Comparing test systems to measure the salinity tolerance of freshwater invertebrates

Ben J Kefford[1]*, Carolyn G Palmer[2], Larisa Pakhomova[2,#] and Dayanthi Nugegoda[1]

[1] *Biotechnology and Environmental Biology, School of Applied Sciences, RMIT University, Bundoora, 3083, Victoria, Australia*
[2] *Unilever Centre for Environmental Water Quality, Institute for Water Research, Rhodes University, Grahamstown, 6139,
Eastern Cape, South Africa*

Abstract

There have recently been several studies into acute salinity tolerance of freshwater invertebrates using different methods, making comparisons between studies difficult. The alternatives focus on experimental flow regimes and ionic proportions. In this study non-rheophilic riverine taxa collected in South Africa and south-east Australia were variously exposed to solutions of sodium chloride (NaCl) and the artificial sea salt, Ocean Nature, in flowing and still water. South African species: *Euthraulus elegans* (Ephemeroptera: Leptophlebiidae), *Micronecta piccanina* (Hemiptera: Corixidae), *Burnupia stenochorias* (Gastropoda: Ancylidae) and *Caridina nilotica* (Decapoda: Atyidae); Australian collected species: *Daphnia carinata* (Cladocera: Daphniidae), *Micronecta annae* and *Physa acuta* (Gastropoda: Physidae). The main findings were:
- The salinity tolerances of a range of taxa were not affected by flow regimes
- Taxa were less sensitive to the artificial sea salt than NaCl
- There was, however, a direct relationship between the LC_{50} values from both salts. This relationship was used to compare the LC_{50} values from studies testing (artificial or natural) sea-water or NaCl.
- The comparison indicated variation in the mean LC_{50} between studies that is probably, at least in part, due to the range of taxonomic groups and rarities of species tested.

When comparing the acute salinity tolerance of non-rheophilic invertebrates, the salt source and criteria for choosing species affect the results, but the flow environment probably does not.

Keywords: stream invertebrates, acute salinity tolerance, test system

Introduction

Salinity in rivers and wetlands is increasing in many arid and semi-arid regions of the world including Southern Africa and Australia (Williams, 1987). There is considerable uncertainty about the effect of this increase on aquatic biota and detailed investigations of salinity tolerance are needed (Hart et al., 1991; Clunie et al., 2002). A number of studies have used a variety of different methods to investigate the acute salinity tolerance of macroinvertebrates making comparisons difficult.

A variety of different salt sources and experimental systems have been used in laboratory salinity tolerance experiments. In both Australia (Kefford et al., 2003; 2004) and South Africa (Kefford, 2002) non-flowing water has been used as a simplified and stand-ardised system for rapidly testing many species. Other studies in South Africa, Palmer et al. (1996), Goetsch and Palmer (1997), Palmer and Rossouw (2000) and Palmer and Scherman (2000), have used a flowing environment to mimic a natural stream. Kefford et al. (2003, 2004) used artificial sea-water because in Australia most inland waters have ionic proportions similar to sea-water (Bayly and Williams, 1973: 1; Williams and Buckney, 1976a; b; Herczeg et al., 2001). Palmer and co-workers used sodium chloride (NaCl) and sodium sulphate (Na_2SO_4) because most agriculture-induced

salinisation in South Africa is NaCl dominated and most saline industrial and mine effluents are SO_4^{2-} dominated (Dallas and Day, 1993). Other studies have also used NaCl (Clemens and Jones, 1954; Williams et al., 1999; Blasius and Merritt, 2002) or sea-water (Shirgur and Kewalramani, 1977; Mills and Geddes, 1980; Williams, 1984; Williams and Williams 1998) to investigate the salt tolerance of freshwater macroinvertebrates. Although Na^+ and Cl^- are the most common ions in sea-water, the presence of other ions may result in differences in the tolerance of macroinvertebrates to NaCl and (natural or artificial) sea-water. For example, *Daphnia magna* 48 h LC_{50} values for various salts ranged from 0.63 to 7.98 g/ℓ for various salts (Mount et al., 1997). Direct comparisons between studies using different salts are therefore difficult.

There are also differences in the criteria for choosing species to investigate. Palmer and co-workers chose one to six species per publication, sometimes considering the same species collected from different locations in different publications, and only included species collectable in high numbers. They mostly tested Ephemeroptera but tested fewer species of Trichoptera and Gastropoda. Ephemeroptera, especially Baetidae, are salt-sensitive compared with other macroinvertebrates (Clemens and Jones, 1954; Hart et al., 1991; Short et al., 1991; Williams and Williams, 1998; Kefford et al., 2003). The tolerances of species from this order are therefore unlikely to reflect the salinity tolerance of most members of natural communities (see Forbes and Calow, 2002). Kefford (2002) and Kefford et al. (2003) attempted to select species from orders in approximate proportion to which the orders were found in the locality where they were collecting macroinvertebrates. This resulted in a relatively large number of taxa (49 and 57, respectively) from many higher taxonomic groups (9 and 16 orders, respectively),

including taxa found at low abundances. Other studies have given a range of explanations for how species were selected. In Canada, Williams et al. (1999) chose six abundant species for testing, four of which were generally associated with low salinity sites while the remaining two species were associated with high salinity sites. In the USA, Blasius and Merritt (2002) chose six species from five orders to represent a number of different trophic levels, habitat requirements, respiration systems and taxonomic groups.

