SPECIAL ISSUE-LETTER

Single-species acute lethal toxicity tests are not predictive of relative population and community effects of two salinity types

Ben J. Kefford ⓘ,[1]* Ross V. Hyne,[2] Andrew J. Brooks ⓘ,[1,3] Jonathan P. Bray,[1,4] Mark Shenton,[1] Kasey Hills,[1,5] Susan J. Nichols[1]

[1]Centre for Applied Water Science, Institute for Applied Ecology, University of Canberra, Canberra, Australian Capital Territory, Australia; [2]Department of Planning, Industry and Environment, Environment Protection Science, Lidcombe Laboratories, Lidcombe, New South Wales, Australia; [3]Department of Planning, Industry and Environment – Water, Wollongong, New South Wales, Australia; [4]Gisborne District Council, Gisborne, New Zealand; [5]New South Wales Environmental Protection Authority, Parramatta, New South Wales, Australia

Scientific Significance Statement

The predominant approach to setting environmental quality guidelines for chemical contaminants to protect aquatic biodiversity involves the use of single-species toxicity tests, which often measure short-term mortality on a limited number of species. It is recognized that the results of these tests may not adequately protect biodiversity in ecosystems; however, it is implicitly assumed that these tests do indicate the relative effects of toxicants in ecosystems. Here, we test this implicit assumption by conducting an experiment in model freshwater ecosystems (mesocosms). We found this relative toxicity assumption was not supported for effects on stream macroinvertebrates comparing two salinity types, sodium bicarbonate ($NaHCO_3$) and salinity with the ionic composition of seawater, which are common contaminants from coal bed effluents and agriculture, respectively. Consequently, extrapolation from single-species toxicity estimates (representing effects on test populations) to the relative effects on natural populations and communities in nature is problematic, at least for $NaHCO_3$ and salinity with the ionic composition of seawater. Studies should test the implicit assumption of relative effects between other combinations of chemical stressors.

Abstract

Human-mediated salinity increases are occurring in freshwaters globally, with consequent negative effects on freshwater biodiversity. Salinity comprises multiple anions and cations. While total concentrations are typically used to infer effects, individual ion concentrations and ion ratios are critical in determining effects. Moreover, estimates of toxicity from single-species laboratory tests, may not accurately predict relative effects on populations and communities. Here, we compare salinity increases from synthetic marine salts and sodium

*Correspondence: ben.kefford@canberra.edu.au

bicarbonate in an outdoor mesocosm experiment in southeastern Australia. We found different effects of salt types on stream macroinvertebrates at the population and community levels, where similar effects were predicted from single-species laboratory tests. Our results caution against the use of single-species laboratory-derived toxicological data to predict both environmentally safe salinity levels and the relative effects of different salt sources on freshwater biodiversity.

Increased salinity, or salinization, in freshwaters caused by various human actions is a major concern globally, given negative consequences for freshwater biodiversity (Cañedo-Argüelles et al. 2019). Salinity is the total concentration of dissolved inorganic ions (Williams and Sherwood 1994), with the proportions of major ions in freshwaters varying with the cause of salinization (Sauer et al. 2016). The composition of major ions can be important, or even more important, than total ion concentrations in determining the toxicity (Kunz et al. 2013; Cañedo-Argüelles et al. 2016a) and acclimation (Orr et al. 2021) in freshwater biota.

For regulatory purposes, the toxicity of a substance is measured in single-species laboratory tests (Buchwalter et al. 2017), most commonly measuring acute lethality (Poteat and Buchwalter 2014), although chronic and/or sublethal responses are possible. Biodiversity protection aims to preserve viable populations and communities (e.g., community structure and taxa richness; Poteat and Buchwalter 2014). Measures of the toxicity of a substance (e.g., concentration lethal to x% of a test population or LC_x) may not predict *absolute effects* in nature, that is, an LC_x (or any other metric of toxicity, e.g., EC_x, NEC) of 5 mg L^{-1} does not predict environmental safe concentration to protect biodiversity (Schäfer et al. 2012; Beketov et al. 2013). However, we suggest it is implicitly assumed for biodiversity protection that such measures of toxicity (e.g., LC_x values) predict *relative effects* of toxicants in nature. We define relative effects of a toxicant to mean that the magnitude of the effects of multiple substances is relative to each other. For example, if two substances, A and B, have LC_x values of 5 and 10 mg L^{-1} respectively, A can be considered twice as toxic as B. Following this assumption, if metrics of toxicity are to be environmentally relevant, then two toxicants at concentrations that produce the same toxicological effect (e.g., LC_x) should cause similar effects, on populations and community structure when exposure occurs in natural environments. Laboratory-derived toxicological estimates are a *common currency* (Liess et al. 2020) expected to predict the relative effects of a toxicant and are often applied for biodiversity protection. But the assumption of whether toxicity remains relative in natural systems has been rarely tested. Here, we aim to determine whether population and community level effects in mesocosms from two salts are predictable based on their relative acute toxicity derived from single-species tests (Hills et al. 2019). We also examine whether the ecosystem process, leaf-litter breakdown, is affected because synthetic marine salts (SMS) and sodium bicarbonate (NaHCO$_3$) have different effects on microbial leaf-litter breakdown (Sauer et al. 2016).

