



Internal presentation on  
toxicity of  $\text{MgSO}_4$  to  
Magela Creek, NT:  
laboratory and field  
results to date

---

CD McCullough, CL Humphrey,  
A Hogan, C Camilleri,  
MM Douglas, P Gell, R Shiel  
& R van Dam

March 2003

# **Internal presentation on toxicity of $\text{MgSO}_4$ to Magela Creek, NT: laboratory and field results to date**

Clint McCullough, [clinton.mccullough@ea.gov.au](mailto:clinton.mccullough@ea.gov.au), Northern Territory University, c/- Environmental Research Institute of the Supervising Scientist, Locked Bag 2, Jabiru, NT 0886, Australia.

Chris Humphrey, [chris.humphrey@ea.gov.au](mailto:chris.humphrey@ea.gov.au), Ecosystem Protection Program, Environmental Research Institute of the Supervising Scientist, GPO Box 461, Darwin NT 0801, Australia.

Alicia Hogan, [alicia.hogan@ea.gov.au](mailto:alicia.hogan@ea.gov.au), Ecological Risk Assessment Program, Environmental Research Institute of the Supervising Scientist, GPO Box 461, Darwin NT 0801, Australia.

Caroline Camilleri, [caroline.camilleri@ea.gov.au](mailto:caroline.camilleri@ea.gov.au), Ecological Risk Assessment Program, Environmental Research Institute of the Supervising Scientist, GPO Box 461, Darwin NT 0801, Australia.

Michael Douglas, [michael.douglas@ntu.edu.au](mailto:michael.douglas@ntu.edu.au), School of Biological, Environmental & Chemical Sciences, Northern Territory University, Darwin, NT 0909, Australia.

Peter Gell, [peter.gell@adelaide.edu.au](mailto:peter.gell@adelaide.edu.au), Department of Geographical and Environmental Studies, University of Adelaide, South Australia 5005.

Russel Shiel, [russell.shiel@adelaide.edu.au](mailto:russell.shiel@adelaide.edu.au), Department of Environmental Biology, University of Adelaide, South Australia 5005.

Rick van Dam, [rvandam@skm.com.au](mailto:rvandam@skm.com.au), Ecotoxicology Laboratory, Sinclair Knight Mertz, 100 Christie St, St Leonards, NSW 2065, Australia.

# Contents

<b>Abstract</b>	<b>5</b>
Background	5
Research findings	5
Conclusion	6
<b>Slides</b>	<b>7</b>
<b>References</b>	<b>33</b>



# Abstract

## Background

Magnesium sulphate ( $\text{MgSO}_4$ ) is a common mine contaminant for many mines through increased rates of weathering of waste rock. This is also true for the waste rock of the Ranger Uranium Mine (RUM) where  $\text{MgSO}_4$  is a major constituent of waste waters. A discharge containing leachate with elevated solutes from this waste rock, flows from Retention Pond One (RP1) of the Mine into the Magela Creek via Coonjimba Billabong during the wet season. However, the salinity arising from this salt has received very little ecotoxicological assessment and results of recent surveys of waterbodies receiving RUM run-off indicated that differences in macroinvertebrate community composition may be correlated with increased salinity arising from  $\text{MgSO}_4$  (O'Connor et al 1995, O'Connor et al 1996). Therefore, a study has been initiated to examine its effects on the ecology of Magela Creek downstream of RUM through laboratory ecotoxicity tests in natural creek water on specific species, and field mesocosm experiments on macroinvertebrate communities.

## Research findings

Single species laboratory ecotoxicological experiments using a range of local endemic species determined that magnesium sulphate is toxic at a Lowest-Observed-Effect-Concentration (LOEC) range of 6.3 mg/L for *Hydra viridissima* through to 302 mg/L for *Chlorella* sp. At this threshold concentration *Hydra* (the most sensitive species) begin to demonstrate a lack of muscular control, reduced feeding and reproduction rates and eventually death. However, it was not clear whether this toxicity was due to the cation (magnesium ion,  $\text{Mg}^{2+}$ ) or the anion (sulphate,  $\text{SO}_4^{2-}$ ).

In order to determine the contribution of  $\text{SO}_4^{2-}$  to  $\text{MgSO}_4$  toxicity, an experiment was conducted where *Hydra* were exposed to  $\text{Na}_2\text{SO}_4$  over the same range of  $\text{SO}_4^{2-}$  concentrations tested in the  $\text{MgSO}_4$  experiments. The *Hydra* were far less sensitive to  $\text{Na}_2\text{SO}_4$  (LOEC 366 mg/L) compared to  $\text{MgSO}_4$  (21 mg/L) indicating that the toxicity was not due to the anion, sulphate.

To further clarify this, another experiment looked at the toxic effect of the magnesium ion cation ( $\text{Mg}^{2+}$ ) on *Hydra* in the absence of sulphate, using Magnesium chloride ( $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ). This was found to produce a LOEC of 6.1 mg/L; indicating that the cation  $\text{Mg}^{2+}$  was responsible for the toxic effect of magnesium sulphate.

The physiological mechanism of this toxicity is thought to be through an inhibition of calcium channels at a cellular level. However, qualitative surveying revealed that *Hydra* still occur in water bodies receiving mine outflow, such as RP1 itself, at levels exceeding this threshold for  $\text{Mg}^{2+}$  (e.g. 31.6 mg/L at the RP1 spillway and 20.6 mg/L for Coonjimba Billabong in April 2000). This appeared to be through an amelioration by the elevated  $\text{Ca}^{2+}$  levels in this discharge (4.1 mg/L at this same time and place). However, it could also be argued that this tolerance over predicted RP1 water toxicity could be due to a different variety of *Hydra* inhabiting these impacted waters.

To test for this, another series of experiments were undertaken. *Hydra* from Coonjimba billabong were tested for their tolerance to elevated  $\text{Mg}^{2+}$  levels, whilst both Coonjimba Billabong and *eriss* stocks of *Hydra* were exposed to a series of dilutions of Coonjimba

water. At the time of collection of Coonjimba Billabong *Hydra*, the billabong  $Mg^{2+}$  and  $Ca^{2+}$  concentrations were 20.6 and 2.5 mg/L respectively (a Mg:Ca ratio of 8.2:1). Both strains (*eriss* and Coonjimba Billabong) demonstrated no LOEC for Coonjimba water, yet Coonjimba Billabong *Hydra* displayed a NOEC and LOEC of 5.5 and 16.5 mg/L respectively, indicating very slight, if any, adaptation to elevated  $Mg^{2+}$ .

*Hydra* tests undertaken where  $Mg^{2+}$  concentration were maintained at 10 mg/L while  $Ca^{2+}$  concentrations were increased with each treatment showed that as long as the Mg:Ca ratio was maintained at 10:1 or below, then  $Mg^{2+}$  should not be of significant toxicity. The major mechanism allowing *Hydra* to exist in waters elevated in  $Mg^{2+}$  therefore appeared to be through the calcium cations also present in the RP1 discharge.