Directly comparing the salinity tolerance of species tested using different methods will not indicate whether any differences observed are due to real differences in tolerances or simply a reflection of the method used. Even if different methods of testing do not affect salinity tolerance, the criteria for selecting species may result in different studies showing differences in the range of tolerances where none exist. It is therefore not always possible to ascertain whether two locations tend to have species that are more or less tolerant, making it difficult to test hypotheses (Nielsen and Hillman, 2000; Kay et al., 2001) about spatial variation in salinity tolerance. In this paper we compare several methods of testing the salinity tolerance of macroinvertebrate species:

- The effect of flow (flowing water vs. standing water)
- The effect of salt source (NaCl vs. the artificial sea salt, Ocean Nature [Aquasonic, Wauchope, New South Wales, Australia]). We hypothesise that if flow or salt source affects salinity tolerance then LC_{50} values should differ between flow systems/salt sources.
- The effect of test species selection, which we explore by comparing the mean and the range of LC_{50} values from selected studies.

As a secondary aim we compare the LC_{50} values obtained from 72 h and 96 h tests.

Methods

General methods

Macroinvertebrates were collected in the field and brought back to the laboratory in the water that they were collected in and the experiments were started once that water was approximately the ambient air temperature of the laboratory (20°C). No other acclimation was conducted. The control and diluting water used in the experiments was Melbourne or Grahamstown tap-water, for the Australian and South African experiments respectively, that had been carbon-filtered and in the case of the Australian experiments also sand-filtered.

Animals were regarded as dead if they were not moving and failed to respond to probing. Snails that had retracted into their shell were placed in freshwater for 30 min and if they failed to respond were regarded as dead. Dead individuals were removed when survivorship had been checked.

Except where noted, salinity in this paper is measured in terms of electrical conductivity or EC (mS/cm; note 1 mS/cm = 1 000 uS/cm = 100 mS/m) adjusted to 25°C, as it is the most common measure of salinity and is also rapidly, accurately and reliably measurable. Experiments were conducted over a variety of periods. The experiment with *Daphnia carinata* was conducted over 48 h as 24 h or 48 h as is standard with this genus (Walker et al., 1996: 127). Experiments with most other species were conducted over 96 h but results are also reported at 72 h to ensure comparability with studies reporting over this period. Experience has shown that some *Micronecta* have high mortality between 72 and 96 h and the

experiments with *Micronecta annae* were performed over 72 h. As is standard, animals were not fed during acute experiments (OECD, 1996; ASTM, 1998).

Standard logistic regression (Agresti, 1990) was used, with the dependent variable the probability of survival and the independent variable the EC. The concentrations lethal to 50% of individuals (LC_{50}) and their 95 % confidence intervals (CI) were calculated from this regression giving an alpha level of 0.05.

The effect of flow

The effect of the two different flow-environments: flowing channels and non-flowing aquaria on the salinity tolerances of four South African species were determined concurrently in August 2002. *Euthraulus elegans* (Ephemeroptera: Leptophlebiidae) and *Micronecta piccanina* (Hemiptera: Corixidae) were collected from the Kat River at Amherst (S 32 ^{o} 38'; E 26 ^{o} 41'). *Burnupia stenochorias* (Gastropoda: Ancylidae) were collected from the Botha River at Visgat Pool (S 33° 13'; E 26° 30'). See Kefford 2002 and Kefford et al. (2004) for site information. *M. piccanina* were caught with a sweep net from slow-flowing pools, *E. elegans* were picked off cobbles in a riffle with a fine brush and *B. stenochorias* were carefully removed from stones from a large pool. *Caridina nilotica* (Decapoda: Atyidae) were obtained from a laboratory colony that was originally stocked from Mpisini Stream in Richards Bay, KwaZulu-Natal (see Kefford et al., 2004). No species was rheophilic (living only in fast-flowing water). *E. elegans* and *B. stenochorias* are regularly found in both fast-flowing riffles and slower-flowing sections of streams, while the other two are predominately found in slower-flowing parts and within fringe vegetation (Personal observations). The four species were chosen because they could be obtained in numbers sufficient to perform the experiment (> 160 individuals) and represented a range of taxonomic groups, modes of locomotion and assumed salinity tolerances.

