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Toxicity of single magnesium pulse exposures to tropical freshwater species

AC Hogan, RA van Dam,

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Contents

Executive summary

Concentrations of solutes originating from the Ranger uranium mine do not occur at constant levels in Magela Creek. They can fluctuate quite widely through time due to changes in creek discharge, mine water discharge and mine water source. Continuous monitoring of electrical conductivity (EC) in Magela Creek since 2006 has shown the extent of variability in creek water quality associated with mine water discharges.

Electrical conductivity is the key signature variable for water originating from the minesite, and is dominated by the concentration of magnesium sulfate ($MgSO_4$). Consequently, concentrations of $MgSO_4$ can be confidently predicted based on EC measurements. A large body of research has been undertaken by the Supervising Scientist Division on the toxicity of $MgSO_4$ to local aquatic species. This work has led to the derivation of a site-specific water quality trigger value (TV) for magnesium (Mg) in Magela Creek of 2.5 mg/L (Mg being the primary toxic ion in $MgSO_4$; van Dam et al 2010). However, the Mg toxicity data and associated TV were derived from tests conducted using continuous constant exposures over days (3 to 6 days depending on the species) as recommended by ANZECC/ARMCANZ (2000). This exposure regime is not representative of the majority of conditions in Magela Creek as evidenced by the continuous monitoring data.

This work was started several years ago. At that time comparison of the proposed chronic exposure Mg TV with the EC continuous monitoring data from the 2005-06 to 2008-09 wet seasons revealed 43 exceedances of the TV. The medians for Mg concentration and exceedance duration were 3.4 mg L^{-1} and 6.1 h, respectively. All except one of the exceedances were of durations shorter than the duration of the chronic toxicity tests. Therefore, they were considered unlikely to be causing detrimental effects downstream of the mine, but there were no quantitative data to support this assumption. Given the high conservation value of the Magela Creek catchment, it was considered necessary to better understand the potential effects of short-term exceedances (pulse exposures) of the Mg TV.

The aims of the study were to (i) assess the toxicity to local freshwater species of Mg pulse exposures relevant to those seen in Magela Creek, and (ii) use the data to develop a model from which Mg or EC TVs could be derived for any given exposure duration.

Six local freshwater species (green alga, *Chlorella* sp.; duckweed, *Lemna aequinoctialis*; snail, *Amerianna cumingi*; cladoceran, *Moinodaphnia macleayi*; green hydra, *Hydra viridissima*; and northern-trout gudgeon, *Mogurnda mogurnda*) were exposed to single Mg pulse exposures of 4 h, 8 h and 24 h duration (at a constant Mg:Ca ratio of 9:1, as per van Dam et al [2010]). At the end of each exposure period the test organisms were transferred to clean water and their responses monitored for the remainder of the standard toxicity test durations (3 to 6 days, depending on species). At least two toxicity tests were undertaken for each combination of species and pulse duration. A limited number of continuous Mg exposure toxicity tests were completed to confirm the responses and toxicity values reported in van Dam et al. (2010).

For all species, Mg toxicity increased as exposure duration increased. However, the extent to which toxicity increased ranged from 2-fold to 40-fold between species. Moreover, the nature of the positive relationship between toxicity and exposure duration differed (linear or exponential) between species. The concentrations of Mg resulting in 10% inhibition of response (IC10) for each species are provided in Table ES.1.

For one species, the cladoceran, *M. macleayi*, increased sensitivity to Mg was observed following pulse exposures at the onset of reproductive maturity (at ~27-h old) compared with

exposure of neonates (<6-h old). This increased sensitivity may be related to the coincidence of the exposure pulse with the physiological processes of moulting and/or reproductive development.

Table ES.1 Magnesium IC10[a] values for each species and Mg pulse exposure duration

Species	IC10 value (mg L^{-1})			
	4-h pulse	8-h pulse	24-h pulse	Continuous exposure [b]
Chlorella sp.	5950	5620	3880	818
Lemna aequinoctialis	4030	1500	80	36
Amerianna cumingi	3030	387	301	5.6
Moinodaphnia macleayi [c]	212	62	128	39
Hydra viridissima	1210	1000	709	246
Mogurnda mogurnda [d]	>4100	>4100	>4100	4010

[a] IC10: concentration at which there was 10% inhibition in response of the organism.

[b] Continuous exposure toxicity data from van Dam et al (2010).

[c] *M. macleayi* data for exposure at onset of reproductive maturity shown only.

[d] *M. mogurnda* data represent concentrations at which there is mortality of 5% of larvae (ie. LC05; due to this test being an acute test).

The IC10 data shown in Table ES.1 were used to derive 99% species protection TVs for each exposure duration, based on the assumption of log-logistic species sensitivity distributions. The resultant TVs for each pulse exposure duration are shown in Table ES.2.

Table ES.2 Trigger values for Mg and EC for different pulse exposure durations

Pulse duration	99% species protection trigger value	
	Mg (mg L^{-1})	EC (µS cm^{-1}) [a]
4 hours	94	1140
8 hours	14	174
24 hours	8	102
Continuous (3–6 days)	3	42

[a] EC calculated based on the Mg trigger value, using an established EC versus Mg relationship.

[b] Continuous exposure trigger values taken from van Dam et al (2010), noting that the value of 3 mg L^{-1} has been rounded up from 2.5 mg L^{-1}.

The functional relationship between the Mg and EC TVs (Table ES.2) and exposure duration is well described by an exponential function. This model provides an interpolation tool by which a TV for any given pulse duration can be calculated. The model will be incorporated into a Mg/EC TV framework that will enable improved interpretation of transient pulses of EC in Magela Creek downstream of the Ranger mine.

It is recommended that further work be done on assessing organism recovery time and the potential for carry-over toxicity in situations where multiple pulses occur in series to enhance interpretation and application of the Mg/EC TV framework.

Toxicity of single magnesium pulse exposures to tropical freshwater species

AC Hogan, RA van Dam, MA Trenfield & AJ Harford

1 Introduction

Environmental contaminants are rarely present at constant concentrations due to the irregular nature of most anthropogenic discharges and the variable hydrology of receiving waters (Handy 1994). Although contaminant concentrations in receiving waters typically fluctuate, they are usually assessed by comparing environmental concentrations to water quality guidelines (limits/criteria) based on laboratory-derived toxicity estimates where organisms are continuously exposed for the full duration of an experiment. Test durations are usually defined within protocols that have been developed for the test species, and are rarely adjusted to reflect environmental exposure durations (Diamond et al 2006, Zhao & Newman 2006, Erikson 2007). The mode (and rate) of action of the chemical being tested is also rarely considered when deciding upon a test duration (Diamond et al 2006, Baas et al 2010). Undertaking toxicity tests to investigate the effects of *multiple* sequential pulses of a contaminant is even more complicated, with frequency of exposure, recovery times between pulses, and the possibility of cumulative effects also needing consideration (Ashauer & Escher 2010). There are models that can be used to predict contaminant toxicity for different exposure scenarios, but these have not yet been widely adopted for regulatory purposes. This is further discussed in Appendix A.

The US Environmental Protection Agency (USEPA) regulates chemical pulses (excursions of water quality criteria) using a Time Averaged Concentrations approach (TACs, Stephan et al 1985). The TAC, being an estimation of exposure over a given timeframe, summarises environmental data to allow comparisons with water quality criteria derived assuming exposure to a constant concentration regime. These regulations limit peak exposures by stipulating that 1-h averages of exposure concentrations do not exceed the Criterion Maximum Concentration (based on acute toxicity data) more than once in three years on average. Additionally, the Criterion Continuous Concentration (based on chronic toxicity data) is used to limit more prolonged exposures by requiring that 4-d averages of exposure concentrations not exceed the CCC more often than once in every three years. This approach assumes that organisms will respond similarly to a short duration, high magnitude pulse as they would respond to longer exposures of lower magnitude pulses (provided that the overall dose is equivalent). Some studies have shown TAC to be a suitable approach (eg. Angel et al 2010). However, other studies have shown either 1) greater toxicity from longer exposures of lower magnitude than equivalent TACs administered over shorter exposures (Diamond et al 2005, Hoang et al 2007), or 2) lower toxicity from longer exposures (Thurston et al 1981, Siddens et al 1986, Curtis et al 1989, McCahon & Pascoe 1990, Hoang et al 2007, Reynaldi et al 2005).

The USEPA recognises the limitations of the TAC approach and is in the process of developing methods to better quantify toxic effects to organisms as a function of the magnitude and time variability of exposures (eg Diamond et al 2006, Erickson 2007, Delos 2008). Other jurisdictions, including Canada and Australia, are yet to provide guidance on the regulation of pulse exposures of pollutants (Uwe Schneider, Environment Canada, Pers. Comm., ANZECC & ARMCANZ 2000).

At Ranger Uranium Mine (Ranger), in Australia's tropical north, waters containing mine-derived contaminants, in particular uranium and magnesium sulfate ($MgSO_4$), enter Magela Creek through both passive and active discharges of mine water and surface runoff. Previous research has demonstrated Mg (present in mine derived water as MgSO4) to be the contaminant of most environmental concern (van Dam et al 2010). Both Mg and sulfur (S) play a vital role in biological processes associated with protein synthesis, enzyme activation, energy transfer and cellular homeostasis (Heaton 1993, Wolf & Cittadini 2003). However, in excess, Mg can be toxic to aquatic organisms (van Dam et al 2010) and moreover the low ionic concentrations of Magela Creek water (MCW; Klessa 2000) provide physico-chemical conditions that enhance the uptake and toxicity of metals and major ions (Luoma & Rainbow 2008).

Waters leaving the Ranger mine site via the small catchments of Corridor and Coonjimba creeks include treated pond water RO permeate and surface runoff from sheeted low grade stockpiles and non-mineralised areas (Figure 1, Supervising Scientist 2009). These small tributaries discharge passively into Magela Creek through waterbodies known as backflow billabongs. The variability of this discharge is exacerbated by the hydraulic interaction between the backflow billabongs and Magela Creek. During periods of high flow in Magela Creek, hydraulic damming retains mine derived waters in the billabongs, which then discharge as creek flow recedes (Frostick et al 2011). In addition, active discharge from Retention Pond 1 (RP1) occurs directly into Magela Creek via pumping to an outfall upstream of the downstream monitoring site (ERA, 2010). More diffuse sources of mine site solutes include wet season surface runoff and shallow groundwater seepage from land application areas that are irrigated with retention pond waters during the dry season (Supervising Scientist 2009). The irregular rates of these numerous potential discharges and the variable hydrology of Magela Creek, (discharge range of 0–3558 $m^3\ s^{-1}$ as reported by Erskine and Saynor 2012), result in fluctuating concentrations of mine-derived contaminants downstream of the mine (Figure 2).

In order to better understand the behaviour of mine-derived contaminants in Magela Creek downstream of Ranger, the Supervising Scientist Division has implemented a continuous monitoring program for the measurement of pH, electrical conductivity (EC) and turbidity. Magnesium sulfate is known to contribute the majority of the ionic content of mine waters entering Magela Creek, and the concentrations of Mg and SO_4 downstream of Ranger can be reliably inferred from EC measurements (Figure 3, Supervising Scientist 2011).

EC measurements in Magela Creek are made at 10–15 minute intervals both up- and downstream of the mine and can be converted to equivalent Mg concentrations to produce a continuous high resolution trace of Mg concentrations (Figure 2). The continuous monitoring time series data provide the basis for designing ecotoxicological testwork that spans a range of environmentally relevant exposure conditions to assess the risk of Mg to local biota. Such a detailed contaminant exposure profile is often unobtainable due to practical difficulties in recording intermittent pollution events in the field (Handy 1994). Knowledge of operational and water management practices at Ranger Mine indicate that contaminant discharge to Magela Creek is likely to remain stable or decrease during the remaining operational life of the mine (Dr D Jones, SSD, pers comm).

A provisional site-specific water quality trigger value (TV) of 2.5 mg L^{-1} had previously been derived for Mg in Magela Creek (van Dam et al 2010). This TV is based on continuous exposure, standard duration (3–6 day) tests conducted in natural creek water using six local species, according to the procedure described in ANZECC/ARMCANZ (2000).

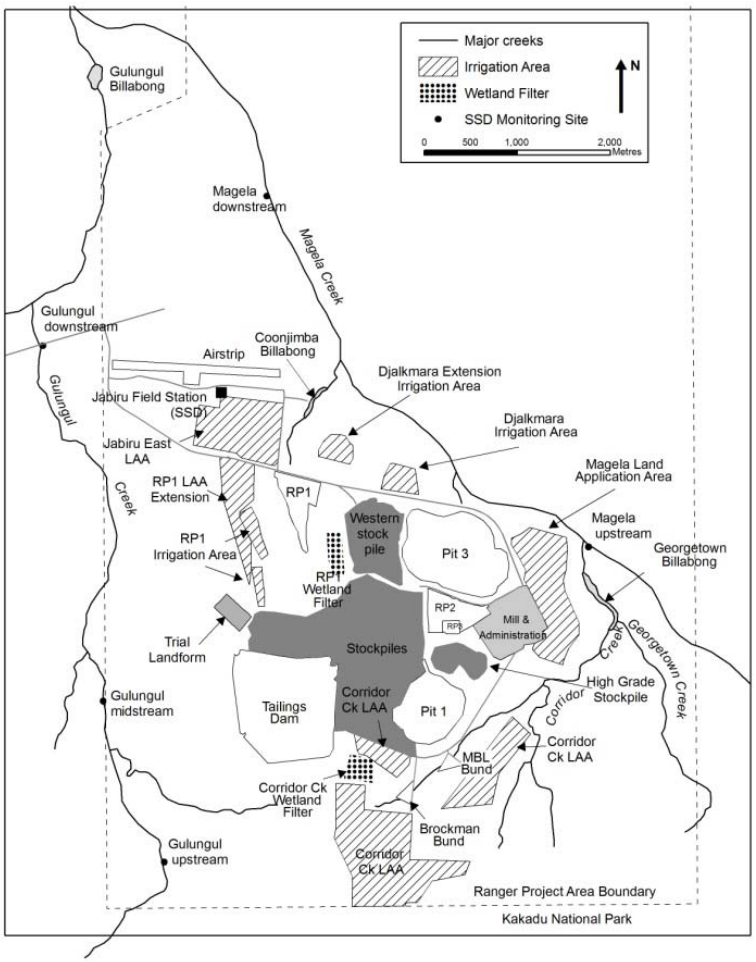


Figure 1 Ranger minesite

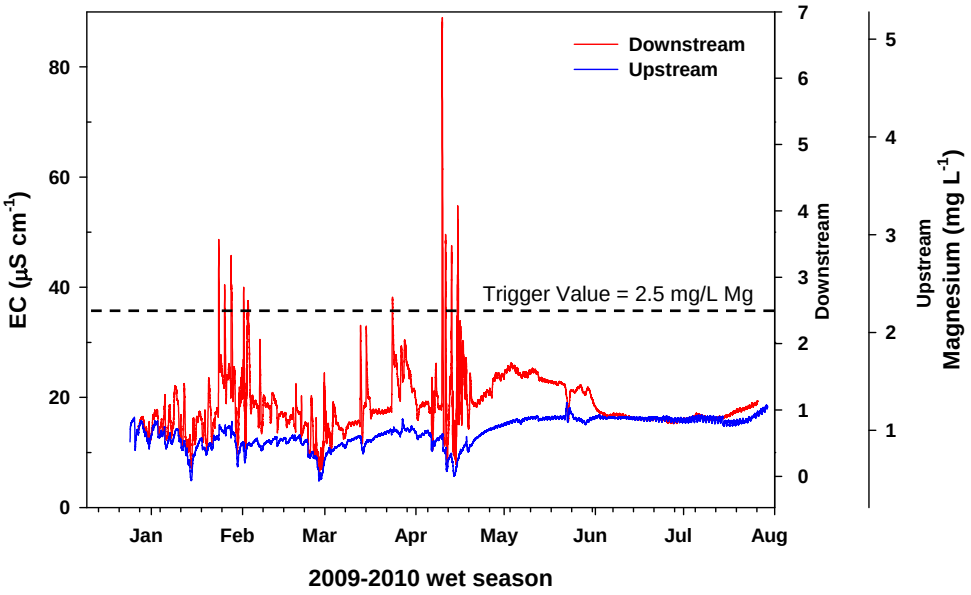


Figure 2 Fluctuations in continuous electrical conductivity (EC) and calculated (from the relationship between EC and Mg shown in Figure 3) magnesium (Mg) concentrations in Magela Creek upstream and downstream of Ranger during the 2009–2010 wet season. Dashed line is the ecotoxicologically derived Trigger Value of 2.5 mg/L Mg.

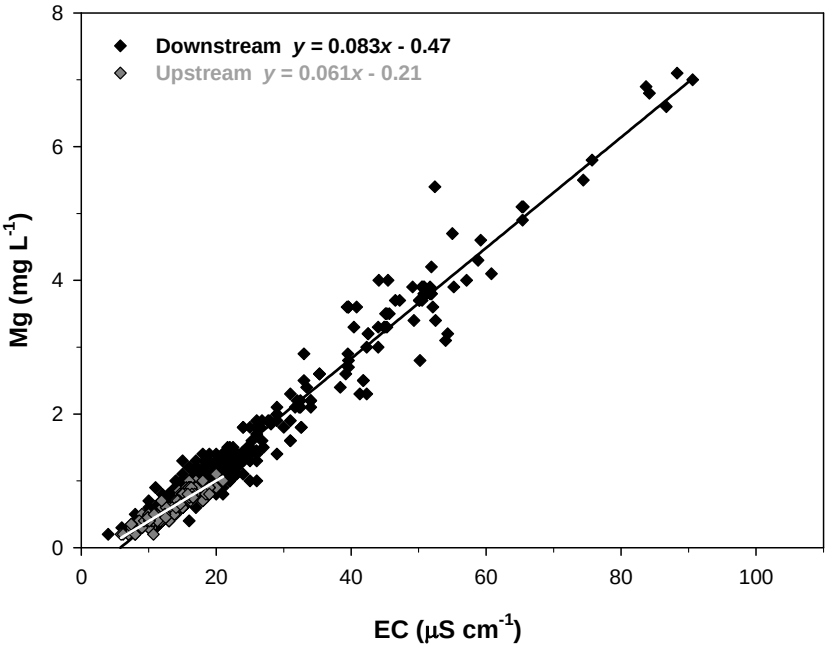


Figure 3 Best fit relationships between electrical conductivity (EC) and magnesium (Mg) concentrations for the downstream (r^2 = 0.96, P<0.0001; black regression line) and upstream (r^2 = 0.84, P<0.0001; white regression line) monitoring stations in Magela Creek (adapted from Supervising Scientist 2011).

However, the continuous monitoring data acquired since the 2005–06 wet season showed that the chronic exposure Mg TV was being exceeded (Figure 2) much more often than revealed by the previous weekly grab sample monitoring regime. Although the vast majority of these exceedances were of durations shorter than the 3–6 day continuous exposures used for the toxicity tests and, therefore perhaps unlikely to be causing detrimental effects downstream of the mine, it could not be concluded with certainty that this was the case. Accordingly, this project was initiated to quantify effects of short-term exceedances of the Mg TV (ie pulses of Mg).

The study's hypothesis was that, for a given concentration, short duration exposures would be less toxic than extended exposures. If this was the case then use of a TV based on extended exposure would be overly conservative for a pulse event.

The aims of the present study were to:

i quantify the effects, on local species, of Mg pulse exposures that are more representative of the actual behaviour of Mg in Magela Creek; and

ii use the data to derive water quality guideline TVs for single pulses of different durations and,

iii subsequently, develop a model from which TVs can be derived for any given exposure duration within the range assessed.

2 Methods

2.1 General laboratory procedures

Equipment that came into contact with test organisms or media was made of either Teflon®, glass or polyethylene. All plastics and glassware were washed by soaking in 5% (v/v) HNO_3 for 24 h before undergoing a non-phosphate detergent wash (Gallay Clean A powder or Dr Weigert Neodisher Laboclean FLA) in a laboratory dishwasher plumbed solely on reverse osmosis and electrically deionised water (Elix, Millipore). All reagents used were analytical grade and stock solutions were made up in high purity water (Milli-Q Element, Millipore).

2.2 Test diluent

Magela Creek water (MCW) was collected approximately monthly from upstream of Ranger (latitude 12° 40' 28", longitude 132° 55' 52") during the wet season and further upstream at Bowerbird Billabong (latitude 12° 46' 15", longitude 133° 02' 20") during the dry season, where good water quality, comparable to wet season quality, persists throughout this period. On each occasion water was collected into 20 L acid-washed plastic containers using an acid-washed bilge pump connected to acid-washed vinyl tubing. The water was either refrigerated immediately at 4 ± 2°C and/or transported to the laboratory where it was stored for no longer than three days before filtration. Once filtered through a 3 μm inline filter cartridge (Sartorius-Stedim or Pall), the water was stored at 4 ± 2°C for up to four weeks before use. Key physicochemical characteristics and major ion concentrations of MCW were measured following each collection (see section 2.5 and 2.6).

2.3 Toxicity test species and general test methods

The toxicity of Mg was assessed using the following six Australian tropical freshwater species: the unicellular green alga (*Chlorella* sp.); the tropical duckweed (*Lemna aequinoctialis*); the green hydra (*Hydra viridissima*); the cladoceran (*Moinodaphnia macleayi*); the pulmonate snail (*Amerianna cumingi*); and the Northern trout gudgeon (*Mogurnda mogurnda*). All test organisms were originally isolated from Kakadu National Park and have been cultured continuously at the Supervising Scientist Division for 10–22 years, depending on the species. Culturing and toxicity test methods are described in detail for five of the species by Reithmuller et al (2003) and for *A. cumingi* by Houston et al (2007). Key information for each test, including the biological endpoint is included in Table 1. Tests examined sub-lethal endpoints over chronic or sub-chronic exposure periods with the exception of the *M. mogurnda* test, which measured acute larval fish survival. Tests were conducted in filtered (3 μm) MCW with the exception of the *A. cumingi* test, for which the high volumes of water used in the test made filtering impractical.

For *L. aequinoctialis* and *Chlorella* sp. tests, nitrate (NO_3) and phosphate (PO_4) nutrients were added at the minimum concentrations that would sustain acceptable growth (Table 1). For *Chlorella* sp. testing, 1 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) buffer was also added to MCW to maintain stable pH. The use of a buffer to control pH was not necessary for the other species.

Table 1 Details of toxicity tests for six Australian tropical freshwater species used to assess the toxicity of Mg pulse durations of 4, 8 & 24 h. Full details are provided in Riethmuller et al (2003) and Houston et al (2007)

Species (common name)	Test duration and endpoint	Control response acceptability criterion	Temperature, light intensity, photoperiod	Feeding/ nutrition	# replicates (Individuals per replicate)	Test volume (mL)	Static/daily renewals
Chlorella sp. (unicellular green alga)	72-h population growth rate	1.4 ± 0.3 doublings day^{-1}; % CV[1] <20%	29 ± 1°C 100-150 μmol m^{-2} sec^{-1} 12:12h	14.5 mg L^{-1} NO_3 0.14 mg L^{-1} PO_4	3 (3 × 10^4 cells)	50	Static
Lemna aequinoctialis (tropical duckweed)	96-h growth rate (based on frond #)	Mean growth rate (k) ≥0.4 d^{-1}; % CV <20%	29 ± 1°C 100-150 μmol m^{-2} sec^{-1} 12:12h	3 mg L^{-1} NO_3 0.3 mg L^{-1} PO_4	3 (4 plants with 3 fronds)	100	Static
Hydra viridissima (green hydra)	72-h population growth rate	Mean growth rate (k) ≥0.27 d^{-1}; % CV <20%	27 ± 1°C 30-100 μmol m^{-2} sec^{-1} 12:12h	30–40 *Artemia* nauplii per day	3 (10)	30	Daily renewals
Moinodaphnia macleayi (cladoceran)	3 brood (120-144 h) reproduction	Mean adult survival ≥80%; mean neonates per adult ≥30	27 ± 1°C 30-100 μmol m^{-2} sec^{-1} 12:12h	30 μL FFV[2] and 6 × 10^6 cells of *Chlorella* sp. per day	10 (1)	30	Daily renewals
Amerianna cumingi (pulmonate snail)	96-h reproduction	Mean eggs per snail pair ≥30	30 ± 1°C 30-100 μmol m^{-2} sec^{-1} 12:12h	2 cm^2 lettuce disc per snail per day	3 (12)	1750	Daily renewals
Mogurnda mogurnda (Northern trout gudgeon)	96-h survival	Mean larval survival ≥80%; % CV <20%	27 ± 1°C 30-100 μmol m^{-2} sec^{-1} 12:12h	Nil	3 (10)	30	Daily renewals

[1] % CV: Percent co-efficient of variation

[2] FFV: fermented food with vitamins. Represents an organic and bacterial suspension prepared by method described in Riethmuller et al (2003).

