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ScienceDirect
Environmental Pollution 151 (2008) 621–630
ENVIRONMENTAL POLLUTION
www.elsevier.com/locate/envpol

Effect of spatial variation on salinity tolerance of macroinvertebrates in Eastern Australia and implications for ecosystem protection trigger values

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Received 6 October 2006; received in revised form 22 March 2007; accepted 22 March 2007

Salinity tolerance of macroinvertebrate communities vary in Eastern Australia hence water quality guidelines should be developed at a local or regional scale.

Abstract

Salinisation of freshwater has been identified as a serious environmental issue in Australia and around the world. Protective concentrations (trigger values) for salinity can be used to manage salinity impacts, though require locally relevant salinity tolerance information. 72-h acute salinity tolerance values were determined for 102 macroinvertebrates collected from 11 locations in four biologically distinct freshwater bioregions in Northeast Australia and compared with sensitivities observed in Southeast Australia. The salinity tolerance of individual taxa was consistent across Northeast Australia and between Northeast and Southeast Australia. However, two distinct communities were identified in Northeast Australia using distributions of the acute tolerance values and a calculated index of salinity sensitivity. Salinity trigger values should therefore be representative of local or regionally relevant communities and may be adequately calculated using sensitivity values from throughout Eastern Australia. The results presented provide a basis for assessing salinity risk and determining trigger values for salinity in freshwater ecosystems at local and regional scales in Eastern Australia.

Keywords: Salinity tolerance; Salinity impacts; Salinisation; Conductivity; Macroinvertebrates; Salinity risk

1. Introduction

Salinisation is one of Australia's most serious environmental issues. Although dissolved salts are natural components of freshwater and some inland aquatic systems have naturally high salinity levels, it is now well recognised that impacts from excessive anthropogenic related increases in concentrations of dissolved salts can have profound and measurable effects on freshwater aquatic ecosystems (Hart et al., 1991; Williams et al., 1991; James et al., 2003; Kefford et al., 2003; Marshall and Bailey, 2004; Dunlop et al., 2005). In Australia, an estimated 5.6 million hectares of land is at high risk from induced dryland salinity and by the year 2050, estimates indicate that 17 million hectares of land may be salt affected (National Land and Water Resources Audit, 2000). In areas already affected, salinity has resulted in social, economic and environmental impacts (Land and Water Australia, 2002). It is therefore clear that there is an urgent need to manage salinity impacts in Australia.

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doi:10.1016/j.envpol.2007.03.020

Establishment of trigger values (guidelines) for salinity can provide a basis for its management but there are currently no widely acceptable, biologically based guidelines applicable at local or regional scales. Probabilistic risk assessment techniques can be used to determine salinity concentrations or trigger values protective of particular taxa or a proportion of taxa (Hart et al., 2003). This can be achieved by modelling a distribution of laboratory derived salinity sensitivity values (species sensitivity distribution (SSD) or taxa sensitivity distributions (TSD)) from which a percentile, or ecosystem protection value (trigger value) can be calculated to ensure the protection of a pre-defined proportion of taxa. Salinity sensitivity data can be derived from either concentration–response laboratory experiments (resulting in ≤96-h LC_{50} values) using a standard composition of dissolved salts and/or the maximum salinity at which taxa have been observed to occur in the field (resulting in F_{max} values). Laboratory experiments provide a direct causal link between a concentration of exposure and a measured effect (Goetsch and Palmer, 1997; Berezina, 2003; Kefford et al., 2003; Kefford et al., 2004) and are preferred for use in the determination of water quality guidelines (ANZECC/ARMCANZ, 2000). Despite a clear need to determine biological effects based ecosystem protection values, there is limited salinity tolerance information available from freshwater organisms in Australia, and in particular there is a lack of such data in Northern Australia as most studies have been conducted either outside of Australia or in Southeastern Australia (Kefford et al., 2003, 2005a, 2006a). It is also not known whether the existing salinity tolerance information provides adequate representation of the variation in salinity tolerance in Eastern Australia. This study evaluates the sensitivity of a broad range of macroinvertebrates in Northeast Australia to a standard synthetic marine salt and compares these tolerance values with those observed across Eastern mainland Australia.

2. Methods

Macroinvertebrates are an important component of freshwater ecosystems forming vital links in aquatic food webs. Macroinvertebrates are known to respond at the community level to salinity impacts (Horrigan et al., 2005) and some are known to be salinity sensitive (Kefford et al., 2003) and are thus useful indicators of salinity impacts. For these reasons macroinvertebrates are an ideal taxonomic group to assess broad geographical trends in salinity sensitivity. To assess macroinvertebrate sensitivity this study applies a rapid assessment approach aimed at collecting and testing the widest possible range of taxa at the lowest possible taxonomic resolution. This approach is a more approximate estimation of salinity tolerance than would be achieved using an intensive method at species level but allows a large number of taxa to be tested with the same test effort (Kefford et al., 2005b). As previous studies have shown that there is wide variation in the salinity tolerance of macroinvertebrate taxa (Kefford et al., 2003), it is important that the sensitivity values of a large number of taxa be used to calculate the species sensitivity distributions (TSDs) used to derive trigger values. This allows a more accurate assessment of the risks at the community level (Kefford et al., 2005b) than would be achieved if data from only a few taxa were used.

2.1. Test method

To investigate salinity tolerance in Northeast Australia, macroinvertebrates were collected from 11 locations (Fig. 1, Table 1). To ensure that a wide variety of taxa were sampled from Northeast Australia, macroinvertebrates were collected from four separate bio-provinces (as shown in Fig. 1). Freshwater bio-provinces (Marshall et al., 2004) reflect spatial dissimilarity between biological communities. For the purposes of this study the Dry Tropics refers to the Northeast portion of the Central freshwater province. Comparisons were made between Northeast Australia (current study) and Southeast Australia using sensitivity information published by Kefford et al. (2003, 2006a).