NaHCO$_3$ is a major constituent of effluents from coal mining and coal bed gas extraction (Dahm et al. 2011; Lopez Vera et al. 2014; Sauer et al. 2016). Australian salinization derived from agriculture commonly has ionic proportions similar to seawater (Herczeg et al. 2001). Single-species acute (Hills et al. 2019) and chronic (Lopez Vera et al. 2014) tests showed that NaHCO$_3$ is consistently more toxic to freshwater invertebrates than SMS or sodium chloride. Indeed, there was a strong regression relationship between the 96-h LC_{10} and LC_{50} values ($r^2 = 0.81$ and 0.91, respectively) of species (representing insects, mollusks and crustaceans) for SMS and NaHCO$_3$ (Hills et al. 2019). Moreover, the rankings of species sensitivities to these salts are similar with regards to both LC_{50} and LC_{10} values, indicating that concentration-response slope functions are similar. So, species sensitive to one of these salts were similarly sensitive of the other, and those tolerant of one were tolerant of the other. A similar relationship exists for 48–96 h LC_{50} values from all freshwater invertebrates and fish between NaHCO$_3$ and NaCl achieved in the ECOTOX database (https://cfpub.epa.gov/ecotox/; Hills et al. 2019), although greater confidence in the relationship between toxicity of NaHCO$_3$ and SMS/NaCl would come from additional studies. The strength of these relationships ($r^2 = 0.81–0.91$) suggest that if additional species, including species from other taxonomic groups, were added, the resulting regression equations would likely be similar, unless these species were drawn from different statistical populations. These regression equations should describe the relative effects of SMS and NaHCO$_3$ and should therefore predict the relative effects of each salt within natural populations and communities. These relative effects should be true, even if it does not describe their absolute effects, such as the declines in density or species richness.

However, there are mechanisms by which toxicity might not predict the relative effect on biota. Toxicity is measured in very few species (Buchwalter et al. 2017) and typically excludes dietary exposure (Poteat and Buchwalter 2014). Moreover, there are longer-term (chronic) and sublethal effects (Hassell et al. 2006; Johnson et al. 2015), reduced tolerance from co-occurring stressors (Beketov and Liess 2005) and indirect effects via ecological interactions (e.g., predation and competition) (Rohr et al. 2006; Bray et al. 2018), which may result in toxicity tests not accurately predicting the relative

effects on populations and communities. We test the hypothesis that concentrations of SMS and $NaHCO_3$, which are reported to have similar toxicity to freshwater invertebrates, have the same effects to the freshwater invertebrate population density and community structure, species richness, and total density.

Methods

We conducted an outdoor experiment using mesocosms holding ~ 900 liters of water. This experiment was conducted at the same location, using identical methods to previous experiments in these mesocosms (Bray et al. 2018, 2019, 2020), and is briefly described here. Two salinity type treatments were used: $NaHCO_3$ (food grade, NUUVEE Essentials) or SMS (Ocean Nature, Aquasonic) dissolved in dechlorinated Canberra tap water. For both treatments, there were low and high levels (see below); plus a control (no salts added). All treatments including the control were replicated in four independent mesocosms (i.e., 20 mesocosms).