2.4 Pulse exposure toxicity testing

The pulse durations to be used for this study were selected based on statistical analysis of the continuous monitoring data for Mg in Magela Creek from the 2005–2009 wet seasons. For this purpose, any exceedance of the ecotoxicologically derived site-specific trigger value for Mg of 2.5 mg L^{-1} (van Dam et al 2010) was considered a pulse. A total of 43 pulses were recorded for this period. The characteristics (magnitude and duration) of each pulse were extracted and a summary of the pulse statistics is presented in Table 2.

Table 2 Summary statistics for exceedances of the site specific trigger value of 2.5 mg L^{-1} Mg (based on exceedance magnitude and duration) inferred from EC continuous monitoring data from 2005–06 to 2008–09 wet seasons.

Statistic	Magnitude (mg L^{-1} Mg)	Duration (hours)
Mean	4.1	9.4
Median	3.4	6.1
80th percentile	4.5	10.5
20th percentile	2.8	2.3
Maximum	16.3	124

All but one of the pulses persisted for 24 h or less. The longest duration pulse of 124 h was considered to be a continuous exposure rather than a pulse (Figure 4). Based on this analysis, it was decided that the durations of greatest interest were between 0 and 24 h and durations of 4, 8 and 24 h were chosen for testing.

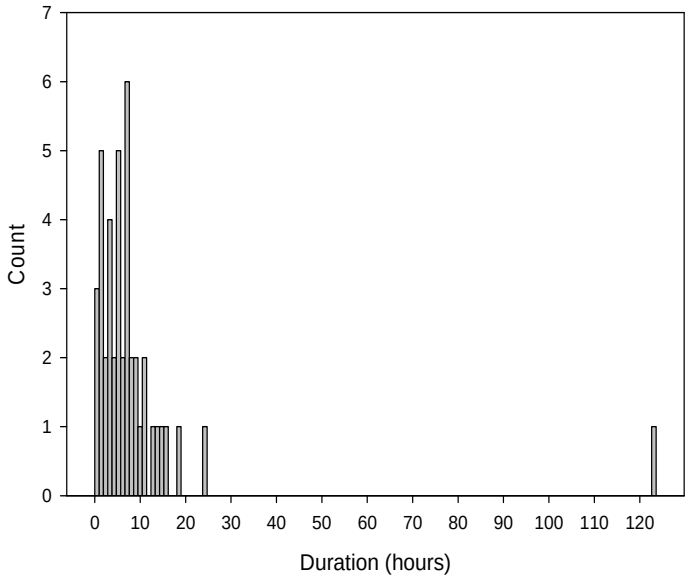


Figure 4 Frequency distribution of exceedances of the site specific trigger value of 2.5 mg L^{-1} as a function of exceedance duration (from 2005–06 to 2008–09 wet seasons)

For each of the pulse durations, test organisms were exposed to a range of Mg concentrations (magnitudes) to obtain a full concentration-response relationship (Table 3). For all species, the Mg pulse was administered at the commencement of the test. At the end of the defined pulse duration the organisms were rinsed and returned to clean test medium for the duration of the standard protocol (Figure 5).

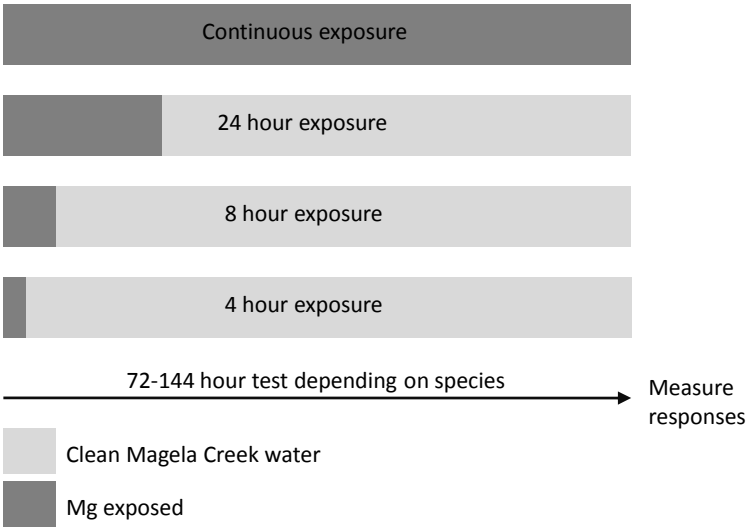


Figure 5 Exposure regimes undertaken for each test species. Note that for *M. macleayi*, additional experiments were undertaken with Mg pulse exposures administered later in the test period, around the onset of reproductive maturity for this species (see text for details).

All exposures to Mg were undertaken in the presence of Ca at a ratio of ~9:1 Mg:Ca (see Appendix B for actual ratios for each test) according to the findings of van Dam et al (2010). The addition of Ca was essential because Ca has been found to have an ameliorative effect on Mg toxicity and these ions occur together in water from the Ranger site. A Mg:Ca ratio of 9:1 was chosen because amelioration of Mg toxicity by Ca was found to be most effective at Mg:Ca ratios of <9:1 and this ratio has not been exceeded in Magela Creek (1985-2009, n = 440, van Dam et al 2010). Magnesium and Ca stock solutions were prepared by dissolving $MgSO_4.7H_2O$ and $CaSO_4.2H_2O$ in MCW. The poor solubility of $CaSO_4$ (~450 mg L^{-1}) limited the maximum Mg concentration that could be tested in this study to 4000 mg L^{-1} (in order to maintain a ratio of 9:1).

For *Chlorella* sp., where there was no toxic effect at 4000 mg L^{-1} Mg, $MgCl_2$ and $CaCl_2$ (which had greater solubility and similar toxicity to $MgSO_4$ up to 4000 mg L^{-1} Mg) were used as substitutes for $MgSO_4$ and $CaSO_4$ (see Appendix C) to permit higher concentrations to be used. The use of the $MgCl_2$ and $CaCl_2$ mix was also trialled for *L. aequinoctialis* and *M. mogurnda*. However, as the toxicity of $MgCl_2$ to these species was found to be greater than that of $MgSO_4$ at concentrations up to 4000 mg L^{-1} Mg (Appendix D and E), only the $MgSO_4$ results were deemed appropriate.

Additional testing was undertaken with *M. macleayi*, because this organism has distinct life stages that could be targeted in order to investigate the effect of when the pulse occurred within its life cycle. To do so, additional tests were undertaken in which pulses were administered so that they occurred when the cladocerans were 27 h of age (ie. 4-h pulse administered at 27 h, 8-h pulse at 25 h or 24-h pulse at 15 h of age, such that the pulse occurs around the onset of reproductive maturity). This age represents the period at which *M. macleayi* reaches reproductive maturity and the first brood of eggs are deposited in the brood pouch. It also corresponds with the second moulting period.

For *Chlorella* sp., the need to rinse algal cells post-pulse presented the challenge of recovering sufficient proportions of undamaged cells following the rinsing process. Following

unsuccessful attempts using centrifugation and dialysis, filtration through 1.2 µm polycarbonate filter papers using a pump with adjusted air pressure resulted in sufficient recovery of cells (80-100%, Appendix F).

Table 3 Ranges of Mg concentrations (mg L^{-1}) tested for each species and exposure duration

Species	Mg concentration range (mg L^{-1})			
	4-h pulse	**8-h pulse**	**24-h pulse**	**Continuous (72-120-h)**
Chlorella sp.	4100–12000[1]	4000–12000[1]	1900–9400[1]	4200
Lemna aequinoctialis	201–4120	199–4150	100–4090	31–3230
Hydra viridissima	212–4230	230–1570	190–1480	54–1640
Moinodaphnia macleayi				
Exposed at test commencement	102–3290	46–2220	32–1020	11–313
Exposed at onset reproductive maturity	50–1710	25–412	4–712	N/A[2]
Amerianna cumingi	49–4170	12–3980	11–3010	5–513
Mogurnda mogurnda	4130	4110	4130	NT[3]

[1] Values for Mg based on tests using magnesium chloride

[2] N/A: Not applicable, as continuous exposures that start at test commencement will also encompass the onset of reproductive maturity

[3] NT = Not Tested. Pulse exposure data compared with continuous exposure data reported in van Dam et al (2010)

2.5 Water quality parameters

Dissolved oxygen (DO), electrical conductivity (EC) and pH were measured on a sub-sample of each batch of MCW upon collection from the field (WTW CellOx 325, TetraCon® 325 & Sentix 41 with Series inolab and Multi 340i meters). For individual toxicity tests, these parameters were measured on a sub-sample of each treatment solution at the start and end of the *L. aequinoctialis* and *Chlorella* sp. static tests, and on sub-samples of fresh and 24-h old treatment waters from each of the daily renewal experiments (ie the *M. macleayi*, *H. viridissima*, *A. cumingi*, and *M. mogurnda* tests). For *A. cumingi* tests, old water of each treatment was measured daily for ammonia using a colourimetric test kit (Merck, Aquamerck® Ammonium Test).

Test water quality was considered acceptable if DO remained higher than 70% saturation, EC remained within 10% of the initial value and pH remained within one unit of the value measured at the test start. For *A. cumingi*, ammonia concentrations were acceptable if they were ≤ 1.0 mg L^{-1}.

2.6 Water chemistry

Water chemistry samples were taken to 1) characterise the test medium, 2) to ensure Mg and Ca test concentrations were accurate, and 3) to check for metal contamination of test solutions, which may confound the results.

Test diluent (MCW) was sampled at each collection from the field into 50 mL glass vials for Total Organic Carbon (TOC) and Dissolved Organic Carbon (DOC, 0.45 µm filtered) and analysed at ***eriss*** within 48 h of collection (Shimadzu TOC-V CSH).

Alkalinity of MCW was analysed by acid titration (APHA 2005, method 2320B) at the Northern Territory Environmental Laboratories (NTEL, Berrimah, Australia) or Envirolab (Chatswood, Australia) within one week of sampling.

Control waters and blank samples were sub-sampled for analysis of a standard suite of metals and major ions (Al, Cd, Co, Cr, Cu, Fe, Mn, Na, Ni, Pb, Se, U & Zn). All other treatments

were analysed for Mg and Ca only. Samples for total metal analysis were collected and acidified to 5% HNO_3, while dissolved metal concentrations were determined from 0.45 µm filtered samples that had been acidified to 1% HNO_3. Filtered samples were sent to NTEL or Envirolab for analysis within one week of collection. Major ions were measured by inductively coupled optical emission spectrometry (ICP-OES; Thermo Iris Intrepid at NTEL; 730-ES Axial, Varian at Envirolab) and trace metals measured using inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7500CE series).

MCW used in algal and *Lemna* tests was supplemented with nutrients and was analysed for NO_3 and PO_4 by NTEL using flow injection analysis (Lachat 8000 series) or by EnviroLab using a discrete analyser (Thermo Scientific, Aquakem 250).

2.7 Data analysis and trigger value derivation

Measured concentrations of Mg were used for the calculation of toxicity estimates. Non-linear regression models were fitted using Sigmaplot v9.01 (Systat Software) to the concentration-response data using pooled datasets for each species at each pulse duration (or duration and life-stage combination for *M. macleayi*). The best fit model was determined by the regression coefficient (r^2) of a limited suite of models (three- and four-parameter sigmoidal and logistic). For each species, Mg concentrations at which there was 10% or 50% inhibition of growth (IC10 or IC50) or 5% or 50% reduction in survival (LC5 and LC50 for *M. mogurnda*) and their 95% confidence limits were calculated for each pulse duration using the model equations.

Growth rates were calculated for each 24-h period of the *L. aequinoctialis* and *H. viridissima* tests, and the final 24-h period of the *Chlorella* sp. tests, to assess whether the organisms were showing signs of recovery post-pulse. Growth rate was based on plant number (rather than frond number) for *L. aequinoctialis* because plant numbers were enumerated daily with frond counts only at the test conclusion.

Site-specific water quality TVs were derived for each pulse duration (4, 8 and 24 h) using the species sensitivity distribution (SSD) approach recommended by ANZECC & ARMCANZ (2000) and described below. Specifically, cumulative probability distributions of the IC10s/LC5 for each species were constructed (Minitab v16.1.1, Minitab Pty Ltd) and fitted to a 2-parameter log-logistic distribution. For *M. macleayi*, which was separately exposed to Mg pulses at two different life stages, the lowest (most sensitive response) IC10 was used in the derivation of the TV. Toxicity data for *M. mogurnda* were not included in the SSDs, as no toxicity was observed up to the highest Mg concentration tested (4100 mg L^{-1}). Hence, the SSDs were based on data from the five remaining species. The TVs for each pulse duration were based on the concentrations of Mg that would be protective of 99% of species, as calculated from the respective SSDs. A 99% protection level was chosen due to the high conservation value of the Magela Creek catchment (van Dam et al 2002).

The TVs for each of the three Mg pulse durations and the continuous exposure (72-144 h) TV reported by van Dam et al (2010) were converted to EC using the EC v Mg linear regression equation for the downstream site on Magela Creek (Figure 3). The subsequent EC TVs were plotted against exposure duration (hours) and polynomial interpolation was used to derive a guideline for any given pulse duration within the range of durations that were assessed. Polynomial interpolation involves fitting a polynomial model of $n - 1$ degrees, where n is the sample size, to the data. Such a model, in this case an inverse 3rd order polynomial, will pass exactly through the data points to provide a smooth approximate interpolation of the data..

3 Results

3.1 Quality control

3.1.1 Test acceptability criteria

All tests met control performance acceptability criteria, with mean (± CV) responses as follows: *Chlorella* sp. growth rate – 1.72 ± 0.2 doublings day^{-1}; *L. aequinoctialis* growth rate (biomass) – 0.47 ± 0.05 d^{-1}; *M. macleayi* reproduction – 35 ± 3 neonates per adult, and parent survival – 85 ± 15%; *H. viridissima* population growth rate – 0.34 ± 0.04 d^{-1}; *A. cumingi* egg production – 91 ± 77 eggs per pair; and *M. mogurnda* survival – 100%.

3.1.2 Physico-chemical composition of test waters

General water quality data (median, 20th and 80th percentiles) are shown in Table 4. They represent water quality from all treatments across all tests. This includes the water quality of both new and old water measured at the start and end of tests for *Chlorella* sp. and *L. aequinoctialis*, and measured daily for the other species. The EC shown represents only that of the MCW used across the tests, as the EC of pulse contaminated water varied greatly between treatments.

Separate measurements taken for each test can be seen in Appendix G and water quality acceptability criteria were met for all species with the exception of several tests with *A. cumingi*. For *A. cumingi*, pH varied by more than one unit for two of the tests (970S and 1776S) and the EC of old water generally also varied more than 10% from that of new water for these tests. These tests were included in final analyses as mean control egg production was still acceptable (168 for 970S and 108 for 1176S). Ammonia concentrations across *A. cumingi* tests were <1.0 mg L^{-1} with the exception of one test (1176S, Appendix G).

The chemical composition of 0.45 µm filtered MCW is shown in Table 5. The range of background Mg in MCW was 0.4–0.9 mg L^{-1} and Ca was 0.2–0.3 mg L^{-1}. Chemical analyses of the blank and procedural blank samples showed that all tests were free from confounding metal contaminants. Alkalinity, hardness and dissolved organic carbon (DOC) of MCW across all collections spanning the three years of testing were considered representative of typical concentrations in Magela Creek (Table 5; Klessa 2000, ***eriss*** unpublished data). *Chlorella* and *Lemna* waters contained the appropriate concentrations of nutrients. Ratios of Mg:Ca in pulse treatments were close to 9:1 across all tests, with 80% of the ratios between 9.0:1 and 10.1:1 (median 9.5:1, Appendix B).

3.2 Magnesium pulse toxicity

The effects of Mg pulse exposure duration on the toxicity of Mg to the six freshwater species are shown in Figure 6 and summarised in Table 6. The relationship between pulse duration and the two key toxicity estimates, the IC10 and IC50, for all species except *M. mogurnda*, are shown in Figure 7, while the details of the resultant regression models are provided in Appendix I. The toxicity of continuous exposures of Mg to the six species during the present study was similar to that reported by van Dam et al (2010; Table 6). For all species, there was a positive relationship between exposure duration and toxicity. The magnitude of the relationship varied markedly between species, while the form of the relationship varied from linear to exponential (Figure 7). Further details of the results for each species are provided below.

Table 4 Physico-chemical variables of test solutions across all tests for each test species. Unless otherwise stated, values are reported as the median (20th, 80th percentile).

Physico-chemical variable (units)	***Chlorella* sp.**[1]	***Lemna aequinoctialis***[1]	***Amerianna cumingi***[2]	***Moinodaphnia macleayi***[2]	***Hydra viridissima***[2]	***Mogurnda mogurnda***[2]
pH	6.4 (6.2,6.6)	6.4 (6.1,6.5)	6.7 (6.3,6.9)	6.7 (6.3,7.0)	6.3 (6.0,6.5)	6.3 (6.2,6.7)
EC (µS cm^{-1})[3]	48 (45,59)	18 (16,21)	27 (21,35)	17 (15,21)	16 (11,17)	17 (16,19)
Temperature (°C)[4]	26-30	26-32	26-31	26-33	26-29	26-28
Dissolved oxygen (%)	95 (93,98)	96 (91,107)	87 (82,94)	96 (92,101)	94 (91,101)	96 (93,104)

[1] Physical parameters were monitored at 0 and 72 h only, *n* = 62 for *Chlorella* sp. and 100 for *L. aequinoctialis*.

[2] Physical parameters were monitored on both new and old water at 24 hourly intervals, *n* = 406 for *A. cumingi*, 688 for *M. macleayi*, 309 for *H. viridissima*; and 42 for *M. mogurnda*.

[3] EC: electrical conductivity of control water.

[4] Only a data range was available for reporting. Measurements of air temperature within test incubators using TinyTag loggers.

Table 5 Measured inorganic composition of 0.45 µm filtered Magela Creek water used across all tests. Values represent the mean ± standard error from control samples taken from each test.

Analyte	DL[1]	Species/test type					
		***Chlorella* sp.[2]**	***L. aequinoctialis*[3]**	***A. cumingi*[4]**	***M. macleayi*[5]**	***H. viridissima*[6]**	***M. mogurnda*[7]**
Ca (mg L^{-1})	0.1	0.3 ± 0.02	0.2 ± 0.04	0.2 ± 0.02	0.2 ± 0.02	0.3 ± 0.05	0.2 ± 0
Mg (mg L^{-1})	0.1	0.9 ± 0.03	0.8 ± 0.06	0.4 ± 0.06	0.8 ± 0.07	0.7 ± 0.09	0.9 ± 0
Na (mg L^{-1})	0.1	8.0 ± 0.14	1.3 ± 0.05	0.9 ± 0.03	1.4 ± 0.1	1.3 ± 0.1	1.1 ± 0.1
SO_4 (mg L^{-1})	0.1	100 ± 2.6[8]	0.5 ± 0.1	0.2 ± 0.02	0.3 ± 0.04	0.2 ± 0.04	0.3 ± 0
Al (µg L^{-1})	0.1	10.1 ± 0.5	35 ± 18.3	42.4 ± 7.6	21 ± 8.0	18.5 ± 7.4	4.7 ± 1.2
Cd (µg L^{-1})	0.02	0.03 ± 0	0.05 ± 0	<0.02	0.06 ± 0	<0.02	<0.02
Co (µg L^{-1})	0.01	0.05 ± 0	0.09 ± 0.03	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.03 ± 0
Cr (µg L^{-1})	0.1	0.2 ± 0.04	<0.1	<0.1	0.2 ± 0.02	<0.1	<0.1
Cu (µg L^{-1})	0.01	0.19 ± 0.03	0.49 ± 0.15	0.27 ± 0.02	0.32 ± 0.05	0.32 ± 0.04	0.26 ± 0.07
Fe (µg L^{-1})	20	72 ± 10.7	46 ± 9.5	89 ± 9.5	82 ± 17	83 ± 18	40 ± 0
Mn (µg L^{-1})	0.01	2.11 ± 0.35	4.12 ± 1.5	2.4 ± 0.5	2.9 ± 0.7	2.5 ± 0.4	1.42 ± 0.04
Ni (µg L^{-1})	0.01	0.16 ± 0.05	0.35 ± 0.13	0.23 ±0.05	0.19 ± 0.03	0.31 ± 0.12	0.14 ± 0.01
Pb (µg L^{-1})	0.01	0.19 ± 0.05	0.05 ± 0.02	0.07 ± 0.03	0.04 ± 0.005	0.04 ± 0.006	0.04 ± 0.01
U (µg L^{-1})	0.001	0.02 ± 0.01	0.02 ± 0.006	0.03 ± 0.007	0.03 ± 0.01	0.02 ± 0.008	0.01 ± 0
Zn (µg L^{-1})	0.1	0.9 ± 0.4	0.9 ± 0.2	0.9 ± 0.2	0.6 ± 0.06	1.1 ± 0.3	1.6 ± 0.9
NO_3 (mg L^{-1})[9]	0.005	2.89 ± 0.02	0.6 ± 0.1	NM[10]	NM	NM	NM
PO_4 (mg L^{-1})[9]	0.005	0.04 ± 0	0.09 ± 0.009	NM	NM	NM	NM
		All species					
DOC (mg L^{-1})[11]	0.004	1.3 ± 0.2					
Alkalinity (mg L^{-1} as $CaCO_3$)[12]	1	5.4 ± 0.7					
Hardness (mg L^{-1} $CaCO_3$)[13]	0.7	1.1 ± 0.3					

[1] DL = detection limit; [2] n = 8; [3] n = 7; [4] n = 10; [5] n = 12; [6] n = 7, [7] n = 2; [8] *Chlorella* sp. test water contained higher SO_4 due to addition of HEPES buffer; [9] nutrients measured for *Chlorella* and *Lemna* only, [10] NM = not measured, [11] DOC: dissolved organic carbon; n = 11, [12] n = 25, [13] n = 50.

3.2.1 *Chlorella* sp.

Chlorella sp. was tolerant of Mg concentrations in the g/L level with IC50s well above 6500 mg L^{-1} for each of the pulse durations examined in this study (Table 6). For all pulse durations, the toxicity of Mg to *Chlorella* sp. was similar whether in the form of $MgSO_4$ and $MgCl_2$ (Appendix C). However, only the $MgCl_2$ data have been presented as they provided a full toxic response for each pulse duration. Based on the IC50 values, the toxicity of Mg to *Chlorella* sp. was similar across 4-h, 8-h and 24-h exposures, with the toxicity of 24-h exposure half that of continuous 72-h exposure (Table 6; Figure 6A). Although the best fitting model (given $n = 4$) to describe the relationship between exposure duration and IC50 values was linear, the true relationship appeared non-linear (Figure 7B) due to the similar IC50s for the three pulse exposures relative to the 72-h exposure. Measurable differences in Mg toxicity between exposure durations were discernible at low effect concentrations (Figure 6). Based on IC10s, Mg toxicity was 7-fold lower for 4-h exposure compared with 72-h exposure (Table 6). There was a strong linear relationship between exposure duration and IC10 value (r^2 = 0.98; Figure 7A).

Algal growth rates for the last 24-h of all pulse experiments (ie algal growth during the 48-72-h period) indicated that, once returned to clean water, algae that survived the pulse period, recovered and grew at a similar or faster rate than the controls, irrespective of Mg concentration and pulse duration (Appendix J, Table J.1).

