



**Toxicity testing of
Gadjarrigamarndah
Creek (Gadji Creek)
water - August 1997**

RA van Dam
B Noller
G Parker
C Camilleri

August 1999



Toxicity testing of Gadjarrigamarndah Creek (Gadji Creek) water - August 1997

R.A. van Dam¹, B. Noller², G. Parker² and C. Camilleri¹

¹ Environmental Research Institute of the Supervising Scientist

Locked Bag 2, Jabiru, NT, 0886

² Northern Territory Department of Mines and Energy

Darwin, NT, 0800

Contents

| | |
|--|-----|
| Summary | iii |
| 1 Introduction | 1 |
| Aims | 2 |
| 2 Materials and Methods | 2 |
| 2.1 Collection of water | 2 |
| 2.2 Preparation of water | 2 |
| 2.3 Preparation of test solutions | 3 |
| 2.4 Toxicity testing procedures | 3 |
| 2.5 Chemical analyses | 5 |
| 2.6 Statistical analysis | 5 |
| 3 Results | 5 |
| 3.1 Physico-chemical and chemical analyses | 5 |
| 3.2 Toxicity testing | 6 |
| 4 Discussion | 8 |
| 5 Conclusions | 10 |
| 6 References | 11 |
| Appendix A | 13 |
| Appendix B | 14 |
| Appendix C | 15 |

Summary

Gadjarrigamarndah Creek (Gadji Creek), in western Arnhem Land, has received groundwater seepage contaminated by spray irrigation of process water from the now decommissioned Nabarlek uranium mine, for a number of years. The major characteristic of the groundwater is its acidity, due to the oxidation of NH_4^+ to NO_3^- , and the subsequent release of H^+ . The major consequence of the groundwater's acidity is the release of aluminium (Al) from accelerated weathering of soil minerals. Thus, since the spray irrigation period, Al has been present in Gadji Creek water at concentrations well in excess of the ANZECC water quality guideline value for the protection of aquatic ecosystems, as well as toxicity values for a large range of aquatic organisms. However, fish community surveys carried out by Pidgeon & Boyden (1995, in prep) between 1986 and 1995 revealed that after an initial decline, few differences could be found in fish abundance and community structure compared to the pre-spray irrigation period. Silica, which is also present in high concentrations in Gadji Creek water, has previously been shown to bind to, and ameliorate the toxicity of Al to fish. Thus, it was hypothesised that high levels of silica in Gadji Creek could be binding with and eliminating the toxicity of Al to aquatic life.

The aims of the present study were to assess the toxicity of Gadji Creek water in the laboratory, to three local aquatic organisms, and attempt to relate this to measured Al and silica concentrations. The three organisms assessed were the fish, *Mogurnda mogurnda*, the green hydra, *Hydra viridissima*, and the cladoceran, *Moinodaphnia macleayi*. Gadji Creek and Cooper Creek (control and diluent water) water were collected on 20 August 1997. Toxicity tests were performed at *eriss*, while the NT Department of Mines and Energy coordinated chemical analyses. Gadji Creek water had no significant effect on survival of *M. mogurnda* and population growth of *H. viridissima* over 4 days. However, reproduction in *M. macleayi* was significantly reduced by approximately 12% when exposed to 100% Gadji Creek water for 5 days. Although statistically significant, this effect was not deemed to be ecologically significant, particularly considering the large overall numbers of offspring produced. Therefore, it was concluded that Gadji Creek water collected in August 1997 exhibited no or very little toxicity to local aquatic organisms.

Chemical analyses revealed that total Al levels in August 1997 were uncharacteristically low compared to previous years. Total Al measured approximately 89 $\mu\text{g/L}$ in August 1997 compared to approximately 200-250 $\mu\text{g/L}$ in previous years. Of the 89 $\mu\text{g/L}$ total Al, filterable Al comprised only 33 $\mu\text{g/L}$. Silica concentrations were again high, at almost 17 mg/L (as SiO_2), and thus, greatly in excess of Al concentration. Therefore, due to the lower than usual Al concentration, and the high silica concentration, it was not surprising that Gadji Creek water exhibited no toxicity to the aquatic organisms in the present study. Other measured compounds that were present at elevated levels in Gadji Creek compared to Cooper Creek, yet apparently also had no effect on the aquatic organisms, were SO_4 , NO_3 , NH_4 , and Mn.

The results of the present study were in partial contrast to those of Rippon & McBride (1994) who assessed the toxicity of Gadji Creek water in 1993. In their study, Gadji Creek water was toxic to *H. viridissima* and *M. macleayi*, but not to *M. mogurnda*. Thus, an improvement of the water quality between the two studies appears to have occurred, and this could potentially be attributable to the reduction in Al concentration. However, the contrasting results may also reflect the seasonally varying water characteristics of Gadji Creek, and do not necessarily represent a long-term temporal decrease in Al concentration.

1 Introduction

The Nabarlek mine lease, in western Arnhem Land, Northern Territory (Figure 1), was mined for uranium in 1979, with milling of the stockpiled ore continuing through to 1989. In 1995, the site was decommissioned and rehabilitated. Part of the rehabilitation included a forest irrigation area, receiving spray irrigation of saline process/tailings water from 1984 to 1990. This resulted in the ammonium (NH_4^+) present oxidising to nitrate (NO_3^-), which was subsequently transferred to the groundwater. Associated with the oxidation of NH_4^+ to NO_3^- was the formation of hydrogen ions (H^+), and therefore substantial acidification of the groundwater. One of the major chemical consequences of groundwater acidification was the release of aluminium (Al) from soil minerals. As a result, Al was found to be present in elevated amounts in the local groundwater. The groundwater at this site seeps from the slope into nearby Gadjarrigamarndah Creek (Gadji Creek), which subsequently also became acidic and contained elevated amounts of Al (DME 1996). Gadji Creek flows into nearby Cooper Creek, a stream not directly affected by acidic groundwater seepage from the Nabarlek mine site.

It is well documented that in acidic waters, Al is acutely toxic to fish (Burrows 1977; Poléo 1997). However, although Gadji Creek water has been acidic and contained elevated levels of Al for some time, recent fish surveys of Gadji Creek revealed that following an initial decline, few differences in fish abundance and community structure could be found compared to the pre-spray irrigation period (Pidgeon & Boyden 1995; in prep). Although not directly compared to Al levels, the results suggested that elevated Al concentrations in Gadji Creek were no longer having an adverse effect on aquatic, or at least fish life, if indeed they ever did.

