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Editor-in-Chief

Dr Reinier Mann, Department of Science, Information Technology, Innovation and the Arts, GPO Box 2454, Brisbane Qld 4000, Australia. email: reinier.mann@qld.gov.au.

Associate Editor

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RESEARCH PAPER

IMPACT OF SALINITY AND IONIC COMPOSITION ON FRESHWATER MACROINVERTEBRATES IN THE FITZROY RIVER CATCHMENT, CENTRAL QUEENSLAND, AUSTRALIA

Rajesh Prasad[1] Sue Vink[2]* and Vinitha Nanjappa[2]

[1] Water Planning Ecology, Department of Science, Information Technology, Innovation and the Arts, Brisbane, QLD 4012, Australia.

[2] Centre for Water in the Minerals Industry, Sustainable Minerals Institute (SMI), The University of Queensland, St Lucia, Qld 4072, Australia.

ABSTRACT

Disposal of high salinity water from coal mines in Queensland is a serious issue for coal mines. This is exacerbated during high rainfall events associated with La Niña conditions as excess water often accumulates in water storages. Release of excess water from mine sites to local streams and rivers is one of the most effective disposal options in order to avoid long term accumulation of saline water. However, if saline water is discharged into the streams and rivers, it is likely to impact the aquatic biota, aquatic ecosystems and ecosystem values. To minimise impacts from environmental releases, the coal industry and environmental regulatory authorities need information related to the potential impacts of salinity on the aquatic environment to help inform appropriate release criteria.

In this study, macroinvertebrates from the Fitzroy River Catchment in Central Queensland, where many Queensland coal mines are situated, were tested for their sensitivity to saline waters. The primary aim was to derive LC50 values, for macroinvertebrates from the Fitzroy Catchment for mine waters of known ionic compositions, which could potentially be used to derive guidelines for mine salts. A second aim was to consider the levels of dilution that would be necessary to reduce salt concentration in mine water to levels tolerable by macroinvertebrates. Test solutions comprised of two artificial mine water types (AMW1 and AMW2), diluted with artificial creek water, all prepared in the laboratory. The results showed that Leptophlebiidae and Baetidae (Order: Ephemeroptera), were more sensitive (or less tolerant) to artificial mine water in comparison with their sensitivity/tolerance to marine salts. Although it was not possible to statistically test whether the difference in toxicity of the two mine waters was significant, it seemed that AMW2 was less toxic than AMW1. This is highly probable as AMW2 contained a much higher proportion of calcium compared to AMW1.

The assays conducted agree with results from earlier studies that show that ionic composition can have ameliorating or confounding effects on the toxicity of saline waters. Further research on the ionic composition of coal mine effluent may provide solutions for management of effluent discharge.

Keywords: Mine water; Salinity; Toxicity; Macroinvertebrate sensitivity; Ephemeroptera.

* Author for Correspondence, email: s.vink@uq.edu.au

INTRODUCTION

Salinisation of freshwater systems has been recognised as a serious environmental issue due to the potential for significant deleterious impacts on freshwater biota and freshwater ecosystems in general (Hart et al. 1991; Clunie et al. 2002; Jasonsmith et al. 2011; U.S. EPA 2011). In Australia, the widespread salinisation of freshwater systems has primarily occurred as a result of the increasing prevalence of 'dryland' or 'secondary' salinisation (Williams 1987). Dryland or secondary salinisation is usually referred to as salinisation of land. It occurs as a result of mobilisation of salts stored in the soil profile and/or in the groundwater by extra water provided through various human activities such as vegetation clearing, irrigation and water impoundment in dams and weirs (Williams 1987; Ghassemi et al. 1995; Hart et al. 1991). Clearing of deep-rooted vegetation leads to reduction of groundwater usage by plants, while irrigation and water impoundment result in increased recharge of groundwater. The extra water raises the water table or increases the pressure of confined aquifers, creating an upward leakage to water table aquifers. Rising water tables bring dissolved salts to within 1 – 2 m of the soil surface. With the water table close to the soil surface, water evaporates, leaving behind salts that lead to land salinisation (Ghassemi et al. 1995). Salinisation of freshwater systems subsequently occurs when salts brought up to the land surface find their way into freshwater systems with runoff, or by direct leakage into freshwater bodies. In more recent times inputs from other anthropogenic sources such as mines and power station wastewaters (Richardson and Bennett 2005; Muschal 2006) have also contributed to at least temporary increases in salinity in the environment.

In the Fitzroy Basin in Queensland (Figure 1), there were more than 40 coal mines in operation as at July 2011 (DEEDI 2011). Licences are issued to mines to discharge wastewater into the environment (DERM 2009). In the recent past, weather patterns associated with El Niño and La Niña have caused either prolonged droughts or very high levels of rainfall. Excess water accumulated in some of the mines in the Fitzroy catchment after extremely high rainfall events during La Niña conditions in the 2007/08 wet season (Hart 2008). Storing this water at the mine sites would have ultimately led to increase in its salinity through evaporation, salt runoff from the surrounding landscape and from groundwater. The most effective option in such a scenario would be to discharge the excess water as quickly as possible when the flow levels in the rivers in the catchment are high (DERM 2009; DEHP 2013).