3.2.2 *L. aequinoctialis*

The toxicity of Mg to *L. aequinoctialis* increased with increasing pulse duration. Based on IC50 values, *Lemna* toxicity of Mg was 4.5× greater following continuous 96-h exposure compared with a 24-h pulse (Table 6). Due to the inability to determine an IC50 for the 4-h pulse using $MgSO_4$, several 4-h exposures were also run with $MgCl_2$ (tests 1243 and 1244L, IC50: 2180 mg L^{-1}, results shown in Appendix D). However, as the *Lemna* were more sensitive to $MgCl_2$ than they were to $MgSO_4$, the $MgCl_2$ results were not combined with those shown in Figure 6B.

Following 8-h and 24-h pulse durations and continuous exposure to Mg, the majority of plants exposed to ≥3000 mg L^{-1} were white or translucent. Following 96-h continuous exposure to Mg at this concentration, most plants had also split from 3-4 fronded plants into single fronded plants. For the plants pulsed for 8 h and 24 h, the splitting of plants into single fronded plants mostly occurred at slightly higher concentrations (4000 mg L^{-1} Mg).

In the last 24 h of the tests (72-96-h) the growth rate (based on plant number) of treatments exposed to >3000 mg L^{-1} for 8 h or > 1500 mg L^{-1} for 24 h were still considerably lower than control growth over the same period, indicating that recovery may be slow or unachievable for this species (Appendix J, Table J.2).

3.2.3 *A. cumingi*

The toxicity of Mg to *A. cumingi* increased with increasing pulse duration with a 24-h pulse being at least twice as toxic as a 4-h exposure, based on IC50 concentrations (Table 6, Figure 6C). A continuous exposure was approximately 20 times more toxic than 24-h exposure and at least 40 times more toxic than a 4-h exposure based on IC50s. This indicates that *A. cumingi* is tolerant of short-term Mg exposures.

For the pulse exposure tests, a reduction in egg numbers at the test conclusion was most commonly associated with the death of an adult snail. Exposure to 4000 mg L^{-1} Mg resulted in ~30% mortality following 4-h exposure and 100% mortality after 8-h exposure. Exposure to 3000 mg L^{-1} resulted in 100% mortality of snails exposed for 24 h. In contrast, continuous exposures resulted in significant reductions in egg production with very few adult deaths. For

example, following continuous exposure to 500 mg L^{-1} Mg for 96-h, reproduction had ceased but there was still 100% survival.

Whilst snails ceased or greatly reduced egg production during the exposure period, once placed in clean water, they recommenced egg production and produced similar egg numbers to the controls during the post-pulse period. This was quantified for one 24-h pulse experiment that had sufficient egg mass numbers and suitable timing of observations. Egg production was reduced during the exposure period by ~ 60% at 25 and 100 mg L^{-1} Mg and ceased above concentrations of 378 mg L^{-1}. Where adult snails survived the exposure period (ie. up to 1560 mg L^{-1}), egg production in the post-exposure period was within 10% of the controls. It was also noted during testing that the snails decreased/ceased feeding during exposure and resumed feeding post-exposure but this was not quantifiable.

Although the toxicity of 8-h and 24-h pulses appeared similar at the 10% effect level, the increased toxicity of the 24-h exposure became apparent at higher Mg concentrations (Figure 6C and see IC50s in Table 6). Even though an IC50 was unobtainable for the 4-h pulse exposure, an IC10 was obtained (3031 mg L^{-1} Mg) so it was not considered necessary to further explore the toxicity of this pulse duration using $MgCl_2$.

3.2.4 *M. macleayi*

M. macleayi was the most sensitive of the six species to Mg (based on IC50s, Table 6). Toxicity increased with increasing pulse duration. Based on IC50 values, toxicity of Mg was 4-times greater following continuous exposure compared with 24-h exposure and 3-times greater again comparing 24-h with 4-h exposure (Table 6).

Effects on reproduction in pulsed treatments were attributable to a combination of parental mortality and delays in parental development and neonate production, compared with controls. For 8-h and 4-h exposures initiated at test commencement, *M. macleayi* tolerated up to 400 mg L^{-1} Mg before effects were observed. A decline in reproduction was observed at 800 and 1600 mg L^{-1} Mg for the 8-h and 4-h pulses, respectively (Figure 6D), and was due to parental mortality (survival data not shown). However, the decline in reproduction observed from 500 mg L^{-1} for the 24-h pulse was attributable to both a reduction in neonate numbers per brood and parental mortality. Reproduction ceased completely following 24-h exposures of 1000 mg L^{-1}, 8-h exposures of 1600 mg L^{-1} and 4-h exposures of 3200 mg L^{-1} (Figure 6), due to parental mortality.

The cladocerans were 2-5 times more sensitive (based on IC50s shown in Table 6) to Mg when exposed at the onset of reproductive maturity compared with exposure at test commencement (Figure 6). The effect of a 4-h exposure of >200 mg L^{-1} Mg at reproductive maturity was entirely associated with parental mortality. Reductions in brood size were commonly observed when *M. macleayi* was exposed for 8 or 24 h, but parental mortality and delays in reproduction also contributed to the lower neonate numbers observed in these experiments. The sensitivity of the cladocerans exposed at the onset of reproduction was similar for 4, 8 and 24-h pulses (Figure 6, IC50 = 247-358 mg L^{-1} Mg), suggesting a mechanism of action that is more dependent on timing of exposure rather than duration.

Most reductions in brood size that were observed for the 8 and 24-h exposures (irrespective of timing of exposure), were carried through to the third brood, well after the adults had been returned to clean Magela Creek water. This indicated that affected parent cladocerans did not recover from the Mg pulse by the test conclusion.

3.2.5 *H. viridissima*

H. viridissima was the second most sensitive species when exposed to Mg pulses. Based on IC50 values, exposure to Mg for 24 h was 1.3-times less toxic than exposure for 96 h, while 4-h exposures were ~2-times less toxic than 96-h exposures (Table 6). Continuous 96-h exposure to Mg at 200 mg L^{-1} resulted in abnormal morphology, specifically thinned, elongated tentacles. At 400 mg L^{-1} Mg, individuals were limp and unable to remain upright, and at 800 mg L^{-1} Mg (close to the IC50 concentration) hydra had ceased feeding. This inability to feed appeared to be due to a loss of tentacle function, where *Artemia* nauplii that came in contact with affected hydra would fall away rather than adhering to the tentacles.

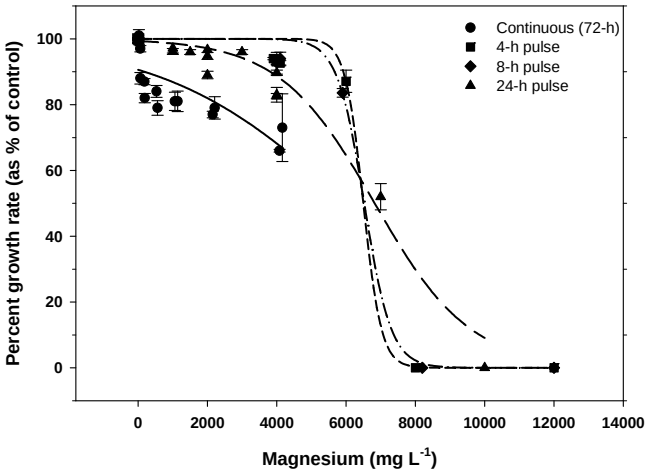
Hydra exposed to a short-term Mg pulse shared similar morphological symptoms as a continuous exposure but they appeared to retain the ability to feed. Individuals that survived the pulse period (up to 1000 mg L^{-1} when exposed for 24 h, 1300 mg L^{-1} for 8 h and 1600 mg L^{-1} for 4 h) showed good morphological recovery by the conclusion of the test. In all Mg concentrations below the IC50 concentrations, the hydra exhibited complete recovery (based on 24-h growth rates) within 24-48 h of being returned to clean water (Appendix J, Table J.3). Hydra that survived exposure to Mg concentrations above the IC50 also appeared to achieve full recovery by the test conclusion, except for after one 24-h exposure where the growth rate of hydra in the highest surviving treatment (1000 mg L^{-1}) was still much lower than that of the control treatment (Appendix J, Table J.3).

3.2.6 *M. mogurnda*

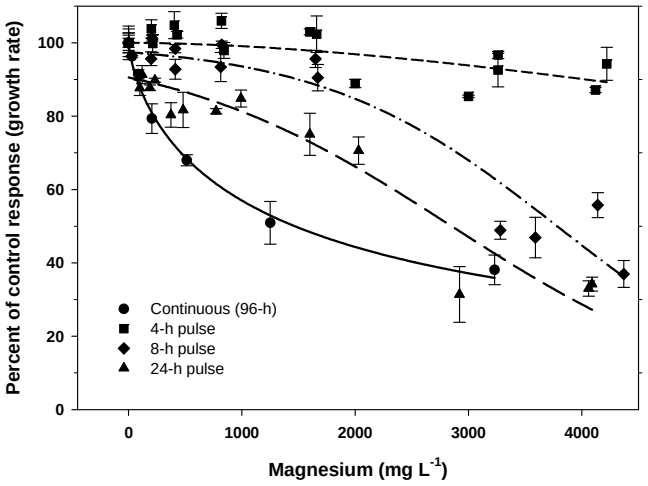
Mogurnda mogurnda was the least sensitive of the six species when exposed to Mg pulses (Figure 6). Fry tolerated exposure to over 4000 mg L^{-1} Mg for 96 h before a 50% decrease in survival occurred (Table 6). Exposure to 4000 mg L^{-1} for 24 h did not affect survival, but fry were noticeably less active. Fry exposed to 4000 mg L^{-1} for 4 and 8-h pulses appeared healthy.

Due to the inability to determine an IC50 for 4, 8 and 24 h pulse using $MgSO_4$, several exposures using $MgCl_2$ were also conducted for each of these pulse durations (tests 1241-1257E, results shown in Appendix E). However, as *M. mogurnda* were more sensitive to $MgCl_2$ than they were to $MgSO_4$ (apparent from the results for the 24-h pulse), the $MgCl_2$ results were not combined with those shown in Figure 6F.

A. *Chlorella* sp.



B. *Lemna aequinoctialis*



C. *Amerianna cumingi*

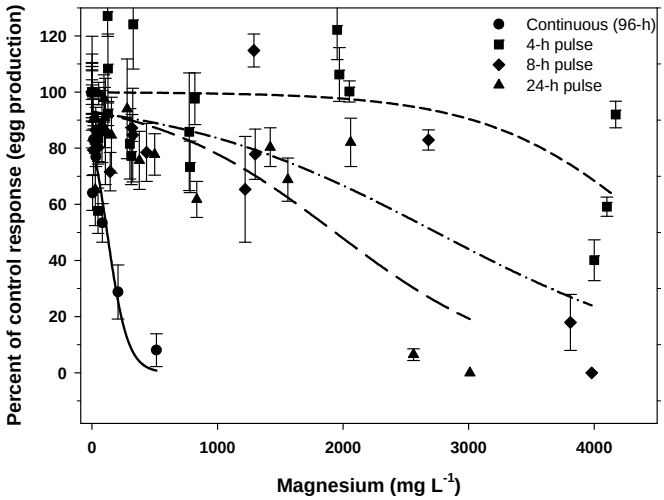
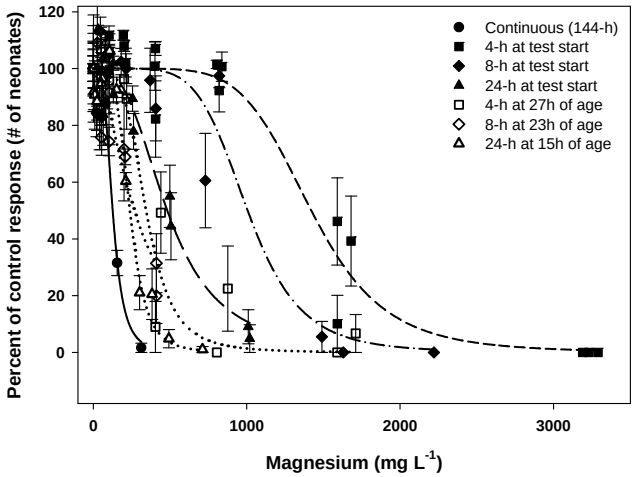
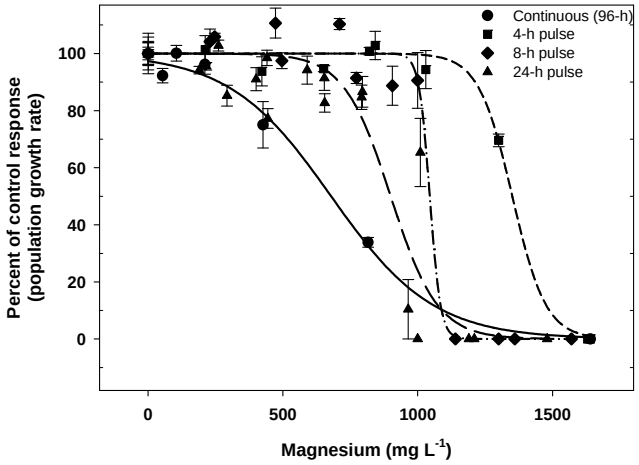


Figure 6 A-C Toxicity of magnesium in Magela Creek Water to three tropical aquatic organisms. Each value represents the mean ± standard error of three replicates. Curve fits are 3 parameter sigmoidal or logistic models: solid line = continuous exposure, long dash = 24-h pulse, medium dash with dot = 8-h pulse and short dash = 4-h pulse. Pulse data shown for *Chlorella* sp. are based on toxicity to $MgCl_2$ (continuous data represents $MgSO_4$ exposure). Control responses are reported in Section 3.1.1.

D. *Moinodaphnia macleayi*



E. *Hydra viridissima*



F. *Mogurnda mogurnda*

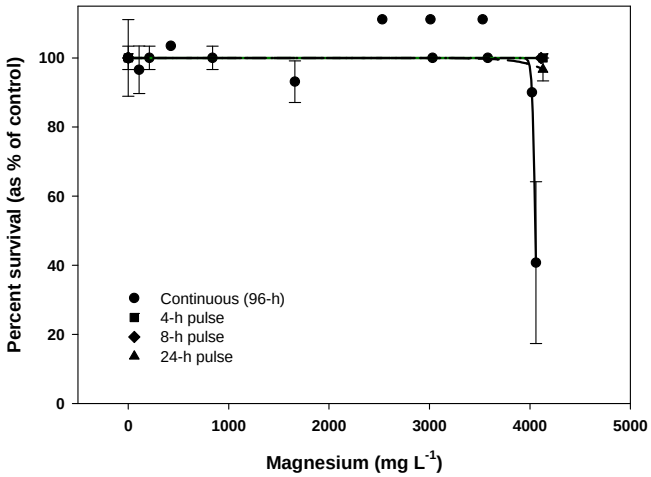


Figure 6 D–F Toxicity of magnesium in Magela Creek Water to three tropical aquatic organisms. Each value represents the mean ± standard error of three replicates, except for *M. macleayi* which has ten replicates. Curves fits are 3 parameter sigmoidal or logistic models: solid line = continuous exposure, long dash = 24-h pulse, medium dash with dot = 8-h pulse and short dash = 4-h pulse, dots = pulses at second moult (*M. macleayi*). Pulse data shown for *Chlorella* sp. are based on toxicity to $MgCl_2$ (continuous data represents $MgSO_4$ exposure). Control responses are reported in Section 3.1.1.

Table 6 Toxicity estimates of pulse exposures of magnesium (mg L^{-1}) to six tropical aquatic organisms

Species	4-h pulse		8-h pulse		24-h pulse		Continuous exposure		
							van Dam et al (2010)		This study
	IC10[1]	**IC50[2]**	**IC10**	**IC50**	**IC10**	**IC50**	**IC10**	**IC50**	**IC50**
Chlorella sp. (unicellular alga)	5954[3] (NC)[4, 5]	6500[3] (NC)	5620[3] (NC)	6526[3] (NC)	3880[3] (3220-4330)	6830[3] (6405-7280)	818 (169-1268)	3435 (2936-3934)	6028 (NC)
Lemna aequinoctialis (duckweed)	4030 (3491-NC)	>4220[6]	1495 (720-2394)	3781 (3412-NC)	80 (NC-677)	2851 (2367-3310)	36 (13-68)	629 (413-956)	1467 (1195-1854)
Hydra viridissima (green hydra)	1213 (1124-1268)	1351 (1321-1454)	1001 (961-1094)	1045 (1014-1111)	709 (532-828)	900 (820-966)	246 (139-322)	713 (646-780)	630 (531-742)
Moinodaphnia macleayi (cladoceran)									
Exposed at test commencement	1017 (707-1354)	1461 (1192-1569)	612 (303-900)	1043 (867-1316)	216 (91-346)	502 (439-604)	39 (17-54)	122 (99-151)	127 (77-202)
Exposed at onset of reproductive maturity	212 (NC-335)	358 (264-420)	62 (NC-162)	296 (231-362)	128 (77-179)	247 (218-278)	NT[7]	NT	NT
Amerianna cumingi (gastropod)	3031 (NC)	>4170[2] (2572-NC)	387 (NC-1972)	2743 (1739-3851)	301 (NC-1260)	1937 (1343-2633)	5.6 (0.6-14)	96 (61-150)	74 (17-379)
Mogurnda mogurnda (fish)	>4100[6]	>4100	>4100	>4100	>4100	>4100	4008 (3850-4025)	4054 (4046-4063)	NT

[1] IC10: magnesium concentration at which there was 10% inhibition in response of the organism

[2] IC50: magnesium concentration at which there was 50% inhibition in response of the organism

[3] Values based on $MgCl_2$ and $CaCl_2$ exposures

[4] NC: not calculable

[5] 95% Confidence limits

[6] Values were reported as "greater than" values where the model could not predict the relevant IC value within the Mg concentration range tested, the maximum of which approximately corresponded to the maximum Mg concentration that could be tested at the specified Mg:Ca ratio of 9:1 without exceeding the solubility limit of $CaSO_4$ (ie. ~4200 mg L^{-1} Mg)

[7] NT: not tested

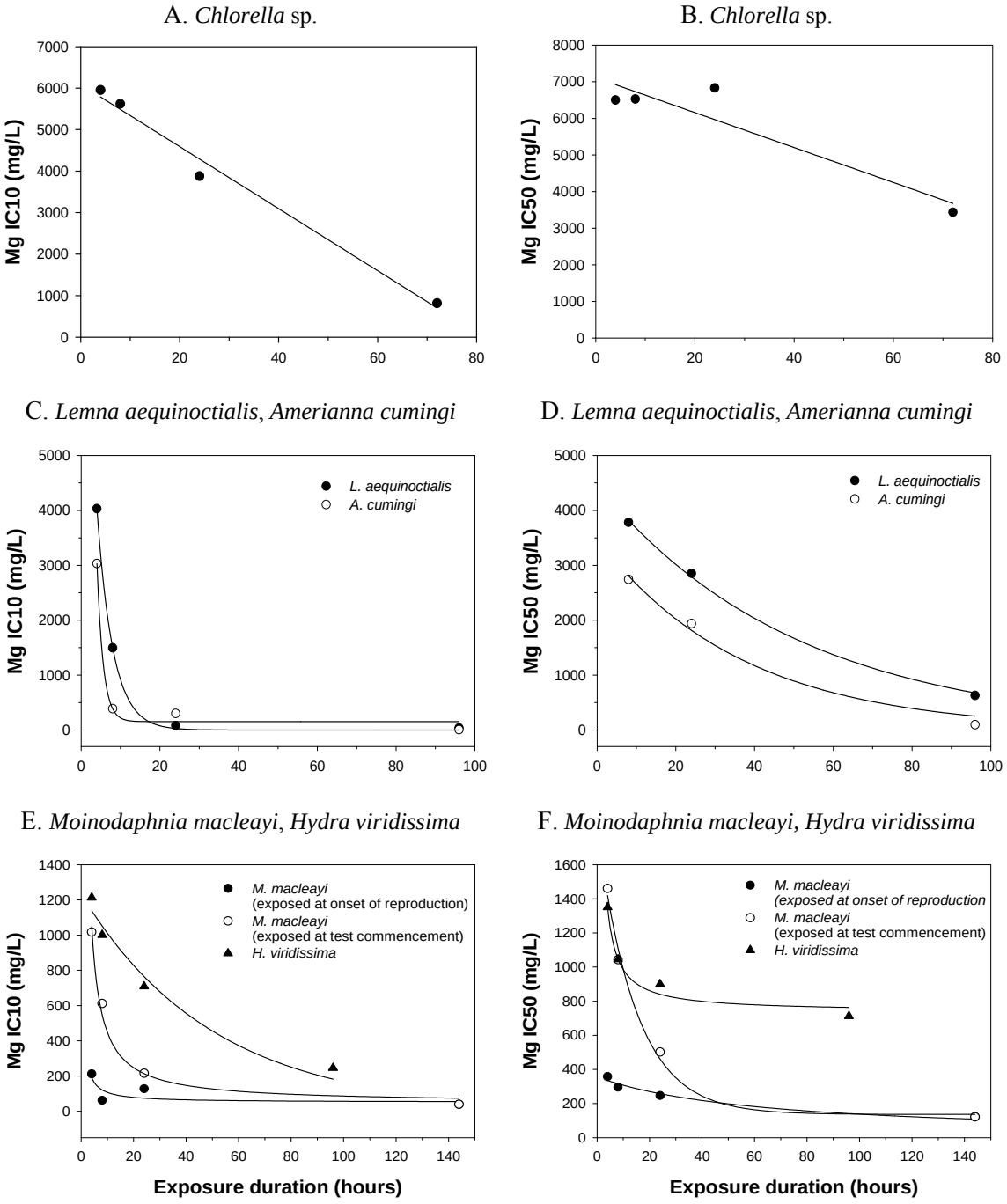


Figure 7 Relationships between Mg pulse exposure duration and the IC10 (left panel) and IC50 (right panel) toxicity estimates for five of the six species tested. Data for the fifth species, *M. mogurnda*, are not shown as insufficient effects of Mg were observed to calculate toxicity estimates. Refer to Appendix H for details of the regression models.

3.3 Mg trigger values and exposure duration versus trigger value model

The SSDs for the 4, 8 and 24-h pulse durations derived 99% species protection TVs (95% CLs) were 94 (6.4–1360) mg L^{-1}, 14 (0.5–384) mg L^{-1} and 8.0 (0.5–144) mg L^{-1}, respectively (Figure 8). The wide confidence limits are due to the low sample size, an unavoidable consequence of deriving site-specific TVs based on a limited number of relevant species. However, the TVs themselves appear reasonable when compared with the toxicity data and also the continuous exposure Mg TV of 3 mg L^{-1} (rounded up from 2.5 mg L^{-1}) from van Dam et al (2010). When converted to EC (using the linear regression equation from Figure 3), the 99% species

protection TVs were 1140 µS cm^{-1}, 174 µS cm^{-1} and 102 µS cm^{-1}, respectively, while the above continuous exposure TV was 42 µS cm^{-1}.

