

Is all salinity the same? I. The effect of ionic compositions on the salinity tolerance of five species of freshwater invertebrates

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Abstract. Salts of marine origin, predominantly consisting of Na^+ and Cl^- ions, are dominant in most Australian inland saline waters. The proportions of other ions, Ca^{2+} , Mg^{2+} , SO_4^{2-} , HCO_3^- and CO_3^{2-} , in the water may influence salinity tolerance of freshwater organisms and thus the effect of increasing salinity may vary with difference in ionic proportions. We exposed freshwater invertebrates to different concentrations of four ionic compositions and compared them with commercial sea salt (Ocean Nature). They were: synthetic Ocean Nature (ONS) and three saline water types (ONS but without: SO_4^{2-} , HCO_3^- and CO_3^{2-} (S1); Ca^{2+} , HCO_3^- and CO_3^{2-} (S2); and Ca^{2+} and Mg^{2+} (S3)), which are considered to be the predominant saline water types in south-eastern Australia and the Western Australian wheatbelt. The 96-h LC_{50} values for the five media were determined for six invertebrate species and sub-lethal responses were observed for two species. There were no differences between responses of invertebrates to various ionic compositions in acute toxicity tests. However, in prolonged sub-lethal tests, animals reacted differently to the various ionic compositions. The greatest effect was observed in water types lacking Ca, for which plausible physiological mechanisms exist. Variation in ionic proportions should be taken into account when considering sub-lethal effects of salinity on freshwater invertebrates.

Introduction

The salinisation of fresh waters is a major environmental concern in all continents with large arid and semi-arid regions, including Australia (Williams 1987). Recently, attention has been given to the lethal (Berezina 2003; Kefford *et al.* 2003) and sub-lethal tolerance (Kefford and Nugegoda 2005) of freshwater invertebrates to increased salinity, whereas other studies have experimentally considered effects of salinity on freshwater invertebrate communities (Nielsen *et al.* 2003; Marshall and Bailey 2004). All of these studies used artificial sea salts, the ionic proportion of which approximates seawater, because it is the most common composition of saline water bodies in south-eastern Australia (Bayly and Williams 1973), which are sodium chloride (NaCl) dominated. However recently it has been acknowledged that there is some variation in the ionic proportion of NaCl-dominated inland saline waters of south-eastern Australia (Radke *et al.* 2002, 2003). The three major saline water types existing in south-eastern Australia (Radke *et al.* 2002) and the wheatbelt region of Western Australia (Pinder *et al.* 2005) were proposed by Drever (1982) and occur due to precipitation out of solution of specific minerals during evapoconcentration of saline waters and result in reductions in the relative concentrations of specific ions. If variations in ionic proportions in NaCl-dominated inland saline waters result in differing biological

effects, then studies investigating the effects of saline water with particular ionic proportions (such as seawater) may not accurately describe the effects of changes in salinity with differing ionic proportions. Consequently, we investigated whether these three common ionic proportions and artificial seawater altered lethal and sub-lethal effects of salinity on freshwater invertebrates. For the common ionic proportions, we used the most extreme cases, where specific ions are eliminated from a saline water source, and therefore refer to the ionic compositions (presence/absence of specific ions), because if the absence of specific ions do not affect salinity tolerance then it is very unlikely that a reduction in the proportions of these ions would affect salinity tolerance.

Materials and methods

Test animals

Six species of freshwater invertebrates were used for acute 96-h LC_{50} toxicity testing (LC_{50} is the concentration of a toxicant lethal to 50% of a population). The protozoan *Paramecium caudatum* Ehrenberg and hydrozoan *Hydra oligactis* Pallas were purchased from Southern Biological (Melbourne, Australia). Other species, collected from central Victoria, in the southern end of the Murray–Darling Basin, were: gastropod *Physa acuta* Draparnaud (Campaspe River, at the Kyneton–Heathcote Rd (37°23'S, 144°31'E)), caddisfly *Notalina fulva* Kimmins, water bug *Micronecta robusta* Hale and mayfly *Centroptilum* sp. (King Parrot Creek, a tributary of the Goulburn River, at Flowerdale (37°23'S, 145°16'E)). These specific species were chosen because they represent a