The flowing channels were small-scale artificial streams consisting of 1 m of plastic gutters (or channels) that overflow at their downstream end into a bucket where a submersible pump in each bucket returned water to the upstream end of its channel (DWAF, 2000). Mesh prevented animals from flowing into the bucket. A strip of mesh and four small stones were placed on the channel bottom to provide the animals with a rough surface for attachment and a range of flow environments, respectively.

Ten salinity treatments were prepared (control, 5.5, 9.6, 16, 18, 21, 23, 30, 35 and 41 mS/cm) by dissolving Ocean Nature salt. For each treatment there was one channel with 20 ℓ of water and three glass aquarium tanks with 6.6 ℓ of water and a third as many animals as the channel, so that densities (animals/ℓ) were equal in both systems. Water was aerated in the aquariums and both systems had high dissolved oxygen (> 80% saturation).

Due to a limited number of animals available and differences in their assumed salinity tolerances, not all species were subject to all salinity treatments. *C. nilotica* were subject to all, while *B. stenochorias* to all ≤ 35 mS/cm and *E. elegans* and *M. piccanina* in to all ≤ 30 mS/cm. Differing numbers of individuals were available for each species: the numbers exposed in each channel and aquarium were 24 and 8, respectively, for *C. nilotica* and *E. elegans*, 7 and 21 for *M. piccanina* in and 6 and 18 for *B. stenochorias*. Individuals emerging as flying adults or otherwise not locatable were excluded from the analysis.

In the tanks different species were prevented from physically interacting by housing them separately in containers that allowed for circulation of water. Due to its larger size, *C. nilotica* was housed unconstrained in the aquaria while the other three species were

housed in separate containers. The channels were divided into two, by means of a mesh barrier, and *E. elegans* and *B. stenochorias* were housed in the upstream section and the other species in the downstream section.

The effect of salts

The tolerances to Ocean Nature salt and NaCl were determined using three species collected from Australia (*Daphnia carinata* [Cladocera: Daphniidae], *Micronecta annae* and the introduced snail *Physa acuta* [Gastropoda: Physidae]) and two South African species (*C. nilotica* and *E. elegans*). The tolerance of *C. nilotica* to Na_2SO_4 was also determined. These species were chosen because they could be obtained in sufficient numbers, represented several higher taxonomic groups and assumed salinity tolerance.

As part of a study into the toxicity of various saline lakes to *Daphnia carinata* (Kefford, 2000a; Kefford et al., 2002) the 48 h lethal tolerance to Ocean Nature and analytical grade NaCl were determined. *D. carinata* were obtained from a laboratory colony originally stocked from the Yarra River (near Melbourne). For further details of the methods see Kefford (2000a).

Micronecta annae and *Physa acuta* were collected from the Barwon River at Pollocksford Bridge (S 38 °09'; E 144 °11') in January 2002 and 2003, respectively, and tested for their tolerance to Ocean Nature and NaCl (Dominion Salt Limited, New Zealand with a minimum NaCl content of 99.9%). *M. annae* was tested in five salinity treatments (control and 6.4, 12.6, 15 and 25.6 mS/cm), while seven salinity treatments (control, 5.0, 7.5, 10, 12.5, 15 and 20 mS/cm) were used with *P. acuta*. These treatments were selected from past experimentation with these species (Kefford et al., 2003). In each treatment there were three 500 mℓ containers with approximately 450 mℓ of water in each and six *M. annae* or three *P. acuta*.

The tolerance of *C. nilotica* from the aforementioned laboratory colony to Ocean Nature, NaCl and Na_2SO_4 was determined in aquaria over 96 h. There were 10 salinity treatments (control, 1, 3, 5, 7, 9, 11, 13, 15 and 18 g/ℓ) for each salt with an additional three salinity treatments (20, 25 and 30 g/ℓ) for Ocean Nature. The EC

of the water in each aquarium was measured. Each treatment consisted of one aquarium with 6.6 ℓ of water and 10 shrimps held unconstrained with no other species.

In October 2002, *E. elegans* collected from the Kat River at Amherst were tested in aquaria to Ocean Nature and NaCl over 240 h (10 d). There were 10 salinity treatments (control, 0.3, 0.5, 0.8, 1.4, 2.3, 3.9, 6.5, 10.8 and 18 g/ℓ) each comprising a single aerated aquarium with 21 individuals. The EC for both salts in each treatment was measured. *E. elegans* were not fed nor was the water changed for the first 96 h of the experiment. At 96 h the water was changed and *E. elegans* were fed 0.02 g of ground Tetramin (fish flakes) per treatment. A second feeding and water change occurred at 192 h (8 d).

The effect of species selection

LC_{50} values from selected studies were compared to determine the similarity of salinity tolerances. Due to the presence of LC_{50} values given as > some value, or censored data (Smith, 2002), the mean LC_{50} was calculated using the Kaplan-Meier method (see Kefford et al., 2003). Where LC_{50} were measured in total dissolved salts (TDS) they were converted to EC in terms of Ocean Nature using the formula EC (in mS/cm) = TDS (in g/ℓ)/0.754 (Kefford et al., 2003). Where multiple LC_{50} values were calculated for a species with the same salt (for different collection locations or dates) the arithmetic mean value for each species was used.