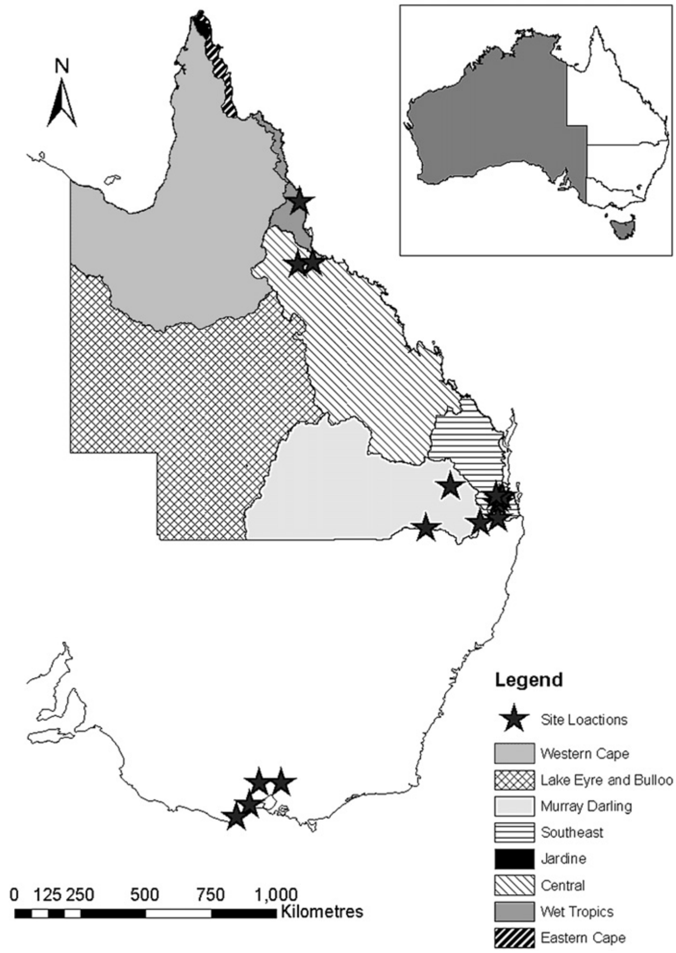

Fig. 1. Map of macroinvertebrate collection locations in Eastern Australia and Queensland bio-provinces.

The salinity tolerance of macroinvertebrates was investigated by measuring the acute response (LC_{50} concentrations) of macroinvertebrates over 72 h to a standard synthetic marine salt Ocean Nature manufactured by Aquasonic Pty Ltd. Tests were conducted using a rapid assessment method described by Kefford et al. (2003, 2005b). Further description is provided here where departure from this method was required for locally specific application or where relevant to the interpretation of results. Controls of filtered laboratory water and collection water were used to confirm that the observed acute responses were due to the influence of salinity alone and not due to relocation and transport into the laboratory. In some instances an individual would have emerged (as a flying adult) over the duration of the test or be missing due to cannibalism and were subsequently excluded from analysis. To account for warmer ambient temperatures in Northern Australia than occur in Southern Australia, tests were conducted at 25 °C (±2 °C) and 20 °C (±2 °C) respectively. After testing, sub-samples of up to ten individuals were preserved and identified to the lowest possible taxonomic classification. For quality assurance 10% of species were re-identified by a separate person.

Two to five collections were made at all sites, the first of which was considered a range finding experiment to establish test treatments suitable for accurate definition of LC_{50} values and subsequent tests refined this range. This technique of data collection provides a mechanism for continual

Table 1
Location of macroinvertebrate collection sites and conductivity at time of collection

Collection location	Latitude (S)	Longitude (E)	EC (mS cm^{-1})[a]
Purga Creek	27° 42′	152° 43′	0.52
Moggil Creek	27° 29′	152° 53′	0.69
Savages Crossing	27° 26′	152° 40′	0.38
Colleges Crossing	27° 33′	152° 48′	0.72
Barney Creek	28° 14′	152° 43′	0.096
Macintyre River at Goondiwindi	28° 32′	150° 15′	0.28
Condamine River at Elbow Valley	28° 22′	152° 8′	0.42
Condamine River at Ranges Bridge	27° 7′	151° 5′	0.57
Burdekin River at Harveys Range Road	19° 26′	145° 51′	0.31
Keelbottom Creek	19° 22′	146° 21′	0.21
Harvey Creek	17° 16′	145° 55′	0.03
Barwon River at Pollocksford	38° 8′	144° 11′	2.45
Campaspe River	37° 23′	144° 31′	1.60
King Parrot Creek	37° 23′	145° 16′	0.10
Broken River	37° 13′	146° 17′	0.22
East Barwon River at Lake Elizabeth	38° 33′	143° 44′	0.20

[a] Maximum EC observed at time of collection.

improvement of the available knowledge about the sensitivity of each taxon. Where testing the influence of pre-exposure to salinity on the short-term acute response of the snail, *Physa acuta*, tests were run with three replicates and a minimum of five treatments.

2.2. Analysis

Where sufficient data was available point estimates of the test concentration associated with a 50% mortality of the test population from that observed under control conditions over the 72-h test duration (72-h LC_{50} values) were determined from a logistic regression using R version 2.1.1 (Venables and Ripley, 2002). In this method the distribution of mortality versus concentration was modelled assuming a continuous concentration-response relationship. Response data at the lowest available taxonomic resolution was used in the analysis. Where the same taxa were collected in multiple bio-regions, response data was pooled for analysis to represent taxa sensitivity across bio-regions. The advantage of using logistic regression is that non-linear datasets can be used and test precision and variability can be calculated. Where sufficient data was not available to estimate a 72-h LC_{50} an approximate estimate of the LC_{50} was determined as non-modelled estimates of the 72-h LC_{50} values. Non-modelled estimates are given as either greater than the maximum conductivity treatment at which test animals were observed to survive, as a range or as the treatment at which 50% of the test animals were observed to survive. To allow comparison between collection locations, data was pooled by location and grouped at the lowest taxonomic level of identification.

A Kaplan and Meier (1958) survival analysis was performed using Statistica 6.0. StatSoft, inc. (2001). It ranks all censored and non-censored sensitivity values of taxa against an electrical conductivity gradient. Sensitivity values at the lowest available taxonomic resolution were used in the analysis hence the survival analysis produced a TSD. From this ranking a TSD of sensitivity values was determined. Tests of difference between two survival functions were performed using a two-sample Cox–Mantel test (Cox, 1959) and tests between multiple groups were performed using Mantel's test (Mantel, 1967).

Water samples were collected at each site and analysed for major ion concentration (bicarbonate, calcium, magnesium, potassium, sodium, chloride, sulphate, and fluoride). Data used in analysis was converted to percent equivalents of major ions (and range-standardised for pH and conductivity). A principal components analysis (PCA) was used to determine differences between sites based on the water quality data recorded at each site using Statistica 6.0. StatSoft, inc. (2001).

Salinity Sensitivity Scores (SSSs) as described by Horrigan et al. (2005) were used to calculate salinity index (SI) scores for sites deemed to be in reference condition in the Wet Tropics (WT), South East Queensland (SEQ), Dry Tropics (DT), and the Queensland Murray Darling Basin (QMDB). Reference sites in this instance are those sites experiencing minimal human disturbance defined as per Steward (2006). SSSs were determined using a sensitivity analysis with predictive artificial neural networks. A total of 2580 edge and riffle habitat macroinvertebrate samples collected between 1994 and 2002 from 1008 sites widely distributed throughout Queensland were used to build the model. An edge habitat refers to stream bank where there is little or no current. A riffle habitat is a reach of relatively steep, shallow, fast flowing and broken water over stony beds (see for full details NR&M, 2001). Samples were collected as part of the Queensland Ambient Biological Monitoring Program (Steward, 2006) using the Australian Rivers Assessment (AusRivAs) protocol.