A number of organic ligands have been demonstrated to prevent Al toxicity to fish (Driscoll et al 1980, Birchall et al 1989, Witters et al 1990). Birchall et al (1989) reported that in the presence of large excesses of silica, as silicic acid (H_4SiO_4), acute Al toxicity to atlantic salmon fry (*Salmo salar*) was eliminated. As a result of this finding, the NT Department of Mines and Energy (DME) have paid particular attention to measured concentrations of both Al and silica in their regular chemical monitoring program of ground- and surface waters at the Nabarlek mine site. Indeed, in Gadji Creek, silica, measured as SiO_2 , has generally been found to be present at approximately 5 to 20 times the molar concentration of Al (DME 1996).

Therefore, it has been hypothesised that silica is forming strong complexes with Al, and thus reducing the risk of Al toxicity to fish. However, the effect of silica on reducing Al toxicity to fish in Gadji Creek is unknown. Therefore, the toxicity of Gadji Creek water to fish needs to be known in order to understand the relationship between the fish survey data (Pidgeon & Boyden 1995; in prep), and the chemical monitoring data. Toxicity testing of Gadji Creek waters has actually previously been carried out by *eriss* in 1991 and 1993 (Hyne 1991; Rippon & McBride 1994). In 1991, Gadji Creek water had no effect on the fish species, *Mogurnda mogurnda*, while bioassays carried out on the cladoceran, *Moinodaphnia macleayi*, and the green hydra, *Hydra viridissima*, were invalid (Hyne 1991). In 1993 significant effects were observed for *M. macleayi* and *H. viridissima*, at 32% Gadji Creek water, while *M. mogurnda* was again unaffected by Gadji Creek water (Rippon & McBride 1994). Unfortunately, as with the fish survey research, the results were not related to Al and silica concentrations.

Therefore, the major comparison of interest in the present study was that between the concentration of labile (bio-available) forms of Al, and the toxicity of the water to aquatic animals, particularly fish.

Aims

The major aims of the proposed research were:

i) to determine the effect of Gadji Creek water collected in the mid to late dry season, when pH is generally at its lowest, to:

- a) the purple spotted gudgeon, *M. mogurnda*,
- b) the green hydra, *H. viridissima*, and,
- c) the cladoceran, *M. macleayi*;

and:

ii) to compare the toxicity data to both the chemical composition of the water, particularly the total and filtered Al and silica concentrations, and both previous toxicity testing and fish survey data recently obtained for Gadji Creek (Hyne 1991; Rippon & McBride 1994; Pidgeon & Boyden 1995; in prep).

2 Materials and Methods

2.1 Collection of water

Water was collected from Gadji Creek (surface water observation station GS8211083 also known as AdjSP29) and Cooper Creek (surface water observation station GS8211061) on 20 August 1997, by Department of Mines and Energy (DME) field officers. Figure 1 indicates the location of Gadji Creek and the AdjSP29 water collection site. Samples were collected in 20 L polyethylene containers that had been washed in 2% Neutracon® and 5% HNO₃, and rinsed with de-ionised water prior to being taken into the field, then rinsed three times with natural water at the collection site. Measurements of pH, conductivity and temperature were taken for both creek waters immediately following collection. For toxicity testing purposes, 20 L of Gadji Creek water and 40 L of Cooper Creek water were collected. Cooper Creek water was to be used as control and diluent water. Three samples at each site were also collected in 250 ml clean amber, glass bottles, for analysis of total organic carbon (TOC) and dissolved organic carbon (DOC). Further water samples were taken for detailed chemical analyses, including Al and silica, as described below. Water for toxicity testing purposes and TOC/DOC analysis were transported to *eriss*' ecotoxicology laboratory on the day of collection.

2.2 Preparation of water

Immediately following arrival at the laboratory, the pH and conductivity of both the Gadji and Cooper Creek samples were measured. They were then filtered through a 10 µm paper filter to remove large particulates and any wild zooplankton and phytoplankton. Following this they were stored in the dark at 4°C, until required for toxicity testing.

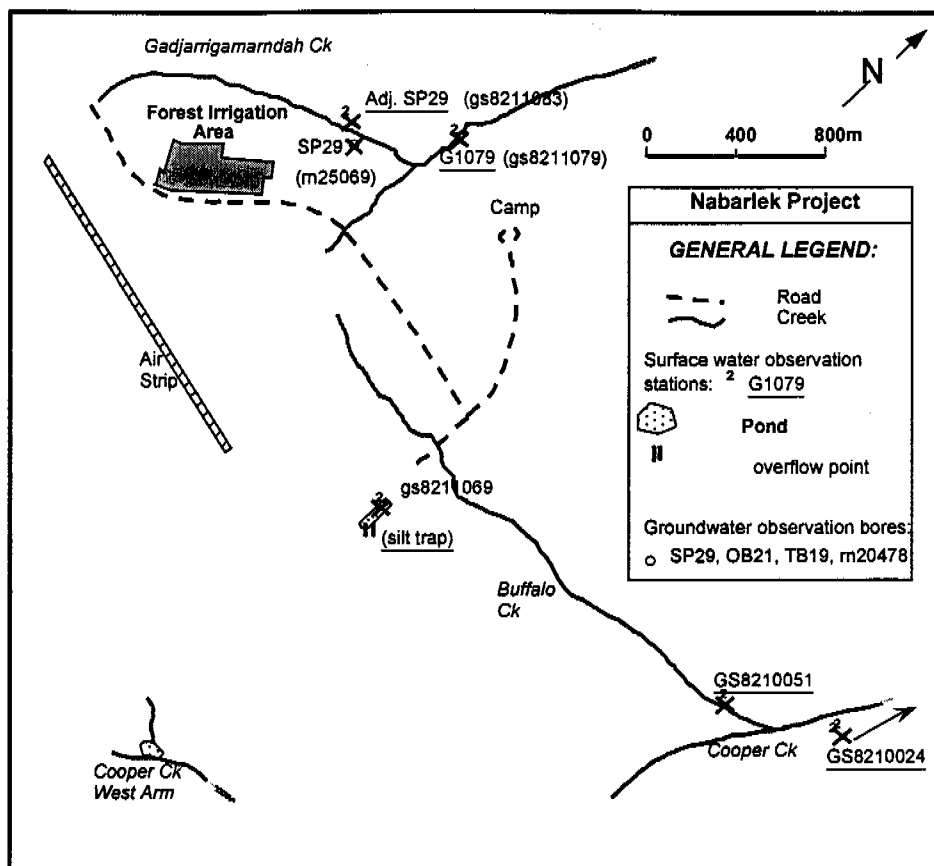


Figure 1 The decommissioned Nabarlek uranium mine site, showing various observation stations, including the Gadji Creek water collection site, AdjSP29. (NB Cooper Creek water collection site, GS821061, is not shown in the figure, but is located between Cooper Creek West Arm and the confluence of Cooper Creek and Buffalo Creek).