The levels of $NaCO_3$ and SMS were selected to have the same toxicity (i.e., 96-h LC_{50}) within the high and low treatments. The low and high SMS treatments were set at nominal levels of 5 and 10 mS cm^{-1} (at 25°C) as these levels occur in southeastern Australia (Kefford et al. 2010; Kefford et al. 2011a), and based on a previous mesocosm experiment, would alter the macroinvertebrate community (Bray et al. 2018). Then, 96-h LC_{50} values for $NaHCO_3$ and SMS subsequently published in Hills et al. (2019)) were used to estimate the relationship between these LC_{50} values between the salts, resulting in: LC_{50} $NaHCO_3$ mS cm^{-1} = $-6.37 + 9.91 \times log10$ (LC_{50} SMS mS cm^{-1}). This regression was used to estimate the corresponding levels of $NaHCO_3$, which should have the same toxicity, that is, nominal electrical conductivity (EC) values of 0.56 and 3.54 mS cm^{-1} for the low and high $NaHCO_3$ treatments, respectively. Therefore, if the single-species toxicity tests (Hills et al. 2019) predict the relative effects in mesocosms, then there should be no difference in the population or community level responses between the low $NaHCO_3$ vs. low SMS and high $NaHCO_3$ vs. high SMS treatments (see Supplementary Fig. S1). Alternatively, if biotic responses differed between the low $NaHCO_3$ vs. low SMS and/or high $NaHCO_3$ vs. high SMS treatments, then it indicates that responses are not predicted by single-species toxicity tests.

Each mesocosm was stocked with macroinvertebrates and microbes by adding three trays (each 36×30 cm) with natural stream substrate (gravels, pebbles, and cobbles) and one 5-mm mesh leaf pack containing 15 g dry mass of *Eucalyptus camaldulensis* leaves (sourced from the bank of the Yass River 34°55′24.6″S 149°10′46.8″E). Leaf packs and the trays were placed in the Cotter River (35°23′10.70″S, 148°51′53.55″E), for 34 d (13 August 2018 to 17 September 2018) to colonize with macroinvertebrates and microbes

before commencing the experiments. The Cotter River is the major source of the Canberra tap water used in the experiment and while regulated by upstream dams, it has a relatively natural catchment, low salinity (≈ 0.03 mS cm^{-1}; Bray et al. 2018) and has a diverse macroinvertebrate fauna. Additionally, two kicknet samples each covering 10 m of riffle/run were placed in each mesocosm 7 d after adding the trays and colonization leaves. On completion of the experiment, all macroinvertebrates in the trays were collected over 3 d (33–35 d since the addition of the kicknet samples). Subsequently, approximately 100 individual macroinvertebrates per tray were identified to species or genus level, where possible, and enumerated.

Three sets of leaf packs (each containing 5 g [dry mass] of *E. camaldulensis* leave as above) in both coarse (5 mm) and fine (< 0.25 mm) bags were added to each mesocosm to assess leaf-litter breakdown. At the end of the experiment, these leaves were removed, dried, and re-weighed.

Differences from previous experiments were: we added an upturned pot (43 cm bottom diameter and ≈ 27 cm at the water surface) at the centre of each mesocosm with a cobble on each pot to hold it in place. The purpose of these pots was to establish more even water velocity (≈ 0.2 m s^{-1}) created by a submersible pump. Additionally, we collected insects emerging as flying adults using floating circular (diameter 35 cm) emergence traps placed over the upturned pot. Adults were funneled into a jar of ethanol, collected periodically to compare differences in emergence between treatments. Adults were identified to family because few individuals of most families emerged (Supplementary Text 01).

Data and metadata are available at https://doi.org/10.5061/dryad.f1vhhmgx5 (Kefford et al. 2021).

Statistical analysis

For each response variable, we undertook the following six planned comparisons between treatments:

1. control vs. SMS low concentration,
2. control vs. SMS high concentration,
3. control vs. $NaHCO_3$ low concentration,
4. control vs. $NaHCO_3$ high concentration,
5. $NaHCO_3$ low concentration vs. SMS low concentration, and
6. $NaHCO_3$ high concentration vs. SMS high concentration

Comparisons 1–4 test whether the salinity concentrations were enough to produce a response. Comparisons 5–6 evaluate whether the two salts have different effects at the concentrations predicted to have the same toxicity (Hills et al. 2019), which is our main interest.

