procedures are available in the operational manual for this protocol.

3.8.2 Fish identification

On occasion there is insufficient time to view a fish to be certain of its identity. When this occurs, the observer should provide the recorder with a possible identity and a query code. After discussion with other observers at the end of the count, or at the completion of all counts, it may be possible to provide a confident identification, particularly if the other

observer(s) have recorded it. However, if an agreed identity cannot be established the fish should remain as an unknown identity and count. These data are currently not included in the analysis, as to date, unknown identifications have been very uncommon.

Quite often there are large schools of very small fish fry at the time of monitoring. These fish can be difficult to identify unless they are in a field of bright sunlight. Most commonly these schools comprise only one species, eg fly-specked hardyhead (*Craterocephalus stercusmuscarum*). However, juvenile rainbowfish (*Melanotaenia* spp), glassfish (*Ambassis* spp) and blue eyes (*Pseudomugil* spp) can also occur in schools of uncertain identity. If at the completion of all visual counts the fish in the school(s) cannot be identified, the observers should obtain a sample of these schools using a fine mesh dip-net to identify the species and quantify the proportions of different species if more than one species is present.

External quality control of visual fish counts is not deemed an important requirement. This is because the streams and billabongs within the Alligator Rivers Region (ARR) have been extensively surveyed and the common fish species are sufficiently well known and distinct from one another to allow accurate identifications by trained observers (with the exception of those species identified in section 3.6).

Confirmation of current nomenclature is achieved by periodic review of species lists in the ARR by a fish taxonomist (NT Museum & Art Gallery or Territory Wildlife Park). Taxonomic revisions can result in fish species being assigned new, or re-allocated, genus and/or species names.

3.9 Observer bias

Visual assessment techniques that involve many different observers over time are particularly prone to observer bias. For correct interpretation of results it is essential that the influence of observer bias is well documented, so differences amongst 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 3.8.1) complemented with the recalibration and correction procedures outlined below.

3.9.1 Fish counts

Only trained fish observers (see section 3.8.1) collect data for this monitoring procedure. Just prior to the commencement of annual monitoring, trained observers recalibrate there observer skills by conducting trial counts. Between trial counts, observers compare and discuss significant recorded differences in species occurrence and abundance estimates. Due to time constraints, limited access to sites and crocodile safety concerns, it is only practical for each observer to complete two trial observations, usually on the morning prior to commencement of monitoring.

Trained and recalibrated observers counts for this monitoring program are compared, using the procedures outlined in section 5.1.2. Biases relating to the fish species present should not occur with trained and recalibrated observers. However, biases in estimates of abundance for each species can still occur. The detection of observer bias is confounded by the mobility of fish. Because observers alternate counts (as it is not possible for them to make counts concurrently), the movement of fish in and out of the transect area will intrinsically result in variations between observer counts. Fish movement, and its influence on the variation between observer counts, varies amongst species. Larger, open water fish species are recorded infrequently and in low abundance as they move in and out of the transect area. Small schooling species are commonly observed and in greater abundances but can also move in and out of the transect area, creating large variations in abundance. While cryptic fish species remain in the transect

area and are recorded in low abundance, they may move in and out of cover without being detected. To reduce the effect of these natural variations in recorded fish abundances, each observer must complete two counts at each transect. Duplication in counts for each observer minimises the effect on observations of fluctuations in fish movement.

3.9.2 Observer boat paddlers

Observer boat paddling is performed by trained personnel (section 3.8.1). Prior to each year's monitoring, boat paddlers practice their skills and are refamiliarised with procedures.

3.9.3 Habitat assessment

Habitat assessment is performed by trained assessors (section 3.8.1). To minimise bias between observers, the measurements on at least the first transect are performed by both personnel each year. Discrepancies between observers are discussed and observers recalibrate against each other.

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 hardcopy datasheets and printed relevant photographs are archived in SEWPAC /SSD registry files.

Fish observations and habitat data are stored electronically in an Access database which is located within the SSD computer network on SharePoint.

> **Environmental Impact of Mining - Monitoring and Assessment\Fish\Ranger\Channel Billabongs\Data\Channel Billabong visual fish observations all years.mdb**

Water chemistry data recorded for these billabongs are currently located in an Excel spreadsheet in the SSD computer network on SharePoint.

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

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

4.2 Data entry QA/QC

Field data QAQC checks must be performed prior to data being entered into the database.

Data entered onto databases are verified by an independent person. Datasheets must be signed and dated on completion of data validation. The person entering the data cannot verify data they have entered onto the database.

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

- Check field blank samples for contamination.
- Compare replicate sample results looking for discrepancy that would indicate contamination (<20%).
- Check results for unusually high measurements.

Further information is available in the SSD's Surface water interpretation and reporting Operational manual.

5 Data analysis

For greater detail of the statistical methods for data analysis, including that of the worked example, refer to the corresponding Operational manual.

5.1 Data preparation

5.1.1 Rejection of data

Data are only rejected if they are not representative of the experimental design and other procedural requirements described in this protocol. For example, if unusual natural environmental conditions prevailed during monitoring that could result in anomalous counts (eg persistent heavy cloud cover reducing underwater light intensity), associated data may not be valid and should be rejected. An example of data falling outside of procedural requirements is provided by counts made in 1996, when monitoring occurred too early in the recessional flow period. In this year, very high abundances of glassfish and chequered rainbowfish were observed in the counts which coincided with the significant upstream migration of these species (upstream migrations of these species are reportedly triggered by pulses in stream flow velocity during the recessional flow period (Bishop et al 1995)). Data representing the anomalously high abundances of these species in 1996 have been removed from analyses that have examined trends in the abundances of these species over time.

5.1.2 Comparison of observer fish counts

In this protocol the primary means of dealing with observer bias is through adequate training to prevent or minimise occurrences, and to identify where improvement is required. However, differences between trained observer counts still need to be checked, and corrected where necessary, to minimise the effect of bias. Ultimately, it is important that the data arising from all observers' counts reflect accurately the impact assessment endpoints that are utilised, ie focus from 1989 to 2010 has been on fish community dissimilarity values (1994–2010) and Chequered rainbowfish abundances (1989–2010). To this end, the focus in training is on correct identification and count estimations for the 10 most abundant species due to their strong influence on the dissimilarity value. For example, Humphrey et al (2006b) identified that from 1994 to 2005, twelve fish species/taxa accounted for 99.5% of the total abundance of fish in both Mudginberri and Sandy billabongs and for 79.2% of the average dissimilarity between the two billabongs. Typically, species outside the top ten in rank abundance are not commonly observed and differences in observers could be due to the natural movement (in and out of the transect area/observer view) or rarity in the transect habitats (littoral zone).

Nevertheless, in reaching these requirements for the key impact assessment endpoints, comparisons between experienced and training (novice) observer counts also focus on other population and community summary response variables, as described below.

Key elements involved in the comparisons of both training and experienced observer counts include:

- Observer counts are compared (preferably for successive years) using the Bray Curtis dissimilarity measure (section 5.2.2) for community structure data, as well as species richness, total abundance and abundance of individual fish species using ANalysis Of Variance (ANOVA).
- Significant discrepancies between any of the analyses generally require correction of the training (or less experienced) observer data by the % difference to the most experienced observer.