## Conclusion

The concentration of  $Mg^{2+}$  in the Magela Creek below the discharge is not expected to exceed 10 mg/L at a  $Mg^{2+}$ : $Ca^{2+}$  ratio of 3.6:1 (ERA environmental data, sourced from ERA Ranger Mine Environment Department). Therefore, from these preliminary data, this discharge may not be causing a detrimental effect to the Magela Creek. However, this result is tentative and awaits further data collection and analysis including bringing the *Mogurnda* fry bioassay up to Australian and New Zealand Environment and Conservation Council (ANZECC) guidelines requirements (ANZECC & ARMCANZ 2000).

# Slides

Slide 1

## The ecological effects of magnesium sulphate in Magela Creek, NT

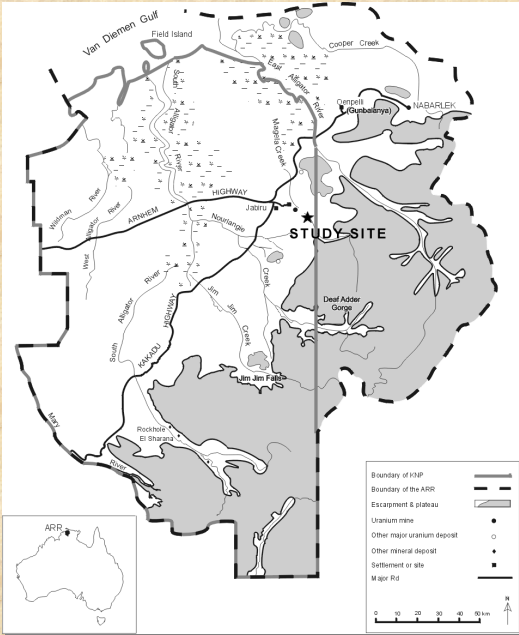
Clint McCullough, Chris Humphrey, Alicia Hogan, Michael Douglas, Peter Gell, Russ Shiel, Rick van Dam



The slide features a textured, parchment-like background. At the top, the title 'The ecological effects of magnesium sulphate in Magela Creek, NT' is written in a large, bold, blue font. Below the title, the authors' names are listed in a smaller, brown font. At the bottom, there are three logos: Environment Australia (Department of the Environment and Heritage), NTU Northern Territory University, and Supervising Scientist.

Slide 2

## Study area – Kakadu National Park



- Wet/Dry season
- Low solute waters
- Very weakly buffered
- Relatively undisturbed ecosystems
- High conservation values

The slide features a textured, parchment-like background. At the top, the title 'Study area – Kakadu National Park' is written in a large, bold, blue font. Below the title is a map of Kakadu National Park. The map shows the park's boundary, major roads, and various geographical features. A star marks the 'STUDY-SITE' location. A legend in the bottom right corner explains the symbols used on the map. To the right of the map, there is a list of five bullet points describing the study area's characteristics.



## Study area – Magela Creek



- Seasonally flowing tributary of the East Alligator River
- Flows into the world Heritage listed Magela Wetlands
- Extremely soft, poorly buffered waters of low conductivity and pH.

## Changing flows through seasons



Wet



Early-Dry



Mid-Dry



## Chemical characteristics of waterbodies, May 1995

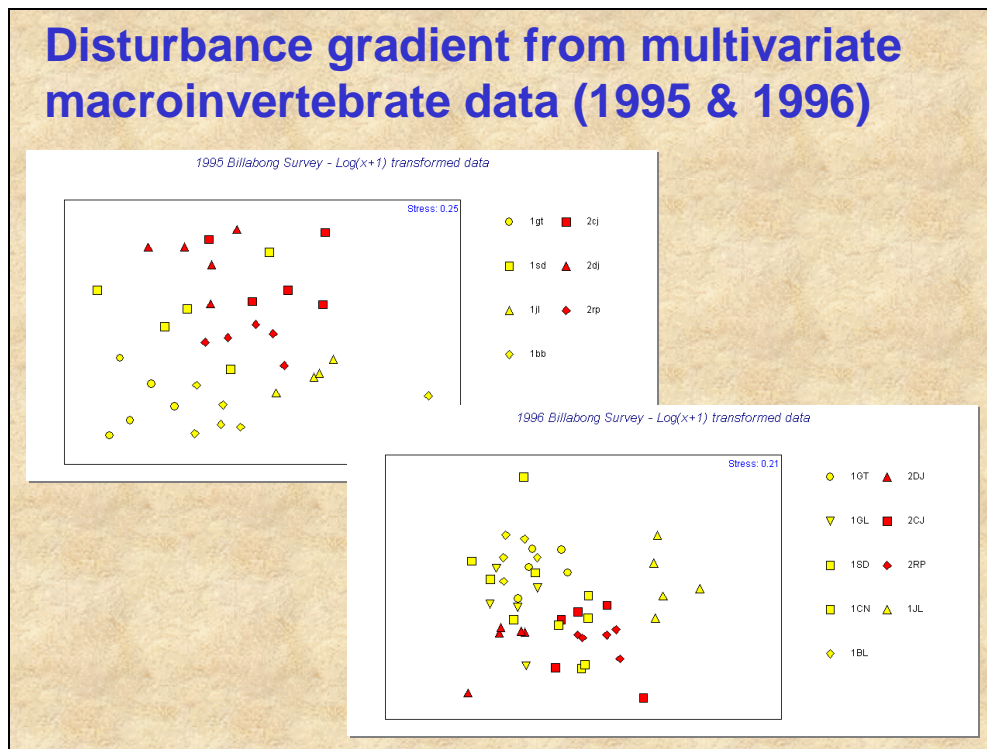
Waterbodies not receiving mine waters	Conductivity (μScm-1)	[Mg] (mg/L)	[SO4] (mg/L)
◆ Buba	41	1.4	0.22
■ Sandy	28	0.38	0.59
● Georgetown	33	1.6	1.6
▲ Town Lake	45	—	—
Waterbodies receiving mine waters			
■ Coonjimba	120	7.5	25
◆ Retention Pond 1	190	18	56
▲ Djalkmara	830	110	320

## Some invertebrate responses in billabongs (May 1995)

### *Waterbodies receiving mine waste waters:*

- **Greater numbers of aerial breathing invertebrates:**
  - surface-dwelling hemipterans (bugs)
  - dytiscid beetles
- **Greater numbers of *Tanytarsus* chironomids**
- **Similar responses in Rockhole Mine Creek (acid-mine drainage)**

Slide 7



Slide 8



## Source of $\text{MgSO}_4$ at RUM



- Primarily due to weathering of the waste rock pile (expected to peak in 2010)