We characterized changes to the community structure of macroinvertebrates. Nonmetric multidimensional scaling ordination using the Bray–Curtis dissimilarity on log_{10} ($x + 1$) transformed densities was used to visualize overall community

patterns. Differences in community composition were tested using one-way PERMANOVA (Anderson, 2017). The effects of the treatments on univariate variables were tested with one-way ANOVA using planned comparisons on $\log_{10} (x + 1)$ transformed data except for taxon richness and leaf breakdown, which were untransformed. Macroinvertebrate counts from individual trays, within each mesocosm, were summed for statistical analysis.
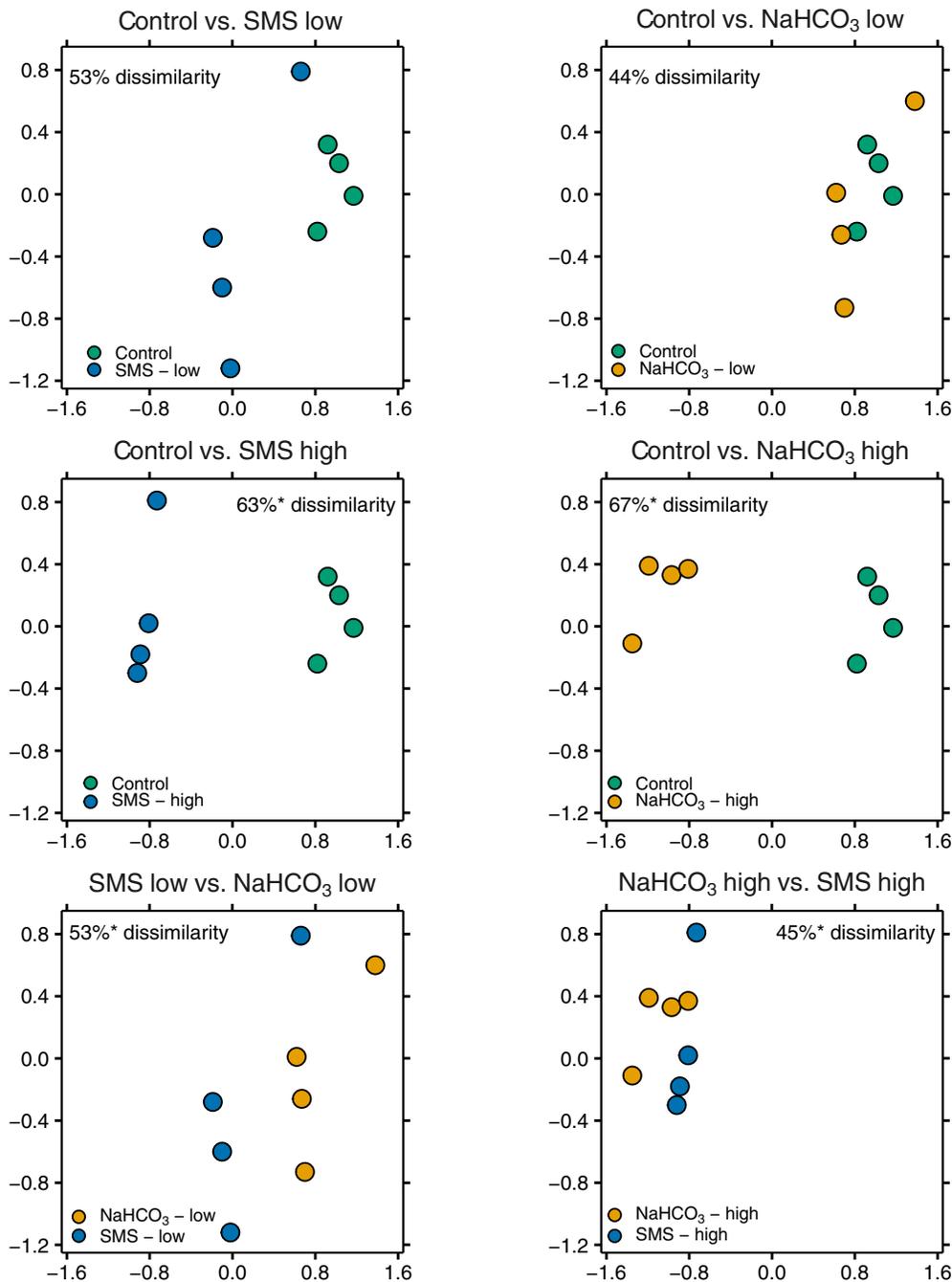

Fig. 1. Nonmetric multidimensional scaling ordination comparing similarity (Bray–Curtis) of benthic macroinvertebrate communities between selected treatments. The percentage values indicate the mean community dissimilarity between treatments and * indicates whether communities were significantly different. Data were analyzed together but comparisons were plotted individually to illustrate patterns. See Table 1 for details of statistical analyses.