- The magnitude of observer differences for individual species can be explored by examining the extent of departure from the 1:1 line in a scatter plot of abundance estimates derived from two observers. Such departure highlights under- and over-estimation of fish numbers for the full range of fish species abundance encountered, thereby identifying improvements and refinements that may be made to training.

5.1.3 Pooling of replicate counts and data transformation

After bias between trained and recalibrated observers has been checked, and corrected if necessary (section 5.1.2), replicate observations (ie four counts completed by two trained observers) for each transect and for each year and billabong, are pooled (averaged) to give one representative count for each transect.

For calculation of dissimilarities and the subsequent use of dissimilarities for ANOVA, regression and multivariate analyses of the community data, abundance data of each fish species are log [x+1] transformed. This transformation is preferred 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 dissimilarity value (Clarke & Warwick 2001).

5.1.4 Random pairing of pooled transect values

For each year of monitoring, Bray-Curtis dissimilarity values are calculated for five independent pairs of averaged transect data between Mudginberri and Sandy billabongs. The transect 'pairs' are selected at random (using a random-without-replacement approach) for each year from the 25 possible pairwise comparisons. The randomly-selected transect sitepairs for each year remain the same in all ensuing analyses relevant to that year of study.

5.2 Impact detection and assessment

5.2.1 An important caveat and background

The possibility of an impact resulting from a change in quality of creek waters due to inputs from the mine site may be inferred from a statistically significant change or trend in the multivariate dissimilarity values 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 upon comparison of the fish community results with other environmental information, especially water chemistry, water flow and habitat data.

Due to an absence of pre-mining baseline data there are no strict 'before' mining data in the design employed for assessing channel billabong fish communities. The simple BACIP design employing just single control and 'impact' billabongs introduces a further deficiency. Both aspects lead to weakened inference that may be made about impacts. Thus where significant statistical change is detected, there may be no way to distinguish between mine-related causes and natural causes that may also have occurred at, say, adjacent non-mining impacted streams. Use of multiple control streams, such as used for macroinvertebrate community-based monitoring for Ranger (***eriss*** 2011 protocol reference), minimises the risk of incorrectly labelling a coincident natural event as an impact (ANZECC & ARMCANZ 2000, Appendix 4). However, it is not possible to implement this multiple-control design for fish community monitoring in channel billabongs (section 3.4) owing to the lack of potential control examples of this type of waterbody in the nearby area.

Given these deficiencies, making a correct inference about mining impacts must rely upon detailed examination of mine and non-mine-related environmental factors that may explain the observed responses, with reference to other lines of monitoring or experimental evidence (including water chemistry, bioaccumulation, laboratory and field-based ecotoxicology, and community studies of macroinvertebrates and fish in shallow billabongs).

Fish community data are analysed for detection of mining impact using a BACIP 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)

(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.

As described in section 2.1, impact detection and assessment for the two scenarios is conducted on the multivariate sitepair or broader dissimilarity matrix using ANOVA, with supportive evidence from PERMANOVA. Where trends in the time series of dissimilarities are evident, regression and, if possible, Analysis of covariance approaches are applied to the sitepair dissimilarities. At the same time, trends and changes over time are sought for the species that are most influential in distinguishing the fish communities from the control and exposed sites. Other multivariate approaches, including ordination, assist in assessment of community results.

5.2.2 Testing of statistical assumptions and other test criteria

Statistical analyses are conducted on the dissimilarity values arising from the observations from each year, with five values for each year being derived from the randomly-paired transects between the control and exposed billabong. It is important to check that the full set of dissimilarity values to be analysed conforms to the underlying assumptions required for application of ANOVA, regression and ANCOVA, including normality, homogeneity of variance and independence (eg Stewart-Oaten et al 1986, Sokal & Rohlf 1995).

Data assumptions of normality, equal variances and independence

Assumptions of **normality** and **equal variances** are checked graphically, as recommended by McGuinness (2002), using plots of the *residuals* or *errors* (ie the difference between a dissimilarity observation and the mean dissimilarity for the group). A worked example of this procedure is provided in the Operational manual using Minitab software. Both assumptions have invariably been met in channel billabong fish community data obtained since 1994.

If the residuals are arranged in time order of data collection, they should succeed each other in a random sequence. If this is observed, then data sets from each year meet the assumption of **independence**. Departure from independence (often termed serial correlation, or autocorrelation) 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 may be used as an initial screening assessment to check for lack of independence, with formal testing conducted using the von Neumann test as detailed in Sokal and Rohlf (1995, 394–396). An example of the use of the latter test is demonstrated in the Operational manual where it is shown that dissimilarity data (from 1994 to 2010) are positively serially correlated. This is not unexpected given the observed trend in dissimilarity over time (section 5.2.4).

Checks of the ANOVA data assumptions should be made annually.

Trend analysis

In an extensive investigation detecting long-term change in marine intertidal communities using the BACIP design, Steinbeck et al (2005) removed from ANOVA analysis any data that had a significant trend (ie with a regression R^2 of >0.5) in the 'Before' period. This criterion was applied to the current dataset (1994 to 2010) using two regressions, (i) mean dissimilarity

(*Y*) against year (*X*), and (ii) each of the 5 replicate dissimilarity values from each year (*Y*) against year (*X*). Because of significant autocorrelation in the dissimilarity time series (from above), regressions were corrected using the Hildreth-Lu procedure, available as a macro in Minitab software. (The Hildreth-Lu procedure uses nonlinear least squares to jointly estimate the regression parameters with a first-order autoregressive process model.)

Significant regressions were found in both cases (i) and (ii) from above. For regression (i), significance of the regression was $P = 0.009$, with $R^2 = 0.36$, and for regression (ii), significance was $P = 0.01$, with $R^2 = 0.07$. Since the R^2 value was less than 0.5 there is insufficient trend in the time series of sitepair dissimilarity values to discount use of ANOVA.

5.2.3 BACIP (ANOVA) test

Lack of independence, such as observed in the present sitepair dissimilarity time series, is common in monitoring data collected over lengthy periods of time. Significant positive autocorrelation can increase the possibility of Type I error, ie lead to false positives in the ANOVA results. In similar situations, some workers have added an autoregressive term to the ANOVA model in order to model the autocorrelated error (eg Steinbeck et al 2005). At this stage, this extended ANOVA approach has not been applied to the present dataset. Instead, scenario (1) testing only, from section 5.2.1, ie ANOVA for the year of interest data ('after' impact) with past years' data ('before' impact), has been applied with the knowledge that, with or without a significant result, further analysis by regression, ANCOVA (if possible) and by abundances of numerically dominant species is required to reduce the risk of accepting a Type I error result.

The BACIP ANOVA analysis has been conducted using data from 1994 to 2010, with 2010 being the year of interest (the 'After' period). Analysis for potential or suspected mine site impact is conducted using the randomly-paired (section 5.1.4) multivariate dissimilarity values, based upon Log [x+1] transformed data (time series of dissimilarity values illustrated in 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).

