BIOLOGICAL EFFECTS OF SECONDARY SALINISATION ON FRESHWATER MACROINVERTEBRATES IN TASMANIA:

THE ACUTE SALINITY TOXICITY TESTING OF SEVEN MACROINVERTEBRATES.

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Original photo courtesy of John Gooderham

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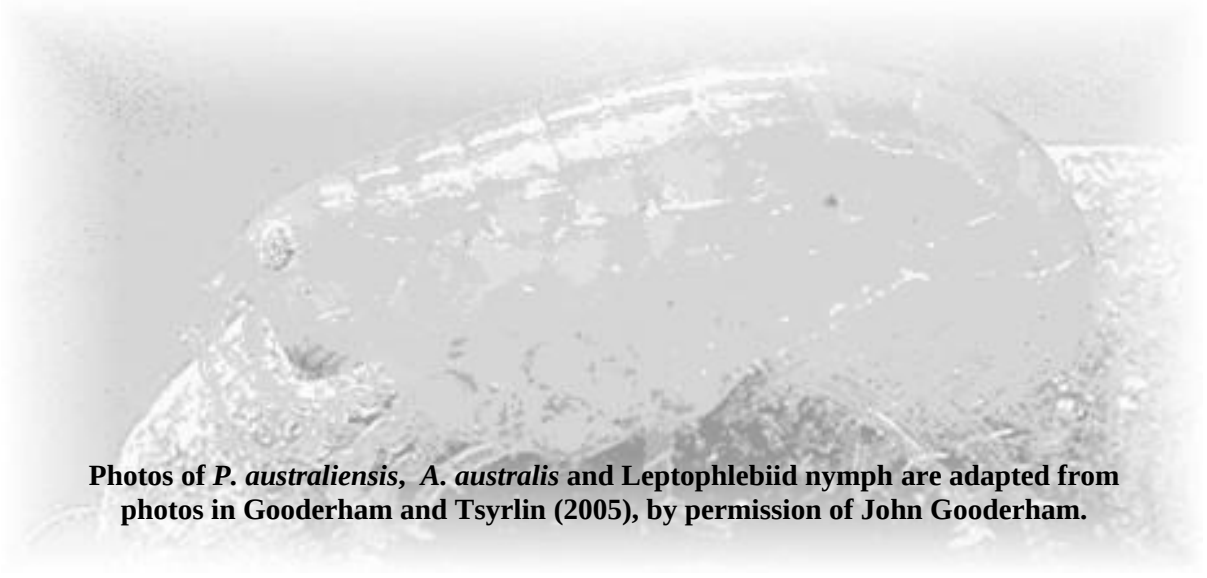


Photos of *P. australiensis*, *A. australis* and Leptophlebiid nymph are adapted from photos in Gooderham and Tsyrlin (2005), by permission of John Gooderham.

TABLE OF CONTENTS

ABSTRACT

Secondary salinisation is a major environmental threat to Australian freshwater rivers, streams and wetlands. In Tasmania there has been limited assessment of the extent and impacts of secondary salinisation, and there has been no research into the biological effects of increasing salinity on freshwater taxa or communities. However expansion of land clearing, cropping and irrigation pose major threats to water balance. High surfacewater and groundwater salinities have been recorded within the Tasmanian NAP region, and high water tables and large sub-surface salt stores have been reported in irrigation areas.

The salinity tolerances of seven macroinvertebrates (*Nousia* sp. AV7, *Dinotoperla serricauda, Eusthenia* spp., *Austrochiltonia australis, Paratya australiensis*, *Physa acuta* and *Glytophysa* sp.) to artificial seawater were determined by measuring the 72 h lethal concentrations required to kill 50% of individuals (LC_{50}). *Nousia* sp. AV7 was also slowly acclimated to increasing salinities over 4 days to determine whether acclimation resulted in a higher acute salinity tolerance. The 72 h LC_{50} values for the seven taxa ranged from between 12.6 to 44.9 mS cm^{-1}, with a mean of 26.4 mS cm^{-1}. The mayfly (*Nousia.* sp. AV7, 12.6 mS cm^{-1}) and the gastropods (*Glytophysa* sp., 14.5 mS cm^{-1} and *P. acuta,* 16.7 mS cm^{-1}) were the most salt-sensitive, followed by the stoneflies (*D. serricauda,* 18.3 mS cm^{-1} and *Eusthenia* spp., 36.7 mS cm^{-1}), and then the macrocrustaceans (*A. australis,* 41.3 mS cm^{-1} *and P. australiensis,* 44.9 mS cm^{-1}). Assessment of the LC_{50} values for each taxon in combination with their known field distribution in south-eastern Australia indicates that small increases in salinity are likely to have adverse affects on the viability of populations of *Nousia* sp. AV7, *Eusthenia* spp., *D. serricauda*, *P. acuta* and *Glytophysa* sp. Populations of these taxa in small headwater streams within the Tasmanian NAP region are particularly at risk.

