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RESEARCH PAPER

ASSESSING THE TOXICITY OF SALINE WATERS: THE IMPORTANCE OF ACCOMMODATING SURFACE WATER IONIC COMPOSITION AT THE RIVER BASIN SCALE

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ABSTRACT

Salinity impacts in freshwater ecosystems are a concern in Australia and around the world. There is a need for greater understanding of the salinity tolerance thresholds of freshwater biota to support the derivation of water quality guidelines that form the basis of policy and regulation aimed at managing salinity impacts. The salinity of freshwater is a mixture of ions (including Na^+, Ca^{2+}, Mg^{2+}, K^+, SO_4^{2-}, CO_3^{2-}, HCO_3^-, and Cl^-) that vary according to factors such as underlying geology and surface/ground water interactions. The composition and concentration of ions is known to affect toxicity making it important to account for such variation when designing toxicity tests used to define water quality guidelines. Test exposures using standard solutions such as marine salts may not be representative of many freshwaters, so may have limited applicability. This study defines a test exposure for salinity based on observed ionic composition at the river basin scale and evaluated the 96-h (acute) response of 10 macroinvertebrate families. It is proposed that such an approach may provide a useful means of defining test exposures for salinity where the aim is to define trigger values for the management of diffuse sources of salinity at a river basin scale.

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INTRODUCTION

Soluble salts occur naturally in freshwater, although elevated concentrations and prolonged periods of exposure may result in negative ecological impacts. Salinity refers to the presence of soluble salts in soils or water and the term 'salinisation' refers to the process of soluble salts accumulating in soils or waters (DNR 1997). Salinisation is linked to natural landscape processes, though it can be affected by human activities resulting in both point and diffuse sources of salinity. Because soluble salts accumulate in the landscape, salinity has become an increasing problem in some parts of Australia (Land and Water Australia 2002) and around the world (Cañedo-Argüelles et al. 2013).

National and State water quality guidelines provide a basis for the regulation and management of potential environmental contaminants. For toxicants, which encompass a large suite of metals, metalloids, inorganic ions and organic compounds, environmental guideline values are based on biological effects data (i.e. toxicity testing) (ANZECC and ARMCANZ 2000). However, for salinity there are no biological effect-based trigger values that have been adopted at national or regional scales in Queensland (DEHP 2009), Australia (ANZECC and ARMCANZ 2000) or around the world. For example, in Europe there are no prescribed environmental quality standards for salt under the European Water Framework Directive (European Commission 2000). Similarly, in South Africa, guidelines for freshwaters do not currently classify salts as toxicants (Cañedo-Argüelles et al. 2013). Although there are no biological effect-based trigger values in Australia, there are guidelines that are based upon reference data collected from recognised reference sites. For those reference-based guidelines, the trigger value is determined as the 80th percentile of reference site monitoring data (see pages 3.3-10 to 3.3-16 of ANZECC and ARMCANZ 2000) or alternatively, the 75th percentile in Queensland (DEHP 2009). Such triggers provide a useful characterisation but often lack spatial resolution, which limits their application. In order to improve the spatial resolution of reference-based guidelines, more data would be required from sites in reference condition. Such locations are increasingly difficult to identify, particularly in lower parts of catchments that may already be impacted by development. As salinity is expected to increase longitudinally along the length of a stream, salinity concentrations observed in the upper parts of catchments may not be reflective of what is observed in the lower parts of catchments. An alternative approach is to define biological effect trigger values based on toxicity testing. Such an approach is preferred under the hierarchy of approaches in the national guidelines (ANZECC and ARMCANZ 2000).

Existing toxicity data that may be used to evaluate the effect of increasing salinity include those describing the effect of individual salts such as data reported in the ECOTOX database (U.S. EPA 2013), and to a lesser extent, multiple salts (Mount et al. 1997; Pillard et al. 2002; Jooste and Rossouw 2002). In these tests salts are typically added to reverse osmosis treated water (i.e. with no other ions present) to derive a test exposure solution. The U.S. EPA ECOTOX database (U.S. EPA 2013) contains a substantial number of citations for salts tested individually. At the time of writing, there were 3347 records for NaCl alone and the database also includes many data records for other salts (U.S. EPA 2013). Although there is a substantial volume of single salt toxicity data, the use of such data may not be representative where multiple salts are present. For example, the results of a study by Kefford et al. (2002) demonstrated NaCl was considerably more toxic to *Daphnia carinata* than standard marine salt. From this it can be inferred that the use of NaCl may overestimate risk for an ionic composition resembling standard marine salts. In contrast, the use of a standard marine salt test exposure may underestimate salinity risk where a specific ion or ions are dominant.

Despite the obvious shortcomings, there is a need for toxicity tests to utilise standardised test formulations, primarily to allow comparison between studies undertaken across geographic boundaries. Marine salts have provided a useful basis for such a purpose and have been used to

evaluate salinity toxicity in a number of studies in Australia, France, Israel and South Africa (Kefford et al. 2003, 2005a, 2006, 2007, 2012; Hassel et al. 2006; Allan 2006; Dunlop et al. 2008; Palmer et al. 2004). As standard marine salts have been identified as having ionic proportions similar to some Australian inland saline waters (Bayly and Williams 1973), it has been regarded as a salt profile that is suitable in representing broadscale salinity increases in natural waters. However, standard marine salts are typically dominated by NaCl and can be lower in HCO_3^- than some freshwaters, suggesting that there may be a need to reassess the appropriateness of this formulation as a surrogate for inland waters that are dominated by high levels of HCO_3^-, Ca^{2+}, or SO_4^{2-} ions. Although the effect of varying test exposures to reflect the ionic composition characteristics of some environmental waters may be expected to influence results, the effects of such variation are not well understood, making it difficult to identify a toxicity data set that may be used to define trigger values for salinity.