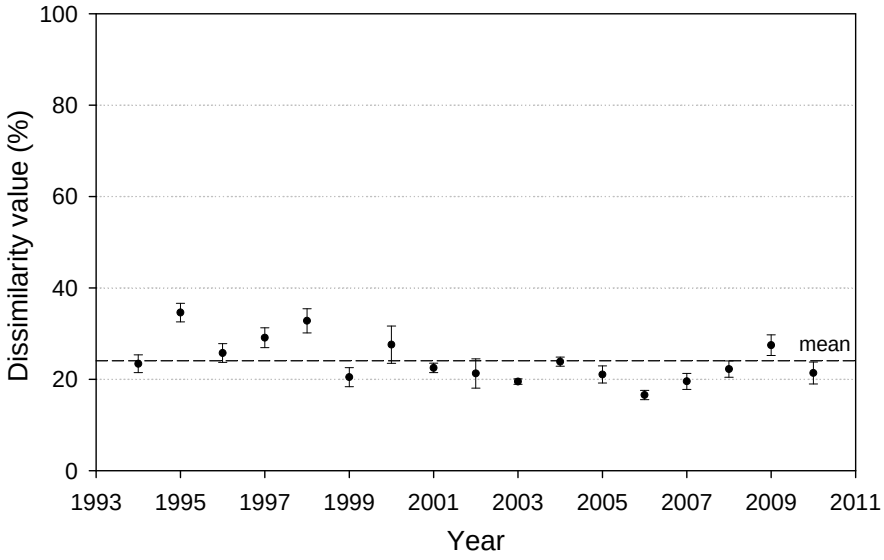

Figure 4 Paired control-exposed dissimilarity values (using the Bray-Curtis measure) calculated for community structure of fish in Mudginberri ('exposed') and Sandy ('control') billabongs over time. Values are means (± standard error) of the 5 possible (randomly-selected) pairwise comparisons of transect data between the two billabongs.

Analyses of the sitepair difference data for species richness or total abundance can be performed in a similar manner. However, these analyses have not been conducted as part of this protocol.

The MINITAB output for the ANOVA results is shown below (Table 5). The details of how to set up the ANOVA model and run the analysis are provided in the Operational manual. The ANOVA results show no significant difference between 2010 and other years ($p = 0.587$). However, the Years factor is significantly different, in the before period ($p < 0.001$) indicating that fish assemblage (dis)similarities between the two billabongs differ amongst years in this period.

Table 5 ANOVA results for channel billabong fish community dissimilarity values using Mudginberri (exposed) and Sandy (control) billabongs. Years Before include 1994–2009, years after are 2010.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
BA	1	38.29	38.29	38.29	0.31	0.587
Year(BA)	15	1862.00	1862.00	124.13	5.33	> 0.001
Error	68	1584.11	1584.11	23.30		
Total	84	3484.40				

The additional scenario testing (2) from section 5.2.1, ie analysis of two or more 'after' years data with those from previous 'before' years, is not deemed appropriate for ANOVA using the data gathered to date. Thus from examination of Figure 4, a significant difference in the BA factor may be found, depending upon which set of Before and After years are used. For example, if results from 2002 to 2010 were considered as the After period, and results prior to 2002 considered the Before period, a very significant BA result is found ($P = 0.006$). While no full assessment of the association of this result with a possible mine-related change has been undertaken, other monitoring data (or lines of evidence) suggest that this is highly unlikely, and indeed, more plausible natural explanations can be attributed (see section 5.2.4/3).

With additional data gathered in the future, it is quite likely that the fluctuating sitepair dissimilarity values, such as observed in Figure 4, will be seen as results consistent with, and correlated with, similar decadal-scale periodicity in annual rainfall and discharge. With a sufficient time-series, therefore, it is conceivable that the scenario of testing between two or more After periods with a series of Before years – such as rehabilitation, closure and operational phases of mining – may validly be conducted and assessed using ANOVA.

5.2.4 Trend analysis and accounting for temporal variation in the BACIP design

In section 2.1.3, the need to identify trends in the sitepair dissimilarity values and, where identified, associate these with natural covariates if possible, was emphasised. This understanding is important in order to ensure that change is not mistakenly attributed to mining in the catchment.

1 Trend analysis using regression

A decline in the sitepair dissimilarity values over time (for the period up to 2004) was first reported in the Supervising Scientist annual report for 2003–04 (Supervising Scientist 2004). As reported above (section 5.2.2), this decline in dissimilarity remains significant over the full dataset (1994 to 2010, $p<0.01$ for pooled dissimilarity values), despite the increases in dissimilarity that have occurred since 2006 (Figure 4). This result indicates that the fish community structures at the two sites are changing relative to one another over time. Without any assessment and potentially, these changes could be occurring at one or both billabongs, and be associated with natural events, or for Mudginberri Billabong, mine-related events.

Possible causal factors for the observed trend were examined by Humphrey et al (2006b) and Buckle & Humphrey (2009). The most likely explanation has been attributed to natural trends in the dominant fish species (see section 5.2.4/3) in response to wet season intensity, or a stepped response due to a change in method (observation canoe to boat) that occurred in 2001 (Buckle & Humphrey 2009). The rise in dissimilarity from 2006 to 2009 supports the hypothesis that trends in fish communities, associated with natural factors at one or both billabongs, are occurring and are more influential, as these years occur within the period of deployment of just one observation method (boat).

Possible causal factors responsible for the observed trend in sitepair dissimilarity and abundances of numerically-dominant fish species are considered in sections 5.2.4/2 and 3 below.

2 ANalysis of COVAriance (ANCOVA)

As described in section 2.1.3/2 above, where natural covariates are found that account for the significant trends in sitepair dissimilarity or fish population abundances, ANalysis of COVAriance (ANCOVA) may be used to test for changes between any of the two time series of results. In the first instance, this requires identification of potential covariates of the trends. This identification procedure is described below for sitepair dissimilarity and for abundance data for individual species.

Seeking correlates of the multivariate space and sitepair dissimilarity

The BIOENV routine from the PRIMER multivariate analysis software (Clarke & Gorley 2001, Clarke & Gorley 2006) and Pearson correlation analysis have been used to assist in identifying potential environmental covariates of the sitepair dissimilarity that may be used in regression and subsequent ANCOVA. BIOENV is 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. Details of the procedure are described in the Operational manual.

For analysis of data to 2010, environmental variables included habitat structure, water clarity and hydrology (from Table 4), with highly correlated variables (≥ 0.95) removed. Only biological and environmental data from, or relevant to, Mudginberri Billabong were included, as multivariate and species-level analysis from sections 5.2.4/3 and 4 below indicated that most of the change in the sitepair dissimilarity arose because of significant changes in the dominant fish species in Mudginberri Billabong. Methods and full results of this analysis are provided in the Operational manual.