Table 1. Summary of effects of salt treatments on the leaf-litter breakdown and benthic macroinvertebrates at the community level—community structure, richness, and total density—and population level—the density of the 12 most abundant macroinvertebrate taxa. Differences in community structure are assessed using one-way PERMANOVA, while one-way ANOVA is used for the other responses. Planned comparisons are used. Degrees of freedom for all analyses = 1.15. Ps-F = pseudo-F. Significant results ($p \leq 0.05$) are highlighted in bold.

			Control vs. SMS (low)	Control vs. SMS (high)	Control vs. NaHCO$_3$ (low)	Control vs. NaHCO$_3$ (high)	NaHCO$_3$ vs. SMS (low)	NaHCO$_3$ vs. SMS (high)
Ecosystem function level—*Eucalyptus camaldulensis* leaf-litter breakdown	Microbial (fine mesh)	t	1.01	2.28	0.17	−9.98	0.84	12.25
		p	0.33	**0.04**	0.87	**<0.001**	0.41	**<0.001**
	Invertebrate (coarse mesh—fine mesh)	t	−1.09	−0.37	0.95	−0.29	−2.04	−0.08
		P	0.29	0.71	0.36	0.77	0.06	0.94
	Total (coarse mesh)	t	−0.60	0.81	1.07	−5.54	−1.67	6.35
		p	0.56	0.43	0.30	**<0.001**	0.12	**<0.001**
Community level	Community structure*	Ps-F	2.2	6.9	1.3	11.0	1.6	3.0
		p	0.06	**0.03**	0.17	**0.03**	**0.03**	**0.03**
	Taxon richness	t	3.5	5.4	1.2	5.9	2.3	−0.6
		P	**0.003**	**<0.001**	0.24	**<0.001**	**0.04**	0.58
	Total density	t	−2.1	−0.5	−0.1	1.8	−2.0	−2.3
		p	0.05	0.62	0.95	0.09	0.06	0.03
Mayflies (Ephemeroptera)	*Austrophlebioides* sp.	t	1.3	1.6	0	1.9	−1.3	**−0.3**
		p	**<0.001**	**<0.001**	0.99	**<0.001**	**<0.001**	0.29
	Offadens sp.	t	2.7	4	0.3	4	2.4	0
		p	**0.02**	**0.001**	0.75	**0.001**	**0.03**	1.00
	Tasmanocoenis sp.	t	1.8	3.3	−0.8	3.3	2.6	0
		p	0.1	**0.005**	0.43	**0.005**	**0.02**	1.00
Caddisflies (Trichoptera)	*Cheumatopsyche* sp.	t	0.3	2.4	2	3	−1.7	−0.5
		p	0.78	**0.03**	0.06	**0.01**	0.1	0.59
	Orthotrichea sp.	t	0.7	2.3	1	2.3	−0.3	0
		p	0.51	**0.03**	0.34	**0.03**	0.76	1.00
	Agapetus sp.	t	8.6	8.6	4.8	8.6	3.7	0
		p	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.002**	1.00
Worms	Oligochaeta	t	0.1	6.4	−1.6	7	1.7	−0.6
		p	0.94	**<0.001**	0.12	**<0.001**	0.11	0.56
Riffle beetles (Coleoptera)	Elmidae	t	1.5	2.6	0.4	3	1.2	−0.4
		p	0.15	**0.02**	0.73	**0.01**	0.27	0.68
Stoneflies (Plecoptera)	Gripopterygidae	t	1.2	3.2	2.4	3.2	−1.2	0
		p	0.26	**0.006**	**0.03**	**0.006**	0.25	0.98
True flies (Diptera)	Chironominae (midge)	t	−2.5	−1.3	−0.01	0.6	−2.5	−1.9
		p	**0.02**	0.20	0.99	0.58	**0.02**	0.08
	Simuliidae (blackfly)	t	−0.6	−0.1	−1.2	−0.6	0.6	0.5
		p	0.55	0.92	0.24	0.54	0.55	0.61
Dragonfly (Odonata)	*Hemigopmphus* sp.	t	0.5	2.0	0.7	0.8	−0.2	1.2
		p	0.61	0.062	0.47	0.43	0.82	0.25

*Based on the Bray–Curtis coefficient on log10 (x + 1) transformed densities.