## Retention Pond 1: Wet Season release



- High salinity ( $>300 \mu\text{Scm}^{-1}$ )
- Primarily due to  $\text{Mg}^{2+}$  and  $\text{SO}_4^{2-}$
- Overflows approximately 4 months of the year (January - April)



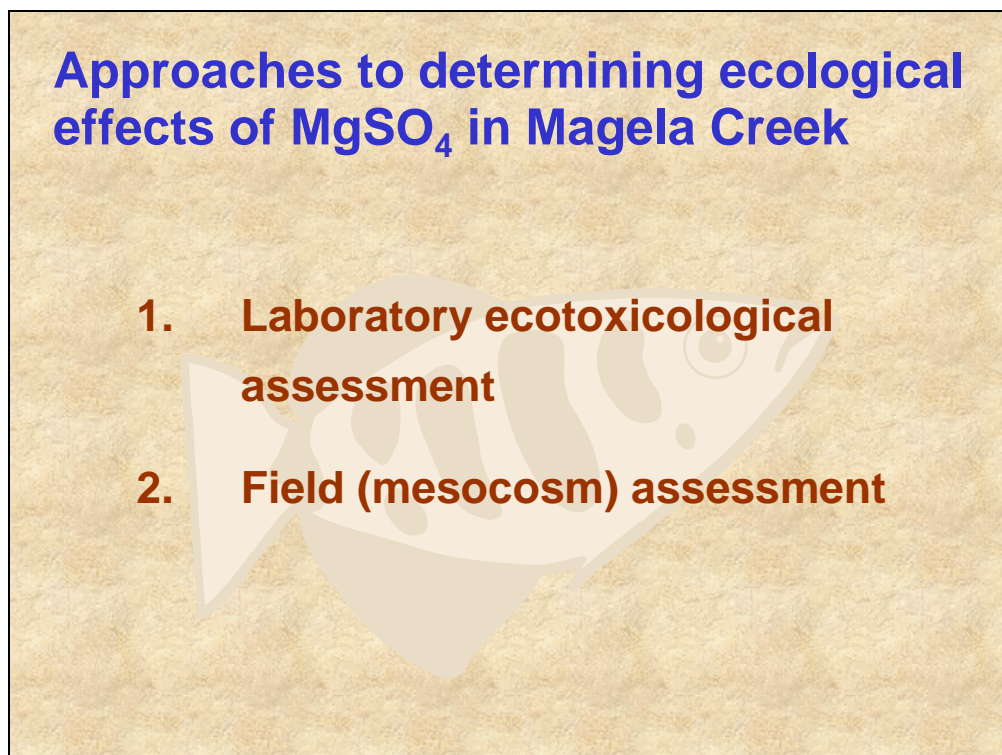
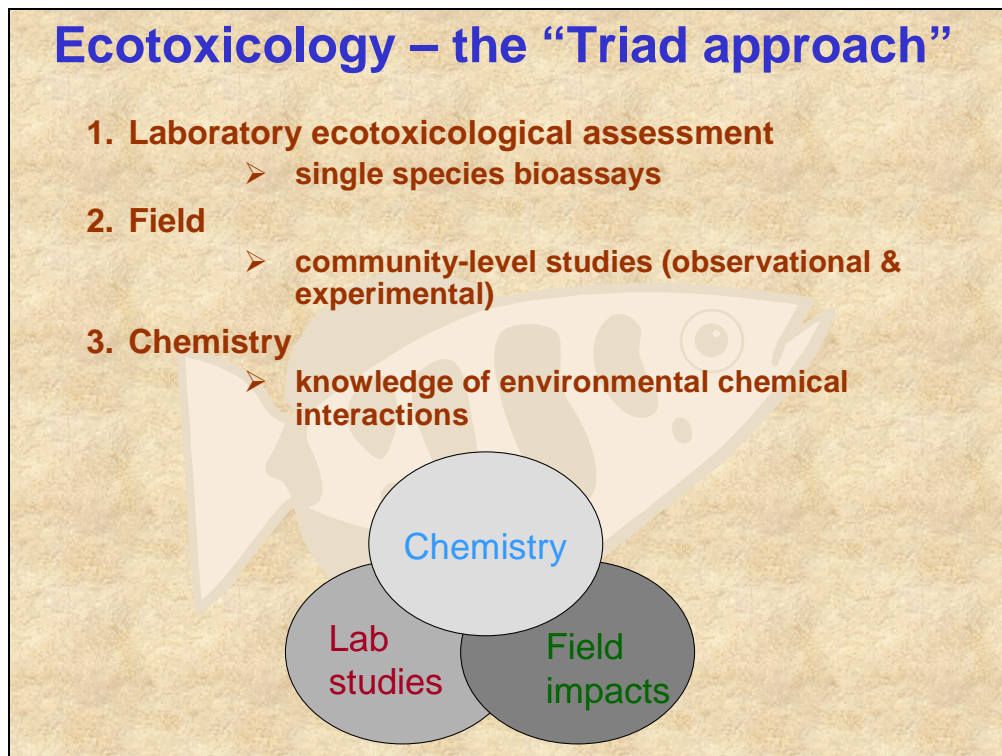
## From RUM to Magela Creek



- Solutes from Waste Rock Pile flow into RP1
- RP1 overflows into Coonjimba Billabong
- Coonjimba Billabong flows to Magela Creek

## Main source of $\text{MgSO}_4$ from RUM







## LABORATORY ECOTOXICOLOGICAL APPROACH

---



### Single-species toxicity tests - background

- Pre-release toxicity tests used historically at Ranger as component of regulatory requirements
- Four species routinely used at **eriss**
- Species selected for: life cycle, ecologically relevant endpoints, ability to be cultured under lab conditions, wide range of taxa and trophic levels
- Standard conditions: 12:12 h light:dark,  $27 \pm 1^\circ\text{C}$ , standard test volumes, etc.