Results

The effect of flow

Survival in the controls was high (> 90%) in all species except *M. piccanina* over 96 h (Figs. 1a-c). For *M. piccanina* control survival was 81 % at 72 h (Fig. 1d) and 66 % at 96 h. This species had considerable mortality in all treatments between 72 h and 96 h making for wide 95% CI of LC_{50} at 96 h (Table 1). Each species in both systems responded to Ocean Nature salt similarly (Fig. 1) with
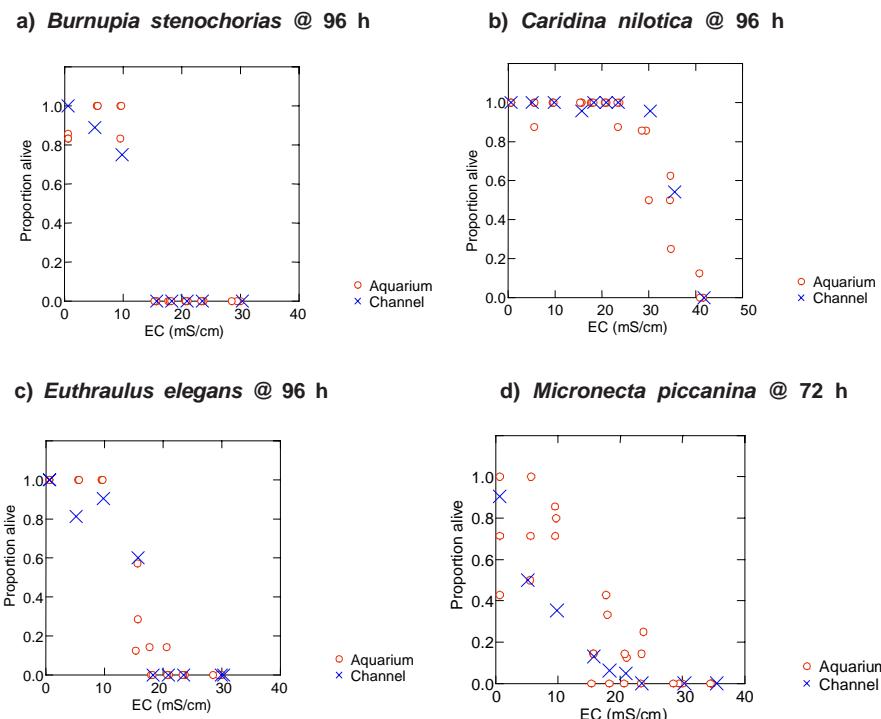
a) *Burnupia stenochorias* @ 96 h

b) *Caridina nilotica* @ 96 h

Figure 1
Salinity tolerance of four species in the two test systems

c) *Euthraulus elegans* @ 96 h

d) *Micronecta piccanina* @ 72 h

LC_{50} values having overlapping 95 % CIs, indicating no significant difference between systems (Table 1). Indeed the difference in LC_{50} values between systems ranged from 0 to 2.7 mS/cm, thus even if a Type two error has occurred, the differences between systems are of little practical consequence. Due to the similarity of the LC_{50} values between systems we also report them calculated from all data (combined).

LC_{50} values for *B. stenochorias* were identical whether calculated over 72 h or 96 h of exposure (Table 1). The 96 h LC_{50} values for *E. elegans* and *C. nilotica* were 87 % and 97 %, respectively, of their 72 h LC_{50} value. For *M. piccanina* the 96 h LC_{50} value was about half the 72 h LC_{50} value; however, as noted above, the high mortality in all treatments between 72 h and 96 h suggests that 72 h of exposure is more appropriate for this species.

The effect of salts

Survivorship in the controls over the acute exposure periods was high (Table 2, Figs. 2 to 6) for all species investigated. In four of the five species the LC_{50} for Ocean Nature was statistically significantly higher than that for NaCl in all time periods. The exception, *M. annae*, had overlapping 95 % CIs but followed the same trend (Table 2, Fig. 3). The LC_{50} value for *C. nilotica* exposed to Na_2SO_4 was lower still than that for NaCl (Table 2, Fig. 5).

The LC_{50} values calculated after both 72 h and 96 h of exposure were identical for *P. acuta* (Table 2). For *C. nilotica* the 96 h values were between 85 and 97 % of the 72 h LC_{50} values, while for *E. elegans* the 96 h values were 92 to 94 % of the 72 h values.

