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RESEARCH PAPER

ARE VARIATIONS IN IONIC PROPORTIONS IMPORTANT FOR THE DERIVATION OF TRIGGER VALUES FOR SALINE MINE DISCHARGE WATERS?

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ABSTRACT

The discharge of saline waters that accumulate in mine pits following seasonal rain presents a toxicity testing challenge, because it is the salt profile of the water that is of concern for release to natural waterways. Recent studies designed to establish site specific electrical conductivity (EC) trigger values through the generation of species sensitivity distributions (SSD) have aimed to address this challenge. Two test waters representing coal-mine pit-water (AMW1 and AMW2) were formulated with chemical salts and differing in the proportions of various ions (Ca^{2+}, Mg^{2+}, Na^+, K^+, HCO_3^-, SO_4^{2-}, Cl^-). Similarly, diluent waters were formulated based on the salinity profile of receiving waters. The use of formulated waters permitted the comparison of salinity profiles representing different coal mining areas in Queensland on an equal footing, without the confounding effects of other contaminants that would be found in natural waters. However, the use of formulated waters presented some practical problems. On the one hand, consistent salinity profiles needed to be maintained across all tests, thereby providing reliable SSDs for deriving trigger values. On the other hand, artificial mine waters needed to be customised to each test species to ensure that the nutritional requirements of each species (*Pseudokirchneriella subcapitata*, *Ceriodaphnia dubia, Melanotaenia splendida, Macrobrachium australiense, Chironomus tepperi*) were not compromised. The data presented here demonstrate that for individual test-species, the toxicities of AMW1 and AMW2 were quite different, with AMW1 being more toxic than AMW2 in prawns and chironomids, and conversely, AMW2 more toxic than AMW1 in fishes and cladocerans. Overall, the TVs derived for the protection of 80% to 99% of species suggest that AMW1 was less toxic than AMW2. However, despite substantial differences in ionic composition, and despite individual differences in sensitivity among different test species, the opposing toxicity indices in individual test-species resulted in SSDs of similar shape and position when salinity was measured as electrical conductivity.

Keywords: Coal-mine discharge; Direct Toxicity Assessment; Salinity trigger value; Species Sensitivity Distribution; Test standardisation

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INTRODUCTION

In 2008 and 2011, many coal mines in Queensland, Australia, were inundated following unusually high rainfall events (Sharma & Franks 2013). In many cases the salinity of the water in mine pits increased over time to be much higher than the receiving streams. Therefore, discharge of these waters to local waterways was perceived by the government regulator as an ecological risk. In Queensland, the Department of Environment and Resource Management (now the Department of Environment and Heritage Protection) sets discharge limits on all industrial processes in the state, but generally requires biological effects data as a basis for those limits.

Two ecotoxicology approaches can be used to establish the risk to ecosystems from saline mine discharges. One approach is to expose test organisms directly to mine effluents in dose response bioassays using diluent waters collected from a suitable reference site. These kinds of direct toxicity assessments (DTA) provide toxicity information on the overall toxicity of the effluent. However, because mine effluents may contain numerous contaminant types, including various metals and organics, DTA does not necessarily provide information of the effects of salinity alone. Lincoln-Smith et al. (2010) performed DTAs on several mine discharge waters from the Hunter Valley in NSW, and compared the toxicities of these waters with the toxicity of NaCl dominated seawater (Ocean Nature™, Aquasonic). The authors reported a range of toxicity indices for the different mine waters for six different taxa, and were able to interpret the data in terms of tolerance or sensitivity to salinity. In particular, the authors noted that the mine waters were far more toxic than NaCl dominated seawater, suggesting that the compositional profile of the mine waters was an important factor in toxicity as had been demonstrated previously (Mount et al. 1997). However, there was also recognition that the various mine waters also differed in the concentrations of various other contaminants, including Al, Fe, Ba, S, and Zn, and that the observed toxicities could not be ascribed exclusively to the salinity profile of the waters.

The alternative approach, as used in the present study, is to formulate saline waters based on specific salinity profiles, but restricted to those ions that generally dictate salinity (i.e., Ca^{2+}, Mg^{2+}, Na^+, K^+, HCO_3^-, SO_4^{2-}, Cl^-). This approach eliminates the various confounding factors and contaminants that may be present in natural waters. This approach also permits the establishment of trigger values specific to salinity, and the manipulation of compositional profiles that permits the performance of toxicity tests that can discriminate between different salinity compositions. However, this approach also excludes many of the micronutrients necessary for the maintenance of tests species that need to be added as supplements to the exposure media. The challenge for ecotoxicology is to provide consistent compositional salinity profiles across all tests/species, and still provide the necessary supplementation for individual tests/species.