Results

Characteristics of experimental treatments

The physicochemical conditions were comparable within treatments throughout the experiment (Supplementary Table S1). The mean-measured EC of SMS treatments was within 8.3% of their target. Calculating target NaHCO$_3$ conductivities using the regression relationship and measured SMS, identified low NaHCO$_3$ treatment had a mean of 0.184 mS cm^{-1}, which was 11.1% less than the target concentration (i.e., 0.207 mS cm^{-1}), and for high treatment mean-measured
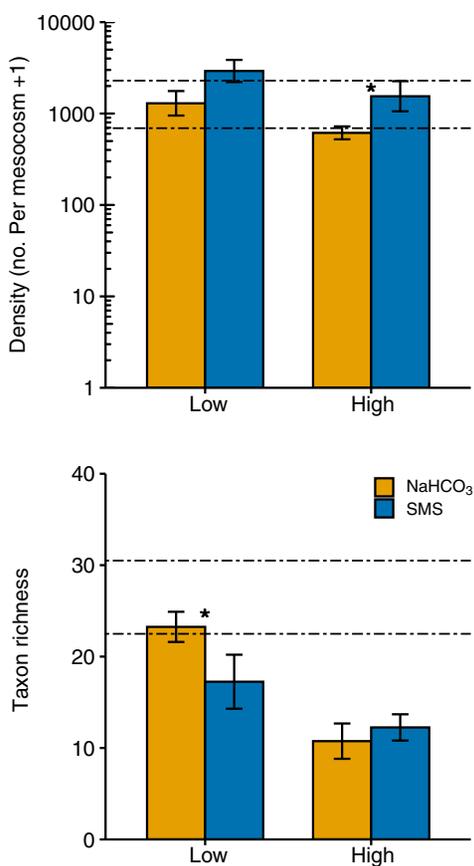
Fig. 2. Mean macroinvertebrate density and taxon richness for each salt treatment (*N* = 4, error bars are standard errors). Dashed lines represent the 95% confidence interval for the control treatment. * indicate significant differences, at the 0.05 level, between the two salts; see Table 1 for further results of statistical analyses.

concentration was 4.15 mS cm^{-1}, which was 31% greater than the target conductivity (i.e., 3.16 mS cm^{-1}).

No measurable carbonate alkalinity was detected except in the high NaHCO$_3$ treatment. In this treatment, of total alkalinity, carbonate alkalinity comprised 12% at the start and 3% at the end of the experiment. The mean water temperature, based on measurements every 15 min, in 10 mesocosms was 21.1°C (mean minimum = 17.1°C, mean maximum = 27.8°C). The highest temperature recorded in any mesocosm was 31.4°C and the minimum was 12.3°C.

Sixty-eight benthic taxa from 11 orders were collected from the 20 mesocosms. The most abundant taxa being Elmidae (riffle beetles), Chironomidae (flies), Baetidae and Leptophlebiidae (mayflies), Gomphidae (dragonflies), worms (Oligochaeta), and Hydropsychidae and Hydroptilidae (caddisflies).

Macroinvertebrate responses to the salt treatments

The high concentration of both salt treatments had statistically significant effects on benthic macroinvertebrate community composition (Fig. 1; Table 1), reducing the overall taxon richness but had no effect on total density (Fig. 2; Table 1). The low concentration of both salts had no effect on the benthic macroinvertebrate community composition, but significantly reduced taxa richness.

Population level effects were considered on the 12 most abundant taxa (Table 1). The densities of the remaining were insufficient and/or were too variable in the control for statistical analysis. Densities of oligochaete worms, caddisflies, mayflies, stoneflies, and riffle beetles were reduced by high concentrations of both salts compared to control treatments (Table 1; Fig. 3). Indeed, two of three abundant mayflies and two of three abundant caddisfly taxa were extirpated by high concentrations of both salts (Fig. 3).

Densities of *Offadens* sp. and *Austrophlebioides* sp. were reduced by low SMS and Gripoterygidae densities were reduced by low NaCO$_3$ concentrations. The low concentrations of both SMS and NaCO$_3$ significantly reduced the density of *Agapetus* sp. In contrast, there were higher densities of Chironominae in the low SMS compared to the control (Table 1).

There were no differences between any treatment for blackfly (Simuliidae) nor *Hemigomphus* sp. (dragonfly) (Table 1).

Macroinvertebrate responses between salt types

There were statistically significance differences in the benthic community structure between the two salts at both the low and high concentrations (Table 1; Fig. 1). There were significantly more taxa in the low NaHCO$_3$ compared to the low SMS (Table 1; Fig. 2) but no difference between the two high levels of these salts. Total density of macroinvertebrates was less in the high NaHCO$_3$ than in the high SMS but no difference between the low levels of these salts (Table 1; Fig. 2).