wide range of different taxonomic groups found in fresh waters and were obtainable in sufficient numbers to experimentally expose to varying salinity and ionic composition treatments. Previous experiments where macroinvertebrate species have been collected from different sites or from the same site on different dates have shown no detectable difference in acute lethal salinity tolerance (Kefford *et al.* 2003, 2005a, B. J. Kefford, D. Nuggeoda, L. Metzeling and E. Fields, unpublished data). This is despite large differences in the acute lethal salinity tolerance between species. We thus assume that any difference in salinity tolerance or response to the different ionic compositions of species obtained from different sources represents differences between the species tested rather than differing responses of animals collected from different sites.

Water quality data from collection sites are given in Table 1 of an Accessory Publication on the *Marine and Freshwater Research* website.

Three species, *P. acuta*, *P. caudatum* and *H. oligactis*, were used in chronic toxicity tests. The results for *P. acuta* will be presented elsewhere (L. Zalizniak, B. J. Kefford and D. Nuggeoda, unpublished data). For hydras and paramecia, the culture growth in different types of treatments was determined as the measure of sub-lethal toxicity and EC_{50} values were calculated (EC_{50} being the concentration of a toxicant that produced the effect in 50% of population). For *H. oligactis*, another sub-lethal end point (tentacle retraction) was used.

Preparation of solutions

Five different solutions were tested. Concentrated stock solution of around 40 mS cm^{-1} of Ocean Nature artificial sea salt (ON) (Aquasonic, Wauchope, NSW) was prepared in Milli-Q water (Millipore, Sydney) and used in preparation of dilutions. Based on both the manufacturers' claimed elemental composition and elemental analysis (inductively coupled plasma mass spectrometry) of Ocean Nature, 'Ocean Nature Synthesised' (ONS) was prepared from analytical-grade reagents. Major ions and trace elements were considered (22 total), and their quantities were calculated (see Table 2 of Accessory Publication on the *Marine and Freshwater Research* website). Ocean Nature was used as a standard to compare with previous investigations using this salt (Kefford *et al.* 2003, 2004a, 2004b, 2005a; Kefford and Nuggeoda 2005), and ONS was used as control for possible effects of various synthesised ionic compositions. Based on ONS preparation, three different ionic compositions were derived to reproduce the three major saline water types described in Radke *et al.* (2002): S1 had the same ionic composition as ONS except there were no sulphates (SO_4^{2-}) or carbonates (HCO_3^- and CO_3^{2-} , referred to as alkalinity (Alk)); S2 was without calcium (Ca^{2+}) and Alk; and S3 excluded Ca^{2+} and magnesium (Mg^{2+}) (Fig. 1; also see Table 2 of Accessory Publication on the *Marine and Freshwater Research* website). Natural S1, S2 and S3 waters have some levels of the elements (see Radke *et al.* 2002) that we excluded. We excluded them in the stock solutions to represent a worst-case scenario. The control and dilution water had enough of these excluded ions to allow high (>85%) survival. Where possible, we tried to use carbon-filtered Melbourne tap water (WLW) as our dilution water and control. However, laboratory cultures required specific media for their maintenance. For paramecia, we used Lozina-Lozinsky medium (Lozina-Lozinsky 1931) and for hydras – M4 medium (Eelndt and Bias 1990). Though M4 medium was designed for daphnids, prolonged culturing of hydras (over several months) using this medium was successful. These media served as culture media, dilution water and control in corresponding experiments. The analysis of major ions for some of these media is presented in Table 3 of the Accessory Publication on the *Marine and Freshwater Research* website.

Animal cultures

Brown hydras, *H. oligactis*, were fed daily with brine shrimps or juvenile *Daphnia carinata* (whichever available, since previous observations showed that the cultures survive equally well with either) *ad lib*. Medium was replaced three times a week.