3. Results and discussion

3.1. Salinity tolerance of macroinvertebrates in Northeast Australia

Sufficient data was available to determine 102 estimates of 72-h LC_{50} values. The acute salinity tolerance of macroinvertebrates was highly variable between those tested ranging from 6.9 to >55 mS cm^{-1}. Modelled sensitivity values with their respective lower 5th and upper 95th percentiles are shown in Table 2 and non-modelled, assigned 72-h LC_{50} values are shown in Table 3. The number of individuals tested is given as an indication of the confidence in the estimate. The mean of all recorded 72-h LC_{50} values in Northern Australia was 25.8 mS cm^{-1}. In terms of the mean sensitivity values as calculated in the Kaplan–Meier function for censored and non-censored data, the most sensitive to most tolerant taxa (at the Order and Sub-Order level) was: Ephemeroptera (10.9 mS cm^{-1}), Basommatophora (17.6 mS cm^{-1}), Veneroidea (18.8 mS cm^{-1}), Gastropoda (19.2 mS cm^{-1}), Integripalpia (20.65 mS cm^{-1}), Hemiptera (21.3 mS cm^{-1}), Diptera (22.4 mS cm^{-1}), Acariformes (22.5 mS cm^{-1}), Epiproctophora (23.3 mS cm^{-1}), Zygoptera (32.4 mS cm^{-1}), Coleoptera (35 mS cm^{-1}), Decapoda (41.9 mS cm^{-1}), Isopoda (>55 mS cm^{-1}). The sensitivity ranking was in general agreement with those of Kefford et al. (2003, 2006a). There was a wide range of salinity tolerances within and across taxonomic groups (Fig. 2). The most sensitive taxon was from the genus *Austrophleboides* (Leptophlebiidae) sampled from Harvey Creek in the WT (72-h LC_{50} of 6.9 mS cm^{-1}) and the most tolerant Families, Cirolanidae and Sphaeromatidae were collected at sites in the QMDB (100% survivorship in all treatments up to and including 55 mS cm^{-1}). Although the most sensitive taxa tested was collected in the WT many tolerant taxa including *Cherax* (Parastacidae) with a 72-h LC_{50} as high as 50 mS cm^{-1} were collected in the WT. In contrast some salinity sensitive taxa were collected in the QMDB including *Cloeon* spp. (Baetidae). The percent survival of *Triplectides australis* (Leptoceridae) was similar when sampled in three bio-regions. When data from the three bio-regions was combined *T. australis* was almost twice as sensitive as that of *Westriplectes* spp. (Leptoceridae) (Fig. 3). There was

Table 2
72-h LC_{50} values (as measured in mS cm^{-1}) of macroinvertebrates with 5th and 95th percentiles of their estimate and the number of individuals tested

Bio-region	Order	Family	Genus species	Authority	72 h acute LC_{50}	Lower 5th percentile	Upper 95th percentile	No. tested
SEQ	Acariformes			Harvey & Growns	21.5	21.1	21.9	150
SEQ	Basommatophora	Physidae	*Physa acuta*	Smith	15.7	15.4	16.0	120
DT	Coleoptera	Dytiscidae	*Necterosoma*	Sharp	37.4	34.1	40.6	16
SEQ	Coleoptera	Hydrophilidae	*Berosus*	Leach	29.0	24.3	33.7	56
WT	Coleoptera	Psephenidae	*Sclerocyphon* type F	Bertrand & Watts	23.4	18.8	28.0	53
SEQ	Decapoda	Atyidae	*Caridinides wilkinsi*	Calman	34.2	32.9	37.2	359
WT	Decapoda	Atyidae	*Caridinides wilkinsi*	Calman	41.3	38.6	44.1	35
DT	Decapoda	Atyidae	*Caridinides wilkinsi*	Calman	33.1	29.2	36.9	30
QMDB	Decapoda	Atyidae	*Paratya australiensis*	Kemp	34.2	31.2	37.2	42
QMDB	Decapoda	Palaemonidae	*Macrobrachium australiense*	Holthuis	42.5	40.3	44.7	70
QMDB	Decapoda	Parastacidae	*Cherax*	Horwitz & Austin	33.5	26.1	41.0	24
QMDB	Diptera	Chironomidae			14.7	9.6	19.9	18
SEQ	Ephemeroptera	Baetidae	*Cloeon*	Leach	13.2	12.5	13.9	70
QMDB	Ephemeroptera	Baetidae	*Cloeon*	Leach	11.7	10.4	12.9	102
WT	Ephemeroptera	Baetidae	*Cloeon*	Leach	8.7	4.1	13.3	29
SEQ	Ephemeroptera	Caenidae	*Wundacaenis*	Suter	13.1	12.4	13.8	120
WT	Ephemeroptera	Leptophlebiidae	*Austrophlebioides*	Campbell & Suter	6.9	5.2	8.6	38
SEQ	Epiproctophora	Gomphidae			21.0	18.9	23.0	21
QMDB	Sorbeoconcha	Thiaridae	*Thiara plotiopsis*	Smith	30.6	27.9	33.4	72
QMDB	Hemiptera	Corixidae	*Micronecta gracilis*	Hale	17.3	12.0	22.6	45
QMDB	Hemiptera	Corixidae	*Micronecta gracilis (juvenile)*	Hale	12.8	9.6	16.1	60
DT	Heteroptera	Notonectidae	*Nychia sappho*	Kirkaldy	10.8	5.7	16.0	35
WT	Integripalpia	Calamoceratidae	*Anisocentropus*	Neboiss	13.9	10.7	17.2	16
SEQ	Integripalpia	Leptoceridae	*Triplectides australis*	Navas	28.5	25.0	32.0	45
WT	Integripalpia	Leptoceridae	*Triplectides australis*	Navas	28.2	25.2	31.2	61
WT	Integripalpia	Leptoceridae	*Westriplectides angelae*	Neboiss	15.1	-31.2	34.3	26
DT	Integripalpia	Leptoceridae	*Triplectides*	Kolenati	18.4	13.2	23.6	15
QMDB	Veneroidea	Corbiculidae	*Corbicula*	Smith	23.1	20.9	25.4	53
SEQ	Veneroidea	Corbiculidae	*Corbicula*	Smith	18.4	18	18.9	765
DT	Zygoptera				40.1	35.3	44.9	24
DT	Zygoptera	Coenagrionidae	*Psuedagrion microcephalum*	Rambur	34.1	28.8	39.5	12

generally more variability between the Orders and Family level taxonomic groups than were observed within them. The variability within groups decreased with increased taxonomic resolution.