In summary and based on analysis using the average (pooled) dissimilarity for each year, the environmental variables measured and used in BIOENV explain moderate proportions of the fish community dissimilarities (best Spearman rank correlation was 0.511), with the difference in electrical conductivity and Mg levels between the downstream and upstream monitoring sites in Magela Creek providing the highest correlations (0.454 & 0.339 respectively). However, when Pearson correlations were calculated between sitepair dissimilarity and the environmental data relevant to Mudginberri Billabong, no significant correlates were identified that could be used in covariance analysis.

Finding a relevant covariate, if indeed such factor exists, for the dissimilarity will be difficult. This is because of the need in the covariate to represent and account for simultaneous biological changes in two independent locations, even though most of the variation in fish communities and the dominant species is occurring at one site (Mudginberri).

Seeking correlates of the abundances of dominant species

Two of the more abundant fish species in Magela and Nourlangie creeks, *Melanotaenia splendida inornata* and *Ambassis* spp, are identified as most influential in determining the magnitude of the sitepair dissimilarity (see SIMPER analysis, section 5.2.4/3 below), with abundances of both species typically higher in Mudginberri Billabong compared with Sandy Billabong. Over the period of record from 1989 to 2010, wet season flow volume in Magela Creek has been found to be negatively correlated with rainbowfish abundance. Explanations for the physical basis for this relationship are provided in section 5.2.4/3. Stream discharge will be used in future ANCOVA analyses for detecting change once the rainbowfish abundance and wet season discharge relationship is better characterised

3 Patterns in dominant fish species

Identifying fish species influential in multivariate space and in the sitepair dissimilarity

The Primer SIMPER routine (Clarke & Gorley 2001, Clarke & Gorley 2006) has been used to examine influential fish species contributing to the dissimilarities between and within Mudginberri and Sandy billabongs. Analysis using the *a priori* group 'billabong' provides a list of fish species in decreasing order of influence upon the average dissimilarity value (averaged over all years). Analysis using *a priori* groups 'billabong' and 'year' provides a list of fish species in decreasing order of influence upon the dissimilarity between billabongs year by year. Influential species can be scrutinised closely for the year of interest or over the time period.

Abundances of the most influential fish species identified by SIMPER analysis are examined closely to determine the possible nature of their influence on the sitepair dissimilarity value. The abundances in both billabongs are examined using descriptive analysis techniques (eg scatter plots), aided by correlation, regression and ANOVA, to determine the nature of trends or differences between periods of interest, as well as to determine environmental correlates of those trends and differences.

The average dissimilarity between Mudginberri and Sandy billabongs (1994 to 2010) was 26.7%, with 62% of that proportion being explained by eight fish species, the most influential being the chequered rainbowfish (*Melanotaenia splendida inornata*) followed by perchlets (*Ambassis* spp). Both the rainbowfish and perchlets can undergo extensive recruitment into stream channels from downstream floodplains in some years and their migration dynamics appear to differ between the two catchments. Of particular note, a decline in rainbowfish abundance over time (1994 to 2005) was observed in Mudginberri Billabong and not Sandy Billabong (Humphrey et al 2006a & 2006b) with population abundances being closely monitored since (see below).

Changes in abundance over time of chequered rainbowfish

The chequered rainbowfish has been identified as the species that has most influence on the sitepair dissimilarity value. This species has declined in abundance in Mudginberri Billabong over the period 1989 to 2010 ($p = 0.008$) (Figure 5). (Data from 1996 were removed from this analysis as monitoring corresponded with a late end of wet season fish migration.) The elevated abundance in rainbowfish in 2009 (Figure 5) provides insights as to the possible cause of population fluctuations, and by association, therefore, the possible cause of interannual changes to the sitepair dissimilarity values (Figure 4).

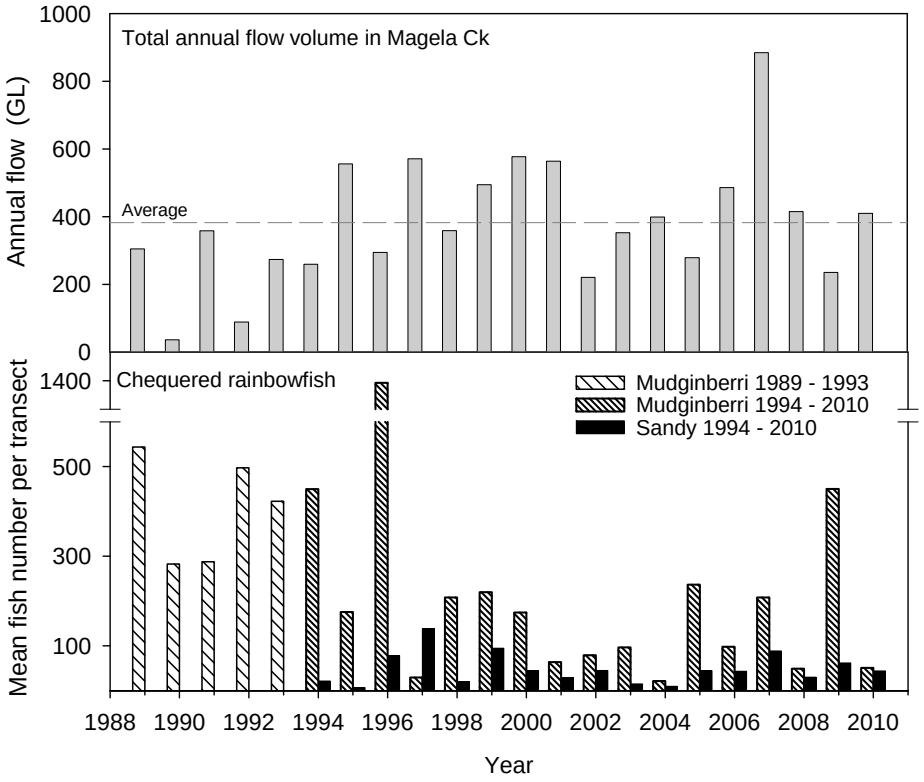

Figure 5 Relative abundance of chequered rainbow fish in Mudginberri and Sandy billabongs from 1989 to 2010. Total wet season discharge for Magela Creek at gauging station G8210009 provided.

Length of previous dry season[2], stream discharge[3], natural solutes concentrations[4] and expansion of floodplain grasses[5] (particularly the aggressive introduced para grass, *Urochloa mutica*) were suggested as possible causes of the decline in chequered rainbowfish abundances by Humphrey et al (2006a,b). These factors, in addition to others, have been systematically investigated on an annual basis (eg Buckle & Humphrey 2009, Buckle et al 2010).

In summary, despite the continued expansion of para grass on the floodplain, rainbowfish abundances in 2009 returned to values akin to those observed pre-1996 (Figure 5). This result suggests that habitat conditions on Magela floodplain, the recruitment source for these fishes,

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[2] Length of previous dry season provides an indication of the duration of floodplain drying prior to the ensuing wet season. The length of drying of floodplains has been linked to the productivity of a river system though a process termed the Flood-Pulse concept (Junk et al 1989). This productivity may in turn influence reproductive success of rainbowfish in this key floodplain nursery area, and subsequent recruitment success to Mudginberri Billabong

[3] The overall amount of water flowing in Magela Creek over a wet season may influence reproductive success of rainbowfish, and subsequent recruitment to Mudginberri Billabong.