Several reports that followed the prolonged release of water from mine sites during and after the floods highlighted the lack of adequate data to determine appropriate salinity water quality guidelines or ecosystem trigger values, and the need for developing water guidelines for the protection of freshwater aquatic ecosystems in the Fitzroy catchment (Hart 2008; Tripodi and Limpus 2010; Delzoppo 2011; Jones and Moss 2011). Most of the salinity toxicity data that are currently available have been derived from organisms collected from permanent streams in NSW, Victoria and from the wet and dry tropics in Queensland (Muschal 2006; Kefford et al. 2003, 2007a; Dunlop et al. 2008). In addition, previous tests have generally been conducted using marine salt composition (e.g., Kefford et al. 2003; Kefford et al. 2004a, b; Kefford and Nugegoda 2005; Hassell et al. 2006; Kefford et al. 2007a, b, c; Dunlop et al. 2008; Prasad 2010), which in many instances is distinctly different from the composition of stream water in the Fitzroy catchment (McNeil et al. 2005; Dunlop et al. 2012).

While salts are essential to the physiology of all plants and animals, high concentrations of salts are toxic to freshwater plants and animals (Bailey and James 2000; Clunie et al. 2002; Kefford et al. 2002). Salt toxicity can be moderated or exacerbated by other factors including the prevailing condition of the aquatic system, ionic composition, exposure regimes including natural salinity variations, adaptations of life history stages or avoidance, and an organism's osmoregulatory mechanism. Osmoregulatory mechanisms are active physiological processes that keep the animal

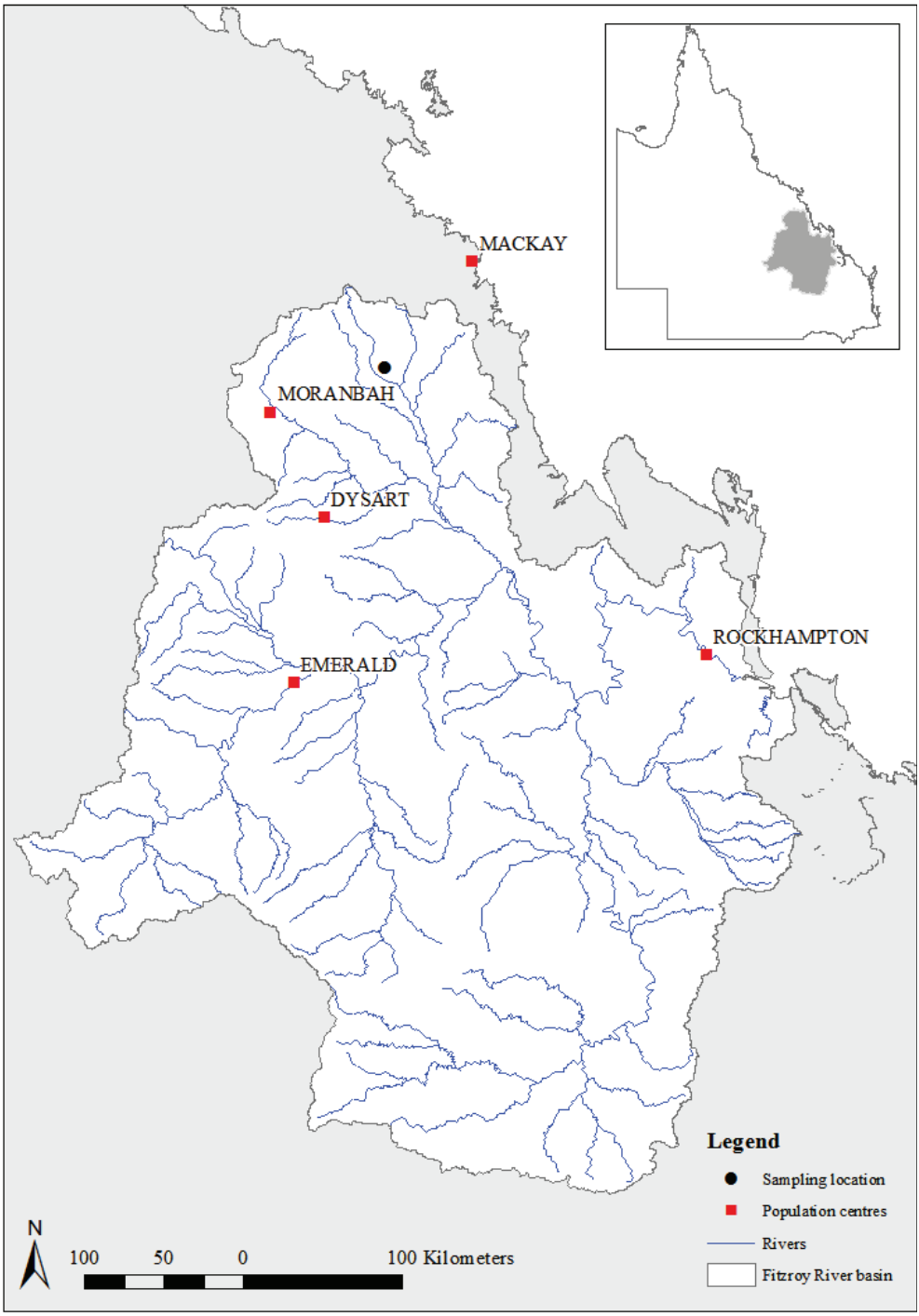


Figure 1. The Fitzroy Catchment and macroinvertebrate sampling site at Nebo Creek.

bodily fluids from becoming too dilute or too concentrated; between an upper and a lower limit within which animal cells can live and perform. Thus, a diverse range of responses to changes in salinity can be exhibited, both by different taxa (e.g., Kefford et al. 2006; Dunlop et al. 2008), and by organisms from the same taxon living in different regions (Dunlop et al. 2008). For example, acute toxicity tests with the freshwater shrimp, *Caridinides wilkinsi*, from the wet tropics in Queensland, resulted in a 72-h LC50 value of 41.3 mS/cm, while acute tests with the same species collected from Queensland Murray Darling Basin and South East Queensland resulted in a 72-h LC50 value of 34.2 mS/cm (Dunlop et al. 2008). Consequently, although the available information can be applied to broadly determine the impacts of saline mine water releases on freshwater ecosystems, there is insufficient specific information to develop robust water quality guidelines for specific regions. ANZECC/ARMCANZ (2000) testing criteria recommend toxicity testing using local taxa and locally relevant solution compositions. Dunlop and McGregor (2007) have further described the approach to be undertaken in setting water quality guidelines for salinity in freshwater streams in Queensland.