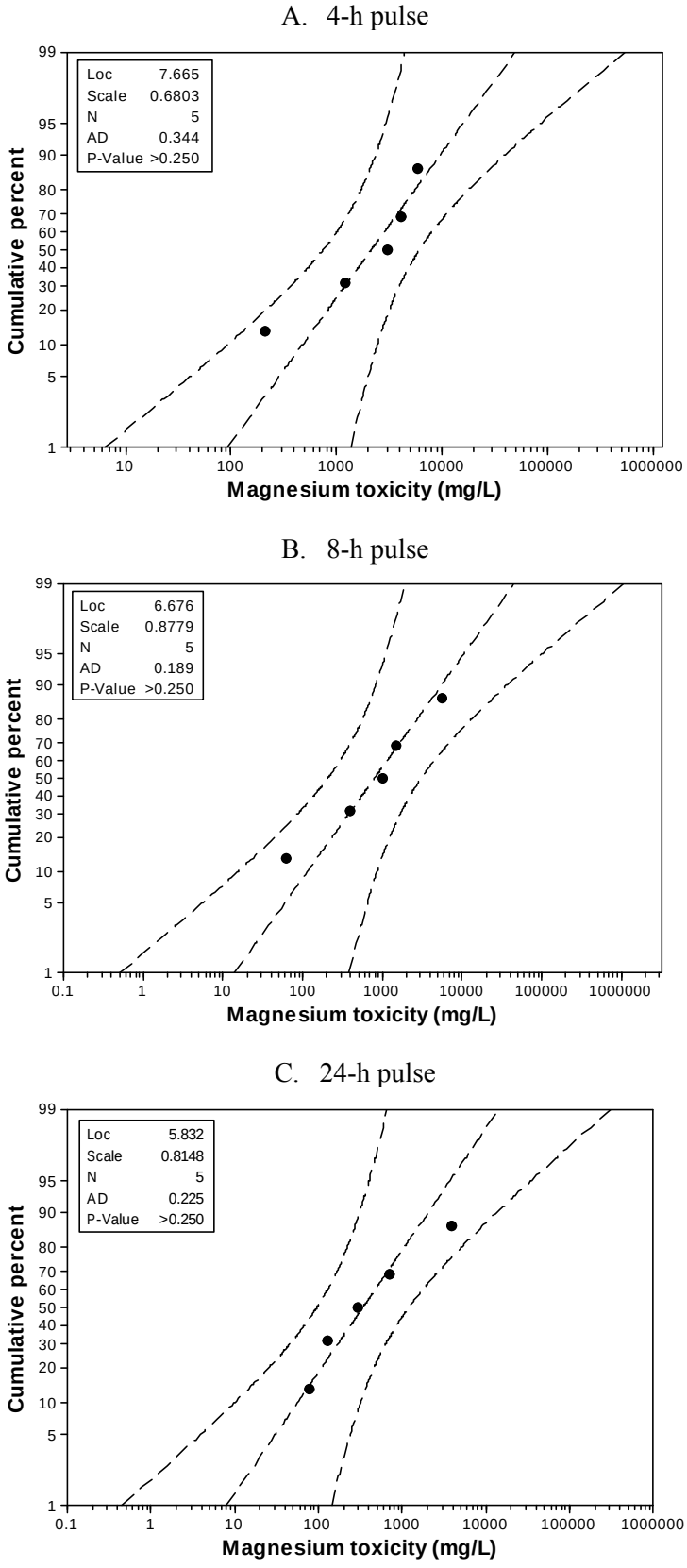


Figure 8 Species sensitivity distributions (SSDs; 2-parameter log-logistic) for Mg based on (A) 4-h, (B) 8-h and (C) 24-h pulse exposure durations

The relationship between the EC TV and exposure duration is shown in Figure 9. The purpose of quantifying this relationship was to enable the prediction of EC TVs for any given exposure duration within the range of durations that have been assessed (ie from 4-h to continuous exposures of at least 72-h). Polynomial interpolation was used to enable the prediction of EC TVs for any given exposure duration within the range of durations that have been assessed (i.e. from 4-h to 72-h).

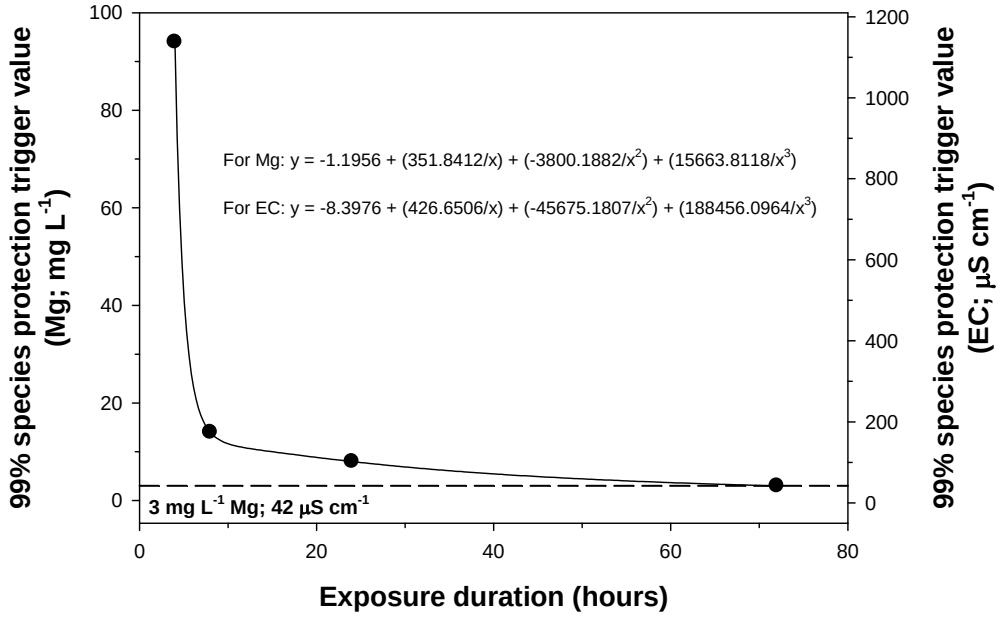


Figure 9 Relationship between trigger value, expressed as Mg (mg L^{-1}) and EC (µS cm^{-1}; after being converted from Mg concentration using the equation in Figure 3 for the downstream site), and exposure duration. The fitted line represents an inverse 3rd order polynomial model as per the equations shown, while the broken line represents the continuous exposure TV of 3 mg L^{-1} Mg or 42 µS cm^{-1}. Details regarding model selection are provided in the text (Section 2.7).

4 Discussion

4.1 Sensitivity to magnesium and effect of exposure duration

In the present study, the sensitivity of the test organisms to continuous exposures of Mg over the full test duration (ie. 3–6 days) was consistent with that that reported by van Dam et al (2010), thus, validating the use of the more comprehensive van Dam et al (2010) continuous exposure data when characterising the Mg toxicity – exposure duration relationships (see Section 4.4). The results for *M. mogurnda* have not been included in this discussion due to the inability to obtain responses following the 4-h, 8-h and 24-h Mg pulses.

It was clear that Mg toxicity to all species increased as exposure duration increased. However, the extent to which Mg toxicity increased, and the nature of the relationship between toxicity and exposure duration, differed between species (Figures 6 & 7). Based on IC50s, the increase in toxicity with increasing exposure duration from 4-h exposure to continuous exposure (72-h to 144-h depending on species) ranged from approximately two-fold for *Chlorella* sp. and *H. viridissima* to over 40-fold for *A. cumingi*. Moreover, the form of the positive relationship between Mg toxicity and exposure duration ranged from linear or near-linear to exponential, and was not necessarily the same for different toxicity estimates (ie. IC10s and IC50s) for the same species (Figure 7 and Appendix I).

Many species-specific factors influence the nature of the relationship between response and exposure duration. The degree of 'true' exposure is influenced by behavioural (eg. avoidance) or biochemical defence mechanisms that may be initiated by an organism upon contact with a toxicant (Calow 1991, Weber 1997). Once exposed, the kinetics of uptake, distribution, depuration and the mode of action all influence the rate at which organisms are affected and can recover from a pulse. Finally, depending on the mechanism of toxicant action, some organisms may experience reversible damage, whilst others may suffer some degree of permanent damage, thus affecting the organism's rate of/ability to recover (eg. Ashauer et al 2010).

Chlorella sp. tolerated exposure to Mg at high concentrations with responses only being observed at gram per litre levels. In addition, exposure duration appeared to have minimal influence on the observed responses. Based on IC50s, *Chlorella* sp. responded similarly to the three pulse durations and was only twice as sensitive as when exposed continuously. These observations may be indicative of *Chlorella* sp. possessing a protective mechanism that enables the alga to minimise exposure to Mg.

Unicellular algae are known to produce exudates consisting primarily of polysaccharides, which have the capacity to bind toxic metals and render them unavailable for uptake into the cell (Kaplan et al 1987, Gonzalez-Davila et al 1995). The release of these exudates has been shown to increase on exposure to toxicants (Pistocchi et al 2000) or as cell growth ceases and cells become nutrient-limited (Mykelstad et al 1989, Kiorboe et al 1990).

In the current work aggregates of cells were commonly observed during continuous Mg exposure experiments. This was manifested as a bimodal size distribution with aggregation confirmed through microscope observations, but was not apparent in the majority of pulse tests. The sticky nature of these exudates are reported as playing an important role in the flocculation of phytoplankton (Eisma 1986, Kiorboe et al 1990) and, as such, lend support to the idea that *Chlorella* sp. possesses such a protective mechanism. However, the role that the saline test waters may play in cell aggregation cannot be disregarded. Should such a

mechanism be triggered rapidly and provide extended protection, it may explain why very similar responses were observed for the 4, 8 and 24-h Mg exposures.

Based on IC50 values, there was little difference in the response of *A. cumingi* between the three pulse durations when compared with their high sensitivity to a continuous exposure. Observations on adult mortality, egg production and snail feeding during the *A. cumingi* tests suggested that this species may be able to withstand short-term Mg exposures using a behavioural and/or biochemical defence mechanism. When exposed to Mg, the snails appear to retract their bodies such that the edge of their shell is in contact with the substrate and they are fully enclosed by their shell. Despite this species lacking an operculum, this behaviour may assist in minimising exposure to the external medium, especially if it coincided with an increase in mucous secretion as is common for aquatic organisms exposed to chemical toxicants (Calow 1991). A snail could only alter its behaviour in such a manner for a short period before needing to resume normal feeding, gas exchange and excretion. The large difference in sensitivity between a 24-h pulse and a 96-h continuous exposure suggests that this point is reached somewhere within this timeframe.

Compared with most of the other test species, *L. aequinoctialis* was also relatively tolerant of short-term Mg exposures. No IC50 was obtained for a 4-h exposure and IC50s of 3781 and 2851 mg L^{-1} were calculated for 8-h and 24-h exposures, respectively. Recovery appeared to be slow or incomplete with this species, in that plants appeared pale and translucent several days after being returned to clean water. In addition, the 24-h growth rates were well below those of control plants during the final day (72 - 96-h) of the 8 and 24-h pulse experiments. This suggests that the uptake, distribution, rate of action and/or depuration of Mg may be a slow process in this species or that irreversible damage has occurred.

Little is known about Mg homeostasis in humans (Changmongpol & Groisman 2002) let alone in aquatic organisms. At the cellular level, it is thought that Mg is transported across membranes through specialised transport systems but that the large size of the Mg hydrated ion in solution makes this a slow process (Flatman 1991). Whether Mg needs to enter an organism/cell in order to elicit a response may be reflected in the rate of the manifestation of symptoms in whole organism toxicity tests. For example, the 4-h IC50 for *H. viridissima* was less than twice that compared with its response to a continuous exposure indicating that the toxic response was relatively rapid. Symptoms displayed by the organisms at all exposure durations included limpness of bodies and elongated tentacles. However, hydra exposed only for a short period (4, 8 or 24-h) retained tentacle function, while those exposed continuously lost their ability to feed.

Tentacle impairment of hydra continuously exposed to Mg has been observed previously and is linked to the inhibition of Ca uptake and subsequent reductions in nematocyst discharge and prey capture (Gitter et al 2003, van Dam et al 2010). It is hypothesised that symptoms such as limpness during short Mg pulses may be rapid osmotic-induced effects caused by saline test solutions. In contrast, the impairment of tentacle function occur only after extended exposures have allowed time for the Mg to interfere with Ca channels in cell membranes. Supporting this hypothesis, hydra have been shown to be highly permeable to water (Lilly 1955), with Steinbach (1963) estimating that *Hydra littoralis* possess a free diffusion space of approximately 30% of their body volume. The rate of recovery observed post-exposure may also depend on whether Mg has been absorbed by, and distributed within, an organism. Although, the rapid recovery of *H. viridissima* during the post-exposure period (Section 4.3) lends support to a simple, reversible mechanism of action such as osmotic stress, without specific knowledge of Mg uptake rates, internal mechanisms cannot be ruled out.

For *M. macleayi*, the time of commencement of exposure (ie at test commencement or around the onset of reproductive maturity) also resulted in markedly different relationships between Mg toxicity and exposure duration (Figure 7E & F). This is discussed in detail in Section 4.2.

4.2 Importance of organism life-stage at point of exposure

Moinodaphnia macleayi exhibited increased sensitivity to Mg (based on neonate production) when exposed at the onset of reproductive maturity (ie at ~27 h old) compared with exposure of newly-released neonates. Notably, the period of exposure at the onset of reproductive maturity also corresponds with the organism's second moult. Consequently, it is not possible to definitively attribute the increased sensitivity to either of these important physiological processes, both of which are recognised as potentially influencing an organism's sensitivity to contaminant exposure (McCahon & Pascoe 1990). The life-stage component of this study was limited to *M. macleayi* only because this species possesses distinct life stages within the duration of the toxicity test protocol, and the test protocol allowed for customised pulse timing. It is possible that other species also possess sensitive life stages.

Increased sensitivity of crustacea to contaminants has been observed when exposure has occurred during critical periods of reproductive activity (McCahon & Pascoe 1988a) and moulting (Emery 1970, Lee & Buikema 1979), or just following moulting (Wright and Frain 1981; McCahon & Pascoe 1988a, 1988b). Increased sensitivity associated with reproductive development or activity has been linked to the increased energy or ionic requirements associated with reproductive processes (McCahon & Pascoe 1988a, Hoang & Klaine 2006), while increased sensitivity due to exposure during or immediately following moulting has been linked to mechanisms associated with Ca regulation (McCahon & Pascoe 1988b). The strong link between moulting and Ca regulation (ie. a substantial amount of Ca is lost from freshwater crustaceans upon moulting, after which they must significantly increase Ca uptake; Greenaway 1985), and the fact that increased Ca reduces Mg toxicity (van Dam et al 2010), lends support to moulting being a key biotic factor in influencing Mg toxicity. The presence of Mg immediately post-moult may also limit the ability for the cladocerans to access Ca from the surrounding water for exoskeleton hardening due to competition at binding sites (Endo et al 2004). However, in the present study, the fact that 24-h Mg exposures coincide with the onset of reproductive maturity and the second moult (IC50 – 247 mg L^{-1}) were still twice as toxic to *M. macleayi* as when exposed for 24-h from test commencement (IC50 – 502 mg L^{-1}), with this latter period including exposure during the first (pre-reproductive) moult (i.e. at ~12-15-h old), suggests that the increased sensitivity is, at least partly, due to the reproductive state. Shorter (eg. 4 - 8-h) pulse exposures coinciding with the first (pre-reproductive) and second (first reproductive) moults would further inform this issue.

4.3 Application to multiple pulse scenarios

Whilst the current study comprehensively addressed the two primary characteristics of a single pulse (magnitude and duration), it did not specifically address multiple pulse scenarios and associated key characteristics such as pulse frequency and length of recovery-time between pulses. In order to apply the findings of this study to real-world situations (where pulses may not occur in isolation), the ability of test organisms to recover from a pulse, and the potential for toxicity to be carried over to subsequent pulses (i.e. carry-over toxicity; Ashauer et al 2010), required consideration.

Three of the five species that responded to Mg in this study showed good recovery by the end of tests. The growth of *Chlorella* sp. in the exposed treatments was equal to or greater than that of the controls in the final 24 h of the all pulse experiments. The appearance and growth

of *H. viridissima* was similar to that of the controls within 48 h of being returned to clean water for concentrations below the IC50, and within 96-h for all but one treatment group exposed to concentrations above the IC50. For *A. cumingi,* individuals that survived a 24-h exposure also exhibited good recovery by resuming feeding and producing egg numbers similar to controls once returned to clean water.

In contrast, *L. aequinoctialis* showed little recovery from an 8-h pulse of >3000 mg L^{-1} or 24-h pulse of >1500 mg L^{-1} based on plant appearance and final day (72–96-h) growth rate. Similarly, for *M. macleayi*, where a true reproductive effect was observed, a reduction in neonate numbers was carried through to the third brood several days after the cladocerans had been returned to clean water. For both these species, it is unknown whether recovery is simply a slow process or if the organisms have experienced irreversible damage that could potentially be accumulated over numerous exposures. Cladocerans have been previously shown to recover (and sometimes exceed control responses) in 21-d growth and reproduction tests after exposure to copper, zinc, selenium, arsenic and ammonia (Diamond et al 2006, Hoang & Klaine 2006). However, there are many examples in the literature of latent toxicity, where effects continue to be observed post-exposure (eg. Holdway and Dixon 1986, Gunn & Noakes 1987, Holdway et al 1994, Brent et al 1998, Angel et al 2010). Some insight into the recovery time for *L. aequinoctialis* and *M. macleayi* after Mg exposure would be gained by conducting further experiments that extend the holding/observation period after the organisms have been returned to clean water.

Carry-over toxicity refers to the phenomenon when an organism exposed to a second contaminant pulse suffers a greater toxic response than that observed after the initial identical exposure, because it carries some accumulated toxicant or damage from the initial exposure (Ashauer et al 2010). It is not possible to address the potential for carry-over toxicity in single pulse studies, because it may occur even if no toxic response is observed during, or a full recovery is observed following, the initial pulse. This issue is complicated further with the consideration of the inter-pulse period, and how to determine the minimum recovery time required by each species (eg. Bearr et al 2005, Diamond et al 2006, Zhao & Newman 2006, Angel et al 2010). Thus, there is a need to assess the potential for Mg carry-over toxicity and determine its significance for the application of the trigger value framework to exceedences of Mg in Magela Creek.

The degree to which empirical data can be used to understand the effect of multiple pulses is limited to the scenarios that are tested in the laboratory. Given the many permutations of pulse magnitude, duration, frequency and inter-pulse period possible in a natural system, there has been a move internationally towards the use of mechanistic effects models (MEMs) for greater predictive ability in setting water quality guidelines. The MEMs use measurements of fundamental kinetic processes such as adsorption, absorption, biotransformation, distribution and elimination to predict the effect of a toxicant on an organism (Ashauer & Escher 2010). However, for numerous reasons such methods are yet to be widely adopted for regulatory purposes. This aspect is further discussed in Appendix A.

An empirical approach to assess Mg pulse exposure toxicity was chosen for the present study because a water quality dataset with high temporal resolution existed to enable the design of laboratory studies that encompassed the range of magnitudes and durations observed in Magela Creek. However, it is acknowledged that this approach may be limited when applied to situations where multiple pulses occur within a short time-frame. This issue is discussed further with respect to the application of the proposed trigger framework in Section 4.4.

4.4 Exposure duration versus trigger value relationship

The Mg toxicity data for 4, 8 & 24 h pulse durations enabled the development of a model to predict TVs for Mg or EC (inferred from the established Mg concentration – EC relationship in Figure 3) based on exposure duration. The exponential nature of the exposure duration – TV relationship was strongly influenced by the exponential nature of the IC10 versus exposure duration relationships for at least two of the species, *L. aequinoctialis* and *A. cumingi*. To our knowledge, this is the first published model enabling adjustment of a trigger value based on exposure duration.

The development of a complete Mg/EC TV framework incorporating the exposure duration – TV model is being undertaken separately to the toxicity study (Tayler & Frostick 2012). Therefore, limited discussion on the application of the framework has been provided here. However, some discussion on the ecotoxicological considerations that must be taken into account when applying the framework is warranted.

As discussed in Section 4.3, the proposed trigger framework is based on single pulse ecotoxicology data and is, therefore, directly applicable to pulses (exceedances) that occur in isolation. However, the continuous monitoring EC data for Magela Creek indicates that multiple exceedances of the current chronic exposure limit have, at times, occurred over relatively short timeframes (days).

Incomplete recovery was observed for two test species in this study and the potential for carry-over toxicity was identified. This is of concern due to the potential for subsequent pulses to become more toxic if insufficient recovery time is allowed between pulses. Caution must therefore be exercised for situations where multiple pulses occur within a short period.

Tayler and Frostick (2012) undertook a detailed analysis of the historical EC continuous monitoring dataset using the Mg/EC TV – pulse duration relationship in order to assess the model's functionality and usefulness within a trigger framework. They found that the peak ECs of all the short-term events in Magela Creek were well below the duration-based TVs interpolated from the model. For example, the highest magnitude pulse of 124 μS cm^{-1} that lasted for 5 h 30 min was only 29% of the duration-based TV of 433 μS cm^{-1}. The longest duration EC pulse of 17 h 20 min, with a peak EC of 60 μS cm^{-1} was 52% of the duration-based TV of 115 μS cm^{-1}. Moreover, an average event, with a duration and magnitude around the mean of all events (4 h 20 min, 50 μS cm^{-1}), was only 5.5% of the duration-based TV.

With the treatment of pond and process water becoming an increasingly significant component of the water management regime at Ranger Mine, the quality of mine water entering Magela Creek should improve such that the frequency of exceedances of the EC Guideline/chronic Limit will decrease in the future, at least until the mine decommissioning phase commences. As such, there is little justification to embark on a large-scale study of multiple pulses at this point in time. However, it is recommended that the additional areas of research proposed in Section 4.3 be pursued to investigate organism recovery time and the potential for carry-over toxicity. This would provide further guidance in applying the trigger framework to multiple pulse scenarios.

5 Conclusions

The present study assessed the toxicity of single pulse exposures of Mg to six freshwater species, with the aim of developing a model to predict water quality trigger values based on exposure duration. This aim was achieved.

For all species, Mg toxicity increased as exposure duration increased. However, the extent to which toxicity increased differed between species, from 2-fold for *Chlorella* sp. to 40-fold for *Amerianna*. Moreover, the nature of the positive relationship between toxicity and exposure duration differed between species, from linear to exponential.

The cladoceran, *M. macleayi*, exhibited differential sensitivity to Mg pulse exposures depending on the life stage that was exposed. Increased sensitivity to Mg was observed following pulse exposures at the onset of reproductive maturity (i.e. at ~27-h old) compared with exposure of newly-released neonates (<6-h old).

Using the pulse exposure toxicity data and associated TVs for each pulse duration, a Mg/EC TV – exposure duration model was developed. The model will be incorporated into a Mg/EC trigger value framework that will enable improved interpretation of transient pulses of EC in Magela Creek downstream of the Ranger mine.

Further work assessing organism recovery time and the potential for carry-over toxicity is recommended to provide guidance on the application of the Mg/EC TV framework to situations where multiple pulses occur within short timeframes.

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Appendix A Models for predicting toxicity of episodic exposure

Efforts to address episodic toxicity exposure have focused on two approaches:

1 Experimental: the development of toxicity tests that attempt to incorporate the episodic nature of exposure to pollutants, and

2 Predictive modelling: the development of models that use measurements of fundamental toxicokinetic processes and toxicodynamic parameters to predict toxicity under episodic exposure conditions Gordon et al. (2010).

With regards to the experimental approach, it has been identified that there are a lack of episodic toxicity data of sufficient quality that could be used for the derivation of water quality guidelines (Gordon et al. 2010). The application of these data is also limited in that the subsequent guidelines derived from such exposure data would only be applicable within the bounds of the pulse characteristics that were tested.

International research into the effects of pulse exposures is predominantly focused on the development of mechanistic effects models (MEMs) or process-based models such as toxicokinetic/toxicodynamic (TK/TD) and Dynamic Energy Budget (DEB) models that predict contaminant effects based on different exposure scenarios (Ashauer et al 2005, Ashauer & Escher 2010, Ashauer et al 2010, Delos 2008, Diamond et al 2006, Jager et al 2006, Kooijman 2010). Prediction of toxicity using modelling relies on empirical measurements of fundamental toxicokinetic processes such as adsorption, absorption, biotransformation, distribution and elimination of a toxicant (Ashauer & Escher 2010). This information, in combination with toxicodynamic parameters related to organism damage, recovery and repair, have been used to predict an organism's response to any given exposure scenario, including fluctuating and pulsed exposure patterns (Ashauer et al 2010, Rubach et al 2010). Models have also been used to extrapolate between untested species and chemicals because many model parameters vary predictably with organism traits (eg. body size), and test data for different species may be combined to produce a coherent set of information on a particular chemical (Jager et al 2006, Ashauer & Escher 2010).