[4] Natural solute concentrations (dissolved salts as inferred by the median electrical conductivity (EC) upstream of the Ranger Mine measured during the routine water quality monitoring program during the wet season) in Magela Creek system have been hypothesised to influence reproductive success of rainbowfish. Previous research has shown that fry of both chequered rainbowfish (Humphrey 1988) and the congener, the black-lined rainbowfish (Supervising Scientist 2005, Section 2.2.3), exhibit reduced survival when exposed to creek waters during wet seasons characterised by low natural solutes (larger wet seasons). Upstream EC values best reflect solute concentrations contributing to downstream wetland water quality conditions during each wet season. (EC data from the downstream site are slightly elevated due to minesite influence and hence do not accurately reflect the overall *natural* solute contribution to the downstream habitats.)

[5] Increases in areal distribution of aquatic grasses (particularly *Urochloa mutica*) may result in reduced rainbowfish abundance in Mudginberri Billabong due to a physical reduction in suitable breeding areas downstream.

may not be overly important. The length of previous dry season and levels of natural solutes (median EC upstream of Ranger) over the wet season are also not significant correlates.

Amongst the original correlates of the decline in rainbowfish abundance (see above; Humphrey et al 2006a,b), only wet season flow remains (negatively) correlated (Figure 6) over the period of record from 1989 to 2010. Correlated components of wet season discharge with fish abundances include: total monthly discharge in January ($p = 0.012$); total monthly discharge in February ($p = 0.023$); and wet season total ($p = 0.027$). These results indicate that higher rainbowfish abundances follow wet seasons of relatively low rainfall (Figure 5). A highly plausible mechanism for this inverse relationship is the stimulus for rainbowfish migration that occurs with flood pulses in Magela Creek (Bishop et al 1995). In wet seasons of low discharge these pulses are typically reduced, which may lead fish to concentrate more in the lowland channel billabongs rather than dispersing more broadly upstream and across the floodplains (Buckle & Humphrey 2009).

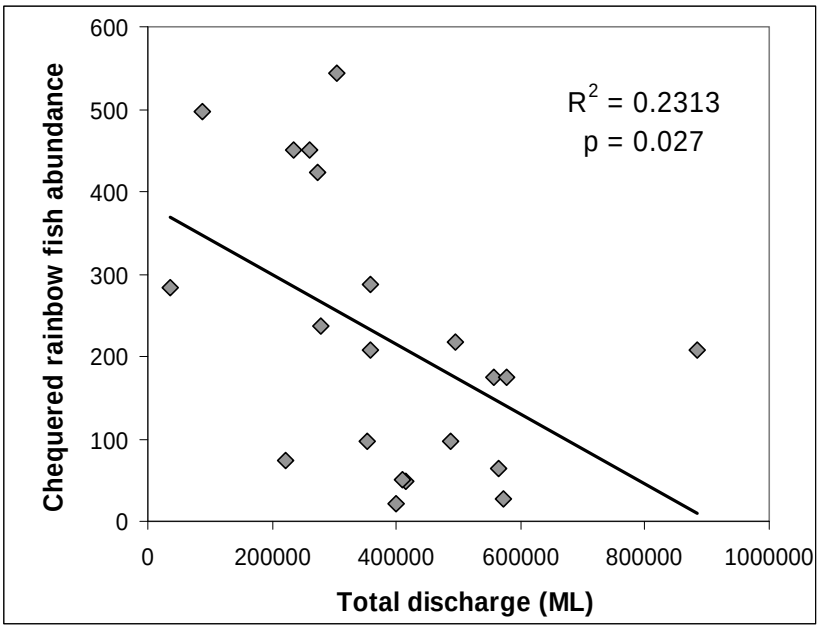

Figure 6 Total wet season discharge (ML) an environmental correlate of rainbowfish abundance in Mudginberri Billabong, 1989–2010. 1996 data removed, see text for reasoning.

Importantly, the abundances of rainbowfish do not appear to be related to any change in water quality over time as a consequence of water management practices at Ranger Mine. The input of magnesium (Mg) from Ranger has been used as a surrogate measure of mine water inputs to Magela Creek (see Humphrey et al (2006a,b) for further information). For the wet seasons encompassing the period of monitoring, from 1988–89 to 2009–10, no significant relationship has been observed between the mine inputs of Mg to Magela Creek (upstream-downstream difference in median Mg (mg/L) concentration data, obtained from routine grab-sampling water chemistry monitoring program) and corresponding rainbowfish abundance in Mudginberri Billabong. This lack of correlation is not surprising as the maximum concentrations of U and Mg measured in Magela Creek at the compliance point downstream of the mine have been at least two orders of magnitude lower than those known to adversely affect larval fishes including, in the case of uranium, chequered rainbowfish (Hogan et al 2005, Cheng et al 2010, van Dam et al 2010).

4 Multivariate analyses to assist in impact assessment

Fish community data (as treated in section 5.1.3) are displayed according to the underlying Bray-Curtis dissimilarity matrix by Multi-Dimensional Scaling (MDS) ordination (Clarke & Gorley 2001, Clarke & Gorley 2006). This graphic depicts the patterns in fish community structure for each billabong transect over time (Figure 7) and the relationship of these transect communities to each other. Points on the MDS with greatest separation represent transects with greatest differences in fish community structure. From Figure 7, the differing proportions of the dominant fish species in each billabong (see section Appendix 2) for the period 1994 to 2010 are evident, since the fish communities in Mudginberri Billabong clearly separate from Sandy Billabong.

The ordination also confirms the greater variability of fish communities in Mudginberri Billabong compared with that observed in Sandy Billabong, as the ordination sample points for Mudginberri are more dispersed in multivariate space (Figure 7).

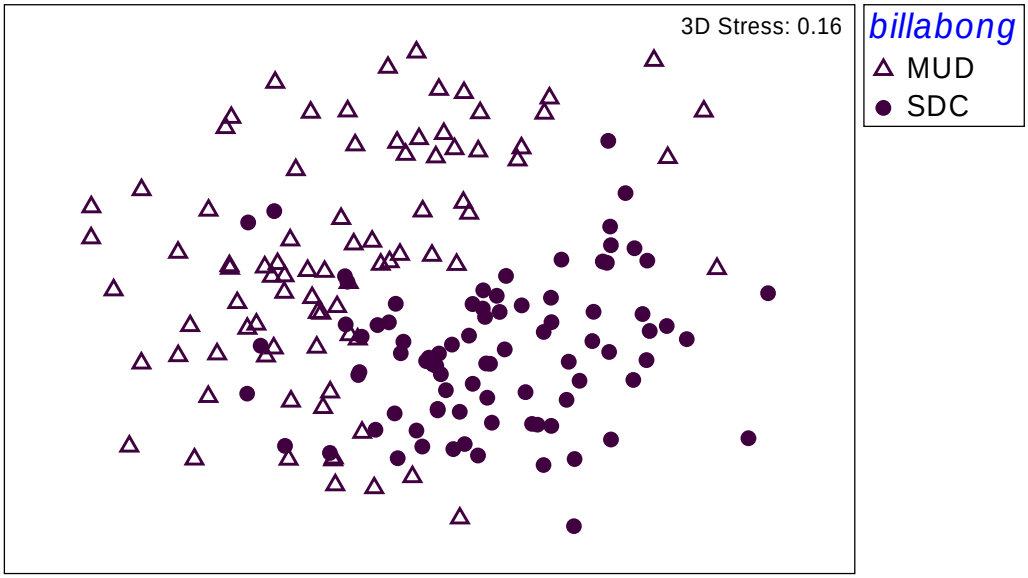

Figure 7 Axis 1 and 3 of a three dimensional MDS ordination plot of fish communities in Mudginberri (MUD) and Sandy (SDC) billabongs, 1994 to 2010. Each point represents the average of four visual counts at each of the five 50 m transects.