As an alternative to laboratory testing data, field observational data of ecological thresholds can be used to assess the risk of salinity (typically measured in the field as electrical conductivity). A study that applied field-observed data to evaluate salinity risk was undertaken by Cormier and Suter (2013a). In that study, field observations of the disappearance of taxa along a gradient of ionic strength were used to identify a threshold of impact at which taxa were rarely observed. This approach was used to develop what the authors of that study refer to as an 'extirpation concentration'. This concept is equivalent to an effect concentration as derived in toxicity test exposures and may be used to derive a species sensitivity distribution to assess risk (Cormier and Suter 2013a). The advantage of using field observational data to derive water quality benchmarks (or triggers) is that it provides improved realism and considers the diversity of ionic compositions present in natural waters, and unlike many laboratory experiments, it provides an indication of the likely response of a community across entire life cycles (Cormier and Suter 2013b). Although such approaches are promising, they rely on observing salinity levels in natural systems that are high enough to result in negative impacts. Such examples are limited and can only be defined once impacts are observed. Furthermore, there is an assumption that field observations of community abundances can be discounted and the observed response is not influenced by other factors.

Modelling the effects of major ion complexes can provide some indication of potential impacts. Attempts have been made to use single salt data to predict the toxicity of complex ionic solutions. The 'toxicologically important major salts' (TIMS) method discussed in Jooste and Rossouw (2002) identifies the dominant ions present in natural waters on the basis of molarity. Once defined, the TIMS are identified based on published effect data from standard test exposures to single salts (e.g. NaCl). This approach is useful to prioritise the potential effects of salts; however, because it uses single salt toxicity data, it may not provide an accurate means to assess mixtures. A study by Mount et al. (1997) developed a salinity-toxicity relationship to predict the effect of salt mixtures. In that study, Mount et al. (1997) investigated the responses of the cladocerans *Ceriodaphnia dubia*, and *Daphnia magna*, and the freshwater fish *Pimephales promelas* to an extensive array (2 900 ion solutions in total) of single salts (e.g. NaCl) and two salts when combined in solution (e.g. NaCl and $MgSO_4$). While this study provided an indication of the toxicity of a complex ionic solution, the authors reported that as the number of cations and ionic strength increased, the predictive capability of the model is reduced. Another study by Pillard et al. (2002) evaluated the toxicity of multiple ions, particularly the effects of bicarbonate, borate, calcium, magnesium and sulfate, to the mysid shrimp *Americamysis bahia* and developed response models to predict the effects of ions. However, that model is only relevant to marine waters, and therefore, has limited application to freshwater. Although each of these approaches provides some insight into the combined effect of ions, existing models have limited capacity to predict the effects of complex ionic compositions and there remains a need for laboratory-based toxicity assessment of salinity impacts.

A more pragmatic approach is to adopt appropriate surrogate salt solutions for laboratory based testing. Test exposures that simulate the specific ionic composition of natural waters provide an accurate approach to assessment of the biological effects of salinity and the determination of trigger values. However, such an approach is unlikely to be practical given the extensive number of potential combinations of ionic compositions present in natural waters. In seeking a balance between the need to evaluate representative test exposures and to provide a standardised basis for toxicity testing to derive trigger values for salinity, this study evaluates the toxicity of an ionic composition representative of the Fitzroy River basin. To trial this approach, ionic composition data from the Fitzroy River basin were analysed to evaluate the variability within the basin and identify a typical ionic composition. This ionic composition was simulated in the laboratory and used as a test exposure for a series of rapid toxicity tests on macroinvertebrates. The results of this study were compared with previously published toxicity data obtained using a standard marine salt test exposure to give an indication of its relative toxicity.

MATERIALS AND METHODS

The approach used here was firstly, to evaluate the diversity of ionic composition present in the Fitzroy River basin, and secondly, to define a single, representative test solution based on observed ionic composition from existing water quality monitoring data. Then, thirdly, to undertake a series of acute toxicity tests in the laboratory using macroinvertebrates collected from the field.

Analysis of ionic composition in the Fitzroy River basin

A study conducted by McNeil et al. (2005) suggested multi-stage cluster analysis was an effective tool to identify broad patters in anion and cation data because it involves few assumptions about the distribution of variables and reduces the influence of outliers. In this study a *k*-means cluster analysis was used to classify the types of water in the Fitzroy Catchment. This analysis partitions observations based on Euclidean distance according to the equation:

$$Distance\ (p,q) = \sqrt{\sum_{i=1}^{n}(p_i - q_i)^2}$$

Where p and q are the data for each sample and the centroids respectively, n is the number of variables and i is the object. Analyses were performed using long term monitoring data collected by the Queensland Government across the basin at 132 monitoring stations for the period 1962-2008. Samples used in the analysis met the following criteria: a) the data were quality assured, b) the sample fell within an electrical conductivity range of 0.8-1.2 mS/cm, and c) the dataset was complete with respect to all major ions. Details of sampling sites are available from the Queensland Government Water Monitoring data portal (https://www.dnrm.qld.gov.au/water/water-monitoring-and-data/portal). The Queensland Government Monitoring and Sampling Manual (DEHP 2013) details the sampling and quality assurance techniques currently used to collect this data, although methods have been updated since 1962. Data records were only included in the analysis where they included data for electrical conductivity (EC) and the concentrations of all major cations (K^+, Na^+, Ca^{2+}, Mg^{2+}) and anions (Cl^-, SO_4^{2-}, HCO_3^-). All samples were collected during low to moderate flow periods and largely represent base flow conditions.