While MEMs have been used in ecotoxicology over the past 30 years to answer specific research questions, their application for regulation has been limited (Preuss et al 2009). Grimm et al (2009) suggested that inconsistency in the MEM approaches used to date have led to a lack of understanding of their benefits by regulators and industry. Another negative factor is that much of the research to date involves the modelling of toxicity test data usually with lethality as the endpoint. The DEB theory approach, which can accommodate continuous sub-lethal data (eg. Muller et al 2010), appear to be the only exception. Handy (1994) noted that because intermittent events are short-lived, acute responses are considered most relevant. However, chronic effects will be important with respect to post-exposure toxicity or repeated sub-lethal exposures. Additionally, sub-lethal endpoints are given preference in guidance documents such as the Australian & New Zealand water quality guidelines (ANZECC & ARMCANZ 2000). Subsequently, most protocols developed for routine ecotoxicological assessments in Australia, including those routinely used at the Supervising Scientist Division, assess sub-lethal endpoints.

Thus, while MEMs show promise as an approach for simulating temporal aspects of toxicity, and hence assessing the effects of pulsed exposures, researchers appear to be some years away from providing a set of tools that can be used for the regulation of chemicals released to the environment.

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Appendix B Toxicity tests included in this report

Species		Test #	Test code	Date	Mg:Ca ratio
Chlorella sp.	($MgSO_4$)	1109G	Alg_Mg@Mg:Ca_07	09/08/2010	9.7:1
		1138G	Alg_Mg@Mg:Ca_08	26/10/2010	9.7:1
	($MgCl_2$)	1189G	Alg_24h Mg pulse_10	19/7/2011	9.0-10:1
		1192G	Alg_24h Mg pulse_11	25/7/2011	9.0-9.7:1
		1199G	Alg_24h Mg pulse_12	2/8/2011	9.3-10:1
		1202G	Alg_4h Mg pulse_01	8/8/2011	9.7:1
		1203G	Alg_8h Mg pulse_01	9/8/2011	10.0:1
		1231G	Alg_24h Mg pulse_13	4/10/2011	9.0-9.7:1
		1232G	Alg_8h Mg pulse_02	17/10/2011	9.1-9.3:1
		1233G	Alg_4h Mg pulse_02	11/10/2011	9.2-9.8:1
Lemna aequinoctialis ($MgSO_4$)		960L	Lem_Mg@Mg:Ca_04	8/12/08	9.0-10.9:1
		961L	Lem_4h Mg pulse_01	2/12/08	9.5-9.9:1
		1011L	Lem_24h Mg pulse_01	13/7/09	9.3-9.6:1
		1018L	Lem_24h Mg pulse_02	18/8/09	9.2-9.8:1
		1097L	Lem_8h Mg pulse_01	7/6/10	9.3-10:1
		1108L	Lem_8h Mg pulse_02	9/8/10	9.6-9.9:1
		1116L	Lem_4h Mg pulse_02	23/8/10	9.7-10.1:1
	($MgCl_2$)	1243L	Lem_4h Mg pulse_03	24/10/11	9-10:1
		1244L	Lem 4h Mg pulse_04	21/11/11	9.3-9.7:1
Moinodaphnia macleayi		945D	Clad_Mg@Mg:Ca_08	9/10/08	8.4-9.2:1
		946D	Clad_4h Mg pulse_01	9/10/08	9.2-9.5:1
		957D	Clad_4h Mg pulse_03	25/11/08	9.2-9.5:1
		958D	Clad_4h Mg pulse_04	25/11/08	9.2-9.5:1
		1003D	Clad_4h Mg pulse_07	5/6/09	9.1-9.8:1
		1012D	Clad_4h Mg pulse_09	9/12/09	9.1-9.9:1
		1101D	Clad_24 Mg pulse_03	16/7/10	8.6-9.8:1
		1105D	Clad_24 Mg pulse_04	30/7/10	9.3-10.1:1
		1111D	Clad_8h Mg pulse_01	13/8/10	8.3-9.0:1
		1115D	Clad_8h Mg pulse_02	27/8/10	9.6-10:1
		1139D	Clad_8h Mg pulse_05	10/11/10	8.7-9.3:1
		1142D	Clad_8h Mg pulse_06	26/11/10	8.7-9.5:1
		1165D	Clad_24h Mg pulse_06	5/5/11	7.5-9:1
		1177D	Clad_24h Mg pulse_07	30/5/11	9-9.7:1
Hydra viridissima		971B	Hyd_Mg@Mg:Ca_04	19/1/09	9.1-9.6:1
		972B	Hyd_4h Mg pulse_01	19/1/09	9.4-9.6:1
		979B	Hyd_4h Mg pulse_02	10/2/09	9.9-10.7:1
		1014B	Hyd_24h Mg pulse_01	20/7/09	9.0-9.4:1

Appendix B (cont.)

Species	Test #	Test code	Date	Mg:Ca ratio
Hydra viridissima (cont.)	1017B	Hyd_24h Mg pulse_02	10/8/09	9.4-9.8:1
	1095B	Hyd_24h Mg pulse_03	31/5/10	9.1-9.9:1
	1100B	Hyd_8h Mg pulse_01	15/6/10	9.4-10:1
	1114B	Hyd_8h Mg pulse_02	16/8/10	8.8-9.0:1
Amerianna cumingi	970S	Amer_Mg@Mg:Ca_05	13/1/09	9.7-10.6:1
	982S	Amer_4h Mg pulse_01	1/9/09	9.1-10:1
	1027S	Amer_4h Mg pulse_02	19/10/09	9.0-10.4:1
	1150S	Amer_4h Mg pulse_03	11/1/11	9.0-9.7:1
	1154S	Amer_24h Mg pulse_01	24/1/11	8.6-9.8:1
	1159S	Amer_8h Mg pulse_01	7/1/11	9.0-9.9:1
	1160S	Amer_24h Mg pulse_02	21/2/11	7.6-9.6:1
	1168S	Amer_8h Mg pulse_02	4/4/11	8.5-9.9:1
	1169S	Amer_8h Mg pulse_03	9/5/11	9.0-9.8:1
	1176S	Amer_24h Mg pulse_03	23/5/11	9.2-9.6:1
Mogurnda mogurnda ($MgSO_4$)	1013E	Fry_4h Mg pulse_01	25/8/09	9.7:1
	1016E	Fry_24h Mg pulse_01	25/8/09	9.7:1
	1125E	Fry_8h Mg pulse_01	13/9/10	10.1:1
($MgCl_2$)	1241E	Fry_24h Mg pulse_02	3/12/11	9-10:1
	1246E	Fry_8h Mg pulse_01	7/12/11	9-10:1
	1247E	Fry_4h_Mg pulse_01	7/12/11	9-10:1
	1252E	Fry_24h Mg pulse_03	19/12/11	9.2-9.3:1
	1253E	Fry_8h Mg pulse_02	25/12/11	9.3-9.5:1
	1254E	Fry_4h Mg pulse_02	6/1/12	9-9.2:1
	1257E	Fry_24h Mg pulse_04	12/1/12	8.9-9:1

Appendix C Sensitivity of *Chlorella* sp. to magnesium sulfate and magnesium chloride

Due to the inability to obtain a full effect for the 4-h, 8-h and 24-h pulses using $MgSO_4$, $MgCl_2$ was trialled. Figure C.1 shows that the toxicity of $MgCl_2$ to *Chlorella* sp. was comparable with that of $MgSO_4$. Hence, in order to obtain full Mg concentration-response relationships for *Chlorella* sp., $MgCl_2$ was used as a substitute for $MgSO_4$.

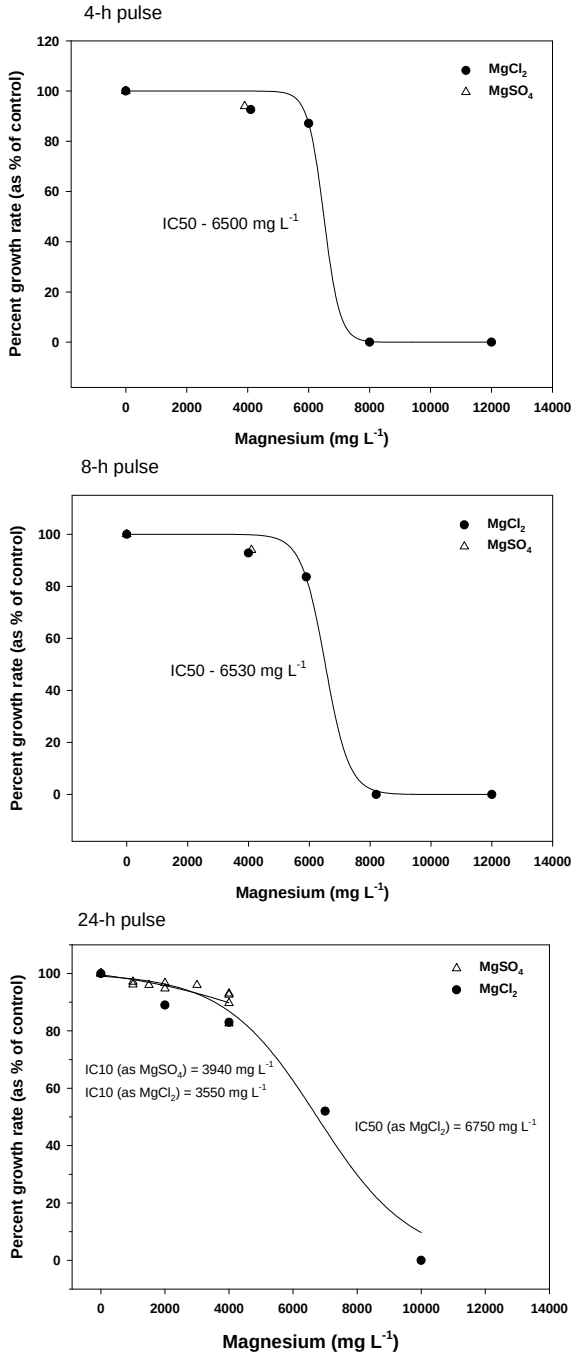

Figure C.1 Toxicity of magnesium chloride ($MgCl_2$) to *Chlorella* sp. relative to the toxicity of magnesium sulfate ($MgSO_4$). Figures represent toxicity over 4-h (top), 8-h (middle) and 24-h (bottom) pulse exposures. Mg IC10s/IC50s were based on 3 parameter sigmoidal models (4-h $MgCl_2$, $r^2 = 0.99$; 8-h $MgCl_2$, $r^2 = 0.99$; 24-h $MgCl_2$, $r^2 = 0.94$; 24-h $MgSO_4$, $r^2 = 0.67$).

Appendix D Sensitivity of *Lemna* to magnesium sulfate and magnesium chloride

Due to the inability to obtain a full effect for the 4-h pulse using $MgSO_4$, $MgCl_2$ was trialled, for which a full toxic response was obtained (Figure D.1). However, due to the difference in toxicity found between the two forms of Mg (Figure D.1), and the local environmental relevance of Mg in the form of $MgSO_4$, it was not appropriate to incorporate the results for $MgCl_2$ with that of $MgSO_4$.

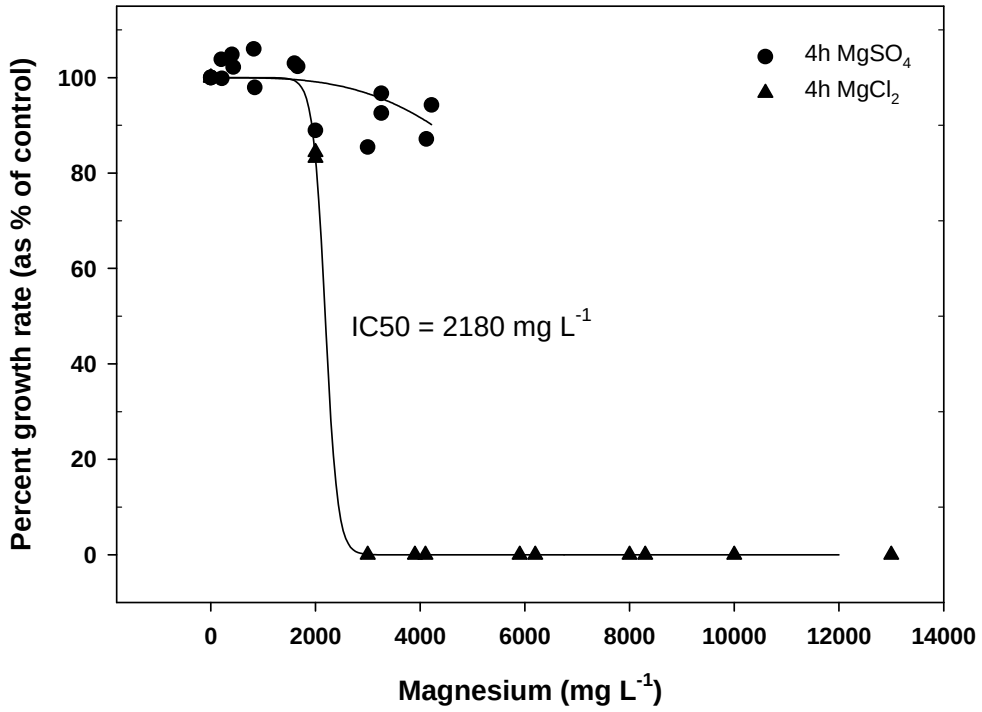

Figure D.1 Toxicity of a 4-h pulse exposure of magnesium chloride ($MgCl_2$) to *Lemna aequinoctialis* relative to the toxicity of magnesium sulfate ($MgSO_4$). The Mg IC50 based on the $MgCl_2$ data was calculated using a 3 parameter sigmoidal model ($r^2 = 0.99$).

Appendix E Sensitivity of *M. mogurnda* to magnesium sulfate and magnesium chloride

Due to the inability to obtain a full toxic effect for *M. mogurnda* for any of the pulse durations of $MgSO_4$ at 4000 mg L^{-1} Mg, trials with $MgCl_2$ were conducted (Figure E.1). While full effects were obtained for each of the pulse durations (Figure E.1), the difference in sensitivity to $MgSO_4$ and $MgCl_2$ evident in the 24-h pulse meant these results could not be incorporated with those of $MgSO_4$ in the main report.

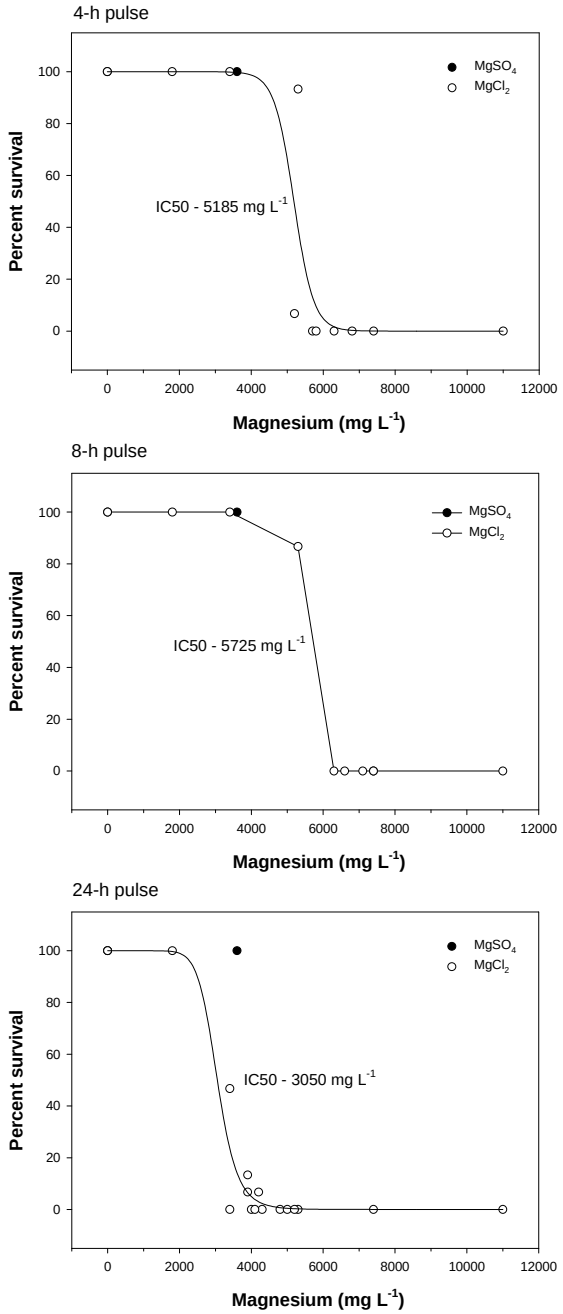

Figure E.1 Toxicity of magnesium chloride ($MgCl_2$) to *Mogurnda mogurnda* relative to the toxicity of magnesium sulfate ($MgSO_4$). Figures represent toxicity over 4-h (top), 8-h (middle) and 24-h (bottom) pulse exposures. Mg IC50s based on the $MgCl_2$ data were calculated using the following models: 4-h – 3 parameter sigmoidal model ($r^2 = 0.79$); 8-h – linear interpolation (a model could not be fitted); and 24-h – 3 parameter logistic model ($r^2 = 0.95$).

Appendix F Recovery of *Chlorella* sp. cells following filtration

For *Chlorella* sp., the need to rinse algal cells post-pulse presented the challenge of recovering sufficient proportions of the cells following the rinsing process. Following unsuccessful attempts using centrifugation and dialysis, vacuum filtration through 1.2 µm polycarbonate filter papers using a vacuum pump with adjustable air pressure proved a successful method. Table F.1 shows the good recovery of algal cells achieved using this method.

Table F.1 Percent recovery of *Chlorella* sp. cells following filtration from Mg pulse exposure solutions using vacuum filtration through a 1.2 µm polycarbonate filter paper.

Test	Mean percent recovery of cells	
	Control	Mg treated cells[1]
1189G	95 ± 1	98 ± 35
1192G	87 ± 6	95 ± 7
1199G	92 ± 3	200 ± 29
1202G	90 ± 7	80 ± 5
1203G	122 ± 8	83 ± 6
1231G	97 ± 10	115 ± 49
1232G	110 ± 12	162 ± 29
1233G	103 ± 9	88 ± 12

[1] Where recovery of magnesium (Mg) treated cells was >100% this resulted from the pre-filtered counts of these treatments being underestimates of the true cell counts (due to cell clumping). This resulted in the post-filtration counts falsely appearing greater than pre-filtration counts.

Appendix G Water parameters across toxicity tests

Table G.1 *Chlorella* sp.

		New water			Old water			Mean
Test	**Treatment**	**pH**	**DO**	**EC**	**pH**	**DO**	**EC**	**Temp**
1189G	0.9	6.39	100	44	6.7	93	42	29.0
	930	6.42	96		6.63	90	46	
	1900	6.4	98		6.64	93	55	
	4300	6.32	99		6.66	93	62	
1192G	0.9	6.35	97	46	6.52	88	46	28.7
	940	6.36	94		6.57	92	47	
	1900	6.41	96		6.61	90	48	
	4200	6.4	98		6.62	93	53	
1199G	0.9	6.35	94	44	6.59	102	44	28.9
	1400	6.42	93		6.6	101	44	
	3200	6.39	95		6.61	101	49	
	4300	6.39	95		6.57	96	52	
1202G	0.8	6.25	98	45	6.52	91	43	27.5
	3900	6.31	104		6.57	93	50	
1203G	0.9	6.26	95	45	6.55	93	42	27.3
	4100	6.29	92		6.54	93	53	
1231G	0.9	6.01	96	46	6.52	96	46	27.9
	1900	6.19	97		6.48	96	48	
	3800	6.17	98		6.42	96	56	
	6700	6.06	94		6.42	97	67	
	9700	6.02	94		6.37	98	76	
1232G	1.1	6.34	93	49	6.71	94	46	26.8
	4000	6.22	94		6.69	93	70	
	5900	6.18	91		6.65	94	60	
	8200	6.18	94		6.54	95	57	
	12000	6	93		6.53	93	61	
1233G	1.1	6.09	98	47	6.7	99	47	28.0
	4100	6.1	97		6.67	97	53	
	6000	6.12	96		6.63	98	59	
	8000	6.01	91		6.52	95	69	
	12000	5.98	92		6.51	92	70	
Median		6.26	95	46	6.57	93	52	28.0
Median (old & new)		6.42	95	48				
Range (old & new)		5.98-6.71	90-104	42-76				26.8-29.0
n		62	62	39				

[a] All treatment concentrations are mg L^{-1} Mg, [b] DO = dissolved oxygen as % saturation, [c] EC = electrical conductivity as µS cm^{-1}, [d] Temperature in °C.

Table G.2 *Mogurnda mogurnda*

			New water			Old water			Mean
Test	**Day**	**Treatment**	**pH**	**DO**	**EC**	**pH**	**DO**	**EC**	**Temp**
1013E	1	0.9	6.22	107	16	6.37	97	17	26.5-27.6
		4130	6.52	104		6.28	97	18	
	2	0.9	6.23	104	16	6.16	95	18	
		4130				6.19	94	18	
	3	0.9	5.95		17	6.23	97	19	
		4130				6.24	95	20	
	4	0.9	6.04		15	6.17	94	19	
		4130				6.45	90	19	
1016E	1	0.9	6.22	107	16	6.45	96	21	26.5-27.6
		4130	6.52	104		6.64	99	NM	
	2	0.9	6.23	104	16	6.08	93	18	
		4130				6.14	92	19	
	3	0.9	5.95		17	6.19	97	20	
		4130				6.21	96	20	
	4	0.9	6.04		15	6.21	95	19	
		4130				6.25	95	19	
1125E	1	0.9	6.50	100	15	6.80	90	20	26.0-28.1
		4110	6.30	89		6.56			
	1	0.9	6.38	109	17	6.74	95	16	
		4110				6.60	98	15	
	3	0.9	6.68	95	16	6.71	93	16	
		4110				6.72	94	17	
	4	0.9	6.48	108	15	6.74	93	17	
		4110				6.82	NM	17	
	5	0.9	6.32	102	16	7.02	88	20	
		4110				6.93	89	19	
	Median		6.27	104	16	6.41	95	19	No mean
	Median (old & new)		6.31	96	17				
	Range (old & new)		5.95-7.02	88-109	15-21				26.0-28.1
	n		42	36	37				

[a] All treatment concentrations are mg L^{-1} Mg, [b] DO = dissolved oxygen as % saturation, [c] EC = electrical conductivity as μS cm^{-1}, [d] Temperature in °C.