The acute lethal tolerances of the individual taxa were broadly consistent with that of members of the same family or genus tested in the Barwon River catchment, south-west Victoria. It is likely that differences in genetic structure between the 'same species' in the two studies account for discrepancies in acute tolerance at the species level (i.e. *P. australiensis* and *P. acuta*). However, variation in salinity tolerance between populations may be reflected in long term and sublethal responses.

The acclimation regime implemented in this study did not result in an increase in acute salinity tolerance for *Nousia* sp. AV7. However an alternative regime incorporating higher conductivities, at least equivalent to the LC_{25} for *Nousia* sp. AV7, may result in an enhanced acute lethal response (LC_{50}). It is vital that future field-based and laboratory-based research be undertaken to quantify the capacity of macroinvertebrates, both at the individual and population level, to adapt to changes in salinity regime, including increases over time, and fluctuations in salinity of different frequencies, magnitudes and duration.

Acute salinity toxicity testing provides useful information on the osmoregulatory, behavioural and acclimatisation capacity of individual taxa to increasing salt concentrations, and as such provides a crude framework for predicting responses of taxa in the field. However, acute toxicity tests in isolation do not represent the complex interrelationships between natural communities and ecosystems. Thus they are often not relevant to, or useful, in predicting the field distributions of individual taxa, or salinities at which populations are sustainable (e.g. *Eusthenia* spp.). A combination of laboratory-based (including sensitive life stages, sublethal and long term effects) and field-based research (assessing patterns of salinity and water quality in association with community structure) is required. In addition the most sensitive of aquatic biota (e.g. microbes, algae and microinvertebrates) and a broad range of taxonomic and functional groups must be tested.

It is recommended that future research also assess the spatial variation in salinity tolerance between populations of the 'same species' and consider taxonomic differences and/or population genetics. This is essential for the development of appropriate regional salinity guidelines. Finally, long term and intensive monitoring of rivers and wetlands within the NAP region, particularly those aquatic systems rated as high risk is required, so that future planning and land management decisions can be appropriately informed.

1. INTRODUCTION

Salt is a natural feature of the Australian landscape (NLWRA 2001; Boulton and Brock 1999). However, over the past 200 years land-use changes have resulted in a dramatic rise in the levels of dissolved salt in Australian soils, surfacewaters and groundwaters (MDBC 1999). Secondary salinity caused by land clearing, irrigation and water diversion (Williams 1999; Hart et al. 1990) has been identified as the greatest environmental threat to land and water resources in Australia (Marshall and Bailey 2004). By 2050 it is predicted that 17 million hectares of Australian land will be either at risk of, or affected by dryland salinity, and up to 41 000 km of streams and lake perimeters significantly salt affected (NLWRA 2001).

1.1 Ecological effects of secondary salinisation on aquatic systems

Adverse biological effects on aquatic and associated biota caused by increases in salinity are well documented (e.g. Brock et al. 2005; Strehlow et al. 2005; Lymbery et al. 2003; Nielsen et al. 2003a; Blinn and Bailey 2001; Skinner et al. 2001). The establishment of salinity thresholds for the protection of aquatic organisms and ecosystem processes is required (Kefford et al. 2003b; Ryan and Davies 1996; Hart et al. 1990). However, the ecological effects of rising salinity are not well understood, particularly impacts on ecosystem processes (Briggs and Taws 2003; Clunie et al. 2002; Hart et al. 1990).