Definition of saline test solution

The salt solution used in toxicity testing was representative of an ionic composition typical for the Fitzroy River basin. This composition was defined as the proportion of ions most frequently observed in the available monitoring data calculated in mEq/L. A frequency distribution was defined for each major anion and cation in 1% data bins. This frequency distribution was used to

identify the percentage at which each major ion was observed to occur with the greatest frequency. The typical ion proportions were compared with the groups defined in the cluster analysis and an ionic proportion deemed to be broadly representative of the Fitzroy River basin was identified as approximately 20% Na^+, 16% Ca^{2+}, 14% Mg^{2+}, 0.5% K^+, 25% HCO_3^-, 22% Cl^-, and 2.5% SO_4^{2-}. These ionic proportions were used to define the base salt solution used for all ecotoxicology experiments.

Preparation of test solutions

Test solutions were prepared by adding analytical grade dry salts to high purity (Milli-Q®) laboratory water to achieve the desired ionic composition for each test. The final ionic composition used in toxicity testing is subsequently referred to as the Fitzroy Composition (FC) as shown in Table 1. The concentration of Mg^{2+} in the initial FC test solution was found to be above the water quality trigger value of 2.5 mg/L suggested by Van Dam et al. (2010). The FC test solution was subsequently re-formulated with Mg^{2+} concentrations below the van Dam et al. (2010) Mg^{2+} water quality guideline to avoid Mg^{2+} related toxicity. This solution is referred to as Fitzroy Composition with Low Magnesium (FCLMg) and its ionic composition is shown in Table 1. Comparisons of the toxicity of test solutions were made with a standard marine salt ionic composition (Ocean Nature™ manufactured by Aquasonic Pty Ltd.). The ionic proportions of each solution are given in Table 1 to allow comparison with the ionic composition of test solutions.

The concentrations of salts added to deionised water to create stock solutions of FC and FCLMg at 10 mS/cm are shown in Table 2. Intermediate treatment concentrations were achieved by either diluting stock solutions with deionised water to reduce salinity to the desired concentration or where higher test concentrations were used, the ionic strength was increased by adding a greater concentration of salts at the proportions described in Table 1.

Chemical analysis of test solutions

Analyses of test solutions were performed in the School of Agricultural and Food Sciences Waters Laboratory at the University of Queensland in accordance with standard American Public Health Association methods (APHA 2005). Major ions (cations and anions) of test solutions were determined using samples of the stock solution. Analyses were performed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (method 3125B), titration (method 2320B) and Ion Chromatography (IC) (method 4110B). Electrical Conductivity (method 2510) and pH (method 4500-H+B) were determined by electrometric methods.

Collection and taxonomic identification of test organisms used in toxicity testing

Benthic invertebrates were collected in the field and transported live to the laboratory where they were sorted into nominal taxonomic groups, then transferred to test treatments within 24 h of

Table 1. Comparison of the ionic composition of six water types including, 1) the initial Fitzroy composition (FC), 2) the Fitzroy Composition with Low Magnesium (FCLMg), and 3) Marine salts (Ocean NatureTM manufactured by Aquasonic Pty Ltd.).

Typical water type	Percent contribution to total ionic strength (determined from concentration in mEq/L)						
	Na^+	Ca^{2+}	Mg^{2+}	K^+	HCO_3^-	Cl^-	SO_4^{2-}
FC	18.4	16.8	14.4	0.4	18.4	26.8	4.8
FCLMg	23	21.1	5.4	0.6	16.3	28.1	5.6
Marine salts	38.7	1.7	8.9	0.8	0.2	45	4.7

collection. All taxa were tested at the same time, and each was tested in separate test chambers. Test organisms were collected from seven sites, four in the Fitzroy River basin, and three within southeast Queensland (Table 3). Sites were selected in the Fitzroy River basin to determine effects of salinity increase using locally collected organisms. Sites were also selected in Southeast Queensland to broaden the range of species tested and allow comparison of sensitivity between this and previous studies. All sites were deemed suitable to collect biota for testing as they were unaffected by mining and not adversely affected by agricultural impacts. The locations, observed EC and pH conditions at the time of sampling are presented in Table 3. All sampling sites had relatively low EC (≤ 670 µS/cm) at the time of collection. Collection for these tests occurred in March and April of 2010 (see Table 4 for test details). Due to its proximity to the laboratory, the majority of collections were from the Pimpama River in Southeast Queensland, although some individuals were collected from the Fitzroy Basin. In total, individuals representing ten macroinvertebrate families were collected from the five sites. Macroinvertebrates used in testing were collected over ten sampling occasions. After testing, sub-samples of ten individuals from each group were preserved and identified to the lowest possible taxonomic classification by a taxonomist from the Water Planning Ecology group within the Queensland Department of Science, Information Technology, Innovation and the Arts. For quality assurance purposes, 10% of species were re-identified by a different taxonomist, and in addition the identifications were verified by nationally recognised experts.