It has been advocated that knowledge of specific ionic compositions is more important than just electrical conductivity (EC) or total dissolved solids (TDS) concentration (Elphick et al. 2011; Soucek et al. 2011). The influence of ionic composition on salinity tolerance has been typically investigated using single salts and simple mixtures (Weber-Scannell and Jacobs 2001; Kennedy et al. 2005; Zalizniak et al. 2006). However, these results are difficult to extrapolate to natural and mine waters, which are more complex in terms of ionic composition and ion combinations. The impact of saline coal mine discharges on freshwater organisms has been investigated by Lincoln-Smith (2010) in the Illawarra and Hunter Valley in New South Wales. This author serially diluted mine water with creek waters to determine the salinity tolerance of macroinvertebrate species. However, the results may have been influenced by additional unidentified compounds present in the mine waters. Dunlop et al. (2012) recently used laboratory prepared solutions with compositions similar to stream waters to test the toxicity of sulfate. The advantage of these artificial test solutions is that other constituents which might affect toxicity are eliminated from the test system, thereby allowing salinity tolerance to be directly tested for combinations of ions representative of waters to which organisms might be exposed.

In this study, two types of artificial mine waters were formulated after samples from mine water from ten mine sites were collected and chemically analysed to determine their ionic components. The formulated mine waters were diluted using a formulated creek water, which was based on analysis of historical data on creek water composition in the Fitzroy catchment. A dilution series of artificial mine water was then used to test the salinity tolerance of aquatic macroinvertebrate families from the Fitzroy catchment by the rapid assessment method described by Kefford et al. (2003, 2005).

The primary aim of the study was to derive LC50 values, for macroinvertebrates from the Fitzroy Catchment for mine waters of known ionic compositions, which could potentially be used to derive guidelines for mine salts. The secondary aim was to consider the levels of dilution that may be necessary to reduce the salinity of mine waters below the tolerance limits of the tested biota.

MATERIALS AND METHODS

Mine water composition

A survey of mine water ionic composition was undertaken so as to formulate saline test solutions that were representative of mine waters in the Fitzroy Catchment. Water samples were collected from 10 mine sites (total of 42 release points) across the catchment. All water samples were collected during the period 9 - 20 August 2011. Major cation and sulfate concentrations were determined by inductively coupled plasma – optical emission spectroscopy (APHA 1995). Chloride was determined

by flow injection (Rayment and Higginson 1992). Carbonate, bicarbonate and total alkalinity were determined using Gran Titration (APHA 1995). Analyses were undertaken by NATA-accredited laboratories contracted by each mine.

Preparation of Artificial Creek Water and Artificial Mine Water

Solutions with an ionic composition representative of mine water and stream waters were prepared in the laboratory. Three artificial waters were prepared. Two mine water compositions (AMW1 and AMW2) were formulated that represented, as near as possible, the range of compositions determined from the mine water survey. A third water type was prepared to represent creek water composition (ACW). This reconstituted water was the same as that used by Dunlop et al. (2012), and was based on analysis of historical data on creek water composition in the Fitzroy catchment. This water was used as a diluent in the preparation of dilutions of mine waters. Solutions were prepared with analytical grade salts and Milli-Q™ water. The ionic compositions of all the artificial solutions and the water from the site of macroinvertebrate collection are given in Table 1. Test solutions for determination of acute toxicity measurements were prepared as a dilution series comprising artificial mine water solutions diluted with artificial creek water to achieve the desired electrical conductivity (EC).

Test species collection

Macroinvertebrates were sourced from Nebo Creek, a freshwater creek located in the Connor River sub-catchment within the Fitzroy Catchment (Figure 1). Nebo Creek, close to the town of Nebo located south-west of Mackay, is not in close proximity to any coal mines or major agricultural area; hence, it was assumed that the creek would be minimally impacted. Therefore, it was highly unlikely that the aquatic biota in this creek had been pre-exposed to mine wastewater and any unnatural elevation of salinity. This creek was found, at first inspection, to contain a high abundance of aquatic insects from the orders of Ephemeroptera (mayflies) and Trichoptera (caddisflies). However, water flow in the creek was observed to decrease dramatically following a dry (no rainfall) period of about three months between August and October, 2011, during which time the numbers of the targeted macroinvertebrates declined.

The macroinvertebrates were live picked in the field, separated into taxonomic families, placed in acid washed polypropylene jars containing aerated collection site water, capped with polypropylene lids and transported to the laboratory on the same day. Two methods were applied in the collection of mayflies. Firstly, stones from the riffle sections of the creek were placed in a clean bucket containing creek water and vigorously shaken to dislodge the mayflies from the stones into the bucket of water. Secondly, a 250-µm macroinvertebrate sampling net was placed downstream of a riffle section of the creek while the stones and pebbles were kicked and dislodged. A third method, using the macroinvertebrate sampling net to sweep along the edges of the creek, collected the caddisflies and

Table 1. Ionic compositions and pH of test solutions. ACW = artificial creek water. AMW = artificial mine water. Marine salt composition is given for comparison.