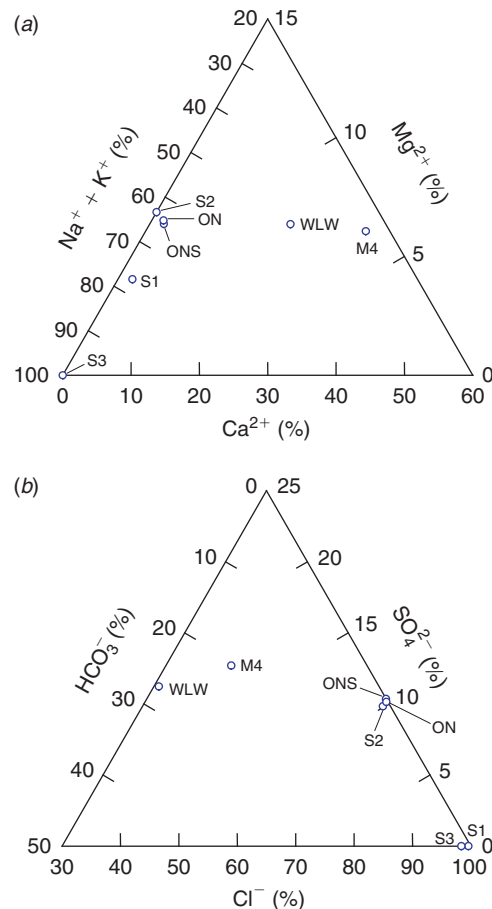


Fig. 1. Measured ionic proportions of the various saline water types, media (M4) and carbon-filtered Melbourne tap water (WLW): (a) cations and (b) anions as a percentage of the total major cations/anions on a mass to volume basis. (For raw data, see Accessory Publication on *Marine and Freshwater Research* website.)

For paramecia *P. caudatum* culturing technique was per Sazonova *et al.* (1997). Lozina-Lozinsky medium was boiled with 0.4 g L^{-1} of dry yeasts and cooled, and then inoculate of culture was introduced. After two days of acclimatising, animals were used for the experiment. Medium was replaced weekly or as necessary.

Animals collected from the field were transported from the site to the laboratory and transferred to the testing solutions as quickly as possible (as per Kefford *et al.* 2003, 2004a, 2005a).

Acute toxicity testing experimental protocols

There was no water replacement or feeding.

Hydras

The protocol published by Pollino and Holdway (1999) was used and is only briefly described. Non-budding hydras were used. To achieve this, hydras were not fed for 1–2 days. Five concentrations of each salt type were used: 4, 6, 8, 10 and 12 mS cm^{-1} replicated 4 times in Petri dishes ($\text{Ø } 54 \text{ mm}$), with five animals per replicate and 15 mL of test solution. Observations were made daily for 96 h; deaths and tentacle retraction of hydras were recorded. For the tentacle retraction, only two rankings were used: 'unaffected' being normal, and 'affected', which is any degree of shortening or disintegration (Pollino and Holdway 1999).

It is not certain in the tulip stage if animals are truly dead; consequently, at the end of experiment, tulip stage animals were transferred to control solution for 48 h. If animals recovered from the tulip stage, they were counted as alive.

Paramecia

Five concentrations of each salt type were used: 2, 4, 6, 8, and 10 mS cm⁻¹ each with 10 animals per concentration held individually in 2-mL wells. Paramecia were fed with the suspension of yeasts (10 g L⁻¹) in Lozina-Lozinsky medium every second day (0.02 mL per well). Mortality and numbers present were recorded and LC₅₀ was determined after 24, 48 and 96 h of exposure.

Insects

The rapid toxicity testing method was used (Kefford *et al.* 2003, 2005a, 2005b). There were 10 animals of each species per treatment of 3 L of water. Exposure concentrations were: for *Centroptilum* sp.: WLW (electrical conductivity (EC) $\approx 0.13 \pm 0.01$ mS cm⁻¹), 5, 10, 15 and 20 mS cm⁻¹, and for other species: WLW, 10, 15, 20, 25 and 30 mS cm⁻¹. Observations were made daily for 96 h.