Tolerance to Ocean Nature salt was well correlated with tolerance to NaCl (Fig. 7). While the sample size was small (n=5) there was a statistically significant relationship between the LC_{50} for NaCl and the log-transformed LC_{50} for Ocean Nature (r=0.91, P=0.033). The best relationship is described by:

$$Log_{10} (LC_{50} \text{ for Ocean Nature}) = 0.83 + 0.035 (LC_{50} \text{ for NaCl}) \quad (1)$$

Where all LC_{50} (mS/cm) are at 72 h except for *D. carinata* where 48 h results are used. These periods were chosen to minimise the difference between exposure periods between species.

Effect of species selection

There are clear differences in the mean salinity tolerances between the three studies using (artificial or natural) sea-water and those using NaCl (Table 3). Assuming a similar difference in the toxicity of NaCl to Ocean Nature as observed in this study, LC_{50} values for NaCl were adjusted using Eq. (1) to give estimates of their value for Ocean Nature (Table 3). While this adjustment reduced the discrepancy between the studies, it did not eliminate it. The mean Ocean Nature adjusted LC_{50} from Blasius and Merritt (2002) and Palmer (unpublished) are about half that observed for Ocean Nature or sea-water by Kefford (2002), Kefford et al. (2003) and Shirgur and Kewalramani (1973) while about a third of that from Williams (1984).

TABLE 1
LC_{50} values in mS/cm (with 95% CI) for four species in two different test system. Combined is calculated using all data from both systems

Species	System	72 h	96 h
Micronecta piccanina	Aquarium	10 (7.7 – 13)	4.8 (1.5 – 7.2)
	Channel	7.3 (4.8 – 9.4)	3.8 (1.4 – 5.8)
	Combined	8.9 (7.1 – 10)	4.3 (2.4 – 5.9)
Burnupia stenochorias	Aquarium	11 (9.6 – 13)	11 (9.6 – 13)
	Channel	11 (9.3 – 12)	11 (9.3 – 12)
	Combined	11 (9.9 – 12)	11 (9.9 – 12)
Euthraulus elegans	Aquarium	16 (15 – 17)	15 (13 – 16)
	Channel	15 (14 – 17)	14 (12 – 15)
	Combined	16 (15 – 17)	14 (13 – 15)
Caridina nilotica	Aquarium	35 (33 – 37)	33 (31 – 35)
	Channel	36 (35 – 38)	35 (34 – 37)
	Combined	35 (34 – 37)	34 (33 – 36)

TABLE 2
LC_{50} values in mS/cm (with 95% confidence interval) for the various salts tested. ON = Ocean Nature.

Species	Salt	Control mortality	48 h	72 h	96 h	240 h
Daphnia carinata	ON	0%	11 (10 –12)			
	NaCl	0%	4.5 (3.8-5.0)			
Micronecta annae	ON	12%		13 (11-16)		
	NaCl	12%		11 (8.9-13)		
Physa acuta	ON	0%		15 (13-18)	15 (13-18)	
	NaCl	0%		8.7 (7.6-9.9)	8.7 (7.6-9.9)	
Caridina nilotica	ON	0%		36 (34 – 41)	35 (33–40)	
	NaCl	0%		18 (16– 20)	16 (14– 18)	
	Na_2SO_4	0%		11 (9.9–13)	9.4 (8.2–11)	
Euthraulus elegans	ON	23% [#]		18 (16-20)	17 (15-19)	12 (9.8 –14)
	NaCl	23% [#]		14 (13-15)	13 (12-15)	7.7 (6.8-8.9)

however 2% over 96 h

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Discussion

Effect of flow

There was a remarkable similarity between the acute salinity tolerances of species tested in flowing and non-flowing environments, suggesting that, in most cases, flow has a minimal effect on the acute salinity tolerance of non-rheophilic macroinvertebrates. Given that tests in still water are much simpler, we recommend this environment be used for acute salinity tolerance testing of non-rheophilic macroinvertebrates. It remains to be shown whether the salinity tolerance of rheophilic macroinvertebrates is affected by flow.

Effect of salt

In contrast, four of five species had significantly higher LC_{50} values for Ocean Nature than for NaCl. Although not statistically significant, the exception had the same trend. The differences between the salts were broadly constant across the different taxonomic groups and from species collected from Australia and South Africa. Sub-chronic exposure of *E. elegans* produced a similar difference in LC_{50} values between salts than did acute exposure. The constancy of this trend suggests that in most cases, acute tolerance to (artificial or natural) sea-water will probably be higher than NaCl. Therefore direct comparison between tests using these salts is problematic. Despite differences in tolerances between the two salts, the LC_{50} for both salts was positively correlated and as measures of relative tolerance between species either should be useful. *C. nilotica* was also exposed to Na_2SO_4, which was more toxic than NaCl and this is in agreement with comparisons on other species (Goetsch and Palmer, 1997; Palmer and Scherman, 2000; Palmer, unpublished)

The presence of calcium and magnesium ions decreases the permeability and increases the integrity of cell membranes and their presence is known to reduce flows of both water and ions across the gills of fish (Rankin and Davenport, 1981: 66). Palmer and Scherman (2000) observed that calcium increased the salinity tolerance of *Tricorythus tinctus* (Ephmeroptera: Tricorythidae) while sulphate decreased its salinity tolerance. Likewise, Dwyer et al. (1992) observed that increased hardness (calcium and magnesium) increased the salinity tolerance of *Daphnia magna* and *Morone saxatilis* (striped bass). Calcium and magnesium in sea-water comprise about 18 and 3 meq % of cations, respectively (Boulton and Brock, 1999), which might reduce sea-water's toxicity relative to that of NaCl.