Table 2. Concentrations of analytical grade salts (mg/L) added to deionised water to generate test dilution waters for the Fitzroy Composition (FC) and the Fitzroy Composition with Low Magnesium (FCLMg) at 10 mS/cm.

Salt	Concentration of salt (mg/L)	
	FC	FCLMg
$NaHCO_3$	5.6	6.25
$MgSO_4.7H_2O$	2.2	0
$MgCl_2.6H_2O$	3.5	0.31
$CaCl_2.2H_2O$	4.5	6.05
KCl	0.11	0.12
$CaSO_4.2H_2O$	0	1.2
$Na_2SO_4.10H_2O$	0	1.02
NaCl	0	1.42

Laboratory testing methods

The toxicity of the test solutions was assessed using short term (96-hour) static, acute macroinvertebrate toxicity tests using the rapid assessment approach described in Kefford et al.

Table 3. Geometric means of electrical conductivity and pH as well as details of the sites from which macroinvertebrate samples were collected.

Catchment name	Site name	Latitude (°S)	Longitude (°E)	Electrical Conductivity (µS/cm)*	pH*
S.E. Qld	Pimpama River	27.82	153.22	405	7.3
	Colleges Crossing	27.55	152.80	321	6.6
	Moggill Creek	27.48	152.88	461	7.5
Fitzroy basin	Parrot Creek	22.90	148.63	670	8.3
	Roper Creek	22.87	148.67	140	7.6
	Middle Creek	22.31	148.20	423	8.0
	Isaac River	21.73	148.01	337	8.0

**In-situ measurements at the time of collection. Where sites were sampled on a number of occasions the geometric means of water quality values are shown.*

(2003, 2005b) and Dunlop et al. (2008) and in accordance with ASTM E729-96 (ASTM 2007). The test endpoint, mortality, was defined as an absence of response by the test animals to gentle prodding. In some instances, flying adults emerged during the tests or were missing due to cannibalism and were consequently excluded from analysis. Tests were undertaken at 25 ± 5 °C. Light conditions were <800 Lux with a 16:8 hour light, dark cycle. All tests were undertaken as static non-renewal tests. Control treatments included river water from the collection locations and were used to confirm that the observed toxicity responses were caused by the test treatment solutions alone, and not as a result of other factors such as stress consequent to relocation to the laboratory. Most tests were un-replicated and included a minimum of five treatments and a control according to the rapid assessment approach described in Kefford et al. (2003, 2005b). Some additional tests were performed using three replicates of a minimum of five treatments and a control.

Statistical analysis of toxicity test data

Acute 96-h LC50 concentrations were generated using log-logistic and probit analyses with log-logistic regressions. A probit regression was used to define LC50 values for tests where only one replicate was available and log-logistic regressions used where there was more than one replicate. Log-logistic analyses were performed using R version 2.1.1 (Venables and Ripley 2002) and probit regressions were performed using ToxCalc™ (ver. 5.0.23F, Tidepool Scientific Software). The distribution of mortality versus concentration was modelled against electrical conductivity as the independent variable and assuming a continuous concentration-response relationship. All 96-h LC50 data were expressed as electrical conductivity (mS/cm). Where there were insufficient data to derive a reliable estimate of the 96-h LC50 using regression approaches, an approximate estimate of the LC50 was identified. Those estimates are presented as censored data where the LC50 represents the test treatment at which all test animals were observed to survive (i.e. > the highest test treatment where 100% survival was observed, or < the treatment at which 100% mortality was observed). Where a comparison was made between the responses of test organisms to two separate test solutions, a three parameter logistic regression was used to compare percentage of mortality responses to test solutions using SigmaPlot 12.5.

Comparison of toxicity data from the current study with previously published results for marine salts

The relative toxicity of the FC test solution was compared to results presented for standard marine salts in Dunlop et al. (2008). The comparison was made between tests where the same methods were used but undertaken at different times. Previous studies have found limited variation between

Table 4. Details of the catchment, site name, test code, date of collection from the field and test water for each of the toxicity tests using the Fitzroy Composition (FC) and Fitzroy Composition with Reduced Magnesium Concentration (FCLMg).

Catchment name	Site name	Test code	Date collected	Test water
S.E. Qld	Pimpama River	PR1	15/03/2010	FC
	Pimpama River	PR2	10/05/2010	FCLMg
	Pimpama River	PR3	30/08/2010	FCLMg
	Colleges Crossing	CC1	29/03/2010	FC
	Moggill Creek	MC1	06/04/2010	FC
Fitzroy basin	Parrot Creek	PC1	13/04/2010	FC
	Roper Creek	RC1	13/04/2010	FC
	Middle Creek	MC1	17/05/2010	FCLMg
	Isaac River	IR1	17/05/2010	FCLMg
	Isaac River	IR2	17/05/2010	FCLMg

collection episodes relative to the variation between species tolerance (as discussed in Kefford et al. 2003, 2005a,b). Therefore, this type of comparison is appropriate given that the variability between data sets is expected to be minimal. It was not possible to compare sensitivity at the species level. Therefore, comparison with the Fitzroy composition was made where a matching sensitivity value was available at the family level of taxonomy. In some cases, toxicity data for standard marine salts reported in Dunlop et al. (2008) were available for multiple genera/species within a family. In these instances, each LC50 value was reported to show the range of reported responses within a family.