Sub-lethal toxicity testing experimental protocols

Hydras

Experimental procedure was as per acute test (Pollino and Holdway 1999) and is only briefly described. However, budding hydras were used. To achieve this, hydras were fed in excess for 4–6 days. Three concentrations of each salt type were used: 1, 2 and 4 mS cm⁻¹. After counting animals and observing tentacle retraction, animals were fed in excess with brine shrimps (0.2 mL per dish). After 1 h all solutions were changed. All parameters for each day were calculated as the geometrical mean between the new and old medium.

The mean relative growth rate of hydras for each treatment concentration was calculated as follows (Pollino and Holdway 1999):

$$K = (\ln N_t - \ln N_{t-1}) / \Delta t$$

where N_t is the number of animals at time t , N_{t-1} the number of animals at time of previous observation and Δt time between two observations.

Paramecia

The experimental protocol is as per acute toxicity testing with paramecia. The culture growth rate (for individual animals) was calculated using a standard formula:

$$\mu = \ln N / T$$

where N is number of animals in the well at time T and T is time from the start of the experiment (days).

Statistics

For each species and treatment type, Probit regression models (see Agresti 1990) were fitted with the x -variable being EC and the y -variable the response variable (survival, population growth or tentacle contraction). From these regressions, LC₅₀ and EC₅₀ values and their 95% confidence intervals were calculated for each treatment type. *Post hoc* comparison of EC₅₀ values was performed using a paired t -test assuming unequal variances.

Results

Acute tests

For all species examined there were no statistically significant differences in their 96-h LC₅₀ values for the different treatments (Table 1). The results for *Centroptilum*

sp. (Table 1) are, however, somewhat inconclusive. For treatments other than ON over 96 h of exposure, they had partial but <50% mortality at the lowest salinity treatment (5 mS cm⁻¹), consequently their 96-h LC₅₀ value is below 5 mS cm⁻¹ for all types of treatments except ON. Since concentrations below 5 mS cm⁻¹ were not tested in this experiment, the error in LC₅₀ calculation is higher than for the other species and thus there is a greater probability of a type 2 error. Across the three species, however, there would appear to be no detectable effect of the different saline water types on acute survival of freshwater invertebrates tested.

Sub-lethal tests

Though there are differences in tentacle retraction of hydras at 24 h, they were eliminated at 72 h (Fig. 2). The EC₅₀ for the S3 salt type initially increased then later decreased. Thus, the hydras appear to adapt to their environment when the initial shock is reduced, and they can return to their 'normal' condition. Interestingly 24- and 48-h EC₅₀ for S1 seemed higher (though not statistically significant) than all the others. It may be that sulphates are more toxic to hydras than chlorides and eliminating them results in a marginally reduced overall toxicity.

Hydra culture growth was partially affected by the variation in ionic compositions (Fig. 3). The 96-h EC₅₀ value for the S2 treatment was significantly lower than for the ONS and S3 types of treatments.

The population growth of the paramecia was significantly reduced (Fig. 4) when calcium was eliminated from the media (S2 and S3 types).

Discussion

General observations

There were no significant differences in toxicity between ON, ONS and S1 (no sulphates and alkalinity) treatments in any of the experiments. Although this was expected with ON and ONS, it also indicated that removal of SO₄²⁻ and Alk did not change the toxicity of salinity in any detectable way. The proportion of these anions is around 13% of the total anion load in ONS, the rest being mostly Cl⁻. When S1 and S2 treatments were prepared, these anions were replaced with Cl⁻, thus increasing its load. Kefford *et al.* (2004a) observed that ON was less toxic to freshwater invertebrates than pure NaCl. The lack of a difference in toxicity between ON, ONS and S1 may indicate that the difference in toxicity between ON and NaCl is not because of Cl⁻ toxicity, but rather lack or difficulty in extraction at high salinity of essential and trace elements, such as calcium, potassium, copper, selenium etc. The 24- and 48-h EC₅₀ (tentacle retraction) for hydras in S1 were slightly higher than in other treatments. We did not specifically test toxicity of Cl⁻ against SO₄²⁻, but other studies with a range of freshwater invertebrate taxa indicate that