Mount et al. (1997) investigated the toxicity of 10 pure salts and their combined toxicity in (mass-based) one-to-one ratios. Their results for *Daphnia magna* and *Ceriodaphnia dubia* show that most combinations of two salts had lower LC_{50} values than one or both of the corresponding single salts. This trend was, however, less apparent in *Pimephales promelas* (fathead minnow). The presence of a single anion and a single cation, as in the case of exposure to NaCl, may have a greater effect of an individual's ability to handle increased ionic concentrations compared with exposed to multiple cation and anions, as in the case of Ocean Nature.

Another possible reason for the difference in tolerance to Ocean Nature and NaCl may be pH. High salinity treatments with Ocean Nature tended to have higher pH than the corresponding NaCl treatment (personal observations). In the *C. nilotica* experiment, for example, at 18 g/ℓ of Ocean treatment the mean pH was 8.2, while 18 g/ℓ of NaCl had a mean pH of 7.9. In freshwater fish and *Daphnia magna*, low pH inhibits sodium uptake and increases sodium loss (Aladin and Potts, 1995), although it is uncertain as to whether such
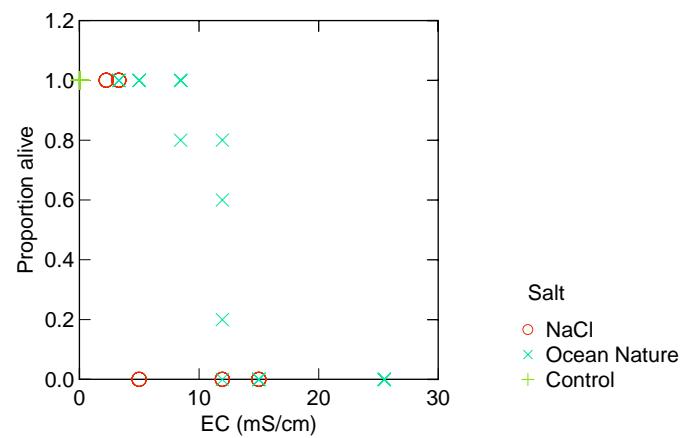

Figure 2
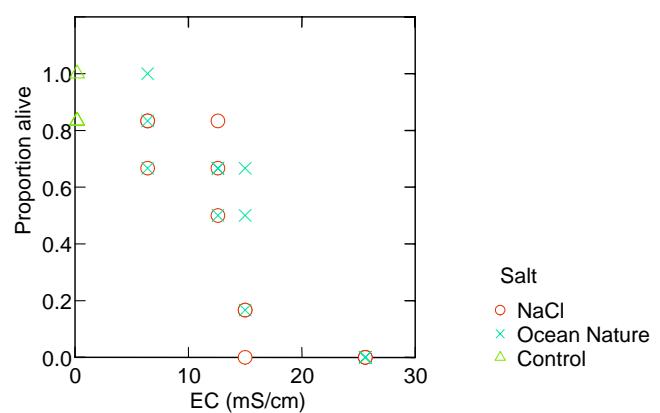*Survival of* Daphnia carinata *over 48 h at different EC levels produced from Ocean Nature salt and NaCl*

Figure 3
Survival of Microcronecta annae *over 72 h at different EC levels produced from Ocean Nature salt and NaCl*
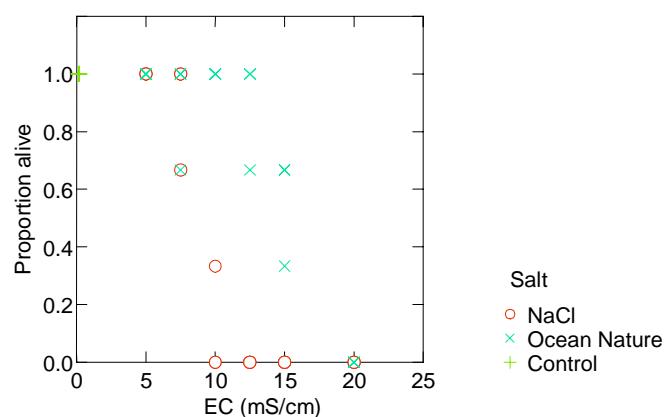

Figure 4
Survival of Physa acuta *over 72 and 96 h (there was no mortality between 72 and 96 h) at different EC levels produced from Ocean Nature salt and NaCl*

an effect would occur in the neutral to slightly alkaline waters of the current study.