Benthic densities of the mayflies *Austrophlebioides* sp., *Offadens* sp. and *Tasmanocoenis* sp., and the caddisfly *Agapetus* sp. were significantly lower in the low SMS treatment compared to the low NaHCO$_3$ treatment (mayflies ≈ −90%, caddisfly = −100%; Table 1; Fig. 3). In contrast, densities of Chironominae were significantly higher in the low SMS than in the low NaHCO$_3$ treatment (Table 1).

For a full description of the effects of the treatments on emerging macroinvertebrates, see Supplementary Text 1, Figs. S3–S5, and Table S2. In general, the high concentrations of both salts significantly altered the community composition and reduced the taxon richness compared to controls. Significant differential effects of the salts were observed in Leptophlebiidae and Simuliidae.

Leaf-litter breakdown

Leaf-litter breakdown was greater in the high NaHCO$_3$ treatment than in controls both for microbial and total breakdown (Table 1; Supplementary Fig. S6). The microbial breakdown was significantly lower in high SMS than in the control. Both microbial and total breakdowns were significantly greater in the high NaHCO$_3$, compared to those in the high SMS treatment. Other comparisons were not significant (Table 1).
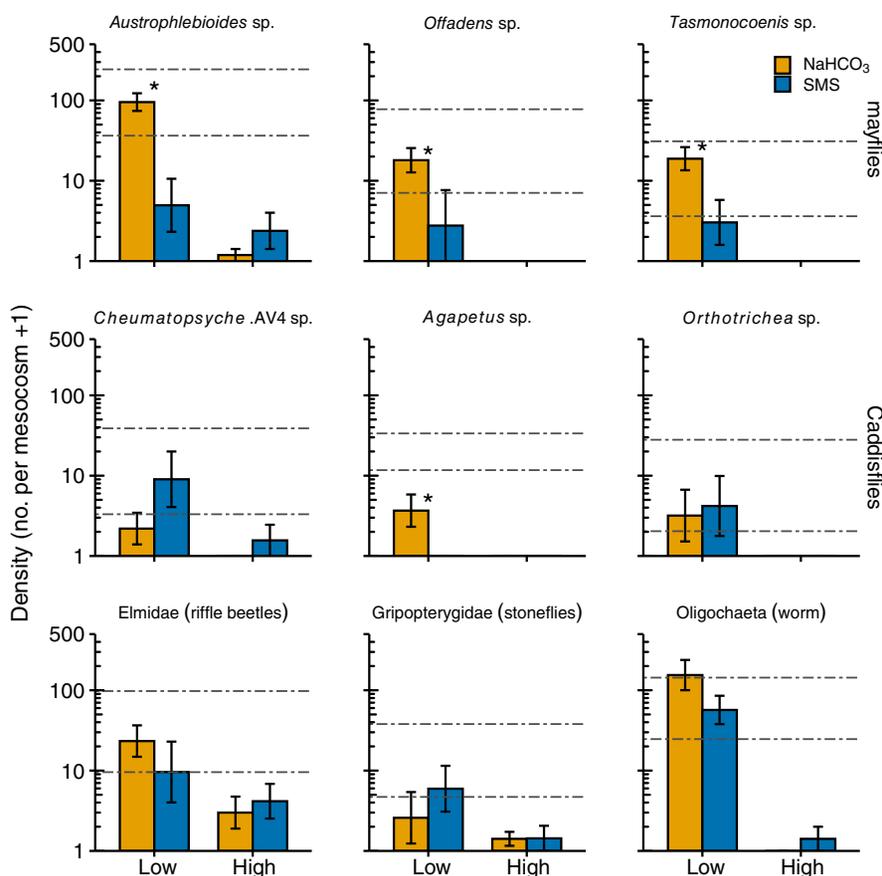
Fig. 3. Mean density of benthic macroinvertebrates for each salt treatment ($N = 4$, error bars are standard errors) that showed significant differences from the control in two or more treatments. The remaining three taxa are graphed in Supplementary Fig. S2. Dashed lines represent the 95% confidence interval for the control treatment. * indicate significant differences, at the 0.05 level, between the two salts; see Table 1 for further results of statistical analyses.