Table 1. The LC₅₀ values for animal species tested in acute 96-h experiments

Species	Type of treatment	LC ₅₀ values (95% confidence intervals)			
		24 h	48 h	72 h	96 h
<i>Paramecium caudatum</i>	ON	8.70 (7.81–9.67)	8.70 (7.81–9.67)	NM	8.70 (7.81–9.67)
	ONS	8.66 (7.77–9.62)	8.66 (7.77–9.62)	NM	8.66 (7.77–9.62)
	S1	9.10 (8.17–10.17)	8.85 (7.93–9.88)	NM	8.85 (7.93–9.88)
	S2	7.24 (6.24–7.82)	7.24 (6.24–7.82)	NM	7.24 (6.24–7.82)
	S3	7.58 (6.65–8.40)	7.38 (6.47–8.15)	NM	7.38 (6.47–8.15)
<i>Hydra oligactis</i>	ON	8.95 (8.50–9.48)	8.75 (8.33–9.32)	8.56 (8.15–9.22)	8.37 (7.91–9.21)
	ONS	9.08 (8.60–9.57)	8.79 (8.37–9.30)	8.61 (8.20–9.15)	8.35 (7.90–8.96)
	S1	9.09 (8.63–9.57)	9.09 (8.63–9.57)	8.90 (8.47–9.40)	8.81 (8.39–9.32)
	S2	9.12 (8.66–9.58)	8.86 (8.43–9.32)	8.86 (8.43–9.32)	8.53 (8.07–9.00)
	S3	9.10 (8.63–9.57)	9.10 (8.63–9.57)	8.92 (8.49–9.39)	8.33 (7.76–8.84)
<i>Notalina fulva</i>	ON	NC	40.55	22.96 (20.32–25.56)	18.46 (16.10–20.94)
	ONS	NC	33.03 (28.91–119.74)	28.17 (24.32–37.90)	19.58 (7.93–23.90)
	S1	NC	32.83	22.55 (19.85–25.55)	18.64 (15.89–21.14)
	S2	60.18	29.51 (26.74–38.27)	24.2	17.97 (14.18–21.54)
	S3	97.46	23.66 (21.28–26.91)	18.27 (15.64–21.04)	15.69 (13.21–17.92)
<i>Micronecta robusta</i>	ON	21.44	19.08	14.51 (–8.27–24.92)	13.44 (–99.73–32.06)
	ONS	25.15 (23.17–27.54)	21.45	18.7	10.51
	S1	29.71 (25.42–45.73)	23.67	17.9	15.78 (2.96–26.14)
	S2	27.61 (25.57–29.67)	23.76	18.02	16.46
	S3	24.15 (15.81–83.88)	19.01	14.11	11.22 (6.65–14.60)
<i>Centropitulum sp.</i>	ON	14.94 (12.58–16.97)	9.25	6.6	5.58
	ONS	13.61	6.33 (2.70–9.26)	2.46 (–3.49–5.33)	1.75 (–3.58–4.21)
	S1	14.37 (12.42–16.76)	10.24	6.57	4.63
	S2	11.32 (8.91–13.49)	7.89	5.17	3.79
	S3	10.19 (7.75–12.53)	6.38 (3.71–8.94)	4.11 (0.07–6.89)	3.57 (–0.95–6.36)

ON, Ocean Nature; ONS, synthetic Ocean Nature; S1, ONS without SO₄²⁻, HCO₃⁻ and CO₃²⁻; S2, ONS without Ca²⁺, HCO₃⁻ and CO₃²⁻; S3, ONS without Ca²⁺ and Mg²⁺; NM, not measured; NC, not calculated (100% survival in all concentrations). For some values, confidence intervals (CI) could not be calculated.