	Ca	Mg	Na	K	HCO_3	SO_4	Cl	pH
				meq %				
Nebo Creek	16	16	18	0	20	1	29	8.1
ACW	12	17	21	0	29	2	18	8.0
AMW 1	2	8	39	0.4	3	15	32	8.4
AMW 2	11	15	23	0.4	12	13	25	8.3
Marine salts	2	9	38	0.8	0.2	5	45	8.2

possibly some mayflies as well. The separation of macroinvertebrates into taxonomic families in the field was done on the basis of morphological similarity. Water quality parameters measured in the field at the time of collection included conductivity, pH, temperature and dissolved oxygen (Table 2). The instruments used were a 'Ultrameter MP6 II' during the first three occasions and a 'Horiba Multiprobe' on the final occasion. Water from the collection site was also taken and subsequently used as one of the control treatments in toxicity tests (CW).

The collected macroinvertebrates were allowed to acclimate to the laboratory conditions overnight for approximately 12 hours. A longer acclimation time was not tenable as animals were not fed at any time during transportation or testing.

Acute toxicity testing with field-collected macroinvertebrates

Salinity tolerances among macroinvertebrates exposed to artificial mine waters were investigated through the performance of acute toxicity tests following the procedures outlined in ASTM E729 – 96 (2007) and ASTM E1192 – 97 (2008). In these tests, macroinvertebrates were exposed to a dilution series of mine waters for 96 hours. The control treatments included creek water from the site of macroinvertebrate collection (CW) and artificial creek water (ACW). The dilutions comprised of 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% dilution (0% dilution was undiluted artificial mine water and 100% dilution was equivalent to diluent only; i.e., only ACW and no artificial mine water). The mortality rate over 96 hours was used to generate 96-h LC50 values. The controls were used to confirm that the observed acute responses were due to the test solutions and not to stress to the macroinvertebrates from collection, transport or other factors. The test end-point of mortality, or more accurately, moribundity (near death) was based on absence of response to gentle prodding. In those instances where test macroinvertebrates emerged (as flying adults) or were missing due to cannibalism, the data from that particular test were not included in the analysis.

The macroinvertebrates tested included two mayfly species from Families Leptophlebiidae and Baetidae (Order: Ephemeroptera), one caddisfly from Family Leptoceridae (Order: Trichoptera), one true bug from Family Pleidae (Order: Hemiptera) and one freshwater shrimp from the Family Atyidae (Order: Decapoda). The test animals of the mayflies and the caddisflies were the larval stages, which are aquatic. The sizes of Leptophlebiidae used in the tests ranged from approximately 5 mm to 20 mm. The Baetidae were generally between 5 mm and 15 mm. The sizes of Leptoceridae were difficult to determine as all were in stick cases, with sticks ranging in size from 5 mm to 30 mm. The Pleidae have a full aquatic life cycle and the sizes of animals tested were largely around 5 mm to 8 mm. The shrimps were all adults in many sizes to a maximum of 40 mm body length.

In the testing procedure, it was difficult to have the same number of animals in each test chamber and have replicates of each test type. This was due to difficulty in collecting large number of animals every time, largely constrained by variability in abundance of animals and time availability for

Table 2. Water quality parameters determined during macroinvertebrate collection in Nebo Creek. na = determination not available at the time of collection.

Date	Electrical Conductivity (mS/cm)	Dissolved O_2 (mg/L)	pH	Temperature (°C)
14/09/2011	0.778	na	7.51	21.3
21/09/2011	0.785	na	8.02	25.8
5/10/2011	0.826	na	7.85	27.8
28/11/2011	0.970	6.68	7.87	32.8

collection. In total, four experiments were conducted to generate sufficient data and to ensure the validity of the experiments. Table 3 shows the number of experiments, number of replicates for each family and the number of individuals of each family in the test chambers.

The ambient temperature during the testing ranged between 18 – 26°C, with a median of 21.2°C. The temperature variation within the laboratory could not be controlled due to the open nature of the laboratory. However, it was assumed that the temperature range was unlikely to influence the results as such temperature variation is common in the natural environment. The temperature range was well within the temperatures that were measured at the collection site (Table 2). The light regime was between 650 - 850 Lux on average, with 16:8 light, dark photoperiod. All tests were undertaken as static non-renewal tests; i.e., the test solutions were not changed for the duration of the experiment. The test chambers were aerated throughout the duration of the testing.

In determining the salinity tolerance of particular taxa to AMW, range finding tests were performed first. In range finding tests, animals were subjected to a wide range of dilutions (different ECs) to estimate the range within which the salinity tolerance was likely to be. Range finding tests were only replicated where sufficient animals were available. Once an estimate of salinity tolerance was obtained, definitive tests were then performed. In definitive tests, the animals were tested within a narrow range of dilutions (or ECs) in order to generate LC50 values. Definitive tests were only undertaken when there were sufficient numbers of test animals to ensure three replicates with a minimum of five individual animals in each test chamber. When enough animals were not available, definitive tests were not conducted. Test chambers were inspected on a daily basis (as a minimum) and dead animals removed. All mortalities in the control and test chambers were recorded.

Statistical analyses

The mortality rate over 96 hours was used to generate 96-h LC50 values. After definitive tests were conducted and where sufficient data were available, point estimates as 96-h LC50 values were determined from a logistic regression using R™ version 2.1.1 (Venables and Ripley 2002). In this method the mortality of the test animals versus EC was modelled assuming a continuous EC-response relationship. A logistic regression was fitted to the data. The data output provides the probability of mortality with a corresponding EC value.

Table 3. The experimental schedule with experiment number, number of replicates per family and number of individual animals per test chamber.