The restrictions on species specific test protocols should not be understated. In Australia, several freshwater species are routinely used for commercial toxicity testing. The protocols that control the consistency of performance of these tests are highly prescriptive, and dictate that diluent waters have specific ionic and nutrient profiles to ensure suitable conditions for each test species. For example, the commercial growth inhibition test employing duckweed (*Lemna* spp.) requires the use of growth media rich in nutrients (OECD 2006). Similarly, chronic toxicity tests with *Ceriodaphnia dubia* (occasionally referred to as *C.* cf. *dubia* or *C. dubia sensu stricto*) rely on a laboratory formulated water to ensure survival over the duration of chronic testing. Early studies indicated that the water that resulted in the highest reproductive output in *C. dubia* was 20% Perrier Water® (France) diluted with filtered Sydney tap water to produce a dilute commercial mineral water (DCMW) and amended with selenium at 2 µg/L and vitamin B12 at 10 µg/L (Bailey et al. 2000b). Commercial tests with this species generally follow variations on this protocol.

The *Australian and New Zealand Guidelines for Fresh and Marine Water Quality* (ANZECC & ARMCANZ 2000) provides a framework for obtaining biological effects data through the application of toxicity testing in conjunction with species sensitivity distributions (SSD). SSD require toxicity data from a range of species that theoretically represent the range of sensitivities of species in aquatic ecosystems. When conducting toxicity tests with the intention of using the results for SSD, the current minimum acceptable data requirement for SSD is a dataset of chronic toxicity data for at least five different species from at least four different taxonomic groups (ANZECC & ARMCANZ 2000). Where commercial testing seeks to establish the toxicity of discrete contaminants, the use of prescriptive test methods across a range of different test organisms is not usually a hindrance. However, where the ionic profile of the test water is the parameter being tested, then problems arise because some test species have specific mineral and nutrient requirements. In this study we examined the toxicity of two artificially formulated mine waters that mimicked two extremes of salinity in order to evaluate how changes in ionic profile can affect, not only the toxicity of saline waters to specific test species, but the numerical value of trigger values (TV) derived through SSD. At the same time we have formulated the test solutions with the added constraint that the nutrient and mineral requirements of each test species needed to be met while retaining consistency of proportional ionic profiles across all test species, thereby ensuring that the SSD is built upon consistent toxicity data.

MATERIALS AND METHODS

Test waters

A survey of mine water ionic compositions was undertaken so as to formulate saline test-solutions that were representative of mine waters in the Fitzroy Basin. Water samples were collected from 10 mine sites (total of 42 release points) across the basin. All water samples were collected during the period 9th - 20th August 2011. Similarly, samples of regional receiving environment creek-water were analysed in order to formulate standard artificial creek water that could be used as a diluent in subsequent toxicity tests. Samples of the commercial mineral water used for culture and testing of *C. dubia* were also analysed for all major anions and cations. Major cation and sulfate concentrations were determined by inductively coupled plasma – optical emission spectroscopy. Chloride was determined by flow injection using the method of Rayment and Higginson (1992). Carbonate, bicarbonate and total alkalinity were determined using Gran Titration (APHA 1995).

On the basis of the analyses of mine waters (see Figure 1), two different artificial mine waters (AMW1, AMW2) were formulated that reflected two extremes of ionic compositions in natural mine waters within the Fitzroy River Basin. Similarly, on the basis of the analyses of creek waters and a dilute commercial mineral water (DCMW), an artificial creek water (ACW) was formulated that reflected the average composition of waters in the receiving environment (see Fig.1). Those compositions are summarised in Table 1.