As species level sensitivities to salinity can be variable at higher taxonomic groupings, comparisons between locations have greatest certainty at the species level. As many species have limited spatial distribution, species level comparisons between bio-regions was possible for only two species *Triplectides australis* (Leptoceridae) and *Caridinides wilkinsi* (Atyidae). *T. australis* was collected in three bio-regions and had a consistent tolerance in each with a 72-h LC_{50} of 28.5 mS cm^{-1} in SEQ, 28.2 mS cm^{-1} in the WT, and an approximate 72-h LC_{50} of >20 mS cm^{-1} in the QMDB. *C. wilkinsi* was also found to have consistent tolerance when collected in the four bio-regions (Fig. 4). However, a small difference between the upper 5th and 95th percent confidence intervals was observed between the highest and lowest estimates from the four bio-regions and in this instance the occurrence of a Type 2 Error cannot be ruled out. Further comparisons across multiple bio-regions were possible between two genera (*Caridinides* and *Paratya*) of the same family (Atyidae). Estimates of the 72-h LC_{50} values of *Caridinides* and *Paratya* were consistent across all four bio-regions. In addition the family Corbicuilidae was found to have consistent 72-h LC_{50} values in the QMDB and SEQ (23.1 mS cm^{-1} and 20.2 mS cm^{-1} respectively). A consistent trend in sensitivity was also observed for the family Leptophlebiidae that had a 72-h LC_{50} of 6.1 mS cm^{-1} when collected in SEQ and 7.2 mS cm^{-1} in the WT.

Comparisons between salinity tolerance observed in the bio-regions of Northeast Australia were also be made by comparing the TSDs of taxa collected in each of the bio-regions. Differences between TSDs was assessed and chi-squared values based on the sums (for each group) of this score were calculated. Though there were some observable differences between the groups (Fig. 5), there were no statistical differences between the bio-regions tested ($\chi^2 = 5.45$, $df = 4$, $p = 0.142$). However, the plot of the TSDs for the bio-regions indicated that there were two distinct groupings. TSDs of macroinvertebrate tolerance in the DT, was similar to that observed in the QMDB, likewise the TSD for the WT and the

Table 3
Non-modelled assigned estimates of 72 hour acute salinity tolerance of macroinvertebrates (estimates provided in mS cm^{-1})

Bio-region	Order/Sub-Order	Family	Genus species	Authority	Assigned LC_{50}	No. tested
DT	Acariformes			Harvey & Growns	>20	136
SEQ	Acariformes			Harvey & Growns	>25	10
SEQ	Basommatophora	Planorbidae	*Helicorbis*	Benson	>10	25
QMDB	Cirolanidae			Dana	>55	4
DT	Coleoptera	Dytiscidae	*Australphilus*	Watts	>35	8
DT	Coleoptera	Hydrochidae	*Hydrochus*	Leach	>40	4
QMDB	Coleoptera	Dytiscidae	*Hydaticus*	Leach	>30	1
QMDB	Coleoptera	Dytiscidae	*Hyphydrus*	Illiger	>30	2
QMDB	Coleoptera	Dytiscidae	*Laccophilus*	Leach	>30	2
QMDB	Coleoptera	Dytiscidae	*Sternopriscus*	Sharp	>35	20
DT	Coleoptera	Dytiscidae	*Tiporus*	Watts	>35	2
QMDB	Coleoptera	Dytiscidae	*Uvarus*	Guigndt	>35	5
QMDB	Coleoptera	Hydrophilidae	*Paranacaena*	Blackburn	>30	8
QMDB	Coleoptera	Hydrophilidae	*Hydrochus*	Leach	35	7
WT	Coleoptera	Hydrophilidae	*Berosus*	Leach	~35	52
SEQ	Coleoptera	Psephenidae	*Sclerocyphon* type F	Blackburn	>20	2
QMDB	Decapoda	Atyidae	*Caridinides wilkinsi*	Calman	>40	6
QMDB	Decapoda	Palaemonidae	*Macrobrachium australiense*	Holthuis	50	16
WT	Decapoda	Palaemonidae	*Macrobrachium australe*	Guérin-Méneville	>45	22
DT	Decapoda	Palaemonidae	*Macrobrachium australe*	Guérin-Méneville	>45	6
DT	Decapoda	Palaemonidae	*Macrobrachium australiense*	Holthuis	>45	2
SEQ	Decapoda	Palaemonidae	*Leptopalaemon*		>40	5
WT	Decapoda	Parastacidae	*Cherax*	Erichson	50	9
QMDB	Decapoda	Parastacidae	*Cherax*	Erichson	>45	7
DT	Diptera	Tabanidae	*Chrysops*	Meigen	>30	2
QMDB	Ephemeroptera	Baetidae	*Cloeon*	Leach	15	17
DT	Ephemeroptera	Baetidae	*Cloeon*	Leach	>10	4
SEQ	Ephemeroptera	Baetidae	*Cloeon*	Leach	>10	18
QMDB	Ephemeroptera	Caenidae	*Tasmanocoenis*	Lestage	~12.5	2
QMDB	Ephemeroptera	Caenidae	*Wundacaenis*	Suter	>12	5
SEQ	Ephemeroptera	Leptophlebiidae	*Ulmerophlebia*	Demoulin	>15	104
QMDB	Ephemeroptera	Leptophlebiidae	*Atalophlebia*	Eaton	>6.4	2
WT	Ephemeroptera	Leptophlebiidae	*Neboissophlebia*	Dean	>8	20
QMDB	Epiproctophora	Hemicordulidae	*Diplacodes Haematodes*	Burmeister	>30	3
QMDB	Epiproctophora	Gomphidae	*Hemigomphus cooloola*	Watson	>30	6
WT	Epiproctophora	Libellulidae	*Nannophlebia risi*	Tillyard	>25	15
DT	Epiproctophora				>20	7
WT	Epiproctophora	Gomphidae	*Austrogomphus austroepigomphus*	Fraser	>25	8
DT	Epiproctophora	Hemicorduliidae	*Hemicordulia intermedia*	Selys	>20	4
DT	Epiproctophora	Libellulidae	*Nannophlebia risi*	Tillyard	>15	1
SEQ	Sorbeoconcha	Hydrobiidae		Stimpson	>15	140
QMDB	Sorbeoconcha				12	16
QMDB	Hemiptera	Belastomatidae	*Diplonychus eques*	Dufour	>35	2
QMDB	Hemiptera	Corixidae	*Micronecta gracitis*	Hale	15	2
QMDB	Hemiptera	Corixidae	*Agraptocorixia*	Kirkaldy	15	1
QMDB	Hemiptera	Naucoridae	*Naucoris australicus*	Stål	>30	9
QMDB	Hemiptera	Naucoridae	*Naucoris subopacus*	Montandon	>20	2
QMDB	Hemiptera	Notonectidae	*Paranisops inconstans*	Hale	>20	3
DT	Hemiptera	Pleidae	*Paraplea brunni*	Kirkaldy	>30	21
QMDB	Hemiptera	Pleidae	*Paraplea brunni*	Kirkaldy	>30	26
SEQ	Hemiptera	Pleidae	*Paraplea brunni*	Kirkaldy	>20	47
QMDB	Isopoda	Cirolanidae		Dana	>55	16
SEQ	Isopoda	Sphaeromatidae	*Cymodetta*	Bowman & Kühne	>50	70
DT	Cladocera				>7	12
SEQ	Monotocardia	Hydrobiidae		Stimpson	>20	70
QMDB	Podocopida	Notodromidae			0.5–40	9
WT	Tricladida	Dugesiidae	*Dugesia*	Girard	>10	3
QMDB	Trichoptera	Atriplectididae		Mosely	>20	3
SEQ	Trichoptera	Calamoceratidae	*Anisocentropus*	McLachlan	>25	22
WT	Trichoptera	Helicopschidae	*Helicopsyche haecota*	Mosely	>15	2
WT	Integripalpia	Leptoceridae			>20	11
QMDB	Integripalpia	Leptoceridae	*Oecetis*	Leach	>20	1
WT	Integripalpia	Leptoceridae	*Oecetis*	Leach	>20	4