Experiment No	Taxon	No. of individuals per test chamber in each replicate		
		Rep 1	Rep 2	Rep 3
1	Leptophlebiidae	4 -6	No rep	No rep
	Baetidae	10 -11	No rep	No rep
	Pleidae	10	No rep	No rep
	Leptoceridae	7 -9	No rep	No rep
2	Leptophlebiidae	9 -12	8 -11	No rep
	Baetidae	8 -10	7 - 9	5 - 9
	Leptoceridae	4 -5	No. rep	No rep
3	Leptophlebiidae	10	8 - 15	5 - 6
	Leptoceridae	8 - 9	No rep	No rep

"Modelled Estimates" were calculated when there were sufficient test animals to have at least five individuals per test chamber, and three replicates of each treatment. "Assigned Estimates" were calculated when there were insufficient macroinvertebrates to conduct definitive tests with at least three replicates. Assigned Estimates are not precise tolerance values but give an approximate indication of salinity tolerances, often maximum tolerance levels. (Assigned Estimates are equivalent to Non-modelled Estimates used in Dunlop et al. 2008, see original document for detailed explanation).

RESULTS

Mine water composition and electrical conductivities

Results from the survey of mine water composition are shown in a Piper diagram (prepared with Geochemist's Workbench®, Aqueous Solutions LLC) (Figure 2). It can be seen that the relative proportion of cations in the majority of mine waters varied between 88% Na, 8% Mg, 4% Ca to 52% Na, 30% Mg, and 17% Ca. Anion composition was considerably more variable.

96-hour acute toxicity tests

Modelled estimates of artificial mine water salinity tolerances were obtained for two families, Leptophlebiidae and Baetidae (Table 4). The LC50 value determined for Leptophlebiidae was 6.9 $mScm^{-1}$ (6900 μScm^{-1}), and for Baetidae was 6.2 $mScm^{-1}$ (6200 μScm^{-1}), both in AMW1. Assigned estimates were obtained for three families, Pleidae, Atyidae and Leptoceridae (Table 4). Only

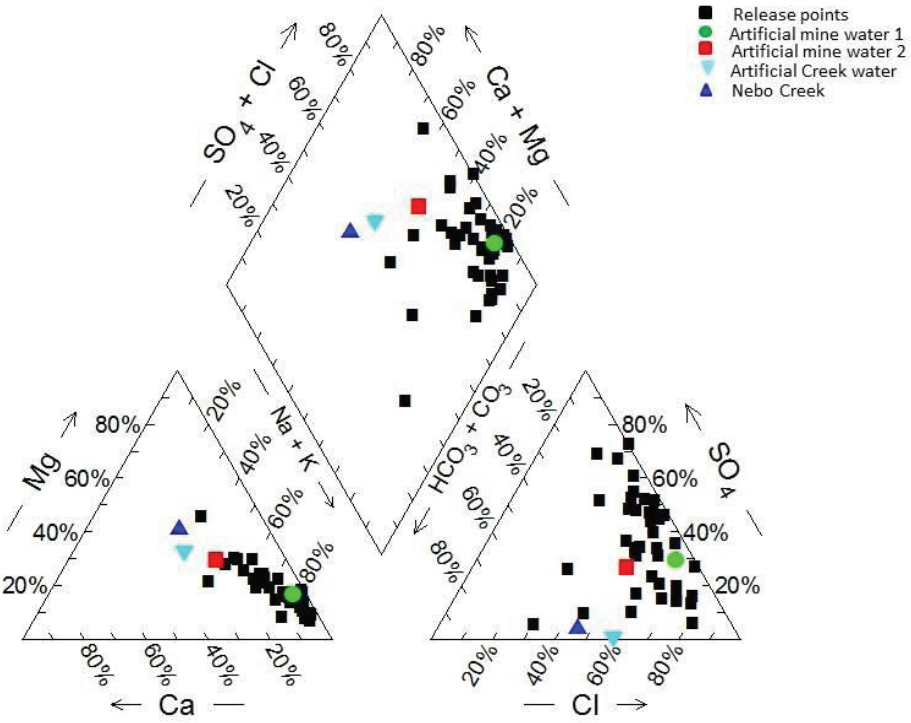


Figure 2. Piper Diagram of mine water composition determined from release points on 10 mine sites located across the Fitzroy.

Leptophlebiidae and Leptoceridae could be tested using AMW2 and the estimation of LC50 values were only Assigned Estimates. This was due to reduced availability of organisms, possibly as a result of reduced water flow in Nebo Creek.

The results from this study showed that Baetidae was the most sensitive taxon while the freshwater shrimp from the Atyidae family was the least sensitive (Table 4). The true bugs, Pleidae, could also be classified in the not-too-sensitive category.

Comparison of LC50 values for Leptophlebiidae and Leptoceridae in AMW1 and in AMW2 (Table 4) show that the LC50 values were slightly lower in AMW2 although the 95 % confidence intervals overlap. No statistical test could be performed to examine if the differences were significant because only one LC50 value was a modelled value, whereas the other three values were assigned estimates.

Comparison of LC50 values of Leptophlebiidae, Baetidae and Leptoceridae from Nebo Creek with those from elsewhere in Queensland, showed that the LC50 values were lower for the ones from Nebo Creek (Figure 3). However, the comparison may not be strictly valid because the LC50 values determined for these families in other parts of Queensland were done with marine salts.

3.3 Mine water dilutions

When the percentages of survival of Leptophlebiidae and Baetidae at different mine water concentrations are plotted (Figures 4 and 5, respectively), it was evident that there was a high level of variability in survival between replicates at each test concentration. Despite the variability, the plots could be used to determine the approximate dilution that would be necessary for different levels of survival (or protection). To achieve 50% survival of Nebo Creek Leptophlebiidae, the artificial mine water had to be diluted by approximately 70% or more (Figure 4). To achieve 50% survival of Nebo Creek Baetids, the mine water had to be diluted by more than 76% (Figure 5).