Test species

Test species that were considered appropriate for the region were selected from commercially available tests offered by Ecotox Services Australasia. The selection of species also met the requirements of ANZECC and ARMCANZ (2000) guidelines for the assessment of toxicants in receiving waters by having at least five species from four trophic levels as part of the testing suite. The suite of tests provided a range of acute and chronic endpoint measurements of toxicity. The use of both the 48-h immobilisation acute test and the three brood *C. dubia* chronic test allowed for the calculation of an acute to chronic ratio (ACR). For this study, a mean ACR of 1.3 was selected. This was calculated from the ACRs determined in the toxicity tests in this study, as well as those of Dunlop et al. (2011) and other unpublished data (ACRs in all studies ranged between
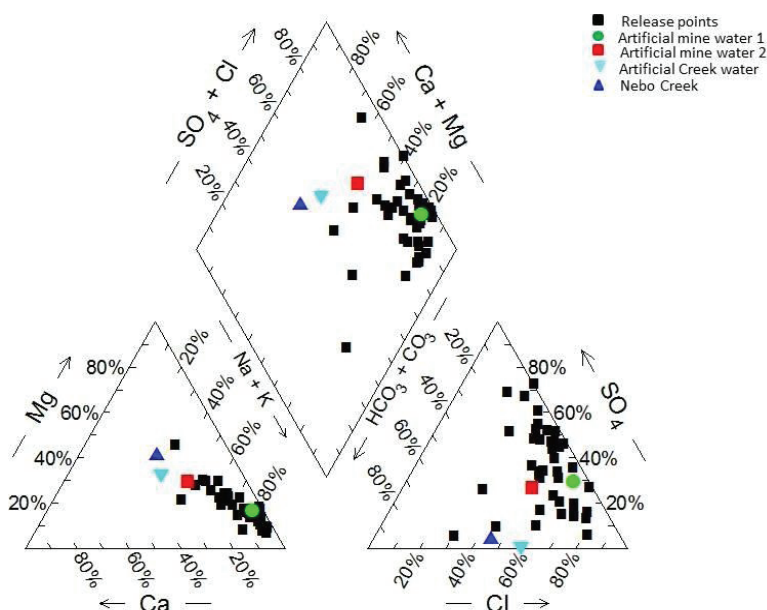
Figure 1. Piper Diagram of mine water composition determined from release points on 10 mine sites (black symbols) located across the Fitzroy Basin. The proportions are calculated as percentage of milliequivalents. The green circular symbol represents the salt composition of AMW1; the red square symbol represents AMW2; the light blue triangular symbol represents ACW (Artificial creek water) and the dark blue triangular symbols represent the natural waters on which ACW is based.

Table 1. Ion composition and pH of test solutions.
AMW = artificial mine water; ACW = Artificial creek water.

	Proportional ionic composition (meq %)							pH
	Ca^{2+}	Mg^{2+}	Na^+	K^+	HCO^{3-}	SO_4^{2-}	Cl^-	
AMW1	2	8	39	0.4	3	15	32	8.4
AMW2	11	15	23	0.4	12	13	25	8.3
ACW	12	17	21	0	29	2	18	8.0

1.0 and 1.5). The ACR was then used to convert the acute data of the fish, prawn, and midge tests to chronic equivalents that were then used to construct an SSD. A description of the tests/species is provided below and a summary of test parameters provided in Table 2.

***Pseudokirchneriella subcapitata* (green alga) growth inhibition test:** Inhibition of growth of marine and freshwater micro-algae has been demonstrated to be sensitive to a wide range of organic and inorganic contaminants (Stauber 1995; Dunlop et al. 2011). *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*) is a freshwater unicellular micro-alga used extensively around the world as a sensitive test species. The 72-h growth inhibition test (cell division test) is based on a U.S. EPA protocol (U.S. EPA 2002) and is described in Stauber *et al.* (1994) with further details in NIWA (1998).

Table 2. Summary of test parameters for the five test species. DCMA = Dilute commercial mineral water; ACW = Artificial creek water; U.S. EPA MHW = U.S. Environmental Protection Agency, Moderately hard water.