The visual arrangement of transect communities shown in the MDS has been formally tested using PERMANOVA (introduced in section 2.1.2) with two factors, i) Exposure (fixed factor, control or impact) and ii) Years (random factor). Results are provided in Table 6. Both factors and the interaction are significant, indicating that fish communities differ between the two billabongs and amongst years, and that differences amongst years between the exposed and control site are not consistent (ie not the same from year to year). Ultimately, these results show that the fish communities of the two billabongs are consistently different from one another and that they each vary from year to year.

An understanding of the fish species and environmental (including habitat) factors contributing to the separation of billabongs can assist in identifying causal mechanisms. The results of associated SIMPER and BIOENV analyses were presented in sections 5.2.4/3 and 5.2.4/2 respectively.

Table 6 Results from a two-factor PERMANOVA (main factors and interactions) for channel billabong fish community dissimilarity values using the exposed (Mudginberri) and control billabongs (Sandy) for all years available (1994 to 2010)

Source	DF	SS	MS	Pseudo-F[1]	P(perm)[2]	Unique perms[3]
Years	16	20699.00	1293.70	8.225	0.0001	9780
Exposure	1	6685.10	6685.10	12.979	0.0002	9941
Years*Exposure	16	8240.80	515.05	3.275	0.0001	9780
Residual	136	21392.00				
Total	169	57016.00				

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). 4 = significance (p) value generated using Monte Carlo sampling, used for situation with limited unique permutations available.

5.2.5 Conclusions

Detailed analyses of the fish community data have indicated that changes in sitepair fish community dissimilarity values and population abundances of the dominant fish species in Mudginberri Billabong to date, are not related to mining activity (Humphrey et al 2006, Buckle et al 2010, with summaries provided above). Other lines of monitoring or experimental evidence (water chemistry, bioaccumulation, laboratory and field based ecotoxicology, and community studies of macroinvertebrates and fish in shallow billabongs), support this conclusion.

Multivariate and univariate analyses seeking to identify species and environmental factors that may influence the sitepair fish community dissimilarity values support the strong influence upon the dissimilarity values of chequered rainbowfish abundances in Mudginberri Billabong (only), and the (negative) relationship between rainbowfish abundances at this site and stream discharge volume.

The analyses highlight some deficiencies of the sitepair dissimilarity approach to impact detection and assessment, including the masking of the direction (ie location exhibiting most change) and cause of interannual variations. An alternative impact detection method, PERMANOVA, circumvents the problem of uncertainty as to the source of change as it analyses the data from the individual sites (section 2.1.2). A comparison between ANOVA based upon sitepair dissimilarity and PERMANOVA using the same dataset is provided in Appendix 1. In summary, 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. With further international testing and confirmation of the robustness of the PERMANOVA method, it is likely that this data analysis method will replace the current sitepair dissimilarity approach in time.

Given the trends observed in fish communities over time in this study, examination of the fish species influencing multivariate patterns will always be required for correct impact assessment.

6 Reporting

6.1 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 during the calendar year:

- 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

6.2 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; and
2) Providing feedback to people on the results of the work and providing assurance that the environment and their lifestyle has 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.

6.3 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 6.4 below), is included in the Report.

6.4 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:

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

Papers and reports produced to address the other (listed in Section 6.1 above) communication requirements for the monitoring and science programs of SSD are also posted to the SSD Website as these become available.

6.5 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 –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 Resources. A full list of recipients is available from the SSD Publications Officer.

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.

6.6 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 6.2 and 6.4 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

A1.1 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 data set. The method of nonparametric 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 not be similar for the before and after periods) yet mask a real change that has occurred 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 5.2.3 from the main report) to results from PERMANOVA analysis on the equivalent data set. This comparison has been carried out for two reasons:

1. To provide complementary results to the BACIP ANOVA conducted in section 5.2.3 (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 data set.

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.

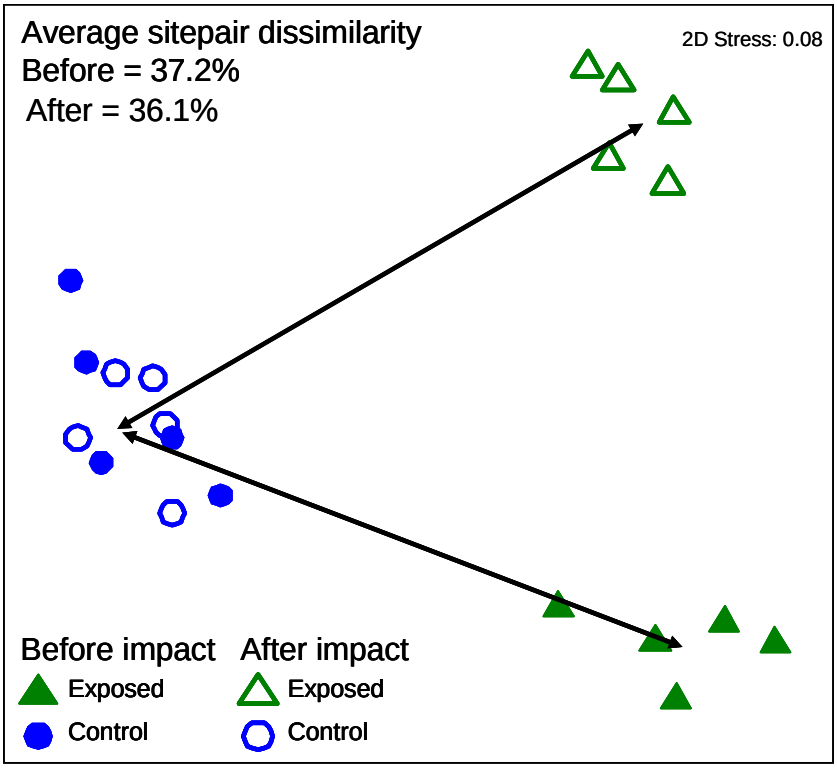

Figure A1 Hypothetical scenario showing that an analysis using a control-Impact sitepair dissimilarity value will not detect all changes that occur in multivariate direction. In this case, while a change has occurred at the exposed site after impact, the sitepair dissimilarity remains similar for the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

A1.2 Comparison of PERMANOVA and BACIP ANOVA Analysis approaches

While PERMANOVA and the BACIP ANOVA use the same original data set, 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 into 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 change in multivariate space. This constraint on the sitepair dissimilarity data limits interpretation of results as change in fish community structure at the exposed site could occur in different directions in multivariate space. Thus, a non-significant before to after dissimilarity value could mask real change that is occurring at the impact site.