DISCUSSION

Members of the Ephemeroptera, Plecoptera and Trichoptera (EPT taxa) are known to be sensitive to stressors (e.g., pollution) in aquatic systems (Kitchin 2005), and are often used as indicators (e.g. EPT Index – see Kitchin 2005; Chessman et al. 2006) in monitoring and assessing aquatic ecosystem 'health' (Growns et al. 1997; Metzeling et al. 2006; Pond et al. 2008; Pond 2010). Leptophlebiidae and Baetidae (mayflies) are families belonging to the taxonomic order of Ephemeroptera, and Leptoceridae (caddisflies) belong to the order Trichoptera. It has been reported that the richness and abundance of Ephemeroptera decline with increasing mining disturbance (Pond et al. 2008;

Table 4. Results of 96-hour acute toxicity tests. AMW = artificial mine water.

Nominal Taxon Identification	AMW solution #	LC50 determination	LC50 (mS cm-1)	95% confidence interval (mS cm-1)
Leptophlebiidae	1	Modelled Estimate	6.9	6.030 - 7.773
Leptophlebiidae	2	Assigned Estimate	<7.6	6.320 - 8.973
Baetidae	1	Modelled Estimate	6.2	5.008 - 7.391
Pleidae	1	Assigned Estimate	<18.5	na
Atyidae	1	Assigned Estimate	<22.7	na
Leptoceridae	1	Assigned Estimate	<9.8	na
Leptoceridae	2	Assigned Estimate	<10.7	na

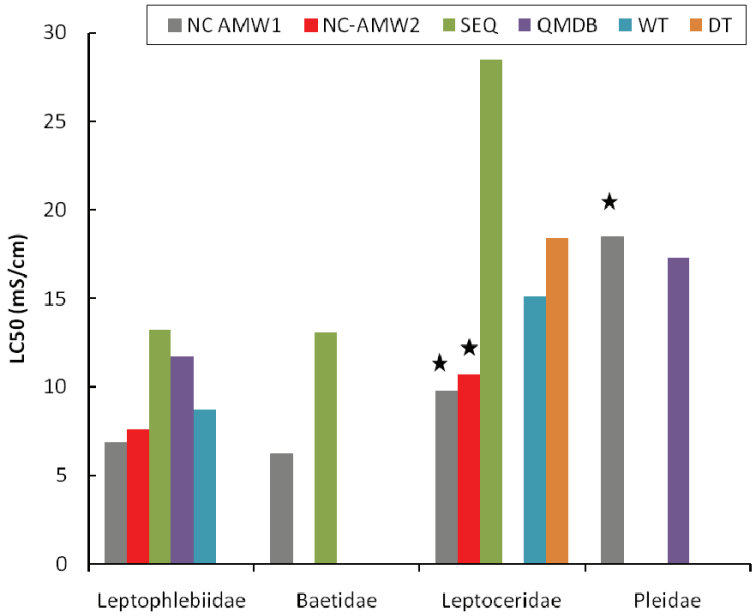


Figure 3. Comparison of LC50 values of taxa collected from Nebo Creek (this study) and other areas in Queensland. NC-AMW1 = Nebo Creek, AMW solution 1; NC-AMW2 = Nebo Creek, AMW solution 2; SEQ = South East Queensland; QMDB Queensland Murray Darling Basin; WT = wet tropics; DT = dry tropics. Test solutions were marine salts for SEQ, QMDB, WT and DT. * indicates assigned values. (SEQ, QMDB, WT and DT data from Dunlop et al. 2008).

Pond 2010). Therefore, data on the relative abundance of these macroinvertebrates from the Fitzroy catchment provide an indication of the present status and may help in determining any future impacts.

Baetidae were the most sensitive taxon followed by Leptophlebiidae. The fresh water shrimp from the family Atyidae was the least sensitive (most tolerant). The true bugs from the family Pleidae could also be categorised as tolerant. A similar order of sensitivities for these taxa has also been reported previously (Kefford et al. 2003; Hassell et al. 2006; Dunlop et al. 2008) when they were tested with marine salts, and with the animals sourced from other parts of Queensland and Victoria. This implies that taxa that are categorised as sensitive or tolerant in one region would very likely be in the same category in other regions.

The results showed that the salinity tolerances exhibited by Leptophlebiidae, Baetidae and Leptoceridae, in both artificial mine water types (AMW1 and AMW2), were lower than their tolerances to marine salts (Figure 3). However, this comparison may not be accurate enough as the tested animals were not from the same location. Ideally, macroinvertebrates from Nebo Creek should also be tested with marine salts, and conversely, macroinvertebrates from other parts of Queensland should be tested with artificial mine waters used in this study to enable a valid comparison. The only other study known to have tested salt tolerance, measured as sensitivity to EC, of macroinvertebrates from anywhere close to the Fitzroy Catchment, was conducted by Dunlop et al. (2008). Those tests used marine salts. However, Kefford et al. (2003, 2005) reported that macroinvertebrates species collected from different sites showed no detectable variability in sensitivity to salinity in acute toxicity tests.