Several factors interact to determine the impact of salinisation on aquatic biota. Taxa vary greatly in their inherent physiological capacity to tolerate the direct toxic and sublethal (e.g. growth and reproduction) effects of salt (Hart et al. 1991). This capacity is shaped by an organism's specific evolutionary history and is largely determined by the solute concentration of the circulatory fluids (haemolymph) and the capacity to maintain the necessary internal balance of ions and water (osmotic regulation) (Bailey and James 2000; Hart et al. 1991). Within a given species this tolerance can vary, both across life stages (e.g. Kefford et al. 2004c & 2003b; Hart et al. 1991) and due to adaptation to differing salinity regimes within different ecosystems (Kay et al. 2001; Hart et al. 1991).

Laboratory-based toxicity testing is thus important to understanding the biological response of an organism to a potentially toxic substance (Chapman 1995). Determining the comparative salt tolerance of different taxa through laboratory testing provides valuable information for biological monitoring (e.g. by identifying indicator species) and ecological risk assessment (Kefford et al. 2003a; Calow and Forbes 2003). It is also vital to establish the osmoregulatory and behavioural responses and acclimatisation capacity, particularly of indicator species, in response to varying salt levels. Such information is a useful first step in establishing minimal acceptable concentrations (Chapman 1995), when developing strategies for the management of saline waste waters (James et al. 2003) and for determining cause and effect relationships in post-impact studies (Chapman 1995). There are many laboratory-based tolerance studies evaluating the effects of salinity on different aquatic species (e.g. Berezina 2003; Kefford et al. 2003a; Hall and Burns 2001; Goetsch and Palmer 1997; Bacher and O'Brien 1989; Mills and Geddes 1980). However in isolation such testing cannot represent the complex interrelationships between natural communities and ecosystems, and is of limited value in predicting ecological impacts (of rising salinity) in nature (Cairns 2003; Calow and Forbes 2003; Cairns 1986). In addition, the majority of this research has been on acute effects, rather than long term effects (Calow and Forbes 2003).

Each species is part of a much larger community, and complex interrelationships between biota maintain a fine trophic balance. Thus indirect effects associated with increases in water salinity can compound direct effects (Clunie et al. 2002; James et al**.** 2003). For example, loss of sensitive riparian vegetation modifies habitat, and the replacement of salt-sensitive species with salt-tolerant species alters biotic interactions (e.g. between competitors, and predators and prey) (James et al. 2003). This can ultimately impact on trophic dynamics, such as productivity, decomposition and the flow of energy and nutrients through food webs (James et al. 2003; Bailey and James 2000).

This picture is further complicated by the fact that increases in salinity rarely occur in isolation from other composite effects linked with modified land use (Halse et al. 2003). For example, saline waste water is associated with the deterioration in other water quality parameters, including nutrients and suspended solids (Kefford 1998b),

and has been found to be more toxic than water of the equivalent conductivity (Kefford 2000). Land clearing and irrigated agriculture adversely affect water quality (e.g. via fertilisers, pesticides and sedimentation), habitat, hydrology and biotic interactions (e.g. the introduction of non-indigenous fishes) (Arthington et al. 2000). These impacts alone result in major trophic changes in aquatic ecosystems (Pusey and Arthington 2003). In addition, unlike the slow gradual rises in salinity associated with past climatic changes (James et al. 2003), secondary salinisation can be associated with comparatively rapid and highly variable changes in salinity, both temporally and spatially (Ryan and Davies 1996). Rapid increases and intense fluctuations may reduce the capacity of an organism to acclimatise, particularly when associated with composite water quality changes. Short pulses of high salt concentrations have been found to be more toxic to macroinvertebrates than the same salt load delivered at a lower concentration but over a longer time period (Marshall and Bailey 2004).

Many authors refer to macroinvertebrates from different locations varying in salinity tolerance as a consequence of adaptation to local salinity conditions (e.g. Pinder et al. 2005; Kefford et al. 2004a; Kay et al. 2001; Metzling 1993). However there are no published studies examining laboratory salinity tolerance of macroinvertebrates across changes in salinity regime, e.g. an increase in average salinities at a site over several generations of macroinvertebrate taxa, and there are very few published studies of the salinity tolerance of the same species collected from different sites with differing salinity regimes. In fish, salt sensitivity has been found to vary with local salinity conditions (Clunie et al. 2002), and increased salinity tolerance has commonly been observed in laboratory tests of fish slowly acclimated (over days) to increasing salinities (Ryan and Davies 1996; Clunie et al. 2002). Laboratory tolerance tests on macro-invertebrates, including *Physa acuta* and *Paratya australiensis* (two of the species tested in this study) indicate that gradual acclimation to higher salinities result in increased salinity tolerance (B. Kefford, unpublished data). However, Kefford (pers. comm., October 2005) has repeatedly found no detectable difference in LC_{50} values when the same species is collected from different sites or from the same site on different dates, despite sometimes large differences in conductivity at the collection locations.