Species	Trophic group	Control exposure waters	Test duration & endpoint (statistical analysis)	Test type	Test Temp.	Acceptability criteria for controls	Reference toxicant
Pseudokirchneriella subcapitata	Micro-alga	U.S. EPA MHW ACW	72-h growth inhibition (Linear interpolation)	Chronic	25°C	≥16×104 cells/mL	KCl
Ceriodaphnia dubia	Micro-crustacean (cladoceran)	DCMW ACW	48-h immobilisation (Max likelihood Probit or Log-Logit interpolation)	Acute	25°C	≥90% survival	KCl
Ceriodaphnia dubia	Micro-crustacean (cladoceran)	DCMW ACW	7-d three brood survival (Max likelihood Probit or Log-Logit interpolation) and reproduction (Linear interpolation)	Chronic	25°C	≥80% non-immobilised. An average of ≥15 young per non-immobilised female. 60% of non-immobilised cladocera must produce ≥3 broods.	KCl
Macrobrachium australiense	Macro-crustacean (prawn)	DCMW ACW	96-h larval imbalance (Log-Logit interpolation)	Acute	25°C	≥90% survival	Cu²⁺
Chironomus tepperi	Insect larvae (midge)	DCMW ACW	48-h immobilisation (Log-Logit interpolation)	Acute	25°C	≥90% survival	Cu²⁺
Melanotaenia splendida splendida	Fish	DCMW ACW	96-h larval imbalance (Max likelihood Probit or Log-Logit interpolation)	Acute	25°C	≥90% survival	Cu²⁺

***Ceriodaphnia dubia* (cladoceran) immobilisation test:** The *C. dubia* freshwater cladoceran 48h acute survival test is one of the most commonly used tests to assess the potential harm a toxicant poses to freshwater aquatic ecosystems. The test is based on, and modified from, a U.S. EPA protocol (Bailey *et al*. 2000a; U.S. EPA 2002). The *C. dubia* acute toxicity test has been demonstrated to be sensitive to heavy metals (Hickey 1989; Hall and Golding 1998; Hickey 2000), organics (Mulhall 1997; Rose et al. 1998), and pesticides (Hickey 1989; Julli 1993; Sunderam et al. 1994). The test has been used routinely in Australia for assessing the toxicity of sewage effluents, mine tailings and pulp/paper mill effluents (e.g. Bailey et al. 2000a).

***Ceriodaphnia dubia* (cladoceran) 3-brood reproduction test:** This test measures chronic toxicity to *C. dubia* using neonates during a three-brood, static-renewal test. The method is based on the *Ceriodaphnia* Survival and Reproduction Test developed by the U.S. EPA (U.S. EPA 2002). The test begins with asexually reproductive neonate female freshwater cladocera. The females are transferred daily to fresh solutions of the same concentration. Each day, observations are made on the survival of each female, the number of neonates produced and neonate survival. The test is terminated at 7 or 8 days when three broods have been produced by each surviving control female.

***Melanotaenia splendida splendida* (eastern rainbowfish) larval imbalance test:** This is a freshwater species with a widespread distribution in north-eastern Australia extending down to central Queensland (Allen et al. 2003). This test uses larval fish that are exposed for 96 h under static-renewal conditions. A larval fish is recorded as affected when imbalance is displayed. Imbalance refers to the loss of swimming ability of the fish such that the fish can no longer remain upright. When a fish is observed to be imbalanced, it is immediately removed from the test vessels and euthanised with an anaesthetic such as Aqui-S® or equivalent. Rainbowfish have been used previously in Australia to assess the toxicity of organic compounds (Humphrey and Klumpp 2003) and metals (Holdway 1992).

***Macrobrachium australiense* juvenile survival test:** This test is a 96-h test undertaken with the freshwater prawn, *M. australiense*. This test is based on the U.S. EPA (1996) Penaeid Shrimp Test Protocol. Exposed prawns are checked daily and recorded as affected and removed from the test when imbalance or immobility is displayed.

***Chironomus tepperi* survival test:** *Chironomus tepperi* is widely distributed throughout Australia and can rapidly colonise freshly flooded environments (Stevens 1994). The method is based on OECD Test Method 219: Sediment-Water Chironomid Toxicity Testing Using Spiked Water (OECD 2004). Second-instar larval stages are exposed for 48 h and checked daily and recorded as affected and removed from the test when immobility is displayed.

Valid toxicity data for use in SSD
The current Australian and New Zealand Water Quality Guidelines use chronic, no observed effect concentration (NOEC) or EC10 data to derive high reliability trigger values, and acute EC50 toxicity data to derive moderate reliability trigger values (section 8.3.4.4, ANZECC & ARMCANZ 2000; Warne 2001). For the purpose of comparison of SSDs with different waters, SSDs were constructed with EC50s, because generally speaking EC50 estimates have tighter confidence intervals and provide a more reliable basis for comparison. In order to obtain chronic EC50s for all test species, it was necessary to convert acute EC50 data to chronic EC50 data by use of an acute to chronic ratio (ACR). As indicated above, an ACR of 1.3:1 was deemed appropriate for saline waters on the basis of data collected in the present and previous studies. Therefore, all acute EC50 data (prawn, fish, chironomid) were divided by 1.3 to obtain chronic EC50 values.