2.3 Preparation of test solutions

The test solutions were prepared on the morning of 21 August 1997. The following dilutions of Gadji Creek water were prepared, using Cooper Creek water as the diluent water; 0% (100% Cooper Creek water), 1%, 3.2%, 10%, 32% and 100% Gadji Creek water. A total volume of 5 L of each dilution was prepared in 5 L polyethylene containers. During the course of the experiments, the solutions were kept in the dark at 4°C, and removed each morning to equilibrate to room temperature, prior to test solution renewal.

2.4 Toxicity testing procedures

The effects of Gadji Creek water on aquatic life were assessed in three standard toxicity bioassays, utilising three species from different trophic levels. The bioassays, which are regularly carried out at *eriss* for toxicity assessment purposes, are summarised in Table 1.

As the bioassay protocols are described in detail in the references cited in Table 1, they are only summarised here. Commencement of the tests was staggered over several days, so as to maximise efficiency, and minimise overlap of labour intensive counting periods. However, all tests were commenced within 96 h following collection of the water.

Table 1: Summary of standard toxicity testing bioassays used for the assessment of the effects of Gadji Creek water.

| Test species | Test endpoint | Test duration (acute/chronic) | Protocol |
|--|-------------------|-------------------------------|--------------------|
| Purple-spotted gudgeon (<i>Mogurnda mogurnda</i>) | sac-fry survival | 96 h (acute) | BTT-E ¹ |
| Green hydra (<i>Hydra viridissima</i>) | population growth | 96 h (chronic) | BTT-B ² |
| Cladoceran (<i>Moinodaphnia macleayi</i>) | reproduction | 3 brood/6 day (chronic) | BTT-D ³ |

¹ Protocol BTT-E is described in Markich & Camilleri (1997), and was approved by NTU AEEC, July 1997 - Ref. No. 97016.

² Protocol BTT-B has been shortened from 6 days to 4 days (96 h) as described in Markich & Camilleri (1997).

³ Protocol BTT-D is described in Hyne et al (1996).

2.4.1 Purple-spotted gudgeon (*M. mogurnda*) 96 h sac-fry survival

Recently-hatched *M. mogurnda* sac-fry (< 10-h-old) were exposed to the above-mentioned dilutions of Gadji Creek water for a period of 96 h. Observations of fry survival were recorded at 24 h intervals. Sac-fry were exposed to 30 ml of test water in glass petri dishes. There were 3 replicate dishes for each dilution, with each containing 10 sac-fry, resulting in a total of 18 test dishes and 180 sac-fry. The test dishes were kept in a constant temperature incubator at $27 \pm 1^\circ\text{C}$, with a photoperiod of 12 h light : 12 h dark. Tests solutions were renewed every 24 h, following recording of sac-fry survival. The sac-fry were not fed prior to, or during the 96 h test period. The test was considered valid if control survival exceeded 80% at the end of the 96 h test period. Conductivity, pH and dissolved oxygen (DO) were measured on fresh (t_0) and 24-h-old (t_{24}) test water daily.

2.4.2 Green hydra (*H. viridissima*) 96 h population growth rate

Asexually reproducing hydra, each with one relatively well developed bud, were exposed to dilutions of Gadji Creek water for a period of 96 h. Observations of any changes to the hydra population (ie one animal equals one hydroid plus any attached buds) were recorded every 24 h. Hydra were exposed to 30 ml of test water in glass petri dishes. There were 3 replicate dishes for each dilution, with each containing 10 initial hydra, resulting in a total of 18 test dishes and 180 hydra. The test dishes were kept in a constant temperature incubator at $27 \pm 1^\circ\text{C}$, with a photoperiod of 12 h light : 12 h dark. Tests solutions were renewed every 24 h, following recording of the number of hydra in each dish. The hydra were fed individually with 3 - 4 live brine shrimp nauplii (*Artemia salina*) per day throughout the 96 h test period. The relative population growth rate (K) was calculated after 96 h, using the following formula:

$$K = \frac{\ln(n_4) - \ln(n_0)}{T}$$

where n_4 = number of hydra at the end of the 4 day period

n_0 = number of hydra at the start of the test period (ie $n_0 = 10$)

T = length of test period in days (ie T = 4)

The test was considered valid if control population growth rate exceeded 0.34 day^{-1} at the end of the 96 h test period. Conductivity, pH and dissolved oxygen (DO) were measured on fresh (t_0) and 24-h-old (t_{24}) test water daily.

2.4.3 Cladoceran (*M. macleayi*) 3 brood/5-6 day reproduction

Female *M. macleayi* neonates (< 10-h-old) were exposed to dilutions of Gadji Creek water until control cladocerans released their third brood offspring. This usually takes 5 - 6 days. Each day, observations were made on the survival of each female, the number of neonates produced, and the number of surviving neonates. The numbers of neonates from all broods are summed for each adult cladoceran, resulting in a count of the total number of offspring per adult. Cladocerans were exposed to 30 ml of test water in plastic 40 ml vials with lids. There were 10 replicate vials for each dilution, with each replicate containing 1 initial cladoceran, resulting in a total of 60 test vials and 60 cladocerans. The test vials were kept in a constant temperature incubator at $27 \pm 1^\circ\text{C}$, with a photoperiod of 12 h light : 12 h dark. Tests solutions were renewed every 24 h, following inspection, and recording of the number and removal of neonates in each vial. The cladocerans were fed 2×10^5 cells/ml of the green alga, *Chlorella* sp. and $1 \mu\text{l/ml}$ of a standard fermented food preparation (see Hyne et al 1996) per day throughout the test period. The test was considered valid if mortality in the controls did not exceed 20%, and reproduction in the controls averaged 30 or more neonates per surviving female over the test period. Conductivity, pH and dissolved oxygen (DO) were measured on fresh (t_0) and 24-h-old (t_{24}) test water daily.