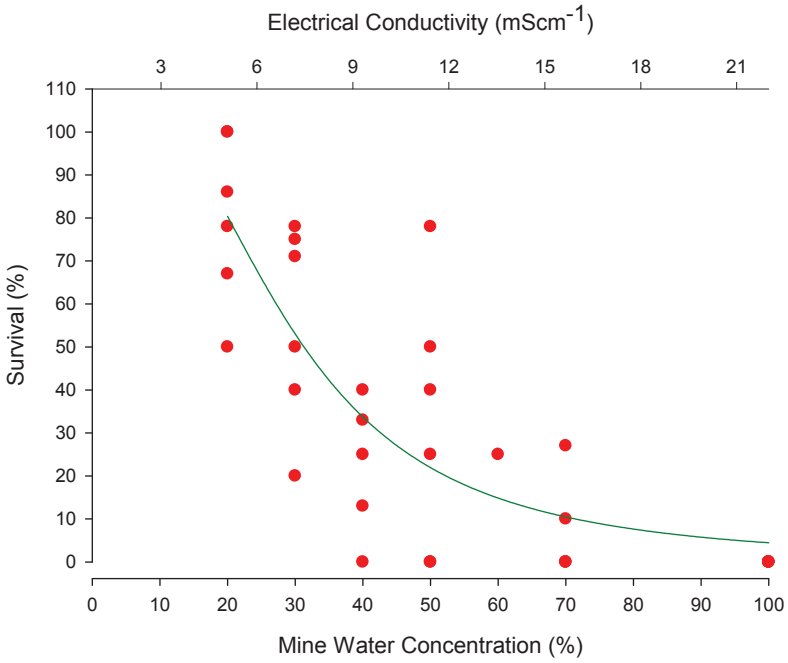


Figure 4. Leptophlebiidae survival at different levels of mine water concentration (dilution) and corresponding conductivities at those concentrations.

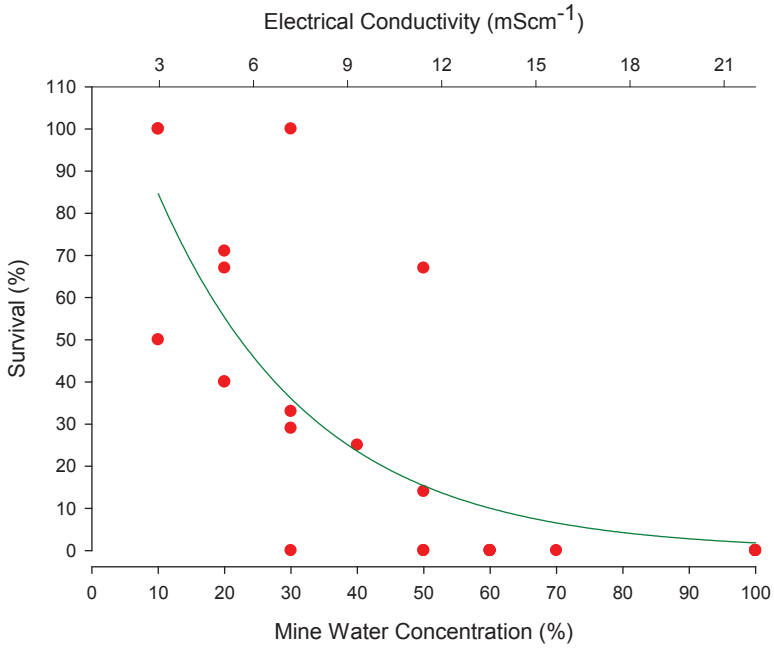


Figure 5. Baetidae survival at different levels of mine water concentration (dilution) and corresponding conductivities at those concentrations.

If the salinity tolerances of macroinvertebrates from different sites do not differ, as reported by Kefford et al. (2003, 2005), then the lower tolerance of macroinvertebrates taxa from Nebo Creek to artificial mine waters could be interpreted in several ways. Firstly, it could mean that Nebo Creek taxa are unusually sensitive. Secondly, it could imply that mine salts are more toxic than marine salts. Thirdly, since the taxonomic resolution used in this and other studies identified the taxa to family level only, the macroinvertebrates sourced from Nebo Creek were possibly different species than species tested elsewhere. It is known that the sensitivity/tolerance of freshwater organisms to changes in salinity is dependent on a variety of factors, including previous exposure and adaptation of life history stages, osmoregulatory mechanisms, the ionic composition of saline solutions and duration of exposure.

Furthermore, a recent study Dunlop et al. (2012) tested the sensitivity of macroinvertebrates to sulfates and reported that the sensitivity of south-east Queensland and Bowen Basin macroinvertebrates were similar. Therefore, assuming that the species tested in this study were the same as those in other studies, and sensitivity/tolerance of macroinvertebrates from different regions are likely similar, the LC50 values for mine waters found in this study suggests that mine waters are more toxic. This conclusion is supported by the results of Kefford et al. (2004a) who found that macroinvertebrate taxa were less sensitive (more tolerant) of marine salts than sodium chloride (NaCl).

The results of this study also seem to indicate that the tested macroinvertebrate taxa were slightly to moderately less sensitive to AMW2 than AMW1. However, more experimental data, modelled LC50 values and statistical tests to determine significant differences are needed before this result could be confirmed. One previous study suggested that responses to ionic compositions do not differ in acute toxicity tests. According to Zalizniak et al. (2006) there were no differences between responses of invertebrates to various ionic compositions in acute toxicity test, but organisms reacted differently to the various ionic compositions in prolonged chronic tests that measured sub-lethal endpoints. Conversely, it has also been documented that salt toxicity is related to specific combinations and concentrations of ions (Chapman et al. 2000). According to Mount et al. (1997) the relative ranking of ion toxicity is: $K^+ > HCO_3^- \approx Mg^{2+} > Cl^- > SO_4^{2-}$. Although in AMW2 the proportion of Mg^{2+} ions were almost two times higher than in AMW1, the proportion of Ca was also more than five times higher in AMW2 (Table 1). According to van Dam et al. (2010), Ca^{2+} ameliorates Mg^{2+} toxicity, so it is possible that the higher proportion of Ca in AMW2 makes this mine water less toxic than AMW1. The salt ions that were most likely to be contributing to the toxicity of AMW1 would be Na^+, SO_4^{2-}, Cl^- and their combinations.