Discussion

We hypothesized that the relative responses of benthic macroinvertebrates should not differ between SMS and NaHCO$_3$ salts, based on their relative (or proportional) single-species acute lethal toxicity (Hills et al. 2019). However, we found differences in the benthic responses to these salts at the community and population levels. Community structure differed between NaHCO$_3$ and SMS treatments at both high and low concentrations. Indeed, the mean Bray–Curtis dissimilarity between these salts (SMS low vs. low NaHCO$_3$ 53%; SMS high vs. high NaHCO$_3$ 45%) was similar to low salinities and the control (53% and 44%), although the high level of the salts had a somewhat greater effect relative to the control (63% and 67%). Low SMS reduced the benthic taxa richness and densities of 4 of the 12 most abundant taxa (*Austrophlebioides* sp., *Offadens* sp., *Tasmanocoenis* sp., and *Agapetus* sp.) to a greater extent than the low NaHCO$_3$ treatment. These differences occurred despite the low NaHCO$_3$ treatment being close to its target concentration (11% less). Chironominae had higher benthic densities in low SMS than in low NaHCO$_3$. Chironominae species hatch and develop at high

salinities of SMS (15 mS cm^{-1}) (Kefford et al. 2007, 2011*b*) and this may explain this subfamily differential response than the other abundant taxa. Hills et al. (2019) tested *Austrophlebioides pusillus* to SMS and NaHCO$_3$, yet we found that a member of this genus did not respond as predicted from those tests. Total benthic density was lower in the high NaHCO$_3$ than in the high SMS, although some caution is needed as the high NaHCO$_3$ was 31% greater than its target. Unlike at the community level, there were no differences at the population level of the effect of high treatments of both salts on the benthic densities of the abundant taxa. This might be because high concentrations of both salts extirpated four abundant taxa and substantially reduced the densities of another five. Relative rates of leaf-litter breakdown between the salts were neither as predicted by invertebrate toxicity (Hills et al. 2019) nor from leaf-litter breakdowns measured in the field and a laboratory experiment (Sauer et al. 2016).

Deviation from an idealized "relative toxic" effect (Hills et al. 2019) can be explained by two nonmutually exclusive mechanisms. (1) The relative sensitivity to NaHCO$_3$ and SMS of the species in our mesocosms are from a different statistical

population than the species Hills et al. (2019) tested. While all species were from southeastern Australia, our mesocosms were stocked from a montane stream, whereas Hills et al. (2019) used species from lowland freshwaters. Ecotoxicological studies commonly assumes that species from anywhere are from the same statistical population (Posthuma et al. 2001; Forbes and Calow 2002) and it would be problematic for regulations if such regional differences caused meaningful differences in relative toxicity, see also Sala et al. (2016). (2) Single-species laboratory toxicity tests do not indicate the relative effects of toxicants in ecosystems.

Decades of research has described, modeled, and/or predicted single-species toxicity from multiple major ions (Mount and Gulley 1992; Mount et al., 1997, 2019; Kefford et al. 2005; Zalizniak and Nugegoda 2006; Hills et al. 2019; Wood et al. 2020). Single-species toxicity tests have the advantage of being inexpensive compared to mesocosm studies, are highly repeatable, and causally link biological effects (e.g., mortality) to the toxicant concentration. Nevertheless, these tests are simplistic (Cairns Jr 1986; Rohr et al. 2006), in that they do not include co-occurring stressors (e.g., food limitations [Beketov and Liess 2005] and hydrologic disturbance [Stampfli et al. 2013]), temperature variability and indirect effects mediated via ecological interactions (Sarma et al. 2002; Becker and Liess 2015; Cañedo-Argüelles et al. 2016b; Bray et al. 2018), and rarely include sublethal or chronic effects, despite their importance (Johnson et al. 2015). Our mesocosm experiment incorporated some of the complexity seen in natural ecosystems, producing outcomes at odds with expectations from single-species tests (Hills et al. 2019). This agrees with the hypothesis that the relative effect of two toxicants at population and community levels in nature cannot necessarily be accurately estimated from simplistic toxicity tests.

Despite the predominance of setting environmental quality guidelines using single-species toxicity test data and significant criticisms regarding their use (Cairns Jr 1986; Forbes and Calow 2002; Calow and Forbes 2003), few studies have attempted to empirically determine if tests can in fact predict effects of chemicals in model or natural ecosystems (Kefford et al. 2004). Our experimental results question the implicit assumption of relative toxicity based on toxicity testing, reinforcing the need to investigate whether similar discrepancies are common. Our findings caution against assuming single-species toxicity data accurately assess relative effects of chemicals on biodiversity.

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