2.5 Chemical analyses

Water samples from Gadji and Cooper Creek were analysed for TOC/DOC at *eriss*' analytical chemistry laboratory. All other analyses were carried out by ChemNorth on behalf of DME, using NATA certified methods.

2.6 Statistical analysis

Due to the nature of the data from the *M. mogurnda* larval survival bioassay, no statistical analysis was required (see Table 2). Data from the *H. viridissima* and *M. macleayi* bioassay were checked for heteroscedasticity and non-normality, however, no transformations were required. Population growth rate (K) of *H. viridissima*, and reproduction of *M. macleayi* were both assessed by 1-way Analysis of Variance (ANOVA), with effects being considered significant if $P \leq 0.05$. Where significant effects were detected, differences among treatment means were located using Tukey's Honestly Significant Difference (HSD) Test. All analyses were performed using the statistical software package, Statistica®.

3 Results

3.1 Physico-chemical and chemical analyses

Major physico-chemical parameters of Gadji Creek and Cooper Creek waters immediately following collection and upon arrival at the laboratory are summarised in Table 2. As can be observed, pH of both creek waters had increased approximately 0.3 - 0.5 units over the 6 h transportation period, and another 0.2 pH units following filtration. This was likely due to the loss of CO_2 from the water samples. The physico-chemical parameters of the test dilutions during the three toxicity bioassays are presented in Appendix A. Briefly, the pH of 100% Gadji Creek water was approximately 6.5, however, when diluted to 32% Gadji Creek water

Table 2: pH, conductivity and alkalinity of Gadji Creek and Cooper Creek water immediately following collection, upon arrival at the laboratory (approximately 6 hours later), and following filtration through 10µm.*

| Water type | Physico-chemical parameter | | | | | | |
|--------------------------|----------------------------|-------------------------|-----------------------|----------------------|-------------------------|-----------------------|-------------------|
| | pH | | | Conductivity (µS/cm) | | | Alkalinity (mg/L) |
| | On-site | Laboratory (unfiltered) | Laboratory (filtered) | On-site | Laboratory (unfiltered) | Laboratory (filtered) | On-site |
| Gadji Creek (AdjSP29) | 5.63 | 5.94 | 6.22 | 287 | 298 | 297 | 15 |
| Cooper Creek (GS8211061) | 6.74 | 7.23 | 7.46 | 66.7 | 66.4 | 65.0 | 142.5 |

* Refer to Appendix A for physico-chemical data during the toxicity bioassays.

increased to approximately 7.4. The pH of the remaining dilutions, including the control (100% Cooper Creek water), was approximately 7.4 - 7.5. Increases in pH of approximately 0.3 - 0.8 units were observed over each 24 h period, prior to solution renewal. Conductivity ranged from just below 70 µS/cm for control (Cooper Creek) water to approximately 300 µS/cm for 100% Gadji Creek water, while D.O. remained at greater than 90% saturation at all times.

The results of the chemical analyses of Gadji Creek and Cooper Creek water are presented in Table 3. Major differences include HCO₃ (ie alkalinity), filterable and labile Al, Ca and Mg (hardness), NO₃, NH₄, SO₄, and Mn.

3.2 Toxicity testing

The results of the *M. mogurnda* and *H. viridissima* toxicity bioassays carried out on Gadji Creek water are shown in Table 4, while those of the *M. macleayi* bioassay are shown in Figure 2 (see Appendix B for raw data). Gadji Creek water had no adverse effect on newly-hatched *M. mogurnda* sac-fry, with all animals surviving at all dilutions, including 100%, over the 96 h test period ($P > 0.05$). Similarly, Gadji Creek water had no adverse effect on *H. viridissima*, with the population growth rate (K) well above the minimum acceptable rate of 0.34 day⁻¹ at every concentration, over the 96 h test period ($P > 0.05$). However, Gadji Creek water did have an adverse effect on *M. macleayi*, with a significant 12% reduction in the number of offspring per adult over 3 broods in animals exposed to 100% Gadji Creek water, compared to control (Cooper Creek water), 1% and 3.2% Gadji Creek water ($P \leq 0.05$). In addition, reproduction in *M. macleayi* exposed to 32% Gadji Creek water was significantly lower than those exposed to 1% Gadji Creek water ($P \leq 0.05$), but not controls. The number of offspring per adult in *M. macleayi* exposed to 0, 1, 3.2, 10, 32 and 100% Gadji Creek water, expressed as the mean \pm SE ($n = 10$), were 44.4 \pm 0.7, 45.7 \pm 0.7, 45.6 \pm 0.8, 43.1 \pm 0.8, 41.8 \pm 0.4, and 39.7 \pm 0.6, respectively.

Table 3 Chemistry of test (Gadji Creek) and dilution (Cooper Creek) waters

| Parameter | Units | Creek (sampling location) | |
|---------------------------|-------|----------------------------|-----------------------------|
| | | Gadji Creek (Adj. SP29) | Cooper Creek (GS8211061) |
| Al (total) | µg/L | 89.09 | 87.29 |
| Al (filterable) | µg/L | 32.98 | 15.94 |
| Al (labile) | µg/L | 27.72 | ND |
| Si (as SiO ₂) | mg/L | 16.9 | 6.9 |
| HCO ₃ | mg/L | 18.3 | 173.7 |
| Na | mg/L | 4.8 | 3.5 |
| K | mg/L | 1.3 | 0.1 |
| Mg | mg/L | 19.8 | 5.6 |
| Ca | mg/L | 13.1 | 1.2 |
| Cl | mg/L | 5.5 | 4.9 |
| NO ₃ | mg/L | 16.75 | 0.05 |
| NO ₂ | mg/L | 0.06 | 0.05 |
| NH ₄ N | mg/L | 4.6 | 0.05 |
| SSO ₄ | mg/L | 102.7 | 0.1 |
| Mn (total) | µg/L | 67.42 | 10.20 |
| Mn (filtered) | µg/L | 55.22 | 1.62 |
| U (total) | µg/L | 0.18 | 0.08 |
| U (filtered) | µg/L | 0.15 | 0.06 |
| DOC | mg/L | 3.37 | 3.4 |
| TOC | mg/L | 3.47 | 3.6 |