RESULTS

Ionic composition of the Fitzroy River basin

Results of the *k*-means cluster analysis used to evaluate the patterns in ion composition in the Fitzroy River basin are shown in Table 5. Six water types (types A-F, Table 5 and Figure 1) were identified.

Toxicity of salt solutions

The results of toxicity testing with the FC and FCLMg are shown in Table 6. A comparison with standard marine salt was possible for six macroinvertebrate families. Each of these showed the FC solution had greater toxicity than standard marine salts (see Table 6).

DISCUSSION

Clustering of sites according to their ionic composition showed that the Fitzroy River basin could be divided into six groups. Water type F is known to be affected by post-mining impacts from the Dee River and was excluded from consideration. The remaining water types were generally dominated by Na^+ and Cl^-/HCO_3^- (Table 5). Proportions of Cl^- and HCO_3^- were variable with water type D high in Cl^-. Water type A had approximately equal proportions of Cl^- and HCO_3^- and groups B, C and E were high in HCO_3^-. The proportions of Ca^{2+} and Mg^{2+} were generally lower than Na^+, but Ca^{2+} dominated in water type E. The proportion of K^+ and SO_4^{2-} were consistently low and did not vary greatly between water types.

Plotting the cluster membership of samples recorded at each site allowed the spatial patterns in ionic composition to be identified between sites and shows the variability observed at each site. The spatial distribution of these water types in the Fitzroy basin is shown in Figure 1. On the whole, an east-to-west gradient was observed with higher inputs of Na+ and Cl- salts towards the coast and weathering of underlying strata producing HCO_3-, Ca^{2+} and Mg^{2+} in the west. A number of factors are likely to influence observed differences in ionic composition including the stage of flow that

Table 5. Ionic composition (on a percentage basis) of the six different water types identified in the Fitzroy basin by a k-means cluster analysis (the occurrence of these water types at water monitoring sites in the Fitzroy River basin is shown graphically in Figure 1).

Water Type	Percent contribution to total ionic strength (determined from concentration in mEq/L)						
	Cations				Anions		
	Na+	Ca^{2+}	Mg^{2+}	K^+	HCO_3^-	Cl^-	SO_4^{2-}
A	21	14	14	0.8	22	25	3
B	20	15	14	1.7	29	17	3.5
C	21	17	10	3.3	36	11	2.2
D	25	10	14	0.6	12	33	4.6
E	14	20	14	2.9	41	6.3	1.6
F	12	15	22	0.4	6	9.8	35