Table G.3 *Hydra viridissima*

			New water			Old water			Mean
Test	**Day**	**Treatment**	**pH**	**DO**	**EC**	**pH**	**DO**	**EC**	**Temp**
971B	0	0.3	5.22	112	10	5.79	95	11	26-29
		53.9	5.74	114		5.97	95		
		105	5.81	115		5.98	95		
		211	5.82	119		5.96	98		
		426	5.83	111		6.01	97		
		816	5.80	110		5.96	94		
		1640	5.83	106		6.01	95		
	1	0.3	5.31		11	5.79	96	11	
		53.9	5.73			6.01	96		
		105	5.78			6.01	95		
		211	5.74			5.96	93		
		426	5.70			5.97	94		
		816	5.82			5.99	95		
	2	0.3	5.26		11	5.74	96	11	
		53.9	5.75			6.02	95		
		105	5.78			6.02	95		
		211	5.76			5.99	96		
		426	5.45			6.04	96		
		816	5.80			6.00	97		
	3	0.3	5.52		10	5.7	93	10	
		53.9	5.80			6.08	91		
		105	5.83			6.10	93		
		211	5.78			6.07	94		
		426	5.81			6.09	94		
		816	5.78			6.08	90		
972B	0	0.3	5.66	114	11	6.00	94	11	26-29
		212	6.18	107		6.28	94		
		422	6.12	111		6.30	97		
		842	6.11	104		6.23	97		
		1640	6.05	103		6.18	97		
		3370	5.98	93		6.03	95		
		4230	5.90	93		5.97	96		
	1	0.3	5.09	118	10	6.07	93	15	
		212				6.01	93	13	
		422				6.02	93	12	
		842				6.18	92	13	
	2	0.3	5.12	112	11	5.78	95	12	
		212				5.72	95	11	
		422				5.70	93	11	
		842				5.75	95	11	

Table G.3 (cont)

			New water			Old water			Mean
Test	**Day**	**Treatment**	**pH**	**DO**	**EC**	**pH**	**DO**	**EC**	**Temp**
	3	0.3				5.81	95	12	
		212				5.84	92	12	
		422				5.76	94	11	
		842				5.78	92	11	
	4	0.3				5.95	93	11	
		212				5.75	95	10	
		422				5.75	91	10	
		842				5.70	91	11	
979B	0	0.4	6.12	110	13	6.40	87	13	26.5-27.5
		651	6.08	108		6.15	91		
		821	6.15	107		6.17	91		
		1030	6.08	107		6.15	92		
		1300	6.05	104		6.14	91		
		1630	6.02	99		6.12	91		
		2060	6.00	101		6.12	91		
	1	0.4	6.13	113	10	6.20	91	11	
		651				6.11	96	12	
		821				6.07	95	11	
		1030				6.13	93	13	
		1300				6.13	89	13	
		1630				6.15	92	12	
	2	0.4	5.76	112	10	6.03	93	11	
		651				6.07	92	13	
		821				6.12	93	12	
		1030				6.11	95	12	
		1300				6.13	92	11	
		1630				6.11	95	11	
	3	0.4	5.75	111	10	5.97	92	12	
		651				6.03	94	12	
		821				6.05	91	12	
		1030				6.04	95	12	
		1300				6.06	96	11	
		1630				6.06	95	11	
	4	0.4				6.08	94	11	
		651				6.04	93	11	
		821				6.07	91	11	
		1030				5.97	93	11	
		1300				5.96	90	11	
		1630				5.85	91	11	
1014B	1	0.8	6.06	102	18	6.31	97	17	26.5-27.3

Table G.3 (cont)

			New water			Old water			Mean
Test	**Day**	**Treatment**	**pH**	**DO**	**EC**	**pH**	**DO**	**EC**	**Temp**
		190	6.38	103		6.59	93		
		293	6.39	102		6.64	94		
		444	6.39	103		6.66	96		
		655	6.41	102		6.67	95		
		965	6.38	102		6.67	95		
		1480	6.37	100		6.70	96		
	2	0.8	6.05	112	15	6.27	92	16	
		190				6.34	96	17	
		293				6.4	90	16	
		444				6.47	92	19	
		655				6.48	93	17	
		965				6.51	93	17	
	3	0.8	6.06	117	15	6.23	92	18	
		190				6.32	95	17	
		293				6.34	93	18	
		444				6.30	91	17	
		655				6.53	90	18	
		965				6.48	91	16	
	4	0.8	6.10	108	18	6.24	92	18	
		190				6.33	91	17	
		293				6.37	93	17	
		444				6.39	94	17	
		655				6.51	89	17	
		965				6.52	96	17	
1017B	1	0.9	6.10	98	15	6.28	95	17	26.1-27.3
		261	6.37			6.49	91		
		401	6.42			6.58	90		
		590	6.43			6.60	93		
		795	6.46			6.52	91		
		1010	6.46			6.59	89		
		1190	6.47			6.62	91		
	2	0.9	6.04	104	15	6.49	112	18	
		261				6.35	115	18	
		401				6.37	109	18	
		590				6.33	108	18	
		795				6.38	110	17	
		1010				6.38	114	17	
	3	0.9	6.29		15	6.46		20	
		261				6.35		18	
		401				6.32		18	

Table G.3 (cont)

			New water			Old water			Mean
Test	**Day**	**Treatment**	**pH**	**DO**	**EC**	**pH**	**DO**	**EC**	**Temp**
		590				6.33		17	
		795				6.31		18	
		1010				6.35		17	
	4	0.9	6.21	108	15	6.67		15	
		261				6.43		16	
		401				6.48		16	
		590				6.48		16	
		795				6.45		16	
		1010				6.42		16	
1095B	1	0.8	6.38	105	17	6.49	92		26-28.2
		216	6.44	107		6.40	89		
		441	6.37	106		6.56	93		
		654	6.38	106		6.51	91		
		792	6.43	103		6.55	90		
		1000	6.52	101		6.59	93		
		1210	6.49	102		6.58	91		
	2	0.8	6.53	101	16	6.50	91	17	
		216				6.51	94	17	
		441				6.53	88	17	
		654				6.48	93	17	
		792				6.54	90	17	
		1000				6.50	93	17	
	3	0.8	6.43	97	16	6.25	92	17	
		216				6.32	92	18	
		441				6.34	93	17	
		654				6.35	92	18	
		792				6.37	93	17	
		1000				6.37	89	16	
	4	0.8	6.01	113	16	6.69	97	17	
		216				6.75	98	16	
		441				6.79	96	17	
		654				6.77	96	16	
		792				6.82	98	17	
		1000				6.79	96	16	
1100B	0	0.8	6.29	94	16	6.49	95	20	26-28.5
		247	6.39	98		6.28	96		
		496	6.42	98		6.33	95		
		773	6.44	97		6.36	97		
		1000	6.47	94		6.40	95		
		1300	6.50	95		6.40	94		

Table G.3 (cont)

			New water			Old water			Mean
Test	**Day**	**Treatment**	**pH**	**DO**	**EC**	**pH**	**DO**	**EC**	**Temp**
		1570	6.53	94		6.40	93		
	1	0.8	6.19	118	16	6.28	101	17	
		247				6.41	101	16	
		496				6.41	102	16	
		773				6.43	98	17	
		1000				6.45	101	17	
		1300				6.46	98	17	
	2	0.8	6.33	107	16	6.49	94	17	
		247				6.53	95	16	
		496				6.54	95	17	
		773				6.53	95	17	
		1000				6.53	89	16	
		1300				6.55	94	17	
	3	0.8	6.26		16	6.66	96		
		247				6.71	97	19	
		496				6.63	97	18	
		773				6.51	99	18	
		1000				6.51	95	17	
		1300				6.47	96	18	
	4	0.8				6.39	92	16	
		247				6.48	95	16	
		496				6.43	96	16	
		773				6.40	94	17	
		1000				6.41	94	16	
		1300				6.45	94	18	
1114B	0	0.8	5.84	90	15	6.51	89	16	27-28.5
		230	6.21	91		6.49	90		
		473	6.20	91		6.48	90		
		710	6.20	87		6.47	88		
		906	6.25	91		6.47	90		
		1140	6.25	93		6.47	89		
		1360	6.30	86		6.45	90		
	1	0.8	6.44	92	15	6.43	93	16	
		230				6.45	91	16	
		473				6.48	92	16	
		710				6.44	91	16	
		906				6.47	91	16	
		1140				6.46	94	16	
	2	0.8	6.4	92	15	6.41	92	16	
		230				6.43	92	16	

Table G.3 (cont)

			New water			Old water			Mean
Test	**Day**	**Treatment**	**pH**	**DO**	**EC**	**pH**	**DO**	**EC**	**Temp**
		473				6.42	89	17	
		710				6.45	90	17	
		906				6.41	92	16	
	3	0.8	6.43	95	15	6.42	94	16	
		230				6.43	94	16	
		473				6.45	95	16	
		710				6.45	98	16	
		906				6.44	94	16	
		1140				6.40	98	15	
	4	0.8				6.32	89	16	
		230				6.29	91	15	
		473				6.34	89	15	
		710				6.27	87	15	
		906				6.34	90	15	
		1140				6.28	90	16	
Median			6.12	103	15	6.35	93	16	No mean
Median old & new			6.32	94	16				
Range			5.12-6.67	86-119	10-20				26-29
n			309	271	181				

[a] All treatment concentrations are mg L^{-1} Mg, [b] DO = dissolved oxygen as % saturation, [c] EC = electrical conductivity as µS cm^{-1}, [d] Temperature in °C.

Table G.4 *Amerianna cumingi*

			New water			Old water				Temp
Test	**Day**	**Treatment**	**pH**	**DO**	**EC**	**pH**	**DO**	**EC**	**NH_3**	**Mean/range**
970S	1	0.3	5.12	90	10	6.77	86	41	0-0.5	29.7
		4.9	5.57	96		6.82	82			28-31
		12.7	5.62	95		6.93	83			
		32.0	5.72	100		6.93	82			
		82.6	5.69	99		6.92	82			
		207	5.75	97		6.90	74			
		513	5.79	94		6.92	73			
	2	0.3	5.39	111	10	6.94	81	62		
		4.9	5.55	113		7.07	86			
		12.7	5.67	113		7.10	83			
		32.0	5.73	113		7.11	85			
		82.6	5.72	109		7.10	80			
		207	5.67	110		7.06	77			
		513	5.85	108		6.99	75			
	3	0.3	5.50	112	11	7.00	86	48		
		4.9	5.57	113		6.96	90			
		12.7	5.70	116		6.99	85			
		32.0	5.76	116		6.98	84			
		82.6	5.73	116		6.92	79			
		207	5.73	115		6.89	82			
		513	5.78	117		6.82	78			
	4	0.3	5.48	112	11	6.84	81	43		
		4.9	5.65	120		6.97	83			
		12.7	5.74	118		7.06	85			
		32.0	5.84	114		7.06	85			
		82.6	5.78	112		7.07	83			
		207	5.80	114		7.00	83			
		513	5.89	103		6.94	85			
982S	1	0.4	6.88	95	21	6.26	87	22	0-0.5	29.3
		54	6.54			6.51	88			27-31
		130	6.53			6.61	87			
		310	6.52			6.52	88			
		770	6.42			6.49	91			
		1600	6.50			6.51	90			
		4200	6.46			6.54	90			
	2	0.4				6.61	85	31		
		54				6.66	82	33		
		130				6.67	85	35		
		310				6.65	81	40		
		770				6.61	77	50		

Table G.4 (cont)

			New water			Old water				Temp
Test	**Day**	**Treatment**	**pH**	**DO**	**EC**	**pH**	**DO**	**EC**	**NH_3**	**Mean/range**
		1600				6.58	73	59		
	3	0.4	5.83	97	17	6.39	85	38		
		54				6.46	82	39		
		130				6.56	83	37		
		310				6.52	80	36		
		770				6.48	83	36		
		1600				6.50	77	36		
		4200				6.51	83	27		
	4	0.4	6.49	92	18	6.80	90	43		
		54				6.76	94	35		
		130				6.74	83	33		
		310				6.62	84	34		
		770				6.65	79	33		
		1600				6.59	83	33		
		4200				6.61	82	27		
	5	0.4				6.57	81	36		
		54				6.87	87	37		
		130				6.86	90	36		
		310				6.80	84	39		
		770				6.82	90	38		
		1600				6.77	85	34		
		4200				6.75	89	27		
1027S	1	0.9	6.37			6.71	94	23	0-1	
		50.7	6.25			6.52	94			28-30
		127	6.28			6.61	87			
		328	6.25			6.62	89			
		819	6.28			6.61	84			
		2050	6.25			6.50	84			
		4170	6.20			6.50	81			
	2	0.9	6.75		31	6.80	84	40		
		50.7				6.74	80	50		
		127				6.77	79	50		
		328				6.71	80	48		
		819				6.61	76	48		
		2050				6.65	76	50		
		4170				6.64	65	50		
	3	0.9	6.10	89	18	6.70	88	44		
		50.7				6.40	78	51		
		127				6.67	81	47		
		328				6.73	82	47		

Table G.4 (cont)

			New water			Old water				Temp
Test	**Day**	**Treatment**	**pH**	**DO**	**EC**	**pH**	**DO**	**EC**	**NH_3**	**Mean/range**
		819				6.73	77	43		
		2050				6.79	75	42		
		4170				6.69		47		
	4	0.9	6.21	100	31	6.67	84	48		
		50.7				6.77	85	43		
		127				6.80	90	41		
		328				6.77	85	38		
		819				6.76	85	38		
		2050				6.69	83	34		
		4170				6.70	82	42		
	5	0.9				6.50	88	33		
		50.7				6.65	87	32		
		127				6.67	88	32		
		328				6.70	88	32		
		819				6.70	86	31		
		2050				6.70	84	29		
		4170				6.68	85	29		
1150S	1	0.4	6.02	92	11	6.53	88	17	0-1	none
		48.5	6.06	95		6.46	89			27-30
		127	6.06	94		6.33	89			
		302	6.01	94		6.25	89			
		775	5.98	93		6.27	88			
		1970	5.97	94		6.10	90			
		4100	5.97	89		6.37	91.5			
	2	0.4	5.88	91	14	6.77	83	20		
		48.5				6.77	84	21		
		127				6.76	83	23		
		302				6.77	84	27		
		775				6.77	81	27		
		1970				6.68	81	30		
		4100				6.82	84	36		
	3	0.4	5.96	98	13	6.84	92	23		
		48.5				6.75	93	23		
		127				6.73	85	25		
		302				6.69	86	24		
		775				6.73	83	25		
		1970				6.65	82	23		
		4100				6.82	83	35		
	4	0.4	5.82	90	10	6.68	85	24		
		48.5				6.71	81	24		

Table G.4 (cont)

			New water			Old water				Temp
Test	**Day**	**Treatment**	**pH**	**DO**	**EC**	**pH**	**DO**	**EC**	**NH_3**	**Mean/range**
		127				6.60	77	28		
		302				6.63	80	25		
		775				6.63	77	26		
		1970				6.59	77	26		
		4100				6.63	80	26		
	5	0.4	6.56	102	10	6.79	88	24		
		48.5				6.63	86	23		
		127				6.63	83	28		
		302				6.67	82	25		
		775				6.66	82	26		
		1970				6.62	82	26		
		4100				6.66	82	25		
1154S	1	0.3	5.86	100	9	6.74	80	28	0-0.5	26.9
		25.7	5.94	99		6.85	83			26-28
		101	5.92	96		6.85	81			
		378	5.89	100		6.65	81			
		1560	5.94	98		6.68	80			
		3010	6.02	96		7.05	83			
	2	0.3	5.83	91	8	6.86	87	26		
		25.7				6.86	85	26		
		101				6.85	81	29		
		378				6.86	84	33		
		1560				6.83	79	37		
		3010				7.07				
	3	0.3	5.73	96	9	6.72	88	25		
		25.7				6.74	89	26		
		101				6.77	84	26		
		378				6.77	87	27		
		1560				6.68	82	27		
	4	0.3	5.99	93	9	6.72	86	29		
		25.7				6.84	89	26		
		101				6.84	88	26		
		378				6.88	86	23		
		1560				6.83	85	24		
1159S	1	0.3	5.66	92	8	6.71	92	20	0-0.5	29.7
		48.5	5.85	94		6.61	95			29-30
		145	5.87	94		6.52	91			
		435	5.85	95		6.36	91			
		1300	5.85	97		6.30	93			
		3980	5.79	97		6.63	95			

Table G.4 (cont)

			New water			Old water				Temp
Test	**Day**	**Treatment**	**pH**	**DO**	**EC**	**pH**	**DO**	**EC**	**NH_3**	**Mean/range**
	2	0.3	5.74	100	9	6.48	82	21		
		48.5				6.59	85	21		
		145				6.62	86	23		
		435				6.67	86	25		
		1300				6.63	82	30		
		3980				6.66		64		
	3	0.3	5.76	94	8	6.75	89	24		
		48.5				6.72	94	24		
		145				6.73	94	24		
		435				6.72	85	24		
		1300				6.71	89	23		
	4	0.3	5.64	96	8	6.81	88	26		
		48.5				6.87	87	26		
		145				6.86	87	27		
		435				6.88	85	24		
		1300				6.75	83	24		
	5	0.3	5.63	93	8	6.86	88	26		
		48.5				6.93	87	26		
		145				6.94	88	26		
		435				6.96	89	25		
		1300				6.94	86	24		
1160S	1	0.3	6.49	95	18	6.94	86	30	0-1	30.1
		10.7	6.63	91		6.94	85			28-31
		30.7	6.46	88		6.87	86			
		95.6	6.33	89		6.75	80			
		280	6.22	93		6.70	80			
		835	6.16	91		6.54	80			
		2560	6.08	90		6.91	85			
	2	0.3	5.94	96	8	6.95	90	32		
		10.7				7.05	85	31		
		30.7				6.99	84	33		
		95.6				6.98	82	38		
		280				6.98	81	39		
		835				6.92	77	44		
		2560				6.95	87	20		
	3	0.3	5.97	95	8	7.01	91	26		
		10.7				6.98	85	28		
		30.7				6.95	95	24		
		95.6				6.96	85	28		
		280				6.92	81	31		

Table G.4 (cont)

			New water			Old water				Temp
Test	**Day**	**Treatment**	**pH**	**DO**	**EC**	**pH**	**DO**	**EC**	**NH_3**	**Mean/range**
		835				6.91	82	26		
		2560				6.85	94	13		
	4	0.3	6.19	97	9	7.17	87	29		
		10.7				7.12	89	29		
		30.7				7.11	89	25		
		95.6				7.04	87	27		
		280				7.04	86	27		
		835				7.01	88	26		
		2560				6.87	90	12		
1168S	1	0.3	6.19	96	9	6.87	89	15	0-1	29.9
		30.6	6.39	98		6.83	89			27-31
		106	6.40	98		6.71	89			
		325	6.24	95						
		1220	6.14	91		6.56	90			
		3810	6.14	94		6.80	90			
	2	0.3	6.15	92	10	6.81	90	23		
		30.6				6.82	85	24		
		106				6.85	86	24		
		325				7.17	87	32		
		1220				6.84	81	31		
		3810				6.83	78	51		
	3	0.3	5.92	91	10	6.94	86	29		
		30.6				6.99	82	29		
		106				6.95	82	28		
		325				6.95	84	28		
		1220				6.87	82	27		
		3810				6.89	85	21		
	4	0.3	6.30	89	10	6.99	87	30		
		30.6				7.02	86	30		
		106				7.08	83	29		
		325				7.02	85	29		
		1220				7.00	82	30		
		3810				6.91	85	15		
	5	0.3				6.92	89	25		
		30.6				6.98	88	26		
		106				6.95	86	21		
		325				6.94	84	22		
		1220				6.92	87	22		
		3810				6.88	88	12		
1169S	1	0.6	6.78	96	11	6.99	87	22	0-1	29.6

Table G.4 (cont)

			New water			Old water				Temp
Test	**Day**	**Treatment**	**pH**	**DO**	**EC**	**pH**	**DO**	**EC**	**NH_3**	**Mean/range**
		11.9	6.47			6.82	87			27-31
		79.8	6.45			6.72	81			
		318	6.38			6.69	85			
		1290	6.35			6.65	84			
		2680	6.35			6.70	85			
	2	0.6	6.38	93	11	6.83	93	22		
		11.9				6.84	86	23		
		79.8				6.82	88	25		
		318				6.80	86	29		
		1290				6.78	83	35		
		2680				6.76	84	46		
	3	0.6	6.26	88	11	7.00	85	30		
		11.9				7.05	85	29		
		79.8				7.04	83	31		
		318				7.05	81	32		
		1290				6.97	82	29		
		2680				6.95	77	34		
	4	0.6	6.99	100	12	6.94	97	27		
		11.9				7.00	94	28		
		79.8				7.03	94	29		
		318				6.95	87	29		
		1290				6.89	94	28		
		2680				6.87	88	30		
	5	0.6				7.20	85	28		
		11.9				7.13	85	26		
		79.8				7.13	85	24		
		318				7.00	83	25		
		1290				7.00	83	25		
		2680				6.99	85	24		
1176S	1	0.6	6.26	93	13	7.01	87	30	0-1.5	29.6
		53.5	6.39	93		6.98	89			26-30
		155	6.33	93		6.91	81			
		499	6.34	93		6.63	78			
		1420	6.35	95		6.78	84			
		2060	6.33	93		6.83	83			
	2	0.6	6.41	96	14	6.94	85	28		
		53.5								
		155				6.96	83	30		
		499				6.92	84	34		
		1420				6.96	84	38		

Table G.4 (cont)

			New water			Old water				Temp
Test	**Day**	**Treatment**	**pH**	**DO**	**EC**	**pH**	**DO**	**EC**	**NH_3**	**Mean/range**
						6.89	74	50		
	3		6.10	86	13	7.03	101	31		
						7.05	99	31		
						7.09	96	34		
						6.99	96	35		
						6.98	92	31		
						6.91	92	32		
	4		6.10	105	12	7.21	85			
						7.02	86	30		
						7.04	89	29		
						6.65	73	32		
						7.04	83	30		
						7.03	88	29		
Median			6.02	96	11	6.82	85	29		29.7
Median (old & new)			6.70	87	27					
Range (old & new)			5.63-7.21	73-120	8-62				0-1.5	26-31
n			406	384	258					

[a] All treatment concentrations are mgL^{-1} Mg, [b] DO = dissolved oxygen as % saturation, [c] EC = electrical conductivity as $\mu S\ cm^{-1}$, [d] Temperature in °C.

Table G.5 *Lemna aequinoctialis*

		New water			Old water			
Test	**Treatment**	**pH**	**DO**	**EC**	**pH**	**DO**	**EC**	**Temp Mean/range**
960L	0.6	5.28	118	21	6.22	92	16	
	30.7	5.45	115		6.54	90		27-31
	85.5	5.41	116		6.56	92		
	205	5.44	114		6.59	88		
	513	5.48	115		6.50	92		
	1250	5.45	113		6.39	92		
	3230	5.45	110		6.18	93		26-30
961L	0.6	5.46	111	21	5.95	90	17	
	201	5.47	110		5.77	89	17	
	404	5.44	110		6.08	90	17	
	820	5.54	107		6.13	90	17	
	1660	5.50	107		6.13	93	17	
	3260	5.47	99		6.14	94	18	
	4020	5.53	97			94	19	
1011L	0.8	6.59	104	22	6.90	92	17	29-31
	120	6.49	102		6.88	91	18	
	233	6.46	101		6.84	92	19	
	482	6.49	103		6.85	91	19	
	992	6.44	101		6.85	91	19	
	2030	6.40	99		6.81	91	20	
	4060	6.41	97		6.78	92	23	
1018L	0.9	6.20	95	22	6.60	96	19	27-32
	99.9	6.49	96		6.47	101	20	
	188	6.48	95		6.48	97	20	
	373	6.47	96		6.47	99	20	
	773	6.43	94		6.52	96	20	
	1600	6.41	95		6.49	101	22	
	2920	6.47	94		6.47	98	24	
	4090	6.24	99		6.48	99	28	
1097L	0.8	6.23	109	21	6.43	89	16	27.8
	199	6.42	114		6.47	91	17	27-28
	413	6.49	114		6.56	91	17	
	814	6.45	111		6.61	90	17	
	1650	6.48	111		6.59	91	16	
	3280	6.39	111		6.58	90	20	
	4150	6.39	110		6.62	91	21	
1108L	0.9	6.03	98	21	6.05	87	16	28.9
	208	6.20	98		5.95	86	16	28.9
	412	6.21	98		6.10	89	16	
	822	6.19	99		6.15	89	15	

Table G.5 (cont)

Test	Treatment	New water pH	New water DO	New water EC	Old water pH	Old water DO	Old water EC	Temp Mean/range
	1670	6.21	96		6.17	90	16	
	3280	6.17	95		6.23	89	19	
	4140	6.17	94		6.27	88	19	
1116L	0.9	6.08	97	22	6.31	100	15	28.6
	213	6.30	95		6.43	107	16	28-30
	429	6.32	96		6.37	98	16	
	842	6.29	95		6.40	107	16	
	1600	6.30	94		6.46	105	16	
	3260	6.20	93		6.48	107	17	
	4120	6.23	94		6.48	105	18	
Median		6.23	99	21	6.47	92	17	28.6
Median (old & new)		6.39	96	18				
Range (old & new)		5.28-6.88	90-115	16-24				26-32
n		99	100	51				

[a] All treatment concentrations are mg L^{-1} Mg, [b] DO = dissolved oxygen as % saturation, [c] EC = electrical conductivity as µS cm^{-1}, [d] Temperature in °C.