Table 4 Effect of Gadji Creek water on the survival and population growth rate (K) of *M. mogumda* and *H. viridissima*, respectively, after 96 h. Results are expressed as the mean (SE, n).

| % Gadji Creek water | Bioassay | |
|---------------------|--|---|
| | Purple-spotted gudgeon, <i>M. mogumda</i> (% Survival) | Green hydra, <i>H. viridissima</i> (Pop ^l . growth rate, K) |
| 0 | 93.3 (2.7, 3) | 0.407 (0.004, 3) |
| 1 | 100 (0, 3) | 0.366 (0.012, 3) |
| 3.2 | 100 (0, 3) | 0.401 (0.016, 3) |
| 10 | 100 (0, 3) | 0.379 (0.009, 3) |
| 32 | 100 (0, 3) | 0.381 (0.014, 3) |
| 100 | 100 (0, 3) | 0.403 (0.012, 3) |

There were no significant differences in the *M. mogumda* and *H. viridissima* bioassays.

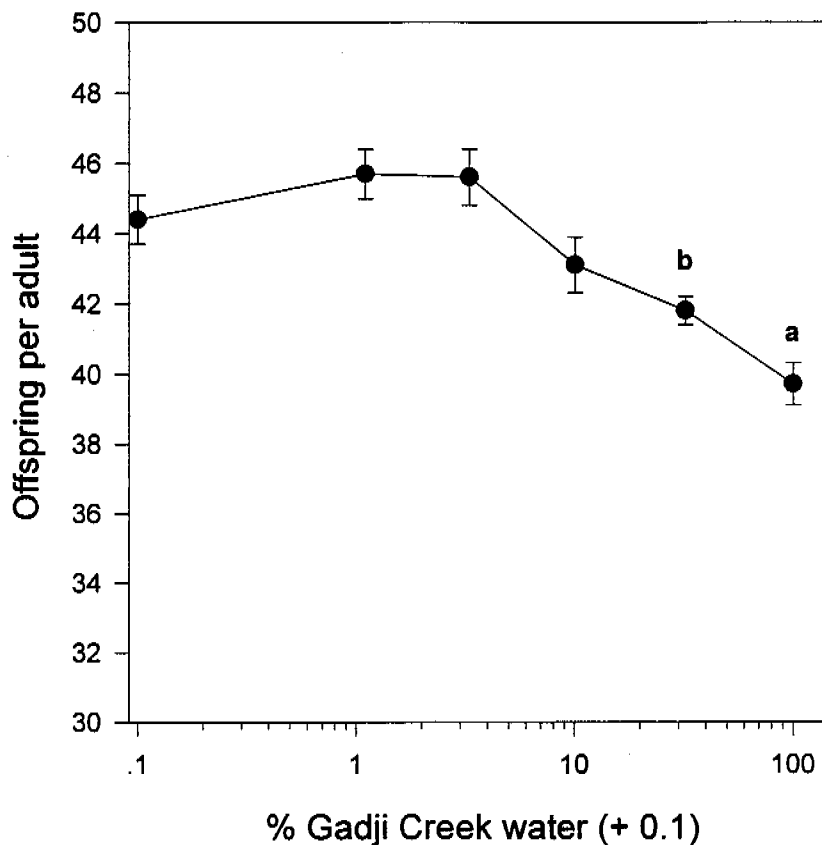


Figure 2 Effect of Gadji Creek water on reproduction in the cladoceran, *M. macleayi*, exposed over 3 reproductive broods (5-6 days). Results are expressed as the mean \pm SE ($n = 10$).

a denotes a significant difference from 0, 1 and 3.2% Gadji Creek water ($P \leq 0.05$).

b denotes a significant difference from 1% Gadji Creek water only ($P \leq 0.05$).

4 Discussion

Gadji Creek water exhibited no, or very little toxicity to the three aquatic organisms assessed in the present study. While a statistically significant 12% reduction in reproduction was observed in *M. macleayi* exposed to 100% Gadji Creek water, one could question the ecological significance of this. Firstly, it has been suggested that such minor changes in effects are often of little environmental concern due to naturally low percentages of recruitment of organisms to their reproductive stages (Underwood & Peterson 1988, Bunn 1995). Secondly, the reduction in mean offspring numbers at 100% Gadji Creek water was a result of slightly smaller third broods in some animals, and it is known that effects on later broods (eg > second brood) generally have much less influence on the intrinsic rate of population increase (r ; van Leeuwen et al 1985). Given this, and the fact that no effects were observed on *M. mogurnda* or *H. viridissima*, it is difficult to conclude that the 12% reduction in reproduction would eventually have led to significant, adverse effects at the population level.

Two previous studies have been carried out on the toxicity of Gadji Creek water, using the same three test species (Hyne 1991, Rippon & McBride 1994). The *M. macleayi* and *H. viridissima* bioassays carried out by Hyne (1991) could not be compared to the results of the present study as they were invalid. In contrast to the results of the present study, Rippon & McBride (1994) found that exposure to 32 and 100% Gadji Creek water collected in April 1993 resulted in approximately 80 and 100% mortality of *M. macleayi*, respectively, following the same test duration (5 - 6 days). In addition, 32 and 100% Gadji Creek water resulted in a 30 and 80% reduction in population growth rate (K) of *H. viridissima*, respectively, following 6 days of exposure. However, as with the present study, Gadji Creek water had no apparent effect on the hatchability and larval survival of *M. mogurnda* in both previous studies (Hyne 1991; Rippon & McBride 1994). Excluding the different times at which the creek water's toxicity was assessed (in terms of both the year and time of year), and slight differences in the test protocols and water collection site (AdjSP29 in the present study compared to GS8211079 in 1993; see figure 1), most variables were similar between the present study and that of Rippon & McBride (1994) (ie diluent/control water, dilutions tested). Rippon & McBride (1994) were unable to attribute the toxicity of the water to any of the chemical water quality parameters measured, except perhaps elevated manganese (Mn) in the case of *M. macleayi*.