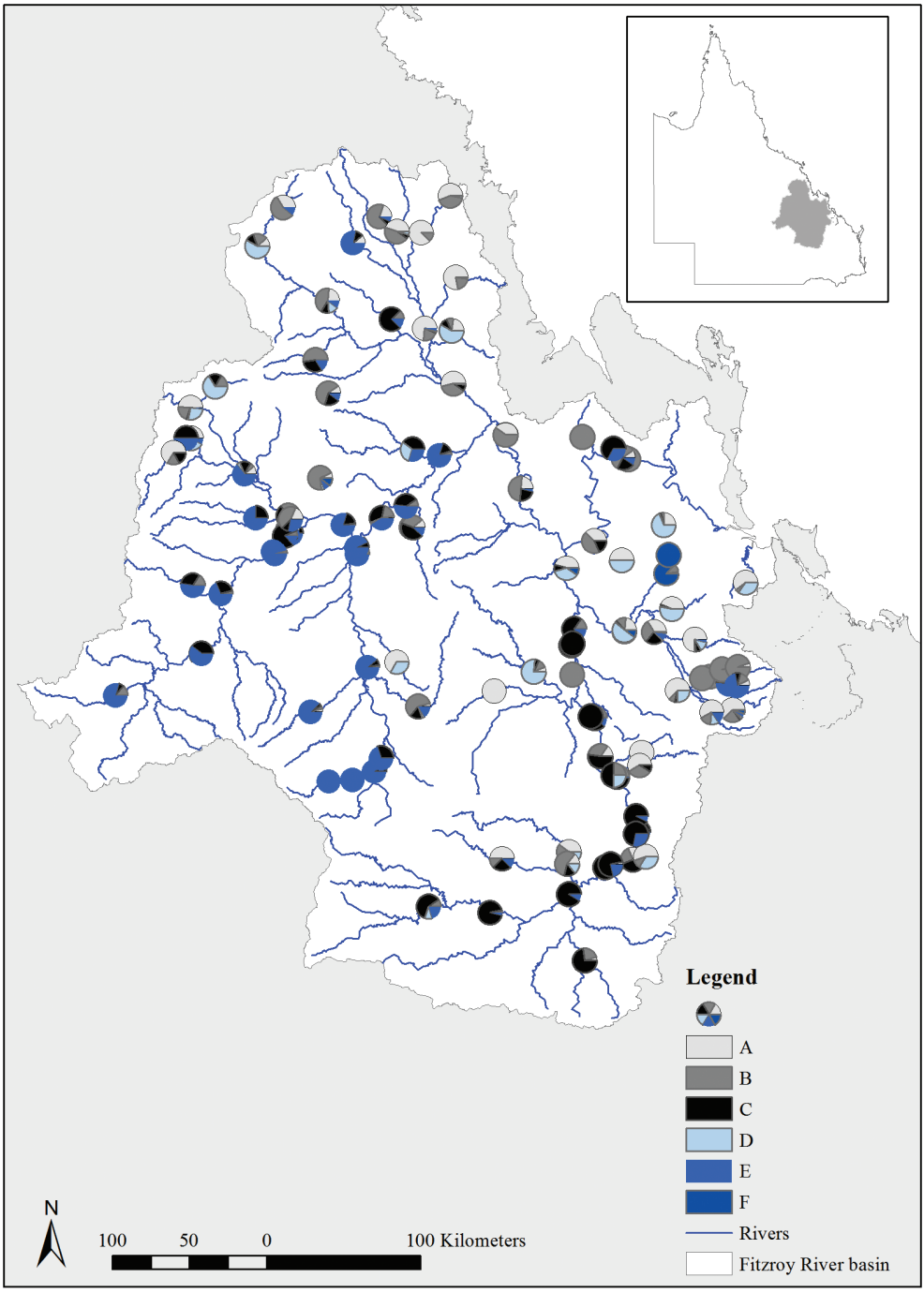

Figure 1. Location of the Fitzroy River basin and its major tributaries and the spatial distribution of water types in the Fitzroy River basin. Each pie chart represents the proportion of observations for which a particular water type was present at each monitoring station in the catchment (refer to Table 5 for a description of water types A-F).

samples were collected, whether the monitoring stations were on the main river channels or smaller tributaries, proximity to coast, underlying geology and localised impacts for point sources. Although there are many factors that influence the ionic composition of surface waters, overall there were distinct differences between waters of the Fitzroy River basin and standard marine salts. The key differences were that the Fitzroy River basin generally had lower Na+ and Cl-, and higher HCO_3- and Ca^{2+} than marine salts (Table 1).

Family level comparisons of the observed LC50 values for the FC solution and those for the Marine Salt (MS) test solution indicated that the FC solution was more toxic for three tests using Leptoceridae, two out of three results using Baetidae, and four using Atyidae (Figure 2).

At the genus level, comparisons between the *Triplectides* sp. (Leptoceridae) showed that the LC50 values for MS solution were up to three times higher than that reported for FC solution. Logistic regressions showing the shape of the dose response curves observed for *Triplectides* sp. to both FC and MS are shown in Figure 3. An exception was for *Cloeon* sp. (Baetidae) collected in the Wet Tropics, that had an LC50 with confidence intervals overlapping that of *Cloeon fluviatile* response to the FC of 6.24 mS/cm (5.92-6.56). However, it is possible that the species tested in the Wet Tropics may be different to the species or sub-species collected in Roper Creek within the Fitzroy River basin.

Although censored data (i.e. where estimates of LC50 are given as > or <) are more approximate and allow less reliable comparisons, it was possible to make comparisons using these data. Comparisons using this data showed the FC exhibited greater toxicity than MS for Hydrobiidae, Sphaeromatidae, Palaemonidae and Atyidae (Table 6). Although based on the information presented, there would appear to be differences between the toxicities associated with the ionic compositions and there is a need to view these results with some caution. It is possible that the observed differences may be attributable to species or sub-species differences or as a result of variation between tests undertaken at different times. However, any difference between species within a genus is unlikely to be large. A study by Dunlop et al. (2008) of the salinity tolerance of a large number of macroinvertebrate taxa showed the variation between genera was greater

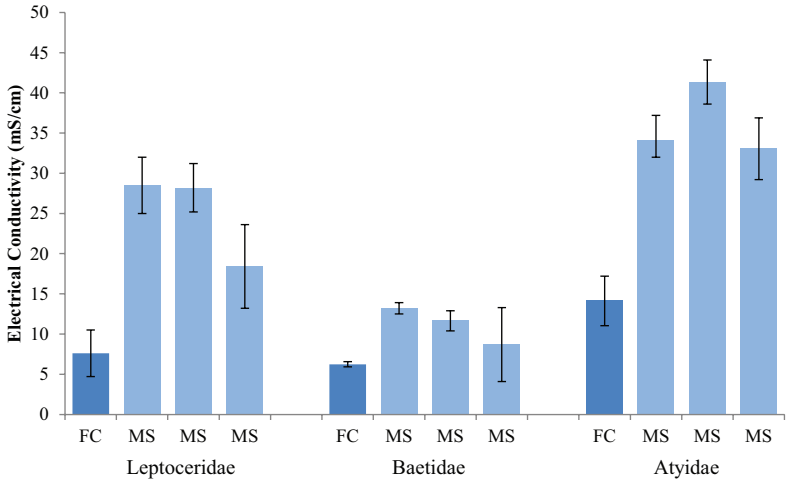

Figure 2. Family level comparison of LC50 results for the Fitzroy Composition (FC) and standard Marine Salts (MS) with error bars showing the upper 95th and lower 5th confidence limits of LC50 estimates. Numerical data taken from Table 6.

Table 6. Results of 96-h acute macroinvertebrate toxicity tests using the Fitzroy Composition (FC) and the Fitzroy Composition Low Magnesium (FCLMg) and FCLMg with increased sulfate.