In nature when salinity increases it will never be due to the addition of pure salts. As previously mentioned, in Australia, the increase in salinity will usually have ionic compositions close to that of sea-water. Experiments performed with NaCl in these circumstances will thus likely over-estimate the effects of salinity increases.

Inland waters are dominated by four major cations (Ca^{2+}, K^+, Mg^+ and Na^+) and four major anions (Cl^-, SO_4^{2-}, HCO_3^- and CO_3^{2-}) (Kalff, 2002: 202) not to mention a large number of minor or trace elements. Even where rises in salinity tend to have ionic proportions similar to sea-water, minor variations in ionic proportions (Radke et al., 2002) may result in variation in the response of organism to salinity changes (see Radke et al., 2003). The acute toxicity of Ocean Nature to *Daphnia carinata* under-estimated the toxicity of three saline lake waters despite those waters having an ionic proportion similar to sea-water (Kefford, 2000 a; b). Toxicity testing of artificial water from one lake (water that was made up to have the identical ionic composition) suggested that the difference in toxicity could be accounted for by minor differences in the proportions of major ions (Kefford, 2000a), while in another lake, differences in the proportions of major ions only partly accounted for differences in toxicity. There is also the potential for increases of salinity to be accompanied by other changes in water quality (Kefford, 1998) and these changes may have effects on aquatic biota or may modify the effect that salinity has on aquatic biota.

Untangling the effects of different ionic concentrations on the salinity tolerance of a range of aquatic organisms would be a challenging task. Mount et al. (1997), for example, tested 1 887 ion solutions using one species, *Ceriodaphnia dubia*, yet only considered combinations of salts in one to one ratios. Until a fuller understanding of the toxicity of differing ionic proportions on a range of species exists, compromises in selecting salt sources will be needed. Where studies are investigating the likely effects of general rises in salinity, a salt source with typical ionic proportions and exposure conditions is likely to be the most practical option. The potential for minor changes in ionic proportion (and other changes in water quality) to affect toxicity should be kept in mind. Where studies are investigating effects of salinity changes from specific sources, where practical, saline water from that source should be used.

An alternative approach would be to use NaCl as a 'worst case scenario'. If species are not affected by rises in salinity caused by NaCl, then the current study suggests that they will not be affected if the ionic proportion is the same as sea-water. While this approach is conservative, it will result in species being over-protected. A balance will have to be struck between the costs and benefits from over- and under- protecting and the costs of determining their salinity tolerance.

72 h vs. 96 h LC$_{50}$

As with Kefford (2002), the LC$_{50}$ values calculated over both 72 and 96 h of exposure were similar, except for *M. piccanina*, which showed high mortality in all treatments between 72 and 96 h and its tolerance is therefore best considered over 72 h. Acute toxicity tests with *Daphnia* species are usually conducted over 24 h or 48 h and 96 h is commonly used for fish (Walker et al., 1996: 127). Therefore conducting 72 h tests with most macroinvertebrates, which are between the size of *Daphnia* and fish, would seem reasonable.
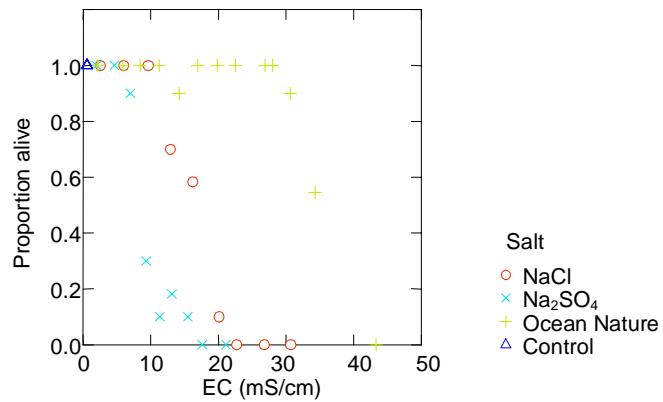

Figure 5
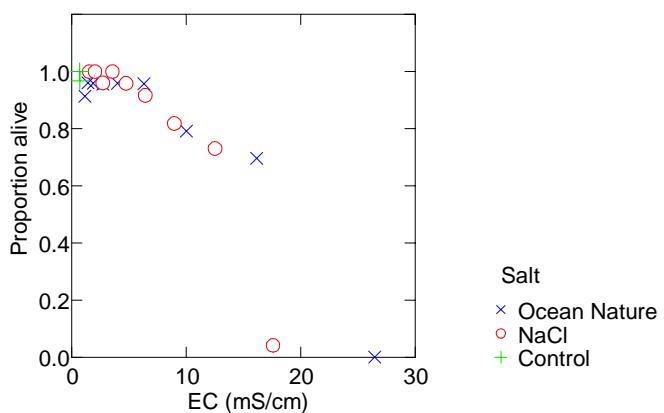*Survival of* Caridina nilotica *over 96 h at different EC levels produced from Ocean Nature salt, NaCl and Na$_2$SO$_4$*