Since the study of Rippon & McBride (1994), there appears to have been a marked improvement in the water quality that has allowed *M. macleayi* and *H. viridissima* to survive and reproduce normally in Gadji Creek water in the laboratory. Comparisons of the measured physico-chemical characteristics of the waters in both studies offer few explanations for this. However, Mn concentration had decreased markedly from 270 µg/L in 1991 (Rippon & McBride 1994) to 67 µg/L in August 1997. While this might account for the reduction in toxicity to *M. macleayi*, it cannot explain the similar reduction for *H. viridissima*. Although Al and silica concentrations were not measured by Rippon & McBride (1994), DME data for early 1993 (DME 1996), indicate that total Al was in the order of 200-250 µg/L compared to 89 µg/L in the present study. This may offer an alternative explanation for the reduction in toxicity of Gadji Creek water from 1993 to 1997, although, as discussed below, high silica levels may have prevented toxicity due to Al on both occasions. Nevertheless, the reduction in Al from previous years (including 1993) to August 1997 represents one of the only visible features that the corresponding difference in toxicity could be attributed to. However, this does not necessarily represent a long term temporal decrease in Al, but rather an anomaly created by an unrelated seasonal or hydrological factor, as discussed below.

The results of the present study, particularly the lack of an observed effect on the fish species, *M. mogurnda*, supported the fish monitoring results of Pidgeon & Boyden (1995; in prep.), whereby following initial declines in fish species richness during the spray irrigation period, fish community structure in Gadji Creek in 1995 had recovered to resemble that prior to the spray irrigation period. There remained, however, a marked difference in fish community structure between Gadji Creek and Cooper Creek that could be correlated with conductivity and sulphate levels (Pidgeon & Boyden 1995). The chemical analyses from the present study show that these physico-chemical differences between the two creeks still exist. However, changes in fish communities within Gadji Creek could not be correlated to any such chemical parameters, and it was suggested that changes may have been due to natural fluctuations in fish populations (Pidgeon & Boyden 1995). As with Rippon & McBride (1994), Pidgeon & Boyden (1995; in prep.) did not measure Al or silica in Gadji Creek water.

Particular physico-chemical characteristics of Gadji Creek water (AdjSP29) collected in August 1997 for the present study, differed markedly compared to 1996 and previous years

(see Appendix C). As stated above, in July 1996, and for that matter for several years prior, filterable Al measured approximately 200-250 µg/L (DME 1996) compared to only 33 µg/L in August 1997. In addition, pH in June 1995 and July 1996 was 4.05 and 4.48, respectively (DME unpublished data; see Appendix C), compared to 5.6 in August 1997. Silica was 27 mg/L in July 1996 compared to 16.7 mg/L in August 1997, although the concentration has not varied too greatly since 1986, and has always been in molar excess of Al (DME 1996). Thus, several physico-chemical parameters of Gadji Creek water in July 1997 appeared to be atypical compared to records for similar periods in previous years. This was most likely due to higher rainfall during the 1996-97 wet season compared to previous years. Another potential reason is a reduction in the ammonium source, resulting in a rise in pH, deceleration of soil weathering, and thus, less solubilisation of Al. While nitrate and ammonium were elevated at the time of this sampling, they were nonetheless lower than pre-decommissioning values.

The ANZECC Water Quality Guideline value (for the protection of aquatic ecosystems) for Al is currently 0.005 mg/L in freshwaters below pH 6.5 (ANZECC 1992). Thus, although the Al levels in Gadji Creek were lower than in previous years, they still exceeded the ANZECC value, yet no toxicity was observed. Previous studies have shown that in waters below pH 5, fish and amphibians can be adversely affected by Al at concentrations as low as 0.02, and 0.01 mg/L, respectively (CCREM 1995). Although a total of 89 µg/L Al was present in Gadji Creek water, only 27.7 µg/L was labile and therefore, presumably, bioavailable. While this still exceeds the ANZECC value, it is lower than the available toxicity values for the majority of fish and invertebrates (CCREM 1995). Furthermore, silica levels were much higher than those of Al, with any potential toxicity perhaps being ameliorated through complexation of silica with Al, as demonstrated by Birchall et al (1989). In their experiments, a silica : Al ratio of 13 resulted in the elimination of acute Al toxicity to Atlantic salmon fry (*Salmo salar*) (Birchall et al 1989). The total Al and silica concentrations were approximately 0.19 and 2.6 mg/L, respectively (Birchall et al 1989), compared to 0.09 and 16.9 mg/L for the present study (ie less Al and more silica than Birchall et al 1989). Thus, given the results of Birchall et al (1989), and the chemistry of Gadji Creek water at the time of sampling, it is not surprising that no, or very little, aquatic toxicity due to Al was observed.

5 Conclusions

Gadji Creek water collected in August 1997 exhibited no toxicity to the fish, *M. mogurnda*, and the green hydra, *Hydra viridissima*, and only slight toxicity to the cladoceran, *Moinodaphnia macleayi*. The effect on *M. macleayi*, a 12% reduction in reproduction, was judged not to be ecologically significant based on the high numbers of offspring that were produced. The toxicity results were mostly in contrast to a previous study on the toxicity of Gadji Creek water to the above three organisms, in 1993. Based on available water chemistry data, it was difficult to identify the reason(s) for the reduction in toxicity of Gadji Creek water between 1993 and 1997. However, the lower than usual concentration of Al measured in Gadji Creek water in the present study compared to previous years may have been a contributing factor to the lack of toxicity of the water. Nevertheless, silica, which has been shown to complex with, and reduce the toxicity of Al to fish, remains at concentrations well in molar excess of Al concentrations. Thus, given the lower than usual Al concentration and the high silica concentration, it is not surprising that Gadji Creek water exhibited no toxicity to the three organisms assessed.