Test Code	Order/Sub-Order	Family/ Sub-Family	Genus/Species	LC50 (mS/cm)[1]				
				FC[1]	No. individ[2]	FCLMg	No. individ[2]	Marine salt[3]
PR1	Integripalpia	Leptoceridae	*Triplectides* sp.	7.60 (4.70-10.5)	42			*Triplectides australis* 28.5 (25.0-32.0) and
MC1	Integripalpia	Leptoceridae				15.6 (12.5-18.7)	24	28.2 (25.2-31.2) *Westriplectides angelae* 15.1
IR2	Integripalpia	Leptoceridae	*Triaenodes* sp.			13.8 (9.8-17.9)	35	(31.2-34.3) *Triplectides* sp. 18.4 (13.2-23.6)
PR1	Ephemeroptera	Leptophlebiidae	*Atalophlebia* sp. AV 13	7.65 (5.76-9.55)	70			no previous data
PR2	Ephemeroptera	Leptophlebiidae	*Atalophlebia* sp. AV 13			9.93 (7.6-12.2)	70	
RC1	Ephemeroptera	Caenidae	*Tasmanocoenis* sp.	<4.97	9			*Tasmanocoenis wundacaenis* 13.1 (12.4-13.8)
PC1	Ephemeroptera	Caenidae	*Tasmanocoenis* sp.	<0.67	9			
IR1	Ephemeroptera	Caenidae	*Tasmanocoenis* sp.			8.8 (5.0-12.5)	20	
RC1	Ephemeroptera	Baetidae	*Cloeon fluviatile*	6.24	28			*Cloeon* sp. 13.2 (12.5-13.9), 11.7 (10.4-12.9)
MC1	Ephemeroptera	Baetidae	*Cloeon fluviatile*	(5.92-6.56)		>5.11	25	and 8.7 (4.1-13.3)
MC1	Monotocardia	Hydrobiidae	*Posticobia brazieri*	6.17 (4.59-7.74)	60			
CC1	Monotocardia	Hydrobiidae	*Posticobia brazieri*	(>0.32 <10.8)	59			Hydrobiidae >15, >20 and ~12
PR2	Monotocardia	Hydrobiidae	*Posticobia brazieri*	>25.2	60			
PR1	Coleoptera	Hydrophilidae	*Berosus* sp.	<22.1	12			*Berosus* sp. 29 (24.3-34.7)
MC1	Coleoptera	Dytiscidae	*Sandracottus* sp.			>26.6	14	*Necterosoma* sp. 37.4 (34.1-30.6)
RC1	Diptera	Ceratopotoninae		>11.6	21			no previous data
MC1	Diptera	Ceratopotoninae		>19.9 (~26.6)	19			
MC1	Isopoda	Sphaeromatidae		(>0.32 <25.8)	60			*Cymodetta* sp. >50
CC1	Decapoda	Palaemonidae		>25.8 <30.4	6			*Macrobrachium australiense* 42.5 (40.3-44.7)
MC1	Decapoda	Atyidae		14.12 (11.05-17.19)	35			*Caridinides wilkinsi* 34.2 (32.0-37.2), 41.3
				(>25.8 <40.2)				(38.6-44.1) and 33.1 (29.2-36.9) *Paratya*
CC1	Decapoda	Atyidae			5			*australiensis* 34.2 (31.2-37.2)
PC1	Hemiptera	Notonectidae	*Paranisops inconstans*	>11.6	21			*Nychia sappho* 10.8 (5.7-16) and >20
IR1	Hemiptera	Notonectidae				17.5 (4.7-30.3)	20	
IR2	Hemiptera	Notonectidae	*Paranisops inconstans*			>19.8	24	
IR2	Coleoptera	no data		>25.4	15			Dytiscidae: *Necterosoma* sp. 37.4 (34.1-30.6)
								Berosus sp. 29 (24.3-33.7) Psephenidae:
								Sclerocyphon type F 23.4 (18.8-28.0)

Note: [1]Upper and lower confidence limits are shown in parentheses. Where > or < symbols or where the ~ symbol are the LC50 is a censored data point (i.e. non-modelled estimate). [2]No. Individ refers to the total number of individuals in each test. [3]Marine salt data (shaded) are those reported in Dunlop et al. (2008).

than within them. Although such comparisons at genus level may be reasonable, where there is a desire to make comparisons with greater accuracy, it would be necessary to utilise a paired test design with multiple test species.

As the Mg^{2+} concentration in test treatments of the FC solution was above the trigger value suggested by van Dam et al. (2010), it was thought that the Mg^{2+} concentration may have increased the toxicity compared with the standard marine salt solution. If this were to occur, it may accentuate any observed differences between the FC and MS solutions. A comparison of toxicity data for the FC and FCLMg showed there were differences between the responses of test biota for some but not all taxa tested (Table 6). The data presented in Figure 4 shows overlapping confidence intervals for one of two results for Leptoceridae, one for Leptophlebiidae and one for Baetidae.

While this suggests there were no differences between the test solutions, there was an exception for one result for Leptoceridae. A possible explanation for the observed differences in LC50 between the two test solutions (i.e. for one of the Leptoceridae comparisons) is that the difference in toxicity may have occurred because the comparison was made between two different genera of Leptoceridae. As differences between the FC and FCLMg test solutions were not consistently observed between genera, it is likely that ionic strength rather than the proportion of Mg^{2+} dominated the toxicity of the FC solution.