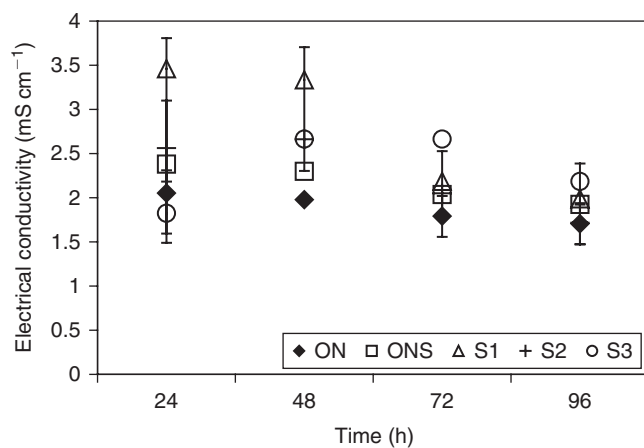


Fig. 2. Values of EC₅₀ (concentration of a toxicant that produced the effect (tentacle retraction) in 50% of the population) for *Hydra oligactis* in different types of treatment (error bars indicate 95% CI).

Na₂SO₄ is more toxic than NaCl (Goetsch and Palmer 1997; Kefford et al. 2004a; Palmer et al. 2004) and that NaCl is more toxic than ON (Kefford et al. 2004a). It would therefore appear that SO₄²⁻ is more toxic than Cl⁻. The replacing

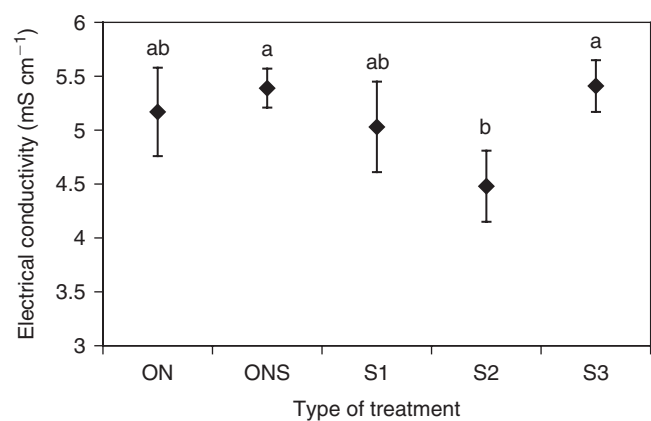


Fig. 3. Ninety-six-hour EC₅₀ values (concentration of a toxicant that produced the effect (culture growth) in 50% of the population) for *Hydra oligactis* in different types of treatment (mean ± s.e., n = 4). Different letters represent significantly different results.

of SO₄²⁻ with Cl⁻ could thus have slightly reduced the overall toxicity to hydras.

The results regarding treatments with calcium deficiencies are discussed in detail below.

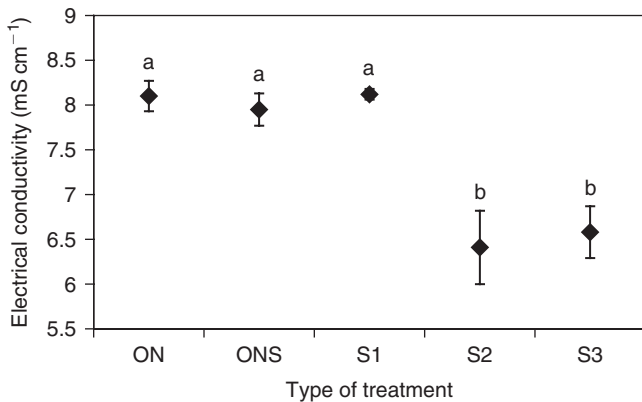


Fig. 4. Ninety-six-hour EC₅₀ values (concentration of a toxicant that produced the effect (culture growth) in 50% of the population) for *Paramecium caudatum* in different types of treatment (mean \pm s.e., $n = 10$). Different letters represent significantly different results.