## Single-species toxicity tests

**Species:** purple spotted gudgeon, *Mogurnda mogurnda*

Test duration: 96 h

Endpoint: sac fry survival

Exposure: acute



## Single-species toxicity tests

**Species:** water flea, *Moinodaphnia macleayi*

Test duration: 5-6 d (production of 3 broods)

Endpoint: reproduction

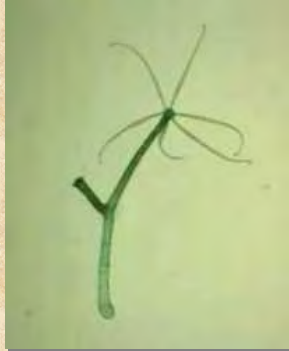
Exposure: chronic





## Single-species toxicity tests

**Species:** green hydra, *Hydra viridissima*



Test duration: 96 h

Endpoint: population growth

Exposure: chronic

## Single-species toxicity tests

**Species:** single-celled green alga, *Chlorella* sp.



Test duration: 72 h

Endpoint: cell division

Exposure: chronic

## Single-species toxicity tests

**Species:** Freshwater snail,  
*Amerianna cumingi*

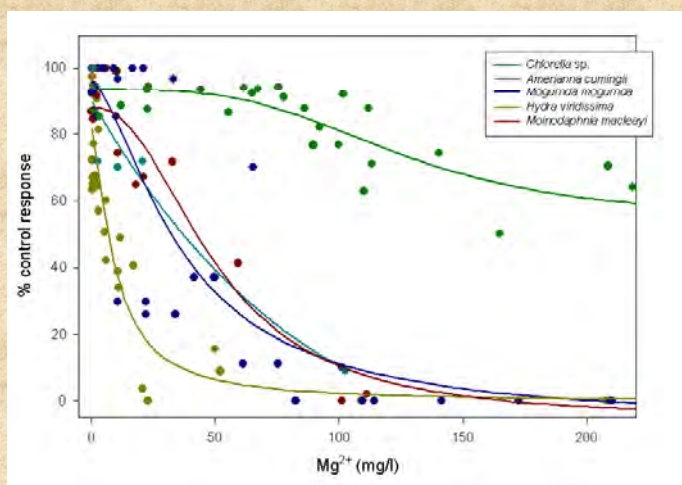


Test duration: 8 days

Endpoint: fecundity (embryos)

Exposure: Chronic

## Summary of ecotoxicity results to date



- *Hydra* highly sensitive - an outlier?
- Initial steep response for *Amerianna*
- *Chlorella* very robust

## Single-species NOECs and LOECs

(geometric means of  $Mg^{2+}$ )

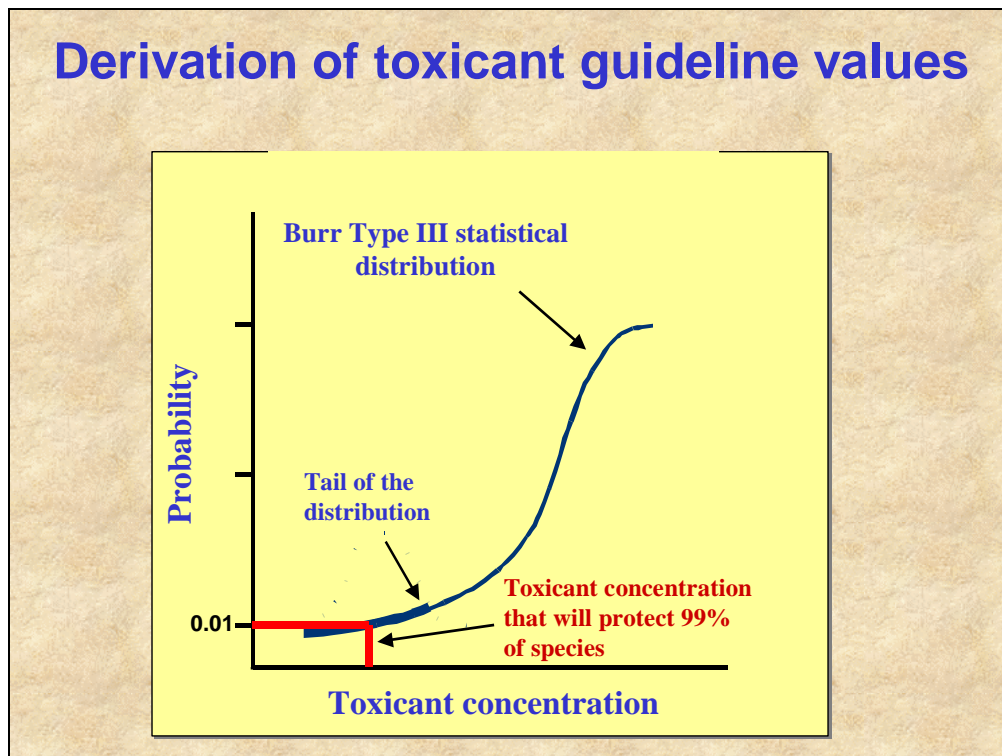
Species tested	NOEC *	LOEC *	No. of tests
<i>Hydra</i>	2.2	4.6	3
<i>Moinodaphnia</i>	10.2	18.0	2
<i>Mogurnda fry</i>	25.2	45.1	4
<i>Chlorella</i>	83.6	143.2	4
<i>Amerianna</i> **	2.0	2.3	1

\*  $Mg^{2+}$  concentration

\*\* IC10 & IC20 results

- ## Deriving toxicant guidelines (single-species toxicity tests)
- **Previously:** assessment factor applied to lowest ecotoxicity value (lowest NOEC)
  - **Now:** Statistical distribution method (ANZECC/ARMCANZ guidelines) – “**BurrliOZ**”
    - Fits ecotoxicity data to the Burr III distribution of curves to protect chosen  $x\%$  of species (e.g. high conservation value sites 99%)
    - Minimum data requirements e.g.  $\geq 5$  species from  $\geq 4$  different taxonomic groups
- More work needed on more taxa





### Magnesium concentrations at or near biological effects

**Billabong studies** (May 1995)

- Waterbodies receiving mine waste waters  
~ 7.5 - 110 mg/L

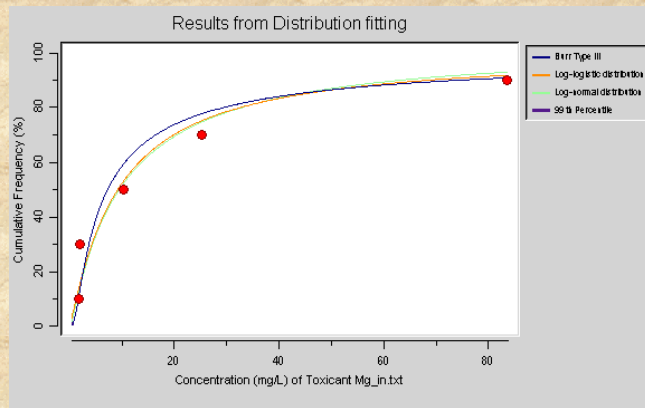
**Ecotoxicological studies** (2001)

- NOECs: 2.0\*, 2.2, 10.2, 25.2, 83.6 mg/L
- LOECs: 2.3\*, 4.6, 18.0, 45.1, 143.2 mg/L
- BurrliOZ 99%: 0.8 mg/L

**Concentrations in field** (e.g. GS0009, 2000-01)

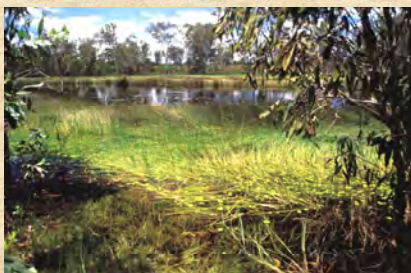
- Max: ~ 4 - 11; frequency exceeded @ GT009 = c.50 %