CONCLUSIONS

The ionic composition of the Fitzroy River basin was shown to be different from standard marine salts. In general it had lower sodium and chloride, and higher bicarbonate and Ca^{2+} than marine salts. The results of toxicity tests demonstrated that the Fitzroy basin test solution was more toxic than standard marine salts, suggesting that marine salts may not adequately represent salinity effects across the basin. It is possible that differences between test solutions were due to the magnesium

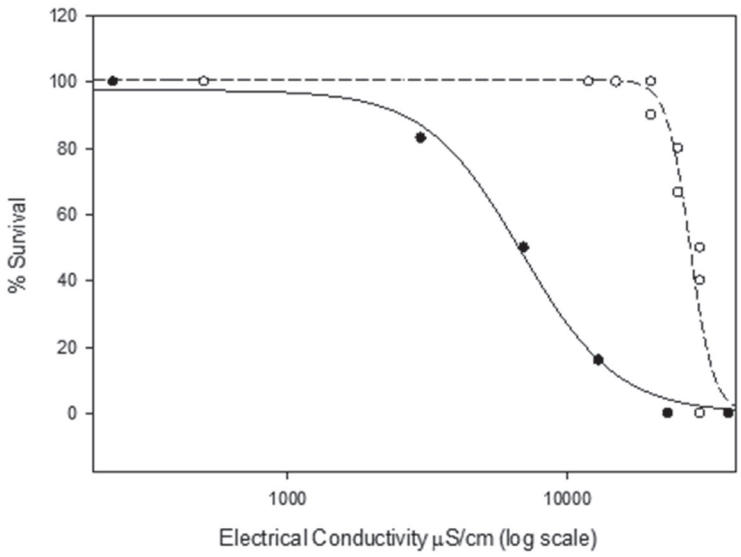

Figure 3. Percent survival of Leptoceridae (*Triplectides* sp.) as a function of increasing electrical conductivity exposed to the Fitzroy composition (solid line and filled circles, r=0.99, df= 7, p<0.0001) and marine salt reported in Dunlop et al. (2008) (dashed line and unfilled circles, r=0.94, df= 15, p<0.0001) showing three parameter logistic regressions.

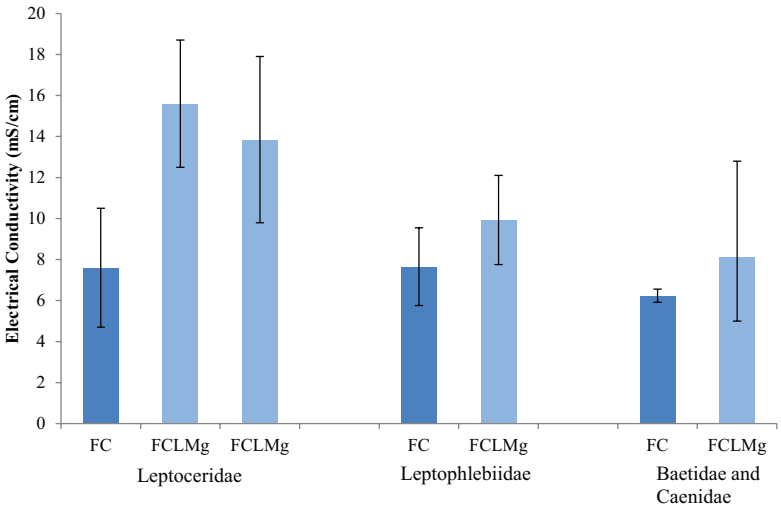

Figure 4. Family level comparison of LC50 results for the Fitzroy Composition (FC) and Fitzroy Composition with Low Magnesium (FCLMg) with error bars showing the upper 95th and lower 5th confidence limits of LC50 estimates. Numerical data taken from Table 6.

concentration of the test solution used to represent the Fitzroy River basin, though differences in toxicity to a solution with lower magnesium proportion were not conclusive. Although further comparative testing is recommended, the use of a standardised test solution that is representative of a regional ionic composition is likely to provide more realistic test conditions and help to overcome the shortcomings of using a standard test exposure such as marine salts or NaCl.

Although the use of an observed ionic composition may be a useful approach to assess salinity impacts and to determine water quality guidelines, before such an approach can be adopted, there is a need to consider further what variation in ionic composition should be represented in testing. The approach used here was to define a single composition at the basin scale. This approach represents a simplification of the complexity in ionic composition present across this river basin. It would be possible to simulate and test a greater number of ionic proportions from across this basin, though this would increase the number of test solutions requiring consideration. Given that the toxicity of saline waters is influenced by hardness, and specifically Ca^{2+}, it is suggested that a useful approach to future testing would be to consider the potential differences in toxicity associated with the east to west gradient where the proportion of Ca^{2+}, Mg^{2+}, and HCO_3^- is higher in the west of the basin. It may also be appropriate to define test exposures according to broadscale patterns in ionic composition similar to those reported in McNeil et al. (2005). While testing additional ionic compositions may be useful, there is likely to be a need for further testing to evaluate the potential for variation in toxicity that may be associated with the observed variations in ionic compositions present in surface water at different scales.

The results of this study showed variation in species tolerance to solutions with the same ionic composition. This was in agreement with a study by Zalizniak et al. (2006) that showed sub-lethal responses to waters with differing ionic composition was species dependent. A study by Mann et al. (2014) also showed that exposure to two different mine waters with different salinity profiles resulted in varying toxicity between species, but interestingly, these differences did not greatly influence resultant species sensitivity distributions for electrical conductivity. Although there may

be differences between the toxicities of test solutions with varying ionic compositions, and this is an important consideration for the derivation of trigger values for salinity, it is also important to ensure an appropriate suite of tests and test end-points are used to define trigger values. In some cases, differences between species may be a greater determinant on resultant trigger values for a given ionic composition than the differences between ionic compositions.

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