It is vital that the salinity tolerance of communities of species, rather than individual species are monitored and maintained within limits that protect key species within communities (James et al. 2003). Field-based studies have been undertaken to examine the relationship between salinity and macroinvertebrate communities. However much of this research is short term and based on the presence or absence of a species (rather than abundance), which does not provide an indication of population sustainability (Bailey and James 2000). In addition, very few studies have examined the effects of increases in salinity in combination with the decline in other water quality parameters (e.g. Kefford 2000), and attributing changes in community structure to salinity or a combination of variables is highly problematic due to the lack of baseline data and many confounding factors (Neilsen et al. 2003b; Mitchell and Richards 1992).

Up until recently most of the field research has focussed on the salt-tolerant or salt-adapted fauna of salt lake systems (e.g. Williams 1998; Williams 1990; Timms 1981; Geddes 1976), or lowland rivers or streams that have been subjected to modified land use and elevated salinities (e.g. Pinder et al. 2005; Kay et al. 2001; Metzling 1993; Bunn and Davies 1992; Mitchell and Richards 1992; Williams et al. 1991). Results from the latter research indicate that the fauna of these systems are relatively salt-tolerant. Importantly, the majority of the sites in these studies are also in areas subject to natural salinisation (in particular, south-west WA), or historically high salinities, such as Deep Ck, Maribyrnong River catchment (Metzling 1993) and Hopkins River sub-catchment (Mitchell and Richards 1992), and thus the taxa were adapted to saline conditions.

Other recent field-based studies in freshwater lowland streams *impacted* by surrounding land use have identified thresholds of approximately 1500 mg L^{-1} ($\approx$ 2.0 mS cm^{-1}) for both reduction in the abundance of salt-sensitive species (Marshall and Bailey 2004), and reduction in taxonomic richness and changes in species composition (Piscart et al. 2005). These communities however are likely to consist of taxa resilient to disturbance and degraded water quality. In addition, these studies do not consider long term population viability.

Combining information from laboratory toxicity testing and field-based studies provides the strongest evidence for the link between observed effects in nature and a

specific toxic substance (Adams and Rowland 2003; Chapman 1995). Such research is crucial to understanding the field distribution of individual species or the links between salinity levels and community composition. A review of laboratory-based and field-based research by Bailey and James (2000) concluded that adverse biological effects are likely to occur for some freshwater species of macroinvertebrates at salinities in excess of 800 mg L^{-1} (≈ 1 mS cm^{-1}). In a more systematic approach Kefford et al. (2003b) integrated research on acute, chronic, sublethal and sensitive life stages with data from the field distributions of macroinvertebrates from one catchment in south-west Victoria. The authors suggested that for lowland rivers with a history of *moderate* salinity that sub-lethal effects (reduction in growth and reproduction) for macroinvertebrates may occur between approximately 0.5 to 1.5 mS cm^{-1} (≈ 400 – 1100 mg L^{-1}), i.e. 10 – 30% of direct lethal effects on older life stages, and that this will likely impact on population sustainability (Kefford et al. 2003b). However the authors acknowledged that they had not considered the composite effects of altered land use. In addition, the most sensitive freshwater taxa (such as microbes, algae, macrophytes and microinvertebrates) (James et al. 2003; Clunie et al. 2002; Hart et al. 1990) were under-represented in the research.

Few field studies investigating the relationship between salinity and macroinvertebrate communties have incorporated relatively undisturbed lotic sites with low salinities. Such research may be particularly important in Tasmania where the majority of aquatic systems are very fresh (91% of waters have salinities < 300mg L^{-1} (≈ 400 µS cm^{-1}) and 78% < 100 mg L^{-1} (≈ 130 µS cm^{-1}) (Buckney and Tyler 1973), and where small headwater catchments are particularly at risk of secondary salinisation (Davies and Barker 2005). Macroinvertebrate taxa in headwater streams may be especially vulnerable because base flow salinities and fluctuations in salinity are likely to be low and potential for acclimatisation to higher salinities reduced (Nielsen et al. 2003b).