(*continued on next page*)

Table 3 (*continued*)

Bio-region	Order/Sub-Order	Family	Genus species	Authority	Assigned LC_{50}	No. tested
DT	Integripalpia	Leptoceridae	*Triplectides parvus*	Baulis	>25	21
QMDB	Integripalpia	Leptoceridae	*Triplectides proximus*	Neboiss	20	8
QMDB	Integripalpia	Leptoceridae	*Triplectides australis*	Navás	>20	22
QMDB	Veneroidia	Hyriidae	*Cucumerunio*	Iredale	>15	1
QMDB	Zygoptera	Coenagrionidae			35	12
QMDB	Zygoptera	Coenagrionidae	*Ishnura aurora*	Brauer	>30	3
QMDB	Zygoptera	Coenagrionidae	*Pseudagrion microcephalum*	Rambur	>35	6
WT	Zygoptera	Diphlebiidae	*Diphlebia nymphoides*	Tillyard	>20	12

SEQ were similar. When sensitivity data from both groups was combined into the two groups, Group 1 (WT and SEQ) and Group 2 (DT and QMDB) a Cox–Mantel (Cox, 1959) test indicated a significant difference between them (test statistic = −2.35, $p = 0.019$).

To investigate whether the observed differences between the TSDs of Group 1 and Group 2 were attributable to community differences between the bio-regions and not simply due to differences between the pool of taxa tested here, SSSs for macroinvertebrate taxa as defined by Horrigan et al. (2005) were used to calculate a salinity index for all reference site samples taken in the four bio-regions. Fig. 7 shows at plot of the median, the 20th and 80th percentiles of the SI at reference sites for each of the bio-regions. Samples of reference sites from Group 1 (WT and SEQ) were found to be comprised of more sensitive taxa than were observed in Group 2 (DT and QMDB). This provides further evidence that there are differences in the salinity sensitivity of macroinvertebrates in these two groups.

3.2. A case study of pre-exposure effects on salinity tolerance

The effect of background salinity (or pre-exposure to salinity) on salinity tolerance was investigated using the freshwater snail *Physa acuta* collected from two sites approximately 2.5 km apart along Purga Creek upstream and downstream of a salinity input. At time of collection the site upstream of the saline water input had a salinity of 0.247–0.568 mS cm^{-1} and the site downstream of the salinity input had a salinity of 3.2–4.4 mS cm^{-1}. At the upstream site the 72-h LC_{50} was 15.7 mS cm^{-1} (lower and upper 95% confidence intervals of 15.4 and 16.0 mS cm^{-1} respectively). At the downstream site the 72-h LC_{50} was 16.0 mS cm^{-1} (lower and upper 95% confidence intervals of 15.5 and 16.7 mS cm^{-1} respectively). The probability of survival (72 h) of individuals collected upstream and downstream of the salinity impact indicates a difference between their response though the 95% confidence intervals of the 72-h LC_{50} estimates overlapped and differences could not be confirmed (as shown in Fig. 6). Given the visual differences in response, it is likely that impacts of a greater magnitude would result in a more pronounced difference in tolerance. *P. acuta* were notably larger at the downstream site suggesting that additional calcium required for shell building had observable sub-lethal effects.

3.3. Water chemistry at sampling locations

The EC data in Table 1 and the concentration of major ions and pH data in Table 4 indicate that water quality was variable between collection locations. Principle components analysis (PCA) was performed to identify groups of collection sites

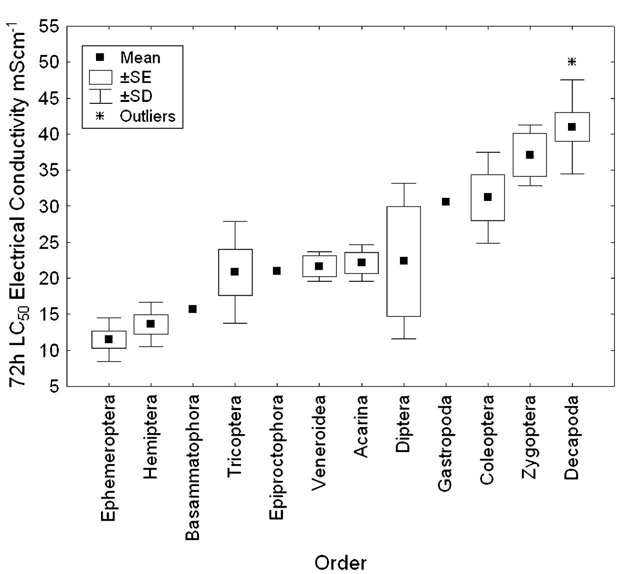

Fig. 2. Box and Whisker plot of non-censored, modelled 72-h LC_{50} values of taxa collected in Northeast Australia at the Order level showing mean values, standard error, standard deviation and outliers.