Construction of an SSD

Species sensitivity distributions (SSD) were constructed using the BurrliOZ software package (Campbell et al. 2000), provided as part of ANZECC & ARMCANZ (2000) package and updated by CSIRO CMIS (http://www.cmis.csiro.au/envir/burrlioz/). BurrliOZ uses a flexible group of distributions, the Burr Type III (Shao 2000). Within the context of ANZECC & ARMCANZ (2000), these distributions are used to estimate the concentrations of discharges such that a given percentage of species will be protected and conversely that a given percentage will be adversely affected.

RESULTS

The proportions of the various major cations and anions varied substantially across different mine sites (Figure 1). The concentrations of anions (HCO_3^-, SO_4^{2-}, Cl^-) were more variable than those of the cations (Ca^{2+}, Mg^{2+}, Na^+, K^+), reflecting the geology across the regions (Figure 1). The two artificial mine waters (AMW1, AMW2) were formulated to represent the range of water types in the basin based on the concentrations of anions (HCO_3^-, Cl^-) and cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) (Table 1, Fig 1).

All toxicity tests conducted satisfied test acceptability criteria (Table 2). The toxicity indices (EC50) generated through toxicity tests are presented in Table 3. Chronic EC50 data, which included some data converted from acute to chronic EC50 data using an ACR of 1.3 are presented in Table 4. Species sensitivity distributions using the chronic EC50 data are presented in Figure 2. There was some species specific variation in the degrees of toxicity measured for each artificial mine water. Freshwater prawns were more sensitive to AMW1 than AMW2. Chironomid midge larvae were also slightly more sensitive to AMW1 than AMW2. Conversely, both fish and cladocerans were more sensitive to AMW2 than AMW1. These variations in sensitivity dictated that some test species (fish, chironomids) held different positions on the SSDs for the two water types (Figure 2). However, overall the differences did not result in SSDs with different positions on the graph, and the SSDs for both AMW1 and AMW2 shared similar regression parameters (Figure 2). On the basis of the SSD constructed using chronic EC50 toxicity test data, TVs for the protection of 80 to 99% of species were calculated (Figure 2).

DISCUSSION

In order to accommodate the nutritional and survival requirements of individual species, it was necessary to begin with known base-formulations. In the case of *C. dubia*, that base was provided

Table 3. Effects data following toxicity tests with AMW1 and AMW2.

Test Species	EC50 (95% confidence interval) mS/cm			
	AMW1		AMW2	
Freshwater prawn survival test (acute)	28.97	(27.74 - 29.82)	35.11	(ID)
Midge survival test (acute)	15.40	(14.81 - 15.80)	17.11	(17.11 - 17.11)
Larval fish imbalance test (acute)	16.15	(15.40 - 17.02)	10.71	(8.19 - 12.53)
Microalgal cell division rate test (chronic)	8.34	(7.42 - 9.17)	9.50	(8.95 - 9.50)
Cladoceran survival test (acute)	4.97	(0.69 - 54.25)	4.24	(3.87 - 6.01)
Cladoceran survival test (chronic)	4.62	(4.24 - 5.83)	5.48	(3.19 - 15.88)
Cladoceran reproduction test (chronic)	3.83	(3.53 - 4.18)	4.21	(3.40 - 4.80)

ID = insufficient data

Table 4. Chronic EC50 values used for derivation of electrical conductivity trigger value.