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 number of 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, PERMANOVA requires an additional factor to partition the two billabong fish communities. For the BACIP ANOVA the years factor is used to partition the replicate control-impact sitepair dissimilarity values. PERMANOVA, being based on the complete dissimilarity matrix, requires the additional exposure factor (fixed) to partition the control and impact billabongs. For PERMANOVA the replicates are values (from the complete dissimilarity matrix) that represent the fish community structure within each billabong (or exposure), 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
Exposure		Fixed	PERMANOVA

Furthermore, because the two analysis models use different datasets (and hence a different number of 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. In PERMANOVA, the BA*Exposure interaction (not available in ANOVA), is the important source of variation to interpret for impact detection. In this analysis the BA factor is not used for impact detection, however can be used to determine the nature of any natural change from before to after when the BA*Exposure interaction is not significant (ie the BA change is consistent between the impact and control billabongs).

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. The Monte Carlo test has been performed due to the low number of possible permutations available for the BA factor.
3. 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 unselecting the 'Fixed effects sum to zero'. See below (section A4) for a brief discussion on the different models.

For both BACIP ANOVA and PERMANOVA analyses years 1994–2009 have been used for the before period, with 2010 as the after period (in this case it is the year of interest).

Table A2 Interpretation of each factor and interaction for the PERMANOVA and BACIP ANOVA analyses on fish community structure data in channel billabongs

Factors	PERMANOVA	BACIP ANOVA
BA	Indicates if the before and after periods differ across exposures (a significant result indicates significant change from the before to after periods). This factor is not directly interpreted for impact detection, however can be interpreted for non-significant BA*Exposure interaction.	**Key factor for impact detection**, A significant BA factor Indicates the magnitude in dissimilarity between control-impact billabongs differs from the before to after period.
Years(BA)	This factor is not important for impact detection as it simply Indicates if years differ within the before or after period across exposures.	This factor is not important for impact detection as it simply Indicates if the magnitude in dissimilarity across all sitepairs differs amongst years
Exposure	Useful to identify differences between the control and impact billabongs. A significant result indicates differences between the two billabongs which could be natural or mining related. In the absence of multiple billabongs within exposures that would better identify natural billabong variation in fish community structure, exploration of correlates to influential fish species and fish community dissimilarities may be required.	N/A
BA*Exposure	**Key interaction for impact detection,** A significant BA*Exposure interaction indicates that the BA result is not consistent between the control and exposed billabongs, suggesting mine site influence as exposed billabong is not responding to natural variation in the same way as control billabong.	N/A
Year(BA)*Exposure	Indicates if the variation amongst Years(BA) is consistent between the control and exposed billabongs. Pairwise comparisons can be used to explore a significant result and ensure variation over time occurs at both the control and exposed billabongs. Variation at just one billabong suggest mine site influence – in this case further investigation with habitat and water quality variables would be required.	N/A

A1.3 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 section 5.2.3 in the main report. For both PERMANOVA and the BACIP ANOVA analysis 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 (169) than the BACIP ANOVA (84) so, theoretically, PERMANOVA should provide greater statistical power due to the inclusion of more data.

Table A3 PERMANOVA results for channel billabong fish community dissimilarity values using exposed (Mudginberri) and control (Sandy channel) billabongs. Years Before include 1994–2009, years after are 2010.

Source	df	SS	MS	Pseudo-F[1]	P(perm)[2]	Unique perms[3]	P(MC)[4]
BA	1	820.43	820.43	0.61909	0.8808	17	0.7475
Years(BA)	15	19878.00	1325.20	2.64970	0.0001	9841	0.0001
Exposure	1	1258.10	1258.10	2.51540	0.0369	9933	0.0163
BA*Exposure	1	738.66	738.66	1.47690	0.1876	9939	0.1772
Year(BA)*Exposure	15	7502.10	500.14	3.17970	0.0001	9807	0.0001
Res	136	21392.00	157.29				
Total	169	57016.00					

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). 4 = significance (p) value generated using Monte Carlo sampling, used for situation with limited unique permutations available.

A1.3.1 Impact detection

Comparative results for the three-factor PERMANOVA and two-factor ANOVA are shown in Table A4.

Table A4 Comparison of results from a three-factor PERMANOVA with the two factor ANOVA for channel billabong fish community dissimilarity values using an exposed (Mudginberri) and control (Sandy channel) billabongs. Years Before include 1994 –2009, years after are 2010.

Factors	PERMANOVA	BACIP ANOVA	Interpretation
BA	0.8808 p(mc)= 0.7475)	0.587	PERMANOVA, across both the control and exposed billabongs no change in fish communities from the before to after period occurs ANOVA, no change in the magnitude of the control-impact sitepair dissimilarity from the before to after period occurs.
Years(BA)	0.0001	<0.001	PERMANOVA, across both the control and exposed billabongs fish communities show differences amongst years in the before period (no replication in the after period). ANOVA, significant differences in the magnitude of the control-impact sitepair dissimilarity is observed amongst years in the before period (no replication in the after period).
Exposure	0.0369		The control and exposed sites differ, however could be natural catchment or billabong differences Exploratory analysis using BIOENV indicates water quality is not a driving influence over fish communities
BA*Exposure	0.1876		The BA result is consistent between control and exposed conditions. This result indicates the exposed site is responding as expected under natural conditions – no mine site influence.
Year(BA)*Exposure	0.0001		The Years(BA) result is not consistent between control and exposed conditions indicating annual variation is not consistent between the two exposure billabongs.

The results and interpretation of the Before and After statistical comparison are essentially the same for both PERMANOVA and ANOVA, showing no significant difference from Before to After (2010, year of interest). However, in PERMANOVA, the BA*Exposure ($p = 0.1876$) interaction shows that the non-significant Before and After periods (BA $p = 0.8808$ ($p(mc)= 0.7475$)) are consistent between billabongs, thus no change in fish community structure has occurred from the before to after period in either the exposed or control site. Thus this result

provides quite specific interpretation of the BA periods comparison in each of the billabongs. The sitepair BACIP ANOVA results are interpreted slightly differently. Here, the BA ($p = 0.587$) shows that the dissimilarity between the two billabongs has not changed from before to after, but there is no specific information available about the response of each billabong – only about the dissimilarity between the sitepair each year. This constraint limits interpretation of the results as any change could be within one or both billabongs. Thus, a non-significant BA result could mask real change that is occurring at the impact site as a result of, for example, coincident and different change at the other site.

Furthermore, for the monitoring of fish communities in channel billabongs the benefits of removing temporal and spatial variation in the BACIP design are reduced. The control versus impact design required for fish study incorporates sites on different (independent) catchments which makes the standardisation of natural factors more difficult than the traditional upstream versus downstream approach. The current sitepair dissimilarity results indicate a trend over time that could be caused by a number of factors, the influences upon fish communities of wet season discharge being the most likely (see section 5.2.4 for further discussion). The trend over time indicates the two fish communities are responding differently to annual influences. As a result, BACIP ANOVA in this protocol, because of its inability to provide information at a site level and being constrained to a univariate (dissimilarity) measure, is limited in its ability to detect changes, outside of natural variation, that could be due to minesite influence.