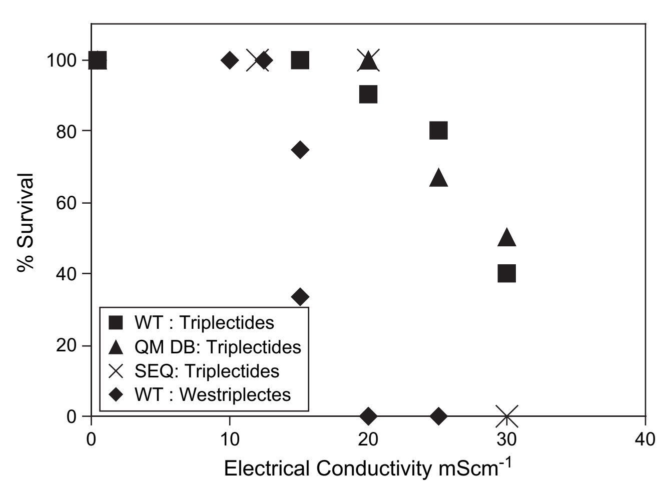

Fig. 3. Percent survival of *Triplectides australis* (Leptoceridae) and *Westriplectes* sp. collected from three Queensland bio-regions. WT, Wet Tropics; QMDB, Queensland Murray Darling Basin; SEQ, Southeast Queensland.

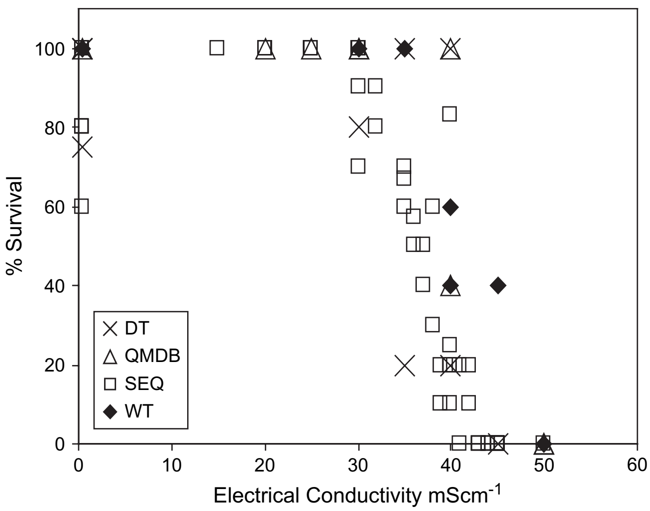

Fig. 4. Comparison between the percent survival of *Caridinides wilkinsi* (Atyidae) for all bio-regions sampled.

based on their water quality. Three components had eigenvalues greater than 1 and explained 86% of the variance. Component 1 explained 43.4% and component 2 explained 30.7% of the total variance within the correlation matrix. Projections of components 1 and 2 are shown in Fig. 8. Variable contributions (loadings) based on correlations and the coefficients of the correlation matrix eigenvectors indicated the variation in component 1 was correlated with the presence of calcium (20%), potassium (18%), chloride (15%), and sulphate (12%). The variation in component 2 was primarily explained by bicarbonate (27%), EC (15%), and sodium (16%). The PCA indicates Harvey Creek (WT), Barney Creek (SEQ) and Colleges Crossing (SEQ) had markedly different water quality than other collection locations in Northeast Australia. The two groups of bio-regions in Northeast Australia that had similar TSDs (refer to Fig. 5) were overlaid in Fig. 8 and can broadly be seen to fit the grouping of sites in the PCA.

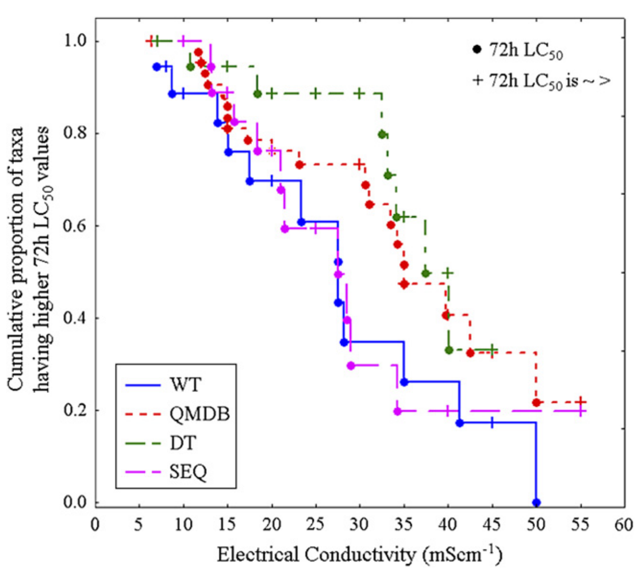

Fig. 5. Kaplan–Meier function of all macroinvertebrate taxa from four bio-regions: SEQ, Southeast Queensland; WT, Wet Tropics; DT, Dry Tropics; QMDB, Queensland Murray Darling Basin.

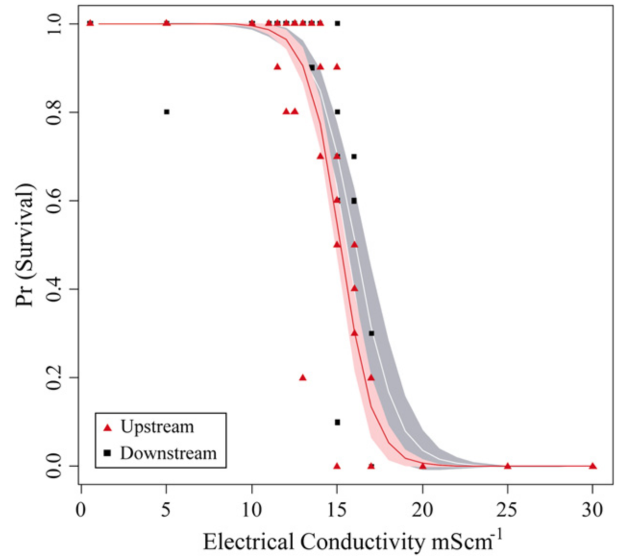

Fig. 6. Probability of survival (72 h) for *Physa acuta* (Physidae) upstream (red line) and downstream (white line) of a salinity impact with respective 95% confidence interval for the modelled probability of survival (For interpretation of the references to colour in figure legends, the reader is refered to the web version of this article).