## BurrliOz modelling of ecotoxicity results

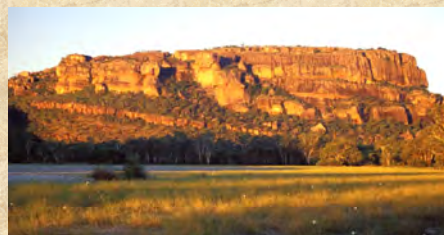


➤ 99% ecosystem protection:  
**Preliminary estimate**  
 $\approx 0.8 \text{ mg/L Mg}^{2+}$

## Field validation of lab tests



Coonjimba Billabong:  
 $\text{Mg}^{2+} \sim 20 \text{ mg/L}$

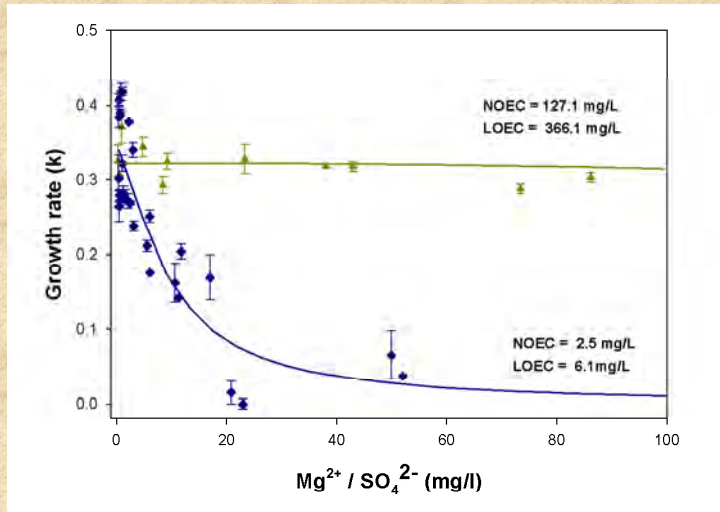


Anbangbang Billabong:  
 $\text{Mg}^{2+} \sim 1.2 \text{ mg/L}$



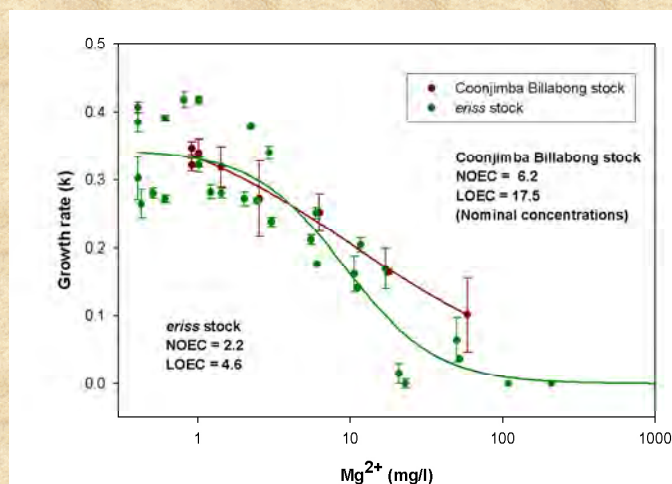
Retention Pond 1:  
 $\text{Mg}^{2+} \sim 30 \text{ mg/L}$

## Cation/anion relative toxicity to Hydra



- Toxicity attributed to  $Mg^{2+}$
- $SO_4^{2-}$  ion already noted in water quality guidelines
- Only high  $SO_4^{2-}$  values toxic

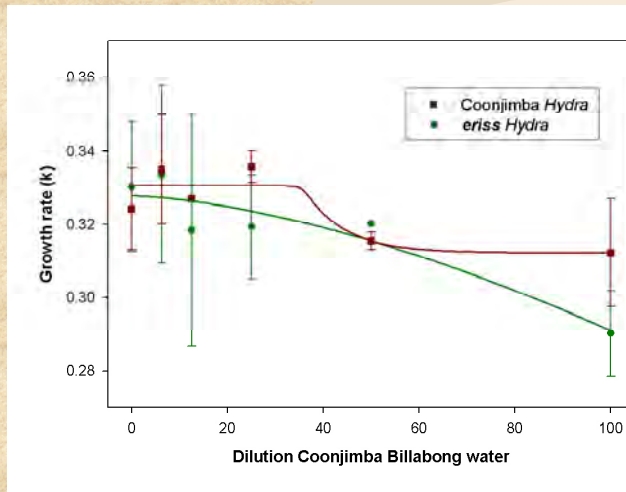
## Population tolerance differences?



- Coonjimba NOEC/LOEC range within approx. 2 – 3 x *eriss* stock
- Possibly “noise” between tests
- Tests on “control” and RP1 stock to follow

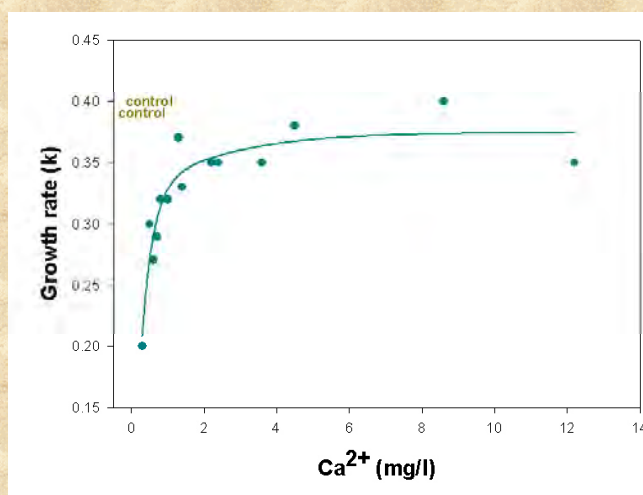


## Physiological tolerance or chemical amelioration?



- Both strains exposed to Coonjimba water
- Similar response of strains
- $Mg^{2+}$  c. 20 mg/L,  $Ca^{2+}$  c. 2.5 mg/L
- Mg:Ca water ratio = 8