Acute tests

Short-term acute toxicity testing is usually conducted in sub-optimal conditions for animals tested: static water regime and no food supply. Though these tests convey very useful information on the range of tolerance of the animals to a particular toxicant (which can be very useful in modelling and management on a wider scale), they give very little information on the mechanisms of action or the effects of a toxicant on organisms subject to long exposures and low sub-lethal concentrations. These experiments are therefore usually regarded as a starting point for more detailed long-term sub-lethal exposures. Though both species were clearly affected by the different ionic compositions in our sub-lethal experiments, it was not so in the acute tests (Table 1). In a short-term exposure with lethal concentrations of salinity, the different ionic compositions had no detectable effect. Osmoregulatory mechanisms may have played a major part in combating the effects of high salinity, rather than fine-tuned biochemical and physiological interactions. Chapman *et al.* (2000) found that there were no differences in the survival or swim-up fry toxicity tests (96-h exposure) of rainbow trout embryos in two saline effluents with different ionic proportions. However, they found that chironomid larvae grew differently in the different effluent (10-days exposure). The same results were obtained for sulphates-dominated saline lakes in the USA (Dickerson *et al.* 1996). Although the researchers stated that undiluted lake water was toxic to *Ceriodaphnia dubia* and attributed this to the differences in ionic composition of major ions, when we recalculated the LC₅₀ (% of dilution) provided by the authors, the LC₅₀ in terms of electrical conductivities was surprisingly similar and not significantly different for *C. dubia* (except in very saline waters) and fat-head minnows. These studies and our results consistently indicate that the short-term lethal toxicity of saline solutions found in nature is not generally affected by different ionic proportion/composition, but longer exposures or sub-lethal

effects can reveal the differences. However, salinity produced from pure salts (e.g. NaCl, Na₂SO₄) and 1 : 1 ratio of pure salts, neither of which occur in nature, do have differing toxicity to that of mixtures of salts (Mount *et al.* 1997; Kefford *et al.* 2004a; Palmer *et al.* 2004).

Sub-lethal tests

There could be several explanations regarding the chronic sub-lethal effects of varying ionic compositions.

- (1) Direct effect of deficiency of the essential element calcium.
- (2) Indirect effect of hardness cations (Ca²⁺, Mg²⁺) and carbonates on the biochemistry of the trace metals.

Direct effects of deficiencies in calcium

Effects on paramecia

Paramecia have around 5000 cilia. Movement of the cilia is controlled by their membrane potential. Stimulation of cilia (chemically or physically) activates a voltage-sensitive Ca²⁺ current associated with the ciliary membrane (Preston and Hammond 1998). This results in avoidance behaviour, making paramecia swim backward (Preston *et al.* 1992). Nakaoka and Ooi (1985) found that in the presence of adenosine triphosphate as a stimulus in the medium, paramecia swim forward if Ca²⁺ concentration is below 10⁻⁶ M (40 μ g L⁻¹) and backward if it is higher than 10⁻⁶ M. This suggests that, though directional swimming is governed by the *intracellular* Ca²⁺ concentration, a minimum amount of calcium in medium is required to maintain normal responses to stimuli. Slightly proportionally higher concentrations of trace metals in S2 and S3 (especially at higher salinities) might have affected animals, but lack of calcium in these media did not allow them to respond adequately. In the case of acute toxicity (Table 1), the differences between various ionic composition types were not evident possibly because the short-term effect of higher salinity *per se* was greater than the effect of ionic composition of media, making the osmoregulatory mechanism primarily responsible for mortality. At lower salinities in sub-lethal exposures, calcium deficiencies might play a greater part in paramecia swimming behaviour, thus making animals in Ca²⁺-lacking media more prone to abnormal behaviour, and consequently expending more energy. In addition, morphogenesis of the complex cell surface during mitosis involves transcellular wave signal, which involves cortical alveoli that act as calcium reservoir in the cell (Laurent and Fleury 1995). Presumably, if there were not enough calcium to initiate the signal, mitosis would be abnormal.

Effect on hydras

External Ca²⁺ ions play a major role in the nematocyst discharge in hydrozoans (Yanagita 1973; McKay and Anderson 1988; Salleo *et al.* 1994a, 1994b; cited in Kawaii *et al.* 1999). Santoro and Salleo (1991) observed that nematocytes do not