Table G.6 *Moinodaphnia macleayi*

			New water			Old water			
Test	**Day**	**Treatment**	**pH**	**DO**	**EC**	**pH**	**DO**	**EC**	**Temp Mean/range**
945D	0	1	6.88	115	21	7.29	97	22	NM
		10.9	6.96	104		7.08	95		26-29
		21	6.94	106		7.09	96		
		40.7	6.92	107		7.22	97		
		79.6	6.96	105		7.19	95		
		156	7.01	101		7.15	96		
		313	6.97	102		7.24	97		
	1	1	6.82	117	21	7.00	94	22	
		10.9	6.78	114		6.93	92		
		21	6.91	115		7.00	92		
		40.7	6.89	115		7.09	92		
		79.6	6.85	117		6.97	95		
		156	6.93	115		7.10	93		
		313	6.90	115		6.94	93		
	2	1			21	7.08	96	22	
		10.9	6.83	117		6.97	95		
		21	6.86			7.03	96		
		40.7	6.83			7.08	96		
		79.6	6.83			7.14	99		
		156	6.85			7.11	100		
		313	6.81			7.02	99		
	3	1	6.84	108	21	6.95	91	23	
		10.9	6.79			6.95	92		
		21	6.75			6.99	97		
		40.7	6.76			6.98	102		
		79.6	6.77			7.06	94		
		156	6.75			7.03	94		
		313	6.73			6.99	94		
	4	1	6.80	105	22	6.94	92	23	
		10.9	6.81			6.96	92		
		21	6.90			6.97	91		
		40.7	6.89			7.02	94		
		79.6	6.87			6.97	93		
		156	6.93			6.92	90		
		313	6.86						
	5	1	7.10	113	24	6.97	93	23	
		10.9	6.95			6.86	95		
		21	6.91			6.92	92		
		40.7	7.08			6.91	93		
		79.6	6.98			6.87	91		

Table G.6 (cont)

Test	Day	Treatment	New water pH	New water DO	New water EC	Old water pH	Old water DO	Old water EC	Temp Mean/range
		156	6.92			6.99	92		
		313	6.91			6.91	93		
946D	0	1	6.89	104	22	7.03	96	28	26-29
		103	6.98	108		7.03	97		
		196	7.05	104		7.04	99		
		402	6.93	104		7.04	99		
		821	6.88	109		7.05	98		
		1590	6.84	108		7.04	98		
		3190	6.80	106		7.02	101		
	1	1	6.82	117	21	7.35	101	22	
		103				7.33	99	34	
		196				7.39	103	38	
		402				7.40	101	31	
		821				7.32	100	39	
		1590				7.40	103		
		3190				7.76	94		
	2	1	6.80	115	22	7.19	96	22	
		103				7.15	95	22	
		196				7.14	101	22	
		402				7.15	94	21	
		821				7.21	97	22	
	3	1	6.88		21	7.11	93	23	
		103				7.09	98	23	
		196				7.07	99	23	
		402				7.11	97	23	
		821				7.11	112	22	
		1590				7.04	110	23	
	4	1	6.92	105	22	6.98	93	23	
		103				6.98	91	22	
		196				6.92	93	23	
		402				6.97	92	23	
		821				6.96	93	23	
		1590				6.98	92	22	
	5	1				7.10	93	23	
		103				7.05	91	23	
		196				7.01	93	23	
		402				7.03	92	23	
		821				7.01	92	23	
		1590				7.04	93	23	
	6	1				7.10	92	24	

Table G.6 (cont)

Test	Day	Treatment	New water pH	New water DO	New water EC	Old water pH	Old water DO	Old water EC	Temp Mean/range
		103				7.02	91	23	
		196				6.99	92	23	
		402				6.96	95	23	
		821				6.98	94	23	
		1590				7.00	93	23	
957D	0	0.5	5.58	101	16	6.02	96	16	27-29
		102	5.87	98		6.10	92		
		201	5.85	98		6.00	98		
		405	5.83	100		5.99	98		
		805	5.82	88		5.97	98		
		1590	5.85	99		6.05	99		
		3230	5.85	100		6.21	97		
	1	0.5	5.44	109	16	6.09	94	16	
		102	5.30	113	16	5.99	92	16	
		201	5.33	112	16	5.85	93	16	
		405	5.38	111	16	5.85	93	16	
		805	5.35	107	16	5.85	94	16	
		1590	5.26	105	16	5.92	91	16	
		3230				5.94	93	16	
	2	0.5	5.35	113	16	5.40	97	15	
		102				5.60	100	15	
		201				5.58	100	15	
		405				5.57	100	15	
		805				5.59	95	15	
		1590				5.59	96	15	
	3	0.5	5.50	105	18	5.79	91	18	
		102				5.72	98	15	
		201				5.70	94	15	
		405				5.63	93	15	
		805				5.65	97	15	
		1590				5.70	101	15	
	4	0.5	5.30	107	17	5.55	99	16	
		102				5.66	97	16	
		201				5.64	96	15	
		405				5.65	97	16	
		805				5.55	99	16	
958D	0	0.5	5.58	101	16	5.88	92	15	27-28
		50.4				5.89	93	15	
		102				5.84	93	15	
		201				5.87	93	15	

Table G.6 (cont)

Test	Day	Treatment	New water pH	New water DO	New water EC	Old water pH	Old water DO	Old water EC	Temp Mean/range
		405				6.08	93	15	
		805				5.77	91	16	
		1590				5.81	90	16	
	1	0.5	5.41	113	16	5.72	99	15	
		50.4	5.78			5.94	100		
		102	5.81			5.98	101		
		201	5.82			5.94	99		
		405	5.75			6.02	97		
		805	5.72			5.91	97		
		1590	5.71			5.89	99		
	2	0.5	5.97	96	17	5.87	97	18	
		50.4				5.92	99	17	
		102				5.91	100	17	
		201				5.96	98	18	
		405				5.96	96	17	
		805				5.99	97	17	
		1590				6.04	99	18	
	3	0.5	5.50	105	18	5.78	92	15	
		50.4				5.87	94	16	
		102				5.79	92	15	
		201				5.87	94	17	
	4	0.5	5.15	105	14	5.66	96	15	
		50.4				5.63	99	15	
		102				5.61	96	16	
		201				5.59	97	15	
		405				5.66	96	15	
	5	0.5	5.30	107	17	5.72	92	16	
		50.4				5.58	92	16	
		102				5.51	90	16	
		201				5.39	94	16	
		405				5.40	92	16	
	6	0.5	5.25	100	17	5.58	96	16	
		50.4				5.58	97	15	
		102				5.61	98	15	
		201				5.59	98	15	
		405				5.61	98	15	
		805				5.62	95	15	
	7	0.5				5.73	94	19	
		50.4				5.90	98	17	
		102				5.84	95	16	

Table G.6 (cont)

Test	Day	Treatment	New water pH	New water DO	New water EC	Old water pH	Old water DO	Old water EC	Temp Mean/range
		201				5.84	94	16	
		405				5.87	92	16	
1003D	1	0.6	6.32	101	19	6.57	104	18	26-28
		103	6.35	108		6.67	109		
		211	6.41	109		6.75	105		
		406	6.37	107		6.77	109		
		838	6.37	108		6.73	105		
		1680	6.35	106		6.68	110		
		3290	6.35	107		6.71	104		
	2	0.6				7.20	99	17	
		103				7.21	96	17	
		211				7.28	96	18	
		406				7.28	99	18	
		838				7.29	99	18	
		1680				7.27	97	24	
		3290				7.28	99	28	
	3	0.6				6.86	104	17	
		103				6.91	104	17	
		211				6.93	105	17	
		406				6.90	103	17	
		838				6.89	104	17	
		1680				6.91	104	17	
	4	0.6				6.63	99	17	
		103				6.73	99	17	
		211				6.82	99	17	
		406				6.79	98	18	
		838				6.80	98	17	
		1680				6.86	99	17	
	5	0.6				7.03	92	18	
		103				6.41	91	18	
		211				7.04	96	17	
		406				6.96	99	18	
		838				6.94	97	17	
		1680				6.89	97	17	
1012D	0	1	6.55	96	21	7.05	97	21	28-29
		51.1	6.70	97		6.93	99		
		104	6.78	98		6.94	98		
		217	6.77	98		7.01	98		
		440	6.67	99		6.97	97		
		887	6.65	99		6.91	96		

Table G.6 (cont)

			New water			Old water			Temp
Test	**Day**	**Treatment**	**pH**	**DO**	**EC**	**pH**	**DO**	**EC**	**Mean/range**
		1710	6.61	98		6.85	98		
	1	1	6.45	97	19	6.76	95	20	
		51.1				6.82	95	21	
		104				6.89	93	21	
		217				6.93	94	21	
		440				6.95	93	22	
		877				6.93	94	22	
		1710				7.04	96	22	
	2	1	6.33	98	22	6.68	92	20	
		51.1				6.71	92	21	
		104				6.72	93	21	
		217				6.75	93	21	
		440				6.87	90	21	
		877				6.79	92	21	
		1710				6.77	94	24	
	3	1	6.32	99	18	6.41	93	20	
		51.1				6.52	98	21	
		104				6.54	96	20	
		217				6.55	97	21	
		440				6.59	97	20	
		877				6.68	97	21	
		1710				6.72	96	20	
	4	1	6.58	99	21	6.41	94	21	
		51.1				6.53	94	20	
		104				6.56	93	20	
		217				6.61	96	20	
		440				6.65	96	20	
		877				6.66	98	20	
		1710				6.79		20	
1101D	0	0.8	6.37	102	21	6.90	95	21	28
		31.8	6.60	103		6.74	95		27-30
		64.6	6.55	112		6.80	95		
		131	6.49	110		6.92	95		
		256	6.49	109		6.85	94		
		506	6.42	106		6.78	96		
		1020	6.52	101		6.74	96		
	1	0.8	6.24	102	17	6.58	96	17	
		31.8				6.74	94	17	
		64.6				6.73	95	17	
		131				6.70	94	17	

Table G.6 (cont)

Test	Day	Treatment	New water pH	New water DO	New water EC	Old water pH	Old water DO	Old water EC	Temp Mean/range
		256				6.71	91	17	
		506				6.71	93	17	
	2	0.8	6.53	92	18	6.49	98	17	
		31.8				6.59	99	17	
		64.6				6.62	100	17	
		131				6.60	98	17	
		256				6.61	99	17	
		506				6.71	100	17	
		1020				6.91	98	17	
	3	0.8	6.20	81	17	6.53	102	17	
		31.8				6.58	100	17	
		64.6				6.55	101	17	
		131				6.78	102	17	
		256				6.75	101	17	
		506				6.72	102	17	
		1020				6.86	98	17	
	4	0.8	6.23	102	17	6.72	102	17	
		31.8				6.83	100	17	
		64.6				6.84	104	17	
		131				6.85	104	17	
		256				6.83	101	17	
		506				6.85	100	17	
		1020				7.07	96	17	
1105D	0	0.8	5.92	97	17	6.08	97	17	28.1
		32.6	6.16	102		6.22	93	17	27-30
		65	6.16	102		6.44	87	17	
		131	6.22	104		6.45	91	17	
		262	6.19	96		6.44	92	17	
		499	6.23	98		6.58	92	17	
		1010	6.24	89		6.61	95	18	
	1	0.8	5.80	97	18	6.25	100	17	
		32.6				6.35	101	17	
		65				6.38	100	17	
		131				6.44	102	17	
		262				6.49	102	17	
		499				6.50	101	17	
		1010				6.53	102	17	
	2	0.8	5.86	110	17	6.32	92	17	
		32.6				6.40	93	17	
		65				6.41	94	17	

Table G.6 (cont)

Test	Day	Treatment	New water pH	New water DO	New water EC	Old water pH	Old water DO	Old water EC	Temp Mean/range
		131				6.45	92	17	
		262				6.46	91	17	
		499				6.47	90	17	
		1010				6.50	90	17	
	3	0.8	6.09	92	17	6.52	91	18	
		32.6				6.56	101	17	
		65				6.59	101	17	
		131				6.54	88	18	
		262				6.55	100	18	
		499				6.50	89	18	
		1010				6.51	93	17	
	4	0.8	6.45	93	17	6.42	88	18	
		32.6				6.49	81	18	
		65				6.54	90	18	
		131				6.56	89	17	
		262				6.55	90	18	
		499				6.48	90	17	
		1010				6.54	91	17	
	5	0.8	6.31	95	17				
1111D	1	0.8	6.78	97	17	6.60	97	18	27.2
		45.7				7.03	98		26-28
		90.1				7.17	97		
		182				7.17	95		
		371				7.20	96		
		731				7.18	97		
		1490				7.23	97		
		2220					95		
	2	0.8	6.10	90	17	6.33	92	17	
		45.7				6.49	93	17	
		90.1				6.50	94	17	
		182				6.51	92	17	
		371				6.52	95	17	
		731				6.54	95	17	
		1490				6.59	89	19	
	3	0.8	6.01	89	17	6.34	87	17	
		45.7				6.38	89	17	
		90.1				6.35	88	17	
		182				6.43	89	17	
		371				6.41	89	17	
		731				6.51	92	17	

Table G.6 (cont)

Test	Day	Treatment	New water pH	New water DO	New water EC	Old water pH	Old water DO	Old water EC	Temp Mean/range
		1490				6.58	93	17	
	4	0.8	6.43	97	17	6.48	89	17	
		45.7				6.48	93	17	
		90.1				6.51	93	17	
		182				6.49	93	17	
		371				6.57	93	17	
		731				6.57	92	17	
		1490				6.55	89	18	
	5	0.8	6.47	90	17	6.48	93	17	
		45.7				6.48	89	17	
		90.1				6.51	93	17	
		182				6.49	89	17	
		371				6.57	90	17	
		731				6.57	92	17	
		1490				6.55	95	17	
1115D	1	0.8	6.86	114	17	6.68	98	17	28.1
		54.6				6.76	97		27-30
		107				6.81	95		
		214				6.78	96		
		407				6.93	97		
		821				6.98	97		
		1630				7.00	97		
	2	0.8	6.27	99	18	6.36	92	17	
		54.6				6.44	99	17	
		107				6.47	92	17	
		214				6.52	92	17	
		407				6.57	91	17	
		821				6.59	91	17	
		1630				6.66	90	17	
	3	0.8	5.94	103	18	6.35	93	18	
		54.6				6.46	95	18	
		107				6.47	93	18	
		214				6.58	95	18	
		407				6.62	94	18	
		821				6.74	96	18	
		1630							
	4	0.8	6.09	95	18	6.40	90	18	
		54.6				6.41	95	18	
		107				6.56	96	17	
		214				6.59	93	17	

Table G.6 (cont)

Test	Day	Treatment	New water pH	New water DO	New water EC	Old water pH	Old water DO	Old water EC	Temp Mean/range
		407				6.67	97	17	
		821				6.66	93	17	
	5	0.8	6.12	93	18	6.66	100	18	
		54.6				7.01	92	18	
		107				7.08	92	18	
		214				7.23	91	18	
		407				7.23	98	19	
		821				7.12	96	19	
1139D	1	1	6.90	100	21	7.10	90	21	29.2
		1				7.07	96	21	26-33
		26.1				7.10	94	21	
		51				7.12	95	21	
		102				7.15	95	21	
		201				7.11	94	21	
		412				7.12	96	21	
	2	1	6.64	101	21	7.21	94	21	
		26.1				7.24	96		
		51				7.23	94		
		102				7.23	97		
		201				7.24	98		
		412				7.20	98		
	3	1	6.53	94	22	6.79	92	20	
		1				6.78	107	22	
		26.1				6.86	106	22	
		51				6.89	98	22	
		102				6.96	103	22	
		201				6.88	101	22	
		412				6.90	98	22	
	4	1	6.43	104	21	6.72	101	21	
		1				6.73	102	21	
		26.1				6.79	103	20	
		51				6.76	104	21	
		102				6.86	105	21	
		201				6.86	104	21	
		412				6.94	103	20	
	5	1	6.60	91	21	6.78	93	21	
		1				6.76	93	21	
		26.1				6.74	94	21	
		51				6.83	92	20	
		102				6.86	95	21	

Table G.6 (cont)

Test	Day	Treatment	New water pH	New water DO	New water EC	Old water pH	Old water DO	Old water EC	Temp Mean/range
		201				6.87	95	21	
		412				6.92	95	21	
1142D	1	1	6.44	112	21	6.76	96	23	27.4
		1				6.73	97	23	26-28
		25.1				6.69	95	23	
		51.8				6.73	93	23	
		103				6.74	95	21	
		208				6.69	94	23	
		412				6.70	93	23	
	2	1	6.32	100	21	6.75	89	23	
		1				6.72	89		
		25.1				6.72	90		
		51.8				6.78	90		
		103				6.76	89		
		208				6.74	90		
		412				6.71			
	3	1	6.73	92		6.66	87	23	
		1				6.69	88	23	
		25.1				6.67	87	23	
		51.8				6.68	88	22	
		103				6.67	89	23	
		208				6.71	87	22	
		412				6.70	91	23	
	4	1	6.50	91	21	6.75	94	23	
		1				6.74	93	23	
		25.1				6.76	91	22	
		51.8				6.75	90	23	
		103				6.72	94	23	
		208				6.71	94	23	
		412				6.77	95	23	
	5	1	6.66	97	22	6.64	91	23	
		1				6.67	91	23	
		25.1				6.67	90	23	
		51.8				6.62	89	23	
		103				6.65	90	20	
		208				6.66	89	23	
		412				6.70	91	23	
	6	1	6.55	94	25	6.72	93	23	
		1				6.65	94	23	
		25.1				6.67	94	23	

Table G.6 (cont)

Test	Day	Treatment	New water pH	New water DO	New water EC	Old water pH	Old water DO	Old water EC	Temp Mean/range
		51.8				6.72	93	23	
		103				6.71	93	23	
		208				6.70	95	23	
		412				6.74	92	23	
	7	1	6.72	96	22	6.64	95	23	
		1				6.71	96	22	
		25.1				7.45	104	21	
		51.8				7.47	99	21	
		103				6.98	98	21	
		208				7.00	99	21	
		412				7.21	100	21	
1165D	1	0.3	6.01	99	11	7.04	90	11	27.6
		0.3				6.41	96		27-28
		3.9				6.48	92		
		9.8				6.49	93		
		24.2				6.53	94		
		60.3				6.63	94		
		151				6.74	96		
		383				6.69	94		
	2	0.3	6.11	111	15	6.25	95	12	
		0.3				6.36	96	12	
		3.9				6.38	95	12	
		9.8				6.41	94	12	
		24.2				6.46	95	12	
		60.3				6.47	94	12	
		151				6.43	95	12	
		383				6.55	96	12	
	3	0.3	6.18	112	15	6.18	93	11	
		0.3				6.28	92	11	
		3.9				6.32	91	11	
		9.8				6.38	93	11	
		24.2				6.38	90	11	
		60.3				6.40	91	11	
		151				6.42	93	12	
		383				6.44	93	11	
	4	0.3	6.2	96	12	6.55	96	11	
		0.3				6.56	95	11	
		3.9				6.59	93	11	
		9.8				6.70	95	11	
		24.2				6.73	94	11	

Table G.6 (cont)

Test	Day	Treatment	New water pH	New water DO	New water EC	Old water pH	Old water DO	Old water EC	Temp Mean/range
		60.3				6.70	95	11	
		151				6.71	93	11	
		383				6.75	96	11	
	5	0.3	6.12	99	12	6.20	104	11	
		0.3				6.37	98	11	
		3.9				6.51	104	11	
		9.8				6.62	103	12	
		24.2				6.67	98	11	
		60.3				6.70	98	11	
		151				6.65	99	11	
		383				6.65	100	11	
1177D	1	0.6	6.42	92	16	7.24	103	14	27.5
		51.1				7.34	101	14	26-28
		105				7.26	103	14	
		212				7.29	101	14	
		302				7.27	101	13	
		493				7.21	102	13	
		712				7.25	102	14	
	2	0.6	6.87	99	14	6.74	90	13	
		51.1				6.56	92		
		105				6.78	92		
		212				6.93	93		
		302				7.06	93		
		493				7.04	91		
		712				7.02	94		
	3	0.6	6.40	106	14	8.10	99	14	
		0.6				7.20	100	14	
		51.1				7.17	101	14	
		105				7.18	101	16	
		212				7.20	101	13	
		302				7.22	100	14	
		493				7.26	102	15	
		712				7.23	100	14	
	4	0.6	6.42	98	16	7.42	106	13	
		0.6				7.49	106	14	
		51.1				7.49	107	13	
		105				7.60	102	14	
		212				7.48	104	14	
		302				7.59	104	14	
		493				7.22	104	15	

Table G.6 (cont)

Test	Day	Treatment	New water pH	New water DO	New water EC	Old water pH	Old water DO	Old water EC	Temp Mean/range
		712				7.82	105	14	
	5	0.6	6.35	100	15	7.21	97	16	
		0.6				7.34	99	14	
		51.1				7.73	100	14	
		105				7.51	95	13	
		212				7.45	95	14	
		302				7.60	99	13	
		493				7.91	102	14	
		712				7.56	99	13	
	6	0.6	6.49	94	16	6.66	94	14	
		0.6				6.76	93	14	
		51.1				6.89	95	14	
		105				6.90	93	14	
		212				6.90	94	14	
		302				6.93	94	14	
		493				6.98	96	14	
		712				7.03	96	14	
Median			6.49	102	17	6.72	95	17	27.8
Median (old & new)			6.71	96	17				
Range (old & new)			5.4-7.91	87-117	11-39				26-33
n			688	657	501				

[a] All treatment concentrations are mg/L Mg, [b] DO = dissolved oxygen as % saturation, [c] EC = electrical conductivity as μScm^{-1}, [d] Temperature in ºC.

Appendix H Inorganic composition of Magela Creek water used in tests

Table H.1 *Chlorella* sp.