3.4. Tolerance of taxa collected in Southeast and Northeast Australia

As some species have limited spatial distribution and as not all taxa are tested using the rapid assessment test protocol, species level comparisons of taxa tolerance in Northeast Australia (this study) and Southeast Australia (Kefford et al., 2003, 2006a) were only possible for *Physa acuta* (Physidae) and *Paratya australiensis* (Atyidae). These taxa had similar sensitivities at both locations. *P. acuta* had a 72-h LC_{50} of 15.5 mS cm^{-1} in Northeast Australia and 14.1 mS cm^{-1} in Southeast Australia *P. australiensis* had a 72-h LC_{50} of 34.2 mS cm^{-1}

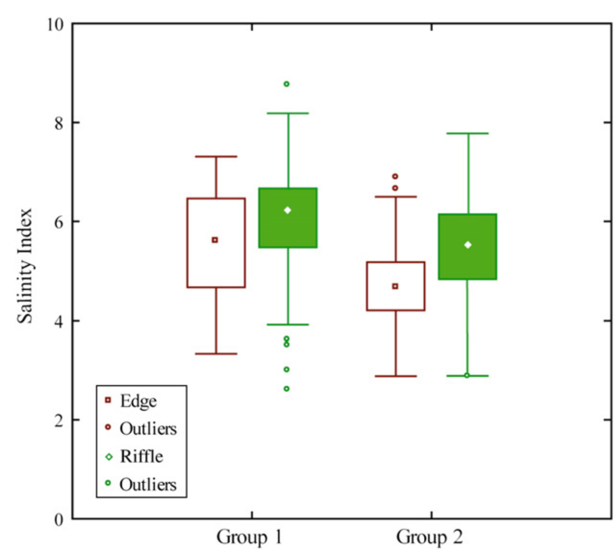

Fig. 7. Box plot showing the median, 25th and 75th percentiles of salinity index values calculated for Edge and Riffle habitats at reference sites in Group 1 (Wet Tropics and Southeast Queensland) and Group 2 (Queensland Murray Darling Basin and Dry Tropics).

Table 4
pH and composition of major ions at collection sites in Northeast Australia

Collection location	pH	$CaCO_3$ (mg L^{-1})	HCO_3 (mg L^{-1})	Ca (mg L^{-1})	Mg (mg L^{-1})	K (mg L^{-1})	Na (mg L^{-1})	Cl (mg L^{-1})	SO_4 (mg L^{-1})	Fl (mg L^{-1})
Colleges Crossing	8.6	99.00	110.00	24.00	20.0	5.50	88.00	150.00	21.00	0.12
Purga Creek (upstream)	8.1	130.00	160.00	30.00	18.0	5.60	51.00	79.00	11.00	0.14
Moggill Creek	7.6	180.00	220.00	43.90	27.3	2.77	68.30	110.00	15.00	0.18
MacIntyre River Goondiwindi	7.8	110.00	134.00	17.20	12.9	3.58	20.90	17.00	3.10	0.15
Burdekin River	8.2	128.00	156.00	20.10	14.6	3.32	19.90	19.00	0.75	0.11
Keelbottom Creek	7.4	55.00	68.00	10.50	5.8	<2.5	22.80	29.00	1.10	<0.02
Harvey Creek	6.7	5.00	6.00	<0.3	<1	<2.5	<5	4.50	0.60	<0.02
Condamine River, Ranges Bridge	8.2	260.00	310.00	41.10	24.2	9.97	46.20	55.00	2.00	0.31
Condamine River, Elbow Valley	7.6	100.00	120.00	19.30	15.4	3.91	32.90	74.00	0.78	0.09
Barney Creek	7.4	27.47	33.41	4.70	2.4	1.10	8.80	9.53	1.25	0.10
Savages Crossing	8.0	95.14	114.73	19.10	13.7	3.38	35.30	58.54	4.26	0.22

and 38 mS cm^{-1} in Northeast and Southeast Australia, respectively.

Although some variation in the tolerance of different species of the same genera has been observed, the variation between genera is greater than within them. It is therefore possible to make comparisons between Northeast and Southeast Australia at the genus level. Three species from the genus *Triplectides* (Leptoceridae) had similar sensitivities across the bio-regions and between Northeast and Southeast Australia. *Triplectides australis* collected in Northeast Australia had an LC_{50} of 28 mS cm^{-1} and *Triplectides australicus* had an LC_{50} of 22 mS cm^{-1}. *Triplectides volda* had an LC_{50} of >25 mS cm^{-1} when collected in Southeast Australia. Another genera, *Cloeon* (Baetidae) was also found to have a similar sensitivity when collected in Northeast and Southeast Australia with a 72-h LC_{50} of 8.7 mS cm^{-1} in Northeast Australia and a 72-h LC_{50} of 5.5 mS cm^{-1} in Southeast Australia with 95% confidence intervals of both estimates overlapping. The TSD of all taxa tested in Northeast Australia and Southeast Australia is shown in Fig. 9. The TSDs from Northeast and Southeast Australia were similar, however a Cox–Mantel test indicated (test statistic = 1.59, $p = 0.07$) that the TSDs were similar, indicating a marginal, statistically significant difference, though in this instance the occurrence of a type II error cannot be ruled out. When interpreting similarities in Physidae tolerance it is important to consider that they are represented by a single exotic species, *Physa acuta*. Since their introduction they have colonised large areas across Northeast and Southeast Australia and originate from a single gene pool (Gooderham and Tsyrlin, 2002). Identification to species level can therefore be confirmed when collected from different locations. This makes them a good species with which to make comparisons across wide geographical areas. However, as they originate from a single and recent ancestry compared with native taxa that have experienced longer adaptation in their respective localities, their tolerance may be expected to be

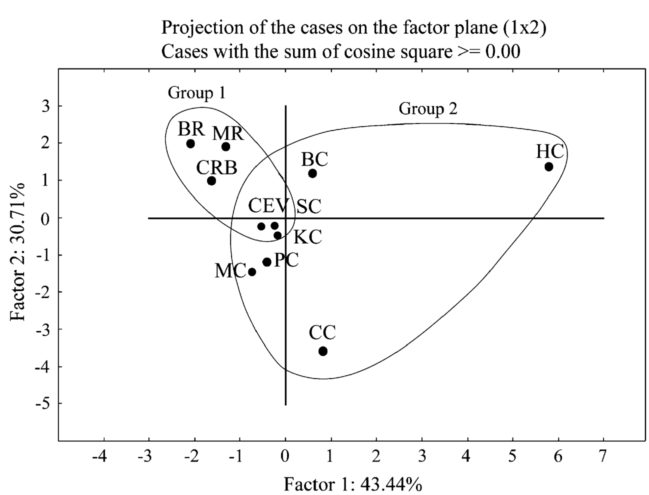

Fig. 8. Principal components analysis of all anions and cations from macroinvertebrate collection locations with the two sensitivity groups overlaid. Group 1 shows sites from the Dry Tropics and the Queensland Murray Darling Basin, Group 2 shows sites from the Wet Tropics and Southeast Queensland.