6 References

- ANZECC 1992. *Australian Water Quality Guidelines for Fresh and Marine Waters*. National Water Quality Management Strategy, Australian and New Zealand Environment and Conservation Council, Australia.
- Birchall JD, Exley C, Chappell JS & Phillips MJ 1989. Acute toxicity of aluminium to fish eliminated in silicon-rich acid water. *Nature* 338, 146-148.
- Bunn SE 1995. Biological monitoring of water quality in Australia: Workshop summary and future directions. *Aust. J. Ecol.* 20, 220-227.
- Burrows WD 1977. Aquatic aluminium: chemistry, toxicology, and environmental prevalence. *CRC Crit. Rev. Environ. Contr.* 7, 167-216.
- CCREM 1995. *Canadian water quality guidelines*, Canadian Council of Resource and Environment Ministers, Ottawa, Canada.
- Department of Mines and Energy (DME) 1996. *Environmental surveillance monitoring in the Alligator Rivers Region*. Report No. 32, for the period 1 April 1996 - 30 September 1996. Mines Division, DME, Darwin, NT, pp. 7-13.
- Driscoll CT, Baker JP, Bisogni JJ & Schofield CL 1980. Effect of aluminium speciation on fish in dilute acidified waters. *Nature* 284, 161-164.
- Hyne RV 1991. *Biological toxicity testing of Gadjarigamundah Creek at Nabarlek*. IR 42, Supervising Scientist for the Alligator Rivers Region, Canberra, ACT.
- Hyne RV, Rippon GD, Hunt SM & GH Brown 1996. *Procedures for the biological toxicity testing of mine waste waters using freshwater organisms*. SSR 110, Supervising Scientist for the Alligator Rivers Region, Canberra, ACT, 67 pp.
- Markich SJ & Camilleri C 1997. *Investigation of metal toxicity to tropical biota: Recommendations for revision of the Australian water quality guidelines*. SSR 127, Supervising Scientist for the Alligator Rivers Region, Canberra, ACT, 87 pp.
- Pidgeon RWJ & Boyden JM 1995. *Assessment of the recovery of Gadjarigamundah Creek from the effects of land application of waste water at Nabarlek Uranium Mine using fish community structure*. Report for project 2220. IR 196, Supervising Scientist for the Alligator Rivers Region, Jabiru, NT, 17 pp.
- Poléo ABS, Østbye K, Øxnevad SA, Andersen RA, Heibo E & Vøllestad LA 1997. Toxicity of acid aluminium-rich water to seven freshwater fish species: A comparative laboratory study. *Environmental Pollution* 96(2), 129-139.
- Rippon G & McBride P 1994. *Biological toxicity testing of Gadjarigamundah Creek water at Nabarlek - final report for project 2108*. IR 142, Supervising Scientist for the Alligator Rivers Region, Jabiru, NT, 7 pp.
- Underwood AJ & Peterson CH 1988. Towards an ecological framework for investigating pollution. *Mar. Ecol. Progr. Ser.* 46, 227-234.
- van Leeuwen CJ, Luttmer WJ & Griffioen PS 1985. The use of cohorts and populations in chronic toxicity studies with *Daphnia magna*: a cadmium example. *Ecotoxicol. Environ. Safety* 9, 26-39.

Witters HE, van Puymbroeck S, Vangenechten JHD & Vanderborght OLJ 1990. The effect of humic substances on the toxicity of aluminium to adult rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J. Fish Biol.* 37, 43-53.

Appendix A

Major physico-chemical parameters (pH, conductivity, dissolved oxygen) expressed as the mean (SD) of the test dilutions during the three toxicity bioassays.

| Parameter | Dilution | <i>M. mogurda</i> | | <i>H. viridissima</i> | | <i>M. macleayi</i> | |
|---|----------|-------------------|---------------|-----------------------|-------------|--------------------|-------------|
| | | t_0^* | t_{24}^{**} | t_0 | t_{24} | t_0 | t_{24} |
| pH | Control | 7.45 (0.03) | 7.63 (0.06) | 7.44 (0.02) | 7.54 (0.11) | 7.46 (0.06) | 7.80 (0.17) |
| | 1% | 7.55 (0.04) | 7.67 (0.06) | 7.53 (0.02) | 7.58 (0.10) | 7.54 (0.05) | 7.83 (0.10) |
| | 3.2% | 7.52 (0.03) | 7.67 (0.04) | 7.51 (0.02) | 7.60 (0.13) | 7.52 (0.05) | 7.90 (0.15) |
| | 10% | 7.48 (0.03) | 7.64 (0.06) | 7.46 (0.01) | 7.49 (0.18) | 7.46 (0.02) | 7.78 (0.12) |
| | 32% | 7.37 (0.02) | 7.54 (0.06) | 7.36 (0.01) | 7.42 (0.12) | 7.36 (0.02) | 7.67 (0.09) |
| | 100% | 6.60 (0.02) | 6.93 (0.10) | 6.60 (0.03) | 6.75 (0.10) | 6.54 (0.06) | 7.08 (0.16) |
| Conductivity ($\mu\text{S}/\text{cm}$) | Control | 64.1 (0.3) | 67.8 (0.2) | 64.2 (0.3) | 67.4 (0.4) | 68.2 (0.2) | 69.4 (0.4) |
| | 1% | 67.4 (0.5) | 70.9 (0.4) | 67.5 (0.4) | 71.1 (0.9) | 70.9 (0.7) | 71.9 (0.4) |
| | 3.2% | 73.2 (0.5) | 76.6 (0.7) | 73.3 (0.4) | 75.8 (0.6) | 76.5 (0.6) | 76.6 (0.5) |
| | 10% | 89.9 (0.3) | 93.6 (0.7) | 90.1 (0.2) | 93.3 (0.8) | 92.7 (0.8) | 94.4 (0.8) |
| | 32% | 142.9 (0.3) | 147.2 (1.4) | 142.9 (0.3) | 147 (0.5) | 145.8 (1.0) | 147.7 (1.4) |
| | 100% | 297.0 (0.6) | 304.0 (3.7) | 297.3 (0.4) | 305.3 (2.6) | 299.8 (1.7) | 303.2 (1.0) |
| D.O. (% saturation) | Control | 106.4 (7.1) | 96.8 (1.9) | 108.0 (7.1) | 90.0 (4.5) | 101.4 (2.1) | 96.4 (2.4) |
| | 1% | 106.6 (7.7) | 95.8 (2.5) | 108.3 (7.8) | 90.3 (4.9) | 101.2 (1.9) | 96.4 (1.0) |
| | 3.2% | 105.2 (5.6) | 95.8 (2.7) | 106.3 (5.8) | 89.0 (1.0) | 100.8 (1.8) | 97.8 (2.3) |
| | 10% | 105.0 (5.8) | 96.8 (2.0) | 106.3 (5.8) | 88.2 (4.0) | 100.4 (1.6) | 95.8 (2.4) |
| | 32% | 105.6 (6.5) | 96.8 (2.8) | 107.0 (6.6) | 90.0 (4.3) | 100.6 (1.4) | 98 (2.8) |
| | 100% | 105.2 (5.7) | 96.8 (2.9) | 106.3 (4.8) | 91.0 (4.3) | 100.6 (1.2) | 95.8 (2.1) |

* t_0 : fresh test water sample prior to daily solution renewal.