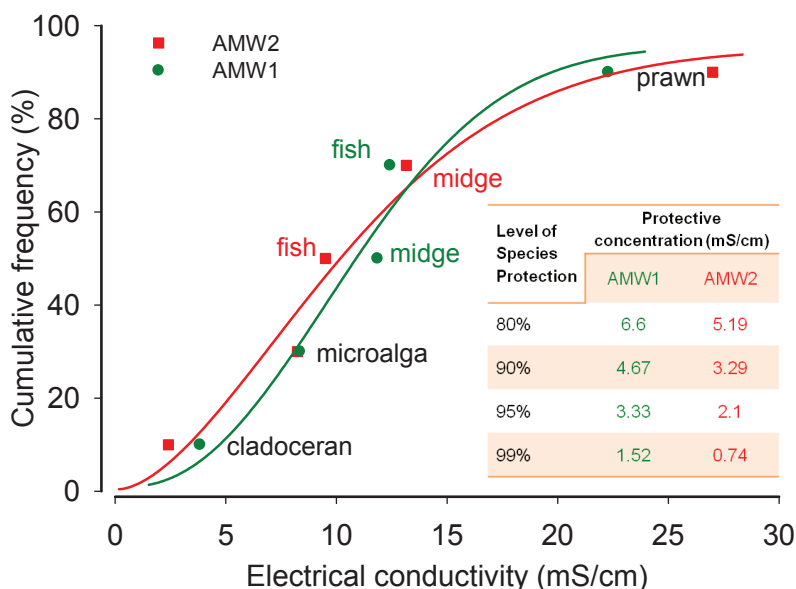| Test Species	Chronic EC50 (mS/cm)	
	AMW1	AMW2
Freshwater prawn survival test	22.28	27.00
Larval fish imbalance test	12.42	8.24
Midge survival test	11.85	13.16
Microalgal cell division rate test	8.34	9.50
Cladoceran reproduction test	3.83	2.41

Figure 2. Species sensitivity distributions (SSD) for test species exposed to artificial mine waters AMW1 and AMW2. The regressions in this plot are based on Burr Type III distributions generated with the BurrliOZ software package (Campbell et al. 2000) and plotted on a single axis.

in the form of a dilute commercial mineral water (DCMW). This same water was used as a base solution for the tests with fish, prawns and chironomids. For the tests with microalgae, the base solution was U.S. EPA moderately hard water (U.S. EPA 2002). Therefore, it can be assumed that the compositional profile of the test water used for microalgae differed slightly from that used for all other tests because there were likely to be several trace elements present in the DCMW that were not present in the U.S. EPA moderately hard water. However, we have assumed that the very low concentrations of these trace elements will have negligible impact on the salinity profile.

In this study, the use of formulated waters permitted the comparison of salinity profiles representing different coal mining areas in Queensland on an equal footing, without the confounding effects of

other contaminants that would be found in natural waters. Furthermore, by carefully customising each test-water for each test-species, we have ensured that the salinity profiles were equivalent across test species.

Saline wastewaters emanating from Queensland coal mines can be highly variable with regard to chemical profile. The two artificial mine waters formulated for this study represented two extremes of mine waters found in the Fitzroy Basin in Queensland. AMW1 was dominated by Na^+, Cl^- and SO_4^{2-}, whereas AMW2 contained higher proportional concentrations of Ca^{2+}, Mg^{2+} and HCO_3^- (Table 1). The compositional profile of saline solutions is known to affect toxicity, and the toxicity of saline solutions is driven by two main factors – osmoregulatory stress (Hainsworth 1981), and toxicity related to specific ions (Mount et al. 1997; Soucek and Kennedy 2005; van Dam et al. 2010). Several previous studies have demonstrated that NaCl dominated waters (seawater) are less toxic to freshwater organisms than the more complex inland water profiles (Kefford et al. 2003; Kefford et al. 2004; Lincoln-Smith et al. 2010). The data presented here demonstrate that for individual test-species, the toxicities of AMW1 and AMW2 were quite different, with AMW1 being more toxic than AMW2 in prawns and chironomids, and conversely, AMW2 more toxic than AMW1 in fishes and cladocerans. Overall, the TVs derived for the protection of 80 to 99% of species suggest that AMW1 was less toxic than AMW2. Interestingly, this is the opposite to conclusions drawn from studies with mayfly nymphs exposed to the same two artificial mine waters (Prasad et al. 2014). However, the data presented in these two studies highlight the caution required when assigning toxicity ranks on the basis of single species. Overall though, despite substantial differences in ionic composition, and despite individual differences in sensitivity among different test species, the opposing toxicity indices in individual test-species resulted in SSDs of similar shape and position (i.e., similar regression parameters), when salinity was measured as electrical conductivity. This result may be co-incidental and specific to our data-sets, or a function of the low number of species used in each SSD. Further studies that evaluate the contribution of individual ions and the importance of osmotic pressure are required to evaluate the importance of ionic composition in the risk to freshwater organisms exposed to saline waters.

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