Analyte[a]	Test												
	1189G	**1192G**	**1199G**	**1202G**	**1203G**	**1231G**	**1232G**	**1233G**	**Mean**	**SD[b]**	**SE[c]**	**Mean (M)[d]**	***n***
Al	12	11	11	7.8	8.4	9.8	10	11	10.13	1.43	0.51	3.8E-07	8
Cd	<0.02	<0.02	<0.02	<0.02	0.022	0.042	0.024	<.02	0.03	0.00	0.00	1.8E-10	
Co	0.05	0.05	0.045	0.042	0.037	0.067	0.063	0.045	0.05	0.01	0.00	8.5E-10	
Cr	<0.1	<0.1	<0.1	<0.1	<0.1	0.33	0.13	0.11	0.19	0.12	0.04	3.7E-09	
Cu	0.16	0.22	0.14	0.068	0.1	0.3	0.35	0.16	0.19	0.10	0.03	2.9E-09	
Fe	60	49	55	56	51	61	120	120	71.50	30.20	10.67	1.3E-06	
Mn	1.5	1.3	1.4	1.5	1.3	3.1	3.5	3.3	2.11	0.99	0.35	3.8E-08	
Ni	0.022	0.026	0.045	0.13	0.14	0.35	0.35	0.18	0.16	0.13	0.05	2.6E-09	
Pb	0.19	0.18	0.032	0.11	0.097	0.47	0.25	0.23	0.19	0.13	0.05	9.4E-10	
Se	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	0.00	0.00	NC	
U	0.019	0.025	0.009	0.008	0.0089	0.01	0.011	0.059	0.02	0.02	0.01	7.9E-11	
Zn	3.4	0.41	<0.1	<0.1	0.11	1.1	0.44	0.18	0.94	1.25	0.44	1.4E-08	
NO_3	2.1	1.8	2.9	3.2	3.2	3.3	3.3	3.3	2.89	0.05	0.02	4.7E-05	
PO_4	0.04	0.04	0.045	0.043	0.043	0.043	0.043	0.043	0.04	0.00	0.00	4.5E-07	
Ca	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	0.2	0.3	0.25	0.07	0.02	6.2E-06	
Mg	0.9	0.9	0.9	0.8	0.9	0.9	1.1	1	0.93	0.09	0.03	3.8E-05	
Na	8.3	8.4	8.3	7.5	7.6	8.2	7.8	7.5	7.95	0.39	0.14	3.5E-04	
SO_4	NM[e]	110	110	96	96	100	92	96	100.00	7.21	2.55	1.0E-03	

[a] All analytes are measured in µg L^{-1} with the exception of Ca, Mg, Na, SO_4 and NO_3 which are in mg L^{-1}, [b] SD = standard deviation, [c] = SE = standard error, [d] M = molar concentration, [e] NC = not calculated, [f] NM = not measured

Table H.2 *Mogurnda mogurnda*

Analyte[a]	Test						
	1013E	**1025E**	**Mean**	**SD[b]**	**SE[c]**	**Mean (M)[d]**	***n***
Al	3.5	5.8	4.65	1.63	1.15	1.7E-07	2
Cd	<0.02	<0.02	<0.02	0.00	0.00	1.8E-10	
Co	0.03	0.03	0.03	0.00	0.00	5.1E-10	
Cr	<0.1	<0.1	<0.1	0.00	0.00	NC[e]	
Cu	0.33	0.19	0.26	0.10	0.07	4.1E-09	
Fe	40	40	40.00	0.00	0.00	7.2E-07	
Mn	1.38	1.46	1.42	0.06	0.04	2.6E-08	
Ni	0.15	0.13	0.14	0.01	0.01	2.4E-09	
Pb	0.03	0.05	0.04	0.01	0.01	1.9E-10	
Se	<0.2	<0.2	<0.2	0.00	0.00	NC[e]	
U	0.005	0.01	0.01	0.00	0.00	3.2E-11	
Zn	2.4	0.7	1.55	1.20	0.85	2.4E-08	
Ca	0.2	0.2	0.20	0.00	0.00	5.0E-06	
Mg	0.9	0.9	0.90	0.00	0.00	3.7E-05	
Na	0.9	1.2	1.05	0.21	0.15	4.6E-05	
SO_4	0.3	0.3	0.30	0.00	0.00	3.1E-06	

[a] All analytes are measured in µg L^{-1} with the exception of Ca, Mg, Na, SO_4 and NO_3 which are in mg L^{-1}, [b] SD = standard deviation, [c] = SE = standard error, [d] M = molar concentration, [e] NC = not calculated

Table H.3 *Hydra viridissima*

	Test											
Analyte[a]	**971B**	**979B**	**1014B**	**1017B**	**1095B**	**1100B**	**1114B**	**Mean**	**SD[b]**	**SE[c]**	**Mean (M)[d]**	***n***
Al	50.8	42.50	5.2	3.5	13	8.5	6.3	18.543	19.58	7.417	6.87E-07	7
Cd	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	0	0	1.78E-10	
Co	0.09	0.11	0.03	0.03	0.1	0.06	0.03	0.0643	0.036	0.013	1.09E-09	
Cr	0.1	<0.1	<0.1	<0.1	0.1	<0.1	<0.1	<0.1	0	0	NC[e]	
Cu	0.29	0.44	0.28	0.33	0.49	0.21	0.22	0.3229	0.106	0.04	5.08E-09	
Fe	80	160.00	40	40	100	120	40	82.857	46.8	17.73	1.48E-06	
Mn	3.2	3.91	1.79	1.38	3.08	2.8	1.37	2.5043	0.995	0.377	4.56E-08	
Ni	0.17	0.19	0.18	0.15	0.33	0.15	1.01	0.3114	0.314	0.119	5.31E-09	
Pb	0.02	0.02	0.03	0.03	0.04	0.05	0.06	0.0357	0.015	0.006	1.72E-10	
Se	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	0	0	NC[e]	
U	0.032	0.02	0.019	0.005	0.015	0.068	0.007	0.0237	0.021	0.008	9.96E-11	
Zn	0.7	0.40	1.5	2.4	1.1	0.3	1.0	1.0571	0.723	0.274	1.62E-08	
Ca	0.1	0.2	0.2	0.2	0.5	0.4	0.2	0.2571	0.14	0.053	6.42E-06	
Mg	0.3	0.4	0.9	0.9	0.8	0.8	0.8	0.7	0.245	0.093	2.88E-05	
Na	0.9	1.7	1.2	0.9	1.9	1.4	1.1	1.3	0.387	0.147	5.65E-05	
SO_4	0.1	0.2	0.4	0.3	0.2	<0.1	0.1	0.2167	0.117	0.044	2.26E-06	

[a] All analytes are measured in µg L^{-1} with the exception of Ca, Mg, Na, SO_4 and NO_3 which are in mg L^{-1}, [b] SD = standard deviation, [c] = SE = standard error, [d] M = molar concentration, [e] NC = not calculated

Table H.4 *Amerianna cumingi*

Analyte[a]	Test														
	970S	**982S**	**1027S**	**1150S**	**1154S**	**1159S**	**1160S**	**1168S**	**1169S**	**1176S**	**Mean**	**SD[b]**	**SE[c]**	**Mean (M)[d]**	***n***
Al	78.6	42	4.3	56.6	54.6	51.4	37.8	65.9	15.7	16.9	42.38	23.88	7.56	1.57E-06	10
Cd	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	0.08	<0.02	<0.02	<0.02	0.00	0.00	1.78E-10	
Co	0.1	0.07	0.01	0.14	0.11	0.09	0.07	0.08	0.05	0.05	0.077	0.04	0.01	1.31E-09	
Cr	0.2	0.1	<0.1	0.2	0.2	0.1	0.1	0.2	<0.1	<0.1	<0.1	0.00	0.00	NC[e]	
Cu	0.3	0.4	0.23	0.33	0.27	0.32	0.24	0.32	0.12	0.17	0.27	0.08	0.03	4.25E-09	
Fe	100	130	20	120	100	80	80	100	80	80	89	29.98	9.49	1.59E-06	
Mn	3.1	2	0.12	5.62	3.37	2.87	2.29	2.07	1.03	1.31	2.378	1.51	0.48	4.33E-08	
Ni	0.14	0.1	0.2	0.29	0.17	0.14	0.19	0.16	0.68	0.21	0.228	0.17	0.05	3.88E-09	
Pb	0.02	0.02	0.35	0.05	0.02	0.04	0.06	0.06	0.03	0.09	0.074	0.10	0.03	3.57E-10	
Se	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	0.4	0.2	<0.2	0.00	0.00	NC[e]	
U	0.022	0.02	0.043	0.025	0.023	0.082	0.012	0.018	0.009	0.009	0.026	0.02	0.01	1.11E-10	
Zn	0.3	<0.1	1.5	2	0.4	0.2	1.1	0.4	0.3	1.6	0.867	0.69	0.22	1.32E-08	
Ca	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.1	0.1	0.18	0.06	0.02	4.49E-06	
Mg	0.3	0.4	0.9	0.4	0.3	0.3	0.3	0.3	0.6	0.6	0.44	0.20	0.06	1.81E-05	
Na	0.9	0.9	NM[f]	1	1	0.8	0.8	0.9	0.9	1	0.911	0.08	0.02	3.96E-05	
SO_4	0.1	<0.5	0.3	0.2	0.3	0.1	0.2	0.2	0.2	0.2	0.2	0.07	0.02	2.08E-06	

[a] All analytes are measured in µg L^{-1} with the exception of Ca, Mg, Na, SO_4 and NO_3 which are in mg L^{-1}, [b] SD = standard deviation, [c] = SE = standard error, [d] M = molar concentration, [e] NC = not calculated, [f] NM = not measured

Table H.5 *Lemna aequinoctialis*

Analyte[a]	Test											
	960L	**961L**	**1011L**	**1018L**	**1097L**	**1108L**	**1116L**	**Mean**	**SD[b]**	**SE[c]**	**Mean (M)[d]**	***n***
Al	88.4	120	6.4	5.2	10.9	8.7	5.5	35.01	48.18	18.25	1.30E-06	7
Cd	0.04	0.06	<0.02	<0.02	<0.02	<0.02	<0.02	0.05	0.00	0.00	1.78E-10	
Co	0.2	0.2	0.02	0.03	0.08	0.04	0.03	0.09	0.08	0.03	1.45E-09	
Cr	0.2	0.5	<0.1	<0.1	0.1	<0.1	<0.1	<0.1	0.00	0.00	NC[e]	
Cu	1.13	0.96	0.23	0.28	0.42	0.18	0.23	0.49	0.39	0.15	7.71E-09	
Fe	80	20	20	40	80	40	40	45.71	25.07	9.50	8.19E-07	
Mn	9.9	9.91	1.05	1.79	3.24	1.76	1.19	4.12	4.01	1.52	7.50E-08	
Ni	0.41	1.09	0.13	0.18	0.25	0.19	0.2	0.35	0.34	0.13	5.96E-09	
Pb	0.04	0.16	0.01	0.03	0.03	0.04	0.04	0.05	0.05	0.02	2.41E-10	
Se	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	0.00	0.00	NC[e]	
U	0.036	0.049	0.003	0.019	0.012	0.008	0.01	0.02	0.02	0.01	8.22E-11	
Zn	1.0	NM[f]	0.7	1.5	0.6	1.0	0.3	0.85	0.41	0.16	1.30E-08	
Ca	0.2	0.2	0.2	0.2	0.5	0.2	0.2	0.24	0.11	0.04	6.06E-06	
Mg	0.6	0.6	0.8	0.9	0.8	0.9	1	0.80	0.15	0.06	3.29E-05	
Na	1.1	1.2	1.2	1.2	1.5	1.3	1.3	1.26	0.13	0.05	5.47E-05	
SO_4	0.7	0.9	0.3	0.4	0.4	0.2	0.3	0.46	0.25	0.09	4.76E-06	
NO_3	NM	0.8	0.535	0.715	0.25	NM	NM	0.58	0.24	0.12	9.27E-06	
PO_4	NM	0.08	0.1	0.11	0.07	NM	NM	0.09	0.02	0.01	9.48E-07	

[a] All analytes are measured in µg L^{-1} with the exception of Ca, Mg, Na, SO_4 and NO_3 which are in mg L^{-1}, [b] SD = standard deviation, [c] = SE = standard error, [d] M = molar concentration, [e] NC = not calculated, [f] NM = not measured

Table H.6 *Moinodaphnia macleayi*

Analyte[a]	Test																
	945D	**957D**	**1003D**	**1012D**	**1101D**	**1105D**	**1111D**	**1115D**	**1139D**	**1142D**	**1165D**	**1177D**	**Mean**	**SD[b]**	**SE[c]**	**Mean (M)[d]**	***n***
Al	4.8	98.4	9.3	4.1	8.5	10.4	14.5	6.8	8.8	16.2	55.1	16.3	21.10	27.87	8.06	7.82E-07	12
Cd	<0.02	0.06	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	0.06	0.00	0.00	1.78E-10	
Co	0.02	0.18	0.08	0.07	0.12	0.04	0.04	0.04	0.05	0.04	0.09	0.06	0.07	0.04	0.01	1.17E-09	
Cr	0.1	0.2	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.2	<0.1	0.17	0.06	0.02	3.21E-09	
Cu	0.33	0.81	0.25	0.22	0.22	0.2	0.28	0.34	0.5	0.3	0.23	0.2	0.32	0.17	0.05	5.09E-09	
Fe	120	NM[e]	220	20	140	40	40	60	40	40	100	80	81.82	59.64	17.24	1.47E-06	
Mn	0.45	8.61	3.97	3.8	5.39	1.73	1.45	1.37	2.56	1.47	2.9	1.29	2.92	2.28	0.66	5.31E-08	
Ni	0.16	0.2	0.12	0.15	0.24	0.19	0.16	0.13	0.14	0.53	0.17	0.14	0.19	0.11	0.03	3.31E-09	
Pb	0.03	0.03	0.01	0.04	0.05	0.07	0.07	0.06	0.05	0.03	0.05	0.03	0.04	0.02	0.01	2.09E-10	
Se	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	0.00	0.00	NC[f]	
U	0.05	0.03	0.009	0.005	0.026	0.006	0.011	0.007	0.125	0.014	0.015	0.012	0.03	0.03	0.01	1.09E-10	
Zn	1	0.3	0.5	0.5	0.5	0.6	0.5	0.8	1	0.4	0.6	0.6	0.61	0.22	0.06	9.30E-09	
Ca	0.2	0.1	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.19	0.05	0.01	4.78E-06	
Mg	1	0.5	0.6	1	0.8	0.8	0.8	0.8	1	1	0.3	0.6	0.77	0.23	0.07	3.15E-05	
Na	1.3	1.1	1.5	1.4	2.8	1.3	1.5	1.2	1.4	1.4	0.8	0.9	1.38	0.50	0.14	6.02E-05	
SO4	0.3	0.6	0.1	0.3	0.1	0.2	<0.1	0.2	0.3	0.4	0.2	0.3	0.27	0.14	0.04	2.84E-06	

[a] All analytes are measured in µg L^{-1} with the exception of Ca, Mg, Na, SO_4 and NO_3 which are in mg L^{-1}, [b] SD = standard deviation, [c] = SE = standard error, [d] M = molar concentration, [e] NM = not measured, [f] NC = not calculated

Appendix I Details of regression models for relationships between Mg pulse exposure duration and the IC10 and IC50 toxicity estimates

Species	Toxicity estimate	Model type	Model equation[a]	Model details (n, r^2, P)	Reference to Figure 7
Chlorella sp.	IC10	Linear	$y = 6086 – 74.74*x$	(4, 0.98, 0.007)	A
	IC50	Linear	$y = 7110 – 47.68*x$	(4, 0.80, 0.069)	B
Lemna aeqinoctialis	IC10	2 parameter exponential decay	$y = 10820*\exp(-0.2470*x)$	(4, 0.99, 0.0002)	C
	IC50	2 parameter exponential decay	$y = 4469.5*\exp(-0.0197*x)$	(3, 0.99, 0.024)	D
Amerianna cumingi	IC10	3 parameter exponential decay	$y = 153.3 + 35427*\exp(-0.6276*x)$	(4, 0.98, 0.086)	C
	IC50	2 parameter exponential decay	$y = 3492.4*\exp(– 0.0273*x)$	(3, 0.98, 0.070)	D
Moinodaphnia macleayi					
At onset of reproduction	IC10	Inverse, first order	$y = 50.87 + (560.7/x)$	(4, 0.42, 0.219)	E
	IC50	2 parameter rational	$y = 1/0.0028 + 0.00005*x$	(4, 0.94, 0.021)	F
At test commencement	IC10	Inverse, first order	$y = 46.46 + (4008.8/x)$	(4, 0.98, 0.006)	E
	IC50	3 parameter exponential decay	$y = 136.12 + 1689.2*\exp(-0.0687*x)$	(4, 0.98, 0.089)	F
Hydra viridissima	IC10	2 parameter exponential decay	$y = 1233.3*\exp(-0.0199*x)$	(4, 0.96, 0.015)	E
	IC50	Inverse, first order	$y = 737.39 + (2480.6/x)$	(4, 0.96, 0.014)	F

[a] x = exposure duration (hours); y = IC10 or IC50 value

Appendix J Growth rate as an indicator of organism recovery from Mg pulse exposure

Table J.1 Algal growth in final 24 h of test

Test code	Pulse duration	Mg (mg L^{-1})	Mean growth rate (doublings d^{-1})	SEM
1202G	4 h	0.8	1.03	0.04
		3900	1.03	0.13
1233G	4 h	1.1	0.97	0.07
		4100	1.06	0.05
		6000	1.66	0.10
1203G	8 h	0.9	0.7	0.07
		4100	0.67	0.04
1232G	8 h	1.1	0.56	0.09
		4000	0.88	0.05
		5900	1.27	0.04
1189G	24 h	0.9	1.09	0.10
		930	1.31	0.13
		1900	1.43	0.09
		4300	1.69	0.09
1192G	24 h	0.9	0.79	0.10
		940	1.23	0.11
		1900	1.27	0.07
		4200	1.52	0.14
1199G	24 h	0.9	0.6	0.03
		1400	0.64	0.04
		3200	0.95	0.20
		4300	1.08	0.15
1234G	24 h	0.9	1.31	0.07
		1900	1.24	0.12
		3800	1.34	0.18
		6700	1.17	0.11

Table J.2 *Lemna aequinoctialis* growth for each 24 h period of the test based on plant number

Test code	Pulse duration	Mg (mg L^{-1})	Mean population growth rate (k), (SEM)			
			0 – 24 h	24 – 48 h	48 – 72 h	72 – 96 h
1097L	8 h	0.8	0.60 (0.04)	0.49 (0.04)	0.29 (0.04)	0.64 (0.01)
		199	0.65 (0.04)	0.45 (0.04)	0.17 (0.06)	0.62 (0.04)
		413	0.65 (0.04)	0.48 (0.04)	0.15 (0.08)	0.61 (0.07)
		814	0.44 (0.14)	0.66 (0.14)	0.17 (0.06)	0.58 (0.04)
		1650	0.73 (0.43)	0.34 (0.41)	0.27 (0.07)	0.57 (0.08)
		3280	0.80 (0.12)	0.04 (0.14)	0.17 (0.07)	0.25 (0.15)
		4150	0.94 (0.08)	0.03 (0.08)	0.10 (0.06)	0.03 (0.03)
1108L	8 h	0.9	0.73 (0.04)	0.37 (0.04)	0.30 (0.08)	0.64 (0.10)
		208	0.76 (0.11)	0.36 (0.09)	0.38 (0.04)	0.55 (0.02)
		412	0.76 (0.11)	0.44 (0.10)	0.27 (0.07)	0.55 (0.01)
		822	0.65 (0.04)	0.53 (0.06)	0.23 (0.08)	0.61 (0.03)
		1670	0.49 (0.14)	0.52 (0.16)	0.26 (0.10)	0.50 (0.11)
		3280	0.32 (0.17)	0.49 (0.10)	0.00 (0.06)	0.25 (0.07)
		4140	0.85 (0.04)	0.16 (0.09)	0.00 (0.09)	0.22 (0.07)
1011L	24 h	0.8	0.28 (0.06)	0.72 (0.08)	0.24 (0.09)	0.58 (0.08)
		120	0.63 (0.15)	0.35 (0.18)	0.34 (0.10)	0.35 (0.05)
		233	0.54 (0.16)	0.53 (0.13)	0.03 (0.07)	0.64 (0.09)
		482	0.51 (0.05)	0.44 (0.04)	0.09 (0.09)	0.51 (0.18)
		992	0.38 (0.16)	0.40 (0.15)	0.34 (0.09)	0.40 (0.05)
		2030	0.34 (0.11)	0.40 (0.14)	0.36 (0.06)	0.11 (0.05)
		4060	0.14 (0.14)	0.84 (0.17)	0.03 (0.06)	0.11 (0.06)
1018L	24 h	0.9	0.07 (0.07)	0.57 (0.12)	0.48 (0.07)	0.41 (0.02)
		99.9	0.14 (0.14)	0.50 (0.16)	0.49 (0.14)	0.32 (0.04)
		188	0.34 (0.06)	0.43 (0.13)	0.37 (0.20)	0.39 (0.01)
		373	0.37 (0.09)	0.46 (0.05)	0.50 (0.07)	0.15 (0.03)
		773	0.32 (0.17)	0.39 (0.05)	0.36 (0.07)	0.31 (0.08)
		1600	0.32 (0.17)	0.56 (0.13)	0.19 (0.06)	0.18 (0.05)
		2920	0.44 (0.14)	0.29 (0.10)	0.14 (0.07)	0.06 (0.08)
		4090	0.14 (0.14)	0.81 (0.12)	0.03 (0.03)	0.00 (0.06)

Table J.3 *Hydra viridissima* growth for each 24 h period of the test

Test code	Pulse duration	Mg (mg L^{-1})	Mean population growth rate (k) ± SEM			
			0 – 24 h	24 – 48 h	48 – 72 h	72 – 96 h
979B	4 h	0.4	0.36 (0.02)	0.39 (0.07)	0.47 (0.05)	0.46 (0.02)
		651	0.26 (0.04)	0.45 (0.09)	0.36 (0.02)	0.53 (0.02)
		821	0.42 (0.08)	0.29 (0.07)	0.45 (0.05)	0.54 (0.06)
		1030	0.33 (0.07)	0.25 (0.11)	0.51 (0.04)	0.50 (0.03)
		1300	0.00 (0.09)	0.36 (0.18)	0.36 (0.08)	0.49 (0.08)
972B	4 h	0.3	0.06 (0.03)	0.60 (0.03)	0.38 (0.04)	0.13 (0.06)
		212	0.10 (0.00)	0.51 (0.02)	0.39 (0.06)	0.24 (0.13)
		422	0.06 (0.03)	0.56 (0.03)	0.29 (0.02)	0.21 (0.11)
		842	0.24 (0.03)	0.37 (0.06)	0.31 (0.02)	0.23 (0.13)
1100B	8 h	0.8	0.25 (0.11)	0.45 (0.03)	0.37 (0.03)	0.43 (0.05)
		247	0.26 (0.04)	0.53 (0.04)	0.38 (0.01)	0.42 (0.02)
		496	0.30 (0.09)	0.42 (0.08)	0.34 (0.10)	0.43 (0.08)
		773	0.24 (0.16)	0.47 (0.09)	0.37 (0.09)	0.33 (0.07)
		1000	0.17 (0.09)	0.31 (0.08)	0.48 (0.05)	0.42 (0.04)
		1300	0.00 (0.37)	0.56 (0.27)	0.14 (0.14)	0.33 (0.04)
1114B	8 h	0.8	0.17 (0.12)	0.49 (0.13)	0.32 (0.02)	0.30 (0.04)
		230	0.22 (0.12)	0.42 (0.12)	0.31 (0.02)	0.38 (0.05)
		473	0.15 (0.08)	0.53 (0.08)	0.33 (0.07)	0.41 (0.05)
		710	0.06 (0.06)	0.63 (0.06)	0.33 (0.06)	0.38 (0.07)
		906	0.00 (0.14)	0.48 (0.04)	0.25 (0.11)	0.49 (0.07)
1014B	24 h	0.8	0.06 (0.03)	0.61 (0.04)	0.30 (0.08)	0.32 (0.08)
		190	0.00 (0.00)	0.69 (0.00)	0.31 (0.01)	0.22 (0.03)
		293	0.00 (0.00)	0.64 (0.03)	0.28 (0.08)	0.18 (0.08)
		444	0.03 (0.03)	0.67 (0.04)	0.05 (0.08)	0.25 (0.04)
		655	0.00 (0.00)	0.66 (0.02)	0.17 (0.04)	0.24 (0.07)
		965	0.00 (0.56)	0.21 (0.32)	0.06 (0.06)	0.21 (0.12)
1017B	24 h	0.9	0.23 (0.07)	0.49 (0.07)	0.19 (0.02)	0.35 (0.08)
		261	0.03 (0.03)	0.69 (0.00)	0.24 (0.07)	0.34 (0.04)
		401	0.03 (0.03)	0.64 (0.08)	0.18 (0.07)	0.29 (0.01)

Table J.3 (cont)

Test code	Pulse duration	Mg (mg L^{-1})	Mean population growth rate (k) ± SEM			
			0 – 24 h	24 – 48 h	48 – 72 h	72 – 96 h
		590	0.00 (0.00)	0.68 (0.02)	0.15 (0.10)	0.37 (0.05)
		795	0.00 (0.00)	0.69 (0.05)	0.03 (0.14)	0.38 (0.05)
		1010	0.00 (0.00)	0.64 (0.06)	0.00 (0.06)	0.43 (0.08)
1095B	24 h	0.8	0.29 (0.05)	0.39 (0.06)	0.25 (0.01)	0.35 (0.01)
		216	0.00 (0.00)	0.71 (0.02)	0.18 (0.04)	0.34 (0.04)
		441	0.06 (0.03)	0.68 (0.04)	0.20 (0.04)	0.33 (0.06)
		654	0.00 (0.00)	0.68 (0.02)	0.07 (0.04)	0.43 (0.03)
		792	0.03 (0.50)	0.61 (0.04)	0.07 (0.03)	0.37 (0.04)