discharge in Ca^{2+} -free medium, and that La^{3+} , Cd^{2+} and Co^{2+} prevented discharge by blocking the Ca^{2+} channel even when some Ca^{2+} was present. Gitter *et al.* (1994) found that discharge of the stenoteles (a type of nematocyst) in *Hydra vulgaris* is regulated by a mechanism that allows intake of Ca^{2+} from ambient solution. This may explain why hydras were not affected in the acute toxicity test (involving no feeding and therefore no nematocysts discharge) (Table 1), but were growing slower in the sub-lethal test (where nematocysts were discharged to capture their prey) in the S2 treatment compared with the ONS control (Fig. 3). As there was some Ca^{2+} present in the M4 medium, which was used as control and dilution water for the range of salinities prepared, at higher salinities the effect of blocking Ca^{2+} by increasing concentrations of Co^{2+} and Ni^{2+} (see Table 2 in Accessory Publication on the *Marine and Freshwater Research* website) may have begun to play a role. Kawaii *et al.* (1999) reported that Mg^{2+} also had an inhibitory effect on atrichous isorhiza (a type of nematocyst) discharge, and that the inhibitory effect of Mg^{2+} increased when the external concentration of Ca^{2+} was lowered. This might explain why the S2 type affected sub-lethal salinity tolerance in hydras. S3 type medium, though lacking Ca^{2+} , may not affect hydras as much as the S2 type (Fig. 3) because it also lacked a powerful Ca^{2+} blocker i.e. Mg^{2+} .

Freshwater hydras reproduce by means of forming buds and developing a foot at the base of a bud and then detaching from the parent. A separated bud was counted as a new animal in our experiments. Zeretzke *et al.* (2002) found that in *Hydra vulgaris* (Zurich strain) foot formation was prevented by lowered concentrations of ambient Ca^{2+} , making animals form branches that persisted on parent's body instead. This would definitely affect the culture growth in our study, because the number of separate individuals has not changed.

Increased toxicity of trace metals

Water quality parameters such as hardness and alkalinity can influence the interactions of ions in ambient solution. Increases in hardness have shown to result in decreased copper toxicity to fish (Pagenkopf 1983) and cladocerans *Daphnia magna* (Schampelaere and Janssen 2002) as a result of competition between the hardness metals (Ca, Mg) and trace-metal species for interaction sites. Welsh *et al.* (2000) also showed that acute copper toxicity was lower in waters containing proportionately more calcium. They also indicated that calcium is more important than magnesium in modifying the toxicity of copper in rainbow trout and chinook salmon. The same applies to uptake of zinc by rainbow trout (Alsop and Wood 1999) and *D. magna* (Heijerick *et al.* 2002), cadmium by *D. magna* (Penttinen *et al.* 1998) and the amphipod *Hyalomma azteca* (Jackson *et al.* 2000), and manganese by brown trout (Stubblefield *et al.* 1997) in the presence of competing Ca^{2+} ions. All water types used in our study contained

essential and trace metals Fe, Mn, Cu, Zn, Mo, Se, Li, Sr, Br, Rb, Co, V and Ni (see Table 2 in the Accessory Publication on the *Marine and Freshwater Research* website), which, at elevated concentrations, can be toxic to aquatic invertebrates. Though the concentration of each trace element was very low, a combined load might be significant in the absence of calcium. Elimination of calcium and/or magnesium out of the solution can result, first, in the relative increase of concentrations of trace elements, especially at higher salinities, and second, in increased toxicity of these elements because in the absence of calcium and/or magnesium, more sites are available for binding at the organism–water interface. The hypothesis of increased trace metal toxicity in Ca^{2+} lacking media remains to be tested.

Metal toxicity can also be reduced by complexation with carbonate, thus decreasing the activity of free hydrated metal ions (Barata *et al.* 1998).

Conclusions

Variation in ionic compositions common in saline inland waters of south-eastern Australia did not affect acute lethal salinity tolerance of any species investigated. However, the different ionic compositions affected the sub-lethal responses of the three investigated species. The water types lacking calcium had the most deleterious sub-lethal effects on the animals. The different responses of invertebrates to various ionic composition types in combination with the sub-lethal range of salinity may be governed by deficiencies in calcium, the chemical interaction of hardness cations, alkalinity and trace metal uptake and toxicity.

In assessing the effects of salinity on freshwater invertebrates, the ionic proportions should be considered in salinity exposures that are likely to induce sub-lethal effects.

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