Figure 6
Survival of Euthraulus elegans *over 96 h at different EC levels produced from Ocean Nature salt and NaCl*
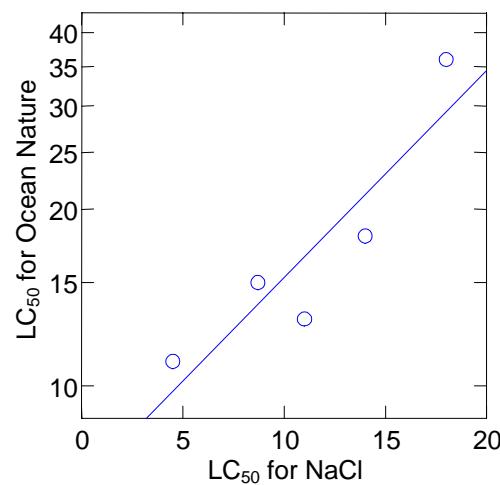

Figure 7
Relationship between LC$_{50}$ values for Ocean Nature salt and NaCl (mS/cm). Values plotted are for 72 h except for D. carinata *where 48 h results are plotted. The straight line is the least squares regression line.*

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Effect of species selection

Various studies (Clemens and Jones, 1954; Shirgur and Kewalramani, 1973; Williams, 1984; Blasius and Merritt, 2002; Palmer, unpublished; Kefford, 2002; Kefford et al., 2003) indicate considerable differences in salinity tolerance, which do not appear to be wholly explainable due to the use of NaCl or (artificial or natural) sea-water. Macroinvertebrate taxa found in low local abundances (or rare) tend to have a higher LC_{50} than common taxa (Kefford, 2002; Kefford, et al 2003). The mean LC_{50} for common macroinvertebrates from Kefford (2002) and Kefford et al. (2003) were 17 and 21 mS/cm, respectively. These values are closer to the mean Ocean Nature adjusted LC_{50} value of 14 mS/cm from both Palmer (unpublished) and Blasium and Merritt (2002) who tested only common species. Two possible reasons for the remaining discrepancy between studies include:

- Real differences in the salinity tolerances of macroinvertebrates from different locations
- In testing relatively few species, only those with a limited tolerance range were selected (see Forbes and Calow 2002).

In particular, Palmer (unpublished) and Williams (1984) selected mostly Ephmeroptera and macrocrustaceans, respectively, for testing; members of the former tend to be salt-sensitive and the latter tend to be salt-tolerant (Kefford et al., 2003). It is therefore possible that differences between studies may be partly caused by the use of different salts and from the taxa chosen for testing.

Criteria by which taxa are included in studies of salinity tolerance are of critical importance. Studies determining the salt sensitivity of specific taxa at a location will probably not reflect the salinity tolerance of the range of taxa present at that location unless a large number of species are chosen from a range of taxonomic groups and rarities. Comparing results from such studies to those that consider a small number of common species or species from restricted taxonomic groups may result in large differences between the studies. Thus apparent differences in salinity tolerances between studies conducted at different locations may not reflect real differences in the tolerances of macroinvertebrates.

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TABLE 3

Summary of LC_{50} values from various studies: mean value (and range) from the suite of species tested in each study. Where n = number of species tested, nd = no determination, ON = Ocean Nature, Ad. ON = NaCl adjusted to Ocean Nature from Equation 1.

Location	Salt source	72 h	96 h	Source
Australia	ON	31 (5.5 – 76) n= 57	nd	1
	Sea-water	nd	43 (31 – 61) n=6	2
S. Africa	ON	32 (11 – 47) n=43	31 (8.5 – 47) n=41	3
	NaCl	nd	8.6 (6.7 – 11) n=6	4
	Ad. ON	nd	14 (12 – 17) n=6	4
	Na_2SO_4	nd	6.6 (3.9 – 11) n=4	4
USA	NaCl	nd	8.4 (4.7 - > 13) n=6	5
	Ad. ON	nd	14 (9.8 - > 15) n=6	5
	NaCl	nd	14 (5.6 – 23) n=4	6
	Ad. ON	nd	26 (11 – 43) n=4	6
India	Sea-water	29 (19-46) n=12 #		7

1 = Kefford et al. (2003), 2 = Williams (1984), 3 = Kefford (2002), 4 = Palmer (unpublished), 5 = Blasius and Merritt (2002) and 6 = Clemens & Jones (1954), 7 = Shirgur & Kewalramani (1973)

Estimated over approximately 72 h from data given in the original paper, which report survival time at various salinity.

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