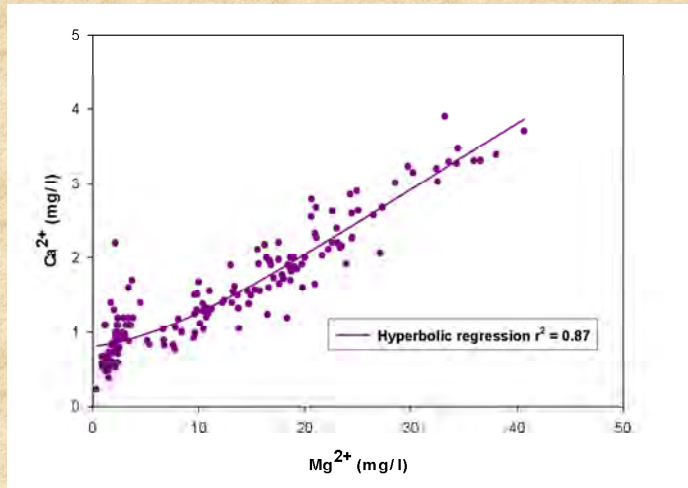
## Calcium amelioration



- Complete recovery @ c. 10 mg/l and greater concentrations of  $Ca^{2+}$
- NOEC geometric mean of 1.2 mg/L
- Mg:Ca ratios of 12:1



## Mg<sup>2+</sup>/Ca<sup>2+</sup> relationship in RUM discharge

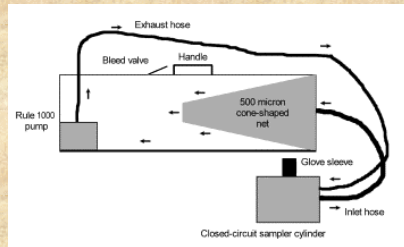


- Arithmetic average  
Mg<sup>2+</sup>:Ca<sup>2+</sup> = 6.76 (< 10 mg/L range)
- Ratio higher at higher Mg<sup>2+</sup> concentrations

## FIELD EXPERIMENTAL APPROACH



## The benthic sampler



- Specifically samples benthic communities
- Functions like a 'vacuum cleaner'
- Quantitative measurements made ( $0.09 \text{ m}^2$ )



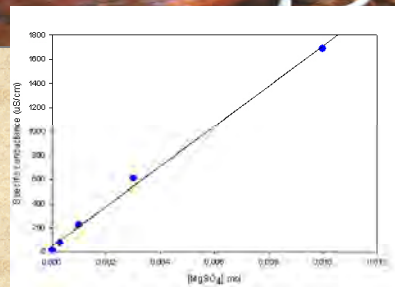
## Pool mesocosm experiments

- Quantitative 'vacuuming' of pool benthic macroinvertebrate communities in creek bed pools



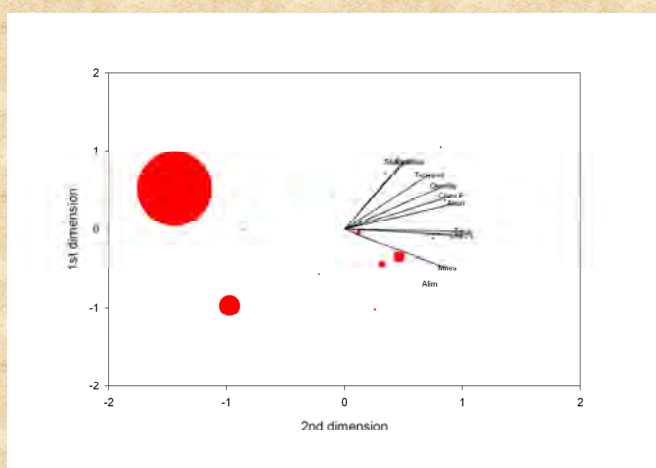


## Spiking pools with epsomite



- Pools spiked with BP grade  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$
- Pools (including control) well mixed
- Concentrations checked with simple conductivity / concentration regression

## 2001 pool spiking data



- Acute impact
- Only 2 pools remaining of 'notable'  $\text{MgSO}_4$  concentration
- All significant taxa correlates away from strongly  $\text{MgSO}_4$  spiked pools

## Current mesocosm experiments



- 3,000 L tubs in drying creek channel in recessional flow 'seeded' with wet creek sediments and waters from the Creek

## Current mesocosm experiments



### Colonisation...

- Aerial dispersal
- Wetted sand
- Leaf litter
- Creek water

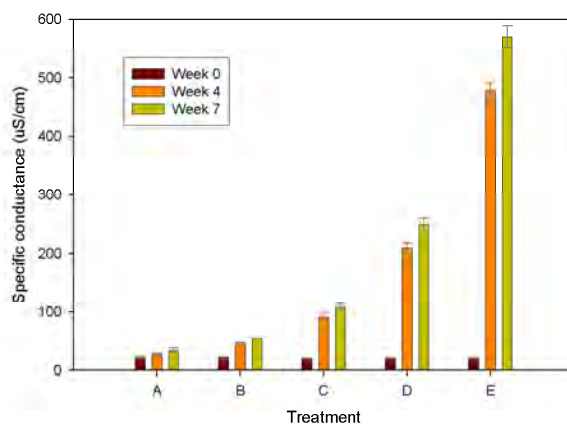


## “BACI” experimental design

### ➤ “Before/After, Control/Impact”

- Sample all tubs before ❖ Week 0
- Spike tubs to varying extents (leave control)
- Sample second time ❖ Week 4
- Repeat post-impact sample ❖ Week 7