A1.3.2 Temporal variation

For both the PERMANOVA and BACIP ANOVA, the Years(BA) factor is significant, indicating that differences occur amongst years in the Before period. The significant Years(BA)*Exposure term ($p = 0.0001$) in PERMANOVA indicates that these differences (amongst years in the before period) are not consistent between billabongs. Neither of these analyses (PERMANOVA and ANOVA) is informative with respect to whether the variability amongst years is occurring at one or both sites. PERMANOVA has additional features, however, (and unlike ANOVA of sitepair dissimilarity) which provide for analysis *within* the datasets of each of the billabongs; in this case (and as noted above, no results are provided here) significant annual variation has been shown to occur at both sites. While true pre-mining data (in this case before 1980) are not available, this finding, nevertheless, provides direct evidence that annual variability is inherent in both exposed and reference sites, and hence provides support that differences amongst years are unlikely to be associated with mining in Magela Creek catchment.

A1.3.3 Spatial variation

Not surprisingly the BACIP ANOVA removes temporal variation between billabong fish communities from the analysis by its control-impact sitepair approach (Table A4).

The PERMANOVA analysis shows significant differences occur between the control and exposed site (Exposure $p = 0.0369$, Table A4) (supporting the visual separation of the two billabong communities in the MDS plot of Figure 5).

A1.4 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 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). 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 and 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 when applied to the current data set[6]. For other comparisons, however, differences could occur and this requires further consideration/advice to determine the most appropriate model.

A1.5 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 channel billabongs, the observed trend over time in the sitepair dissimilarity violates the independence assumption which limits the BACIP ANOVA's ability to detect minesite influence (in the absence of a covariate to account for this variation) from the before to after period. The unconstrained data used in PERMANOVA is less bound by data assumptions.

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

1. 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.
2. The ability to select the analysis model type (restricted or unrestricted model).

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[6] Comparison of restricted versus unrestricted models with the Channel billabong fish community data has been conducted. Results for both the multivariate dissimilarity data analysed using PERMANOVA and the BACIP sitepair dissimilarity data (analysed using PERMANOVA) using restricted and unrestricted models, were similar.

A1.6 References

Anderson MJ 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26, 32–46.

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Appendix 2 Summary of fish biodiversity information arising from studies in Mudginberri and Sandy Billabongs

A2.1 Background

An understanding and assessment of the species and abundances of fishes making up channel billabong communities is important so that informed interpretation of annual results may be made. In particular, relative changes to the abundances of common species occurring in Mudginberri and Sandy billabongs greatly influence the dissimilarity values measured annually and hence examination of species abundance patterns should be conducted regularly. The relative predominance of chequered rainbowfish (*Melanotaenia splendida inornata*) in Mudginberri Billabong, and interannual changes in the abundance of this species in Magela Creek and their effects upon the dissimilarity values (see section 5.2.4/3 in main report, Humphrey et al 2006), provide an example of the need for an understanding of the dynamics of key species within each catchment. The dynamics of these species may be influenced by landscape-wide factors (Humphrey et al 2006), information about which can assist in properly inferring change. The information may also assist land managers of Kakadu National Park in biodiversity assessments of the Park, required for their own corporate reporting needs.

A2.2 Biodiversity summary

Assessment of biodiversity provides a sound understanding of the fish species and abundances which make up the fish community structure. Humphrey et al (2006) provide a summary of the mean abundance (no. fish/50 m) of fish species from both billabongs for the period 1994 to 2005. Since that report, only one additional fish species (One-gilled Eel – *Ophisternon gutterale*) has been observed in Mudginberri Billabong (a single specimen recorded by a training observer and confirmed by the senior observer).

Since 1994, 31 species have been recorded in Mudginberri Billabong and 29 species recorded in Sandy Billabong, with a combined total of 35 species for both sites. The visual census procedure provides a thorough species inventory of the fish community. However, it is biased against nektonic species such as ariid catfish (*Neoarius* spp), plotosid catfish (*Neosiluris ater*), bony bream (*Nematalosa erebi*), and ox-eye herring (*Megalops cyprinoides*) which are more abundant in the open, central waters of the billabongs.

Humphrey et al (2006) showed that total fish density averaged 751 and 545 per 50 metre transect in Mudginberri and Sandy billabongs, respectively, and more than 95% of the average fish abundance was derived from 6 small to medium-sized fish species (see plates 1–6 arranged in decreasing average abundance (1994–2005) in Mudginberri Billabong). With one exception (mouth almighty, *Glossamia aprion*), these are all schooling species and this enhances their detection by visual census. Of the 6 species, the fly-specked hardyhead (*Craterocephalus stercusmuscarum*) was the most abundant species in both billabongs and occurred in very similar densities: 397/50m in Mudginberri and 357/50m in Sandy. The chequered rainbowfish (*Melanotaenia splendida inornata*) was much more abundant in Mudginberri (255/50m) than in Sandy (47/50m). Rainbowfish were largely responsible for the higher overall abundance of fish recorded in Mudginberri. Pennyfish (*Denariusa bandata*) were also more abundant in Mudginberri. Conversely, the abundances of glassfish (*Ambassis agrammus* and *A. macleayi*), banded grunter (*Amniataba percoides*) and mouth almighty (*G. aprion*) were greater in Sandy Billabong.

Plate 1

Scientific name: ***Craterocephalus stercusmuscarum***

Gundjeihmi name(s): **dilebang or dolbo**

Common name(s): **fly-Specked hardyhead**

Plate 2

Scientific name: ***Melanotaenia splendida inornata***

Gundjeihmi name(s): **dilebang or dolbo**

Common name(s): **chequered rainbowfish**

Plate 3

Scientific name: ***Ambassis macleayi***

Gundjeihmi name(s): **na-rranggi**

Common name(s): **reticulated perchlet**

Plate 4

Scientific name: ***Amniataba percoides***

Gundjeihmi name(s): **mandidi**

Common name(s): **banded grunter**

Plate 5

Scientific name: ***Denariusa bandata***

Gundjeihmi name(s): **na -rranggi**

Common name(s): **penny fish**

Plate 6

Scientific name: ***Glossamia aprion***

Gundjeihmi name(s): **na-rranggi or djabelh**

Common name(s): **mouth-almighty**

A2.3 References

Humphrey C, Pidgeon B & Buckle D 2006. Monitoring of fish communities in deep channel billabongs associated with Ranger uranium mine, Northern Territory. In *A guide to monitoring fish stocks and aquatic ecosystems*. Australian Society for Fish Biology Workshop Proceedings, Darwin, Northern Territory, 11–15 July 2005, eds Phelan MJ & Bajhau H, Fisheries Incidental Publication 25, NT Department of Primary Industry, Fisheries and Mines, Darwin, 86–103.