The higher toxicity of the two mine waters compared to marine salt solutions is highly likely to be related to the higher ratios of Mg^{2+} and SO_4^{2-} in the mine waters. Dunlop and McGregor (2007) compared the toxicity of three test solutions and reported that the most toxic solution had the highest concentration of Mg, and the second most toxic solution had the second highest Mg but low HCO_3^- and high Cl^-. The toxicity of double salt solutions is reportedly lower than single salt solutions; in some cases this has been shown to be due to particular ions such as Ca (Mount et al. 1997). These researchers reported decreased toxicity of chloride to *Ceriodaphnia dubia* when tested as a combination of sodium chloride and calcium chloride, compared with sodium chloride alone. Chloride and sodium on their own can be highly toxic when present in high concentrations (Mount and Evans 1993; Valenti et al. 2006).

This study concurs with other studies (Mount and Evans 1993; Mount et al. 1997; Goodfellow et al. 2000; Kennedy et al. 2005; Soucek and Kennedy 2005; Zalizniak et al. 2006; Weber-Scannell and Duffy 2007; Stekoll et al. 2009; Elphick et al. 2011; Soucek et al. 2011) in showing that ionic composition and interactions between different ions determine the toxicity of a saline solution.

Water hardness has also been shown to play a highly significant role in the overall toxicity of salts (Cowgill and Milazzo 1991; Heijerick et al. 2003; Kennedy et al. 2005; Davies and Hall 2007; Soucek et al. 2011; van Dam et al. 2010). Water quality guidelines that are based on the ions present in the anthropogenic inputs and background ionic composition of receiving waters may be more appropriate than guidelines based on salinity or EC alone (Prasad et al. 2012). However, salinity or EC is much easier to measure *in situ* than ionic composition profiles.

Previous work has also shown that mine water salinity and ion composition in the Fitzroy catchment can vary widely due to spatial, seasonal and long term changes to rainfall inputs, spatial variations in geology, soil, overburden and tailings from mining activity, as well as mine water management strategies (Moran et al. 2006; Côte et al. 2007; Gozzard et al. 2009; Vink et al. 2009). Hence, it may be necessary to take into account ambient water types with respect to naturally-occurring ions, rainfall and runoff, and temporal and spatial salinity changes, before the impacts of an increase in salinity on macroinvertebrates can be determined. Furthermore, while accounting for ionic composition it may be important to not only determine what ions are present but also how much of each ion type is present.

A preliminary exploration of data to determine what dilution would be required to keep the salinity below the toxic levels for Leptophlebiidae and Baetidae (determined in this study), shows (Figures 3 and 4) that mine waters would have to be highly diluted. Freshwater is a scarce commodity in the mining areas of the Fitzroy catchment and considering the variety of needs such as mining, power generation, primary production, human consumption and recreation, the availability of more water is highly unlikely. Water management is a serious issue for mining operations (Côte et al. 2010) and this study contributes to our knowledge relating to dilution levels required to ameliorate saline mine waters to make them suitable for safe discharge into streams.

However, the plots presented in Figures 4 and 5 are only useful to obtain dilution values within the range of concentrations of the solutions used in the test. To obtain dilution factors at lower or higher concentrations, testing has to be done with those ranges of concentrations included in the testing regime. Also, more test data would provide more robust point estimations and a stronger regression model. Additionally, more testing at lower concentrations of test water would help in determining dilution values for 90% or 95% survival.

During this study, it was found that the abundance of individuals in all of the tested families declined rapidly at the Nebo Creek collection site when the water levels became low and flow stopped. This suggests that freshwater biota may be under natural stress due to flow variations in the stream. Most of the streams and headwater portions of Australian rivers are ephemeral, resulting in a series of disconnected pools with very little to zero flow at various times of the year. When aquatic systems are reduced to pools, salinity in these pools can rise rapidly as result of evaporation. At times of low water levels, macroinvertebrate taxa such as Ephemeroptera and Trichoptera may be less tolerant to the additional anthropogenic influences that would increase the salinity and alter the ionic composition of a stream.

CONCLUSIONS AND FUTURE RESEARCH

Differences in ionic composition are likely to influence the toxicity of saline solutions to macroinvertebrates in the Fitzroy basin. The LC50 values of AMW2 were slightly lower than those of AMW1 for both Leptophlebiidae and Leptoceridae. AMW2 contained higher proportions of Mg and Ca ions. It is known that a high ratio of Ca to Mg reduces Mg toxicity.

The modelled LC50 values derived in this study could potentially be used as the basis of setting guidelines for mine water discharge in the Fitzroy Catchment. However, it would be desirable to derive water quality guidelines for individual ions.

In this study of acute toxicity tests with mine waters of differing ionic compositions, the LC50 values indicate that mine effluent discharges into natural aquatic systems would require substantial dilution to avoid deleterious effects among sensitive aquatic taxa. Chronic toxicity testing that accounted for the full life-cycles of these taxa would likely generate toxicity measures indicative of even greater sensitivity, and would point to a need for even greater levels of dilution of mine discharges to avoid deleterious effects among sensitive aquatic taxa. Furthermore, the risks are likely to be greater during times of drought or low flow in ephemeral streams, because aquatic biota will be living closer to their sensitivity thresholds.

Further research on the ionic compositions of effluents and of receiving waters, with a focus on reducing toxicity by manipulation of ionic compositions, may lead to optimisation of effluent treatment prior to discharge. More research is also needed to examine the relationship between dilution prior to release, mixing in receiving waters and the eventual toxicity of saline water discharged into the streams and rivers of the Fitzroy catchment.

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