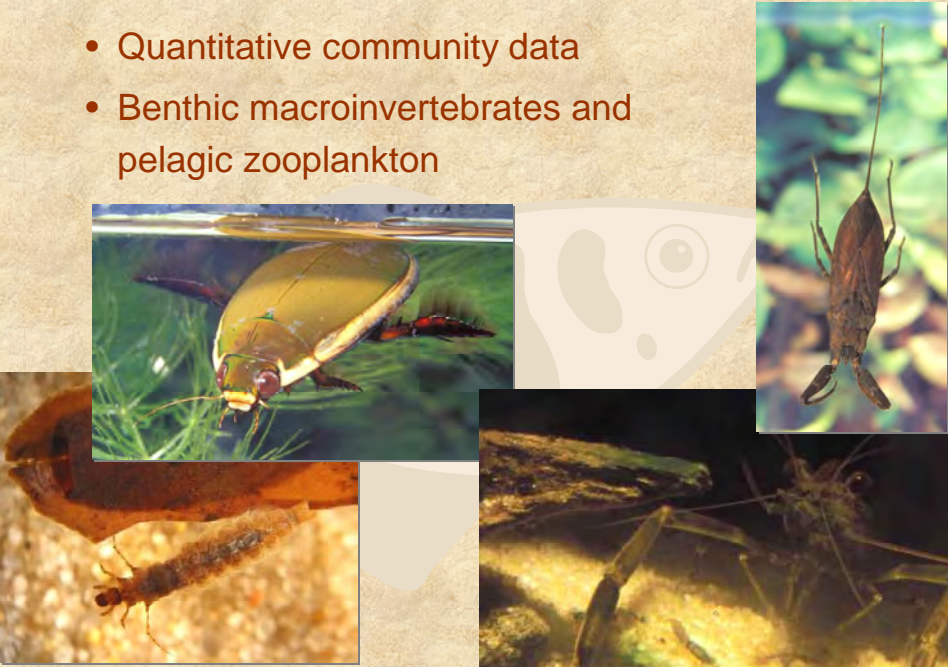
## MgSO<sub>4</sub> concentrations



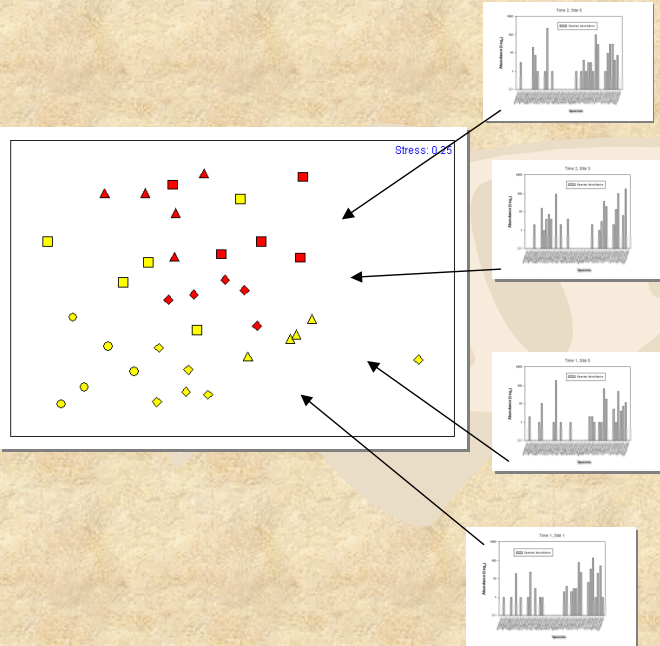
- Conductance increases over time due to evaporation
- Accuracy of spiking good (within c. 10% nominals)

## Mesocosms – (macroinvertebrates)

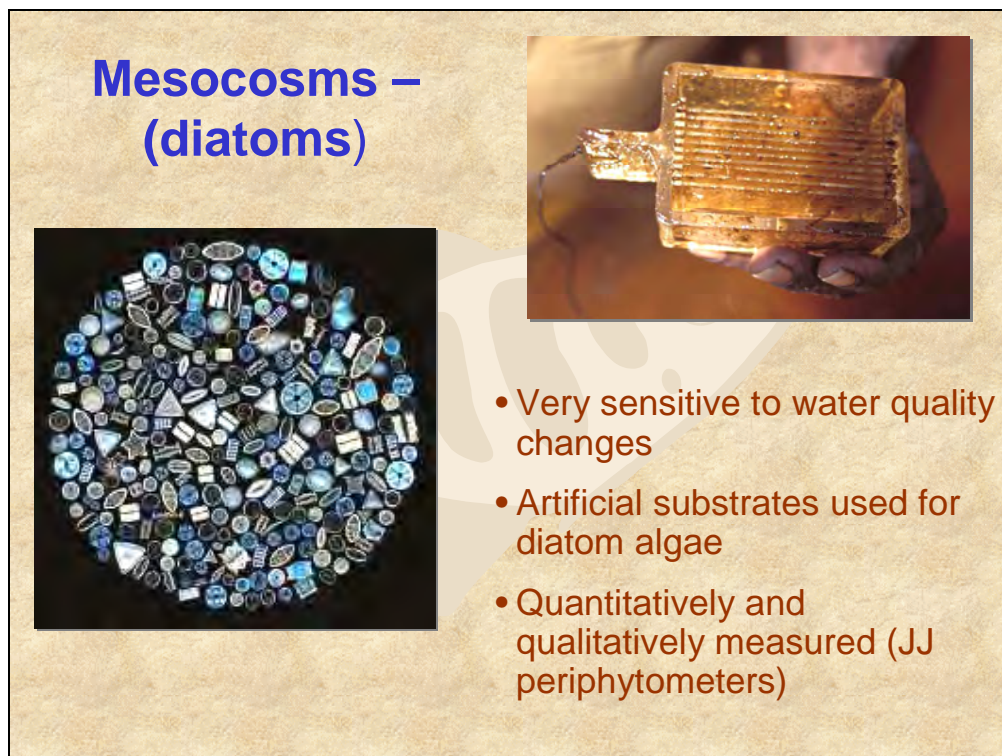
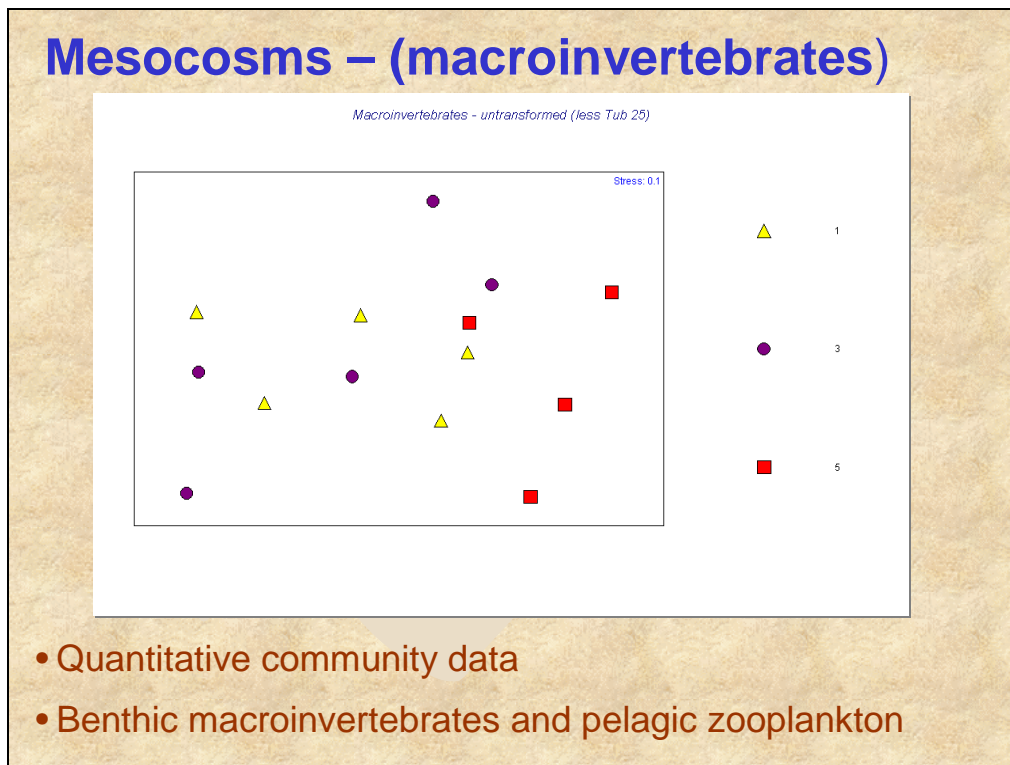
- Quantitative community data
- Benthic macroinvertebrates and pelagic zooplankton