** t_{24} : 24-hour-old test water sample following daily solution renewal.

Appendix B

Raw data for the final day of toxicity tests on Gadji Creek water.

| Species/endpoint/ test duration | Dilution (% Gadji Creek water) | | | | | |
|--|--------------------------------|---------------|---------------|---------------|---------------|---------------|
| | 0 (Control) | 1 | 3.2 | 10 | 32 | 100 |
| <i>M. mogurnda</i> | | | | | | |
| % survival (96 h) | | | | | | |
| Repl 1 | 100 | 100 | 100 | 100 | 100 | 100 |
| Repl 2 | 100 | 100 | 100 | 100 | 100 | 100 |
| Repl 3 | 100 | 100 | 100 | 100 | 100 | 100 |
| Mean % survival (SE) | 100 (0) | 100 (0) | 100 (0) | 100 (0) | 100 (0) | 100 (0) |
| <i>H. viridissima</i> | | | | | | |
| population growth (96 h) | | | | | | |
| Repl 1 | 52 | 45 | 51 | 44 | 45 | 46 |
| Repl 2 | 52 | 46 | 44 | 44 | 42 | 54 |
| Repl 3 | 49 | 39 | 55 | 49 | 51 | 51 |
| Mean growth rate (SE) | 0.407 (0.004) | 0.366 (0.012) | 0.401 (0.016) | 0.379 (0.009) | 0.381 (0.014) | 0.403 (0.012) |
| <i>M. macleayi</i> | | | | | | |
| reproduction (5 days) | | | | | | |
| Repl 1 | 44 | 45 | 44 | 47 | 41 | 41 |
| Repl 2 | 44 | 44 | 46 | 39 | 40 | 41 |
| Repl 3 | 46 | 48 | 44 | 42 | 41 | 41 |
| Repl 4 | 46 | 44 | 50 | 40 | 43 | 39 |
| Repl 5 | 39 | 49 | 48 | 43 | 43 | 40 |
| Repl 6 | 47 | 47 | 45 | 43 | 42 | 39 |
| Repl 7 | 45 | 48 | 47 | 43 | 41 | 43 |
| Repl 8 | 43 | 44 | 45 | 45 | 42 | 39 |
| Repl 9 | 44 | 44 | 46 | 47 | 41 | 37 |
| Repl 10 | 46 | 44 | 41 | 42 | 44 | 37 |
| Mean number of off-spring per adult (SE) | 44.4 (0.7) | 45.7 (0.7) | 45.6 (0.8) | 43.1 (0.8) | 41.8 (0.4) | 39.7 (0.6) |

Appendix C

Available physico-chemical and chemical data for Gadji Creek water (AdjSP29) since March 1995 (Data supplied by DME)

| Parameter | Units | Date | | | | | | | |
|----------------------|-------------------------|----------|----------|-----------|-----------|-----------|-----------|----------|-----------|
| | | 5-Mar-95 | 5-Jun-95 | 26-Sep-95 | 21-Jul-96 | 17-Mar-97 | 19-Aug-97 | 9-Dec-97 | 11-Mar-98 |
| pH | | 5.38 | 4.05 | 6.98 | 4.48 | 7.1 | 5.63 | 6.67 | 7.22 |
| Cond | uS/cm | 120 | 276 | 424 | 462 | 36.1 | 287 | 213 | 40.9 |
| CO ₃ /Alk | mg CaCO ₃ /L | | | | 3 | | 15 | 0 | 0 |
| HCO ₃ | mg/L | | | | | | 18.3 | | |
| Na | mg/L | 1.5 | | | 5.3 | | 4.8 | | 1.9 |
| K | mg/L | 0.22 | | | 1.3 | | 1.3 | | 0.1 |
| Mg | mg/L | 3.7 | | | 27 | | 19.8 | | 2.1 |
| Ca | mg/L | 3.1 | | | 19 | | 13.1 | | 1.3 |
| Cl | mg/L | 2.7 | | | 9.4 | | 5.5 | | 2.2 |
| NO ₃ | mg/L | 18 | | | 71 | | 16.75 | | 1.59 |
| NO ₂ | mg/L | 0.05 | | | 0.05 | | 0.06 | | <0.16 |
| NH ₄ N | mg/L | 1.1 | | | | | 4.6 | | |
| SSO ₄ | mg/L | 15 | | | 130 | | 102.7 | | 8.1 |
| SiO ₂ | mg/L | 8.4 | | | 27 | | 16.9 | | 7.9 |
| Al (filt) | ug/L | 121 | | | 220 | | 32.98 | | 58 |
| Al (total) | ug/L | 187 | | | 220 | | 89.09 | | 102 |
| Cu (filt) | ug/L | 1 | | | | | | | |
| Cu (total) | ug/L | 1 | | | | | | | |
| Pb (filt) | ug/L | 0.5 | | | | | | | |
| Pb (total) | ug/L | 0.5 | | | | | | | |
| Zn (filt) | ug/L | 3.2 | | | | | | | |
| Zn (total) | ug/L | 3.4 | | | | | | | |
| Mn (filt) | ug/L | 27 | | | 90 | | 55.22 | | 8.96 |
| Mn (total) | ug/L | 30 | | | 88 | | 67.42 | | 12.3 |
| U (filt) | ug/L | 0.2 | | | 0.35 | | 0.15 | | 0.032 |
| U (total) | ug/L | 0.3 | | | 0.37 | | 0.18 | | 0.041 |
| Ra (filt) | mBq/L | 32 | | | | | | | |
| Ra (total) | mBq/L | 4 | | | | | | | |