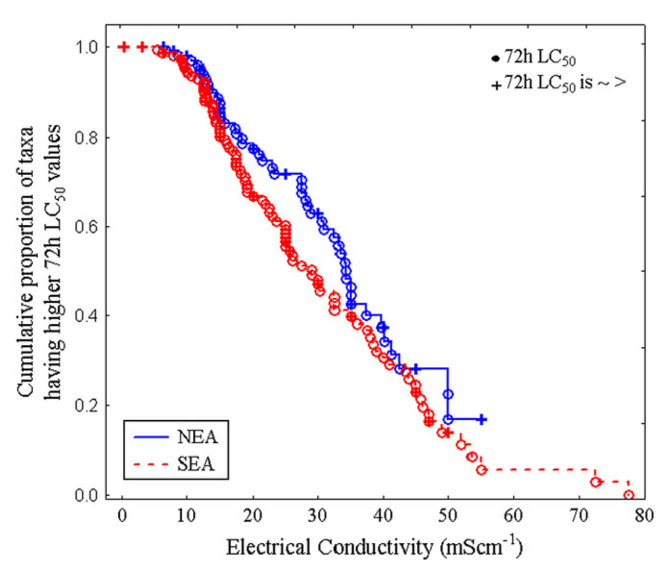

Fig. 9. Kaplan–Meier function showing the sensitivity of macroinvertebrates collected from Northeast Australia (NEA), Southeast Australia (SEA).

similar across regions. However, given the variation in the probability of their survival observed with exposure to slight salinity increases in the case study presented above it is likely that they will respond to changes in background water chemistry making it reasonable to make geographical comparisons with their tolerance.

4. Conclusion

Freshwater macroinvertebrates in Northeast Australia were found to have variable responses to salinity with 72-h LC_{50} values ranging between 6.9 and >55 mS cm^{-1}. This range in tolerance is consistent with previous studies in Southeast Australia by Kefford et al. (2003, 2006a). The large variation in the salinity tolerance observed in freshwater crustaceans and molluscs may be attributable to their classification as either freshwater or marine. The distinction between freshwater animals of marine origin and marine animals is sometimes blurred (Banarescu, 1990) and further research is required to investigate the implications of using sensitivity values for *euryhaline* taxa of primary marine origin in a TSD to represent taxa within a freshwater ecosystem that do not necessarily require protection from salinity. However, it is recommended that all taxa collected in freshwater environments be considered as representative of those ecosystems and represented in a TSD. The broad range of sensitivities observed indicates that some macroinvertebrates collected in freshwater environments can survive short-term (72 h) exposure to salinity concentrations near that of seawater. Although the lethal concentrations of the most sensitive taxon tested are within the upper 90th percentile of all surface water salinity concentrations for all defined salinity zones in Northeast Australia (McNeil et al., 2005), increased salinity resulting from the disposal of saline water, saline water intrusion and run-off from land affected by secondary salinity may exceed lethal concentrations of some freshwater aquatic animals.

We found three species and two families had similar sensitivity when collected in multiple bio-regions in Northeast Australia. A greater proportion of sensitive taxa were tested in the WT and SEQ than in the QMDB and DT. The observed differences between bio-regions were confirmed using macroinvertebrate data from ambient monitoring in Northeast Australia to calculate salinity index (SI) values for reference sites within the bio-regions. Resultant SI values confirmed that a greater proportion of salinity sensitive taxa are found to occur in the WT and SEQ (Group 1) than in the QMDB and the DT (Group 2). Water chemistry data in locations in Group 1 was different from Group 2. The observed spatial variation in community tolerance may be explained by the frequency of stream flows. Streams in Group 1 are more likely to have frequent flows and higher rainfall and more constant salinity. Streams in Group 2 are generally ephemeral and prone to extended periods of drying and periodic flooding. Fauna that have evolved in these locations are therefore more likely to be tolerant of fluctuations in salinity. However, due to the scarcity of freshwater in ephemeral streams, permanent waterholes are often of high ecological significance. Hence a precautionary approach should be used in the development of salinity guidelines in these instances. Given the spatial differences between bio-regions the use of a single TSD for Northeast Australia to calculate ecosystem protection trigger would mask the more sensitive or tolerant extremes of the true distributions of taxa found in Group 1 and Group 2. It is therefore recommended that the WT and SEQ and have separate ecosystem protection trigger values from that used in the QMDB and DT. We also found that two species and two genera had similar sensitivity when collected in Northeast and Southeast Australia and the TSDs of taxa tested in Northeast Australia corresponded well with that observed in Southeast Australia. However, given the observed differences within the Northeast it is possible that the variation within Northeast and Southeast Australia may obscure differences between them hence regionally or locally based ecosystem protection values based on taxa found at the scale of interest, with sensitivity values taken from throughout eastern Australia, are likely to provide the most accurate trigger values for salinity.

Physa acuta were found to have a modest increase in salinity tolerance when exposed to greater background salinity. Although the observed difference in tolerance was not statistically significant, the responses suggest that tolerance is likely to be affected by long-term incremental exposure to increased salinity greater than approximately 3-5 mS cm^{-1}. Further research to develop ecosystem protection values for salinity should consider pre-exposure effects on salinity tolerance. Other considerations include sub-lethal effects as they have been found to occur at lower concentrations than acute effects (Kefford and Nugegoda, 2005; Hall and Burns, 2002; Hassell et al., 2006; Kefford et al., 2006b). The composition of major ions has been shown to affect sub-lethal tolerance (Zalizniak et al., 2006; Mount et al., 1997). Also the presence of contaminants have been shown to reduce have additive or synergistic effects on the toxicity of salinity (Dassanayake et al., 2003; Hall and Anderson, 1995). As some variability in sensitivity has been observed within taxonomic groups it is recommended that assessments of salinity risk be made at the species level of taxonomic resolution to ensure the accuracy of predictions.

This study provides a significant advance in the available information with which to derive ecosystem protection, trigger values for salinity in Eastern Australia. As individual sensitivity was observed to be similar across large geographical areas data the sensitivity data presented here are likely to be relevant for the development of ecosystem protection trigger values across Eastern Australia. However, as broad scale differences in community sensitivity were observed between the bio-provinces in Northeast Australia it is recommended that where possible local or regional guidelines be developed to ensure accurate representation and protection of the taxa observed at the local scale.

Acknowledgements

This study was funded under the National Action Plan for Salinity and Water Quality State level Investment Program.

The program has input from the Queensland Environmental Protection Agency and the Department of Natural Resources, Mines and Water. BJK appreciated the supported by Land and Water Australia and the Murray Darling Basin Commission under the National Rivers Contaminants Program (LWA project No. RMI 12) and from the Queensland Department of Natural Resources, and Water. The authors wish to thank Fran Sheldon and Peter Negus for comments on the manuscript; also Brendan Farthing, Farah Zavahir, and Jaye Lobegeiger for valued contribution to the project.

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