## MDS – Multi-Dimensional Scaling

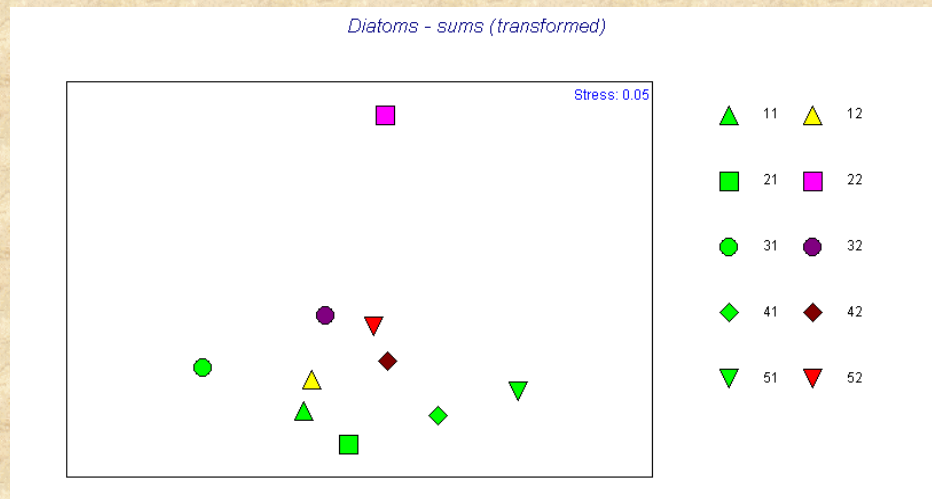


- Reduces extensive community data down to a single scatter plot
- Statistically testable differences



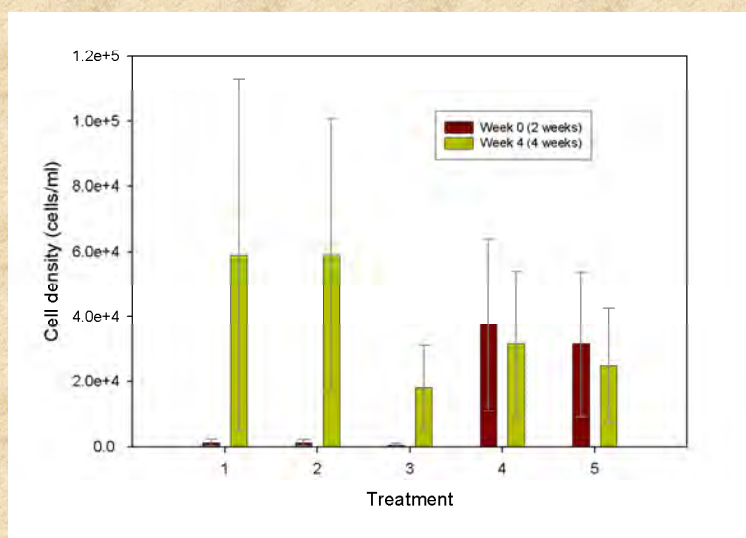


## Mesocosms – (diatoms)



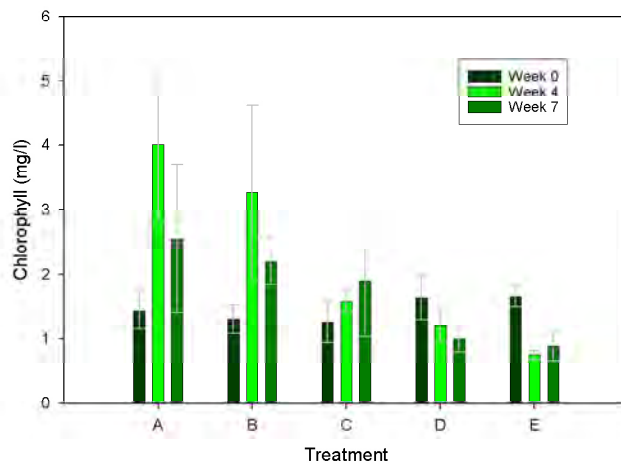
- Community changes over time ( $\alpha = 0.3\%$ )
- No significant difference between treatments

## Mesocosms – (diatoms)



- Low conc's / high conc's behave differently
- Very noisy

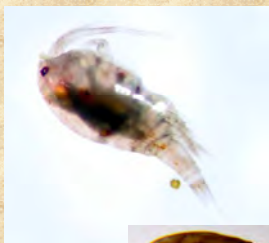
## Mesocosms – (phytoplankton)





- Chlorophyll was significantly higher in the control ( $p = 0.014$ )
- Similar response for other pigments (Cb & Cc)
- High intra-treatment variability

## Mesocosms – microinvertebrates

- Quantitative community data
- Pelagic zooplankton
- Large pelagic phytoplankton



## Future work...

- Inclusion of other species for lab ecotoxicity tests (*Lemna* and black-striped rainbowfish) and repeat some tests (= robust assessment and modelling).  
- Learn more about the influence of  $\text{Ca}^{2+}$  on  $\text{Mg}^{2+}$  toxicity on other species.
- Compare laboratory and field results to derive a suitable  $\text{Mg}^{2+}$  standard for Magela Creek.
  - *At low concentrations, in low solute waters,  $\text{MgSO}_4$  may be a stimulant e.g. freshwater snails*
  - *'High-sensitivity' of Hydra and Amerianna*
  - *New Guidelines place greater emphasis on multi-species test results - field NOECs and LC50s?*

## Thanks to...

- Co-authors / collaborators
- The Traditional Owners of the Ranger Mineral Lease, Gundjehmi Aboriginal Association, for permitting the field experiments
- The numerous workers and volunteers that have assisted in the project



## References

- ANZECC & ARMCANZ 2000. *Australian and New Zealand guidelines for fresh and marine water quality. National Water Quality Management Strategy Paper No 4*. Australian and New Zealand Environment and Conservation Council & Agriculture and Resource Management Council of Australia and New Zealand, Canberra.
- O'Connor R, Humphrey CL, Dostine P, Lynch C & Klessa B 1996. A survey of aquatic macroinvertebrates in lentic waterbodies of Magela and Nourlangie Creek attachments, Alligator Rivers Region, NT: Second year of data 1996. Internal report 242, Supervising Scientist, AGPS, Canberra. Unpublished paper.
- O'Connor R, Humphrey CL, Dostine P, Lynch C & Spiers A 1995. A survey of aquatic macroinvertebrates in lentic waterbodies of Magela and Nourlangie Creek attachments, Alligator Rivers Region, Northern Territory. Internal report 225, Supervising Scientist, AGPS, Canberra. Unpublished paper.