Recently published research on 1008 stream and river sites across Queensland included many sites in very good condition (in the range of 4 – 40 µS cm^{-1}) and the majority of sites had conductivities less than 800 µS cm^{-1} (Horrigan et al. 2005). The research roughly supports the thresholds (0.5 to 1.5 mS cm^{-1}) proposed by Kefford et

al. (2003b) for lowland rivers. Changes in macroinvertebrate community structure (replacement of salt-sensitive taxa with salt-tolerant taxa) were most dramatic as conductivity reached approximately 0.8 – 1 mS cm^{-1} (≈ 600 – 750 mg L^{-1}), and were more pronounced in riffle communities (Horrigan et al. 2005). However, once again, microinvertebrates and small multicellular organisms are likely to be even more salt-sensitive (James et al. 2003).

1.2 Salinity in Tasmania

As a result of past climatic, geological and tectonic processes (Hill and Orchard 1999) Tasmania shares much of its flora (Hill et al. 1999) and aquatic invertebrate fauna with south-eastern mainland Australia (Bunn and Davies 1990; De Deckker and Williams 1982). It is not known whether the salinity tolerance of freshwater taxa are similar between Tasmania and the south-eastern mainland, or if adaptation to perhaps lower salinities in Tasmania may reduce the salinity tolerance of Tasmanian freshwater fauna compared with that of the mainland. However, a comparison of the acute 72 h salinity tolerances of macroinvertebrates from similar hydrological settings across a large spatial scale (Barwon Catchment, Victoria, Australia and south-east Eastern Cape, South Africa) found similar salinity tolerances among members of the same order (Kefford et al. 2005b).

In Tasmania, knowledge of background salinity levels and regimes is very limited (Davies and Barker 2005) and it has not been possible to differentiate between primary and secondary salinity (Hocking et al. 2005a). However, while the majority of Tasmanian waters are very dilute (78% < 100 mg L^{-1} or ≈ 130 μS cm^{-1}) compared with waters of mainland Australia (Buckney and Tyler 1973), shallow subsaline wetlands (400 - 3000 mg L^{-1} or ≈ 0.5 – 4 mS cm^{-1}) and a handful of relictual inland salt lakes and pans exist in the rain-shadow area of the Midlands (Buckney and Tyler 1976). Salinities in these salt lakes were recorded by Tassell (2004) as ranging from 2 to 208 mS cm^{-1}. In addition, the Coal River catchment in the Southern Midlands is known to be naturally saline (Foley and Bobbi 2003), and the majority of sites where surface waters are currently monitored in the catchment have recordings above 1 mS cm^{-1} (≈ 800 mg L^{-1}) (Foley and Bobbi 2003). While most sites have median values below 2.0 mS cm^{-1}, several have recordings above 4 mS cm^{-1}, and the maximum recording in one of these tributaries has reached 18.8 mS cm^{-1} (Foley and Bobbi

2003). Indeed much of eastern Tasmania lies within the Tasmanian National Action Plan (NAP) region for salinity, which extends east from the Central Highlands and from Brighton in the south to Flinders Island in the north.

Limited groundwater monitoring and knowledge of hydro-geological processes that drive dryland salinity (Bastick and Walker 2000; Davies and Barker 2005) have prevented a thorough assessment of the extent and range of impacts resulting from secondary salinisation in Tasmania (NLWRA 2001). Hocking et al. (2005a) suggests that the majority of dryland salinity, at least in the Northern Midlands of Tasmania is a result of historical land clearance, and sampling of high-risk stream sites suggests evidence of secondary salinisation (Davies and Barker 2005). A review of groundwater data across the NAP region suggests a rise in groundwater levels (Hocking et al. 2005a & b). These results are uncertain due to limited groundwater monitoring (Hocking et al. 2005a & b), however Finnigan (1995) reported on the presence of large salt stores at depths up to at least 6 metres and consistently high water tables in the Cressy/Longford, Coal River and other regional irrigation areas. Groundwater conductivities up to 33 mS cm^{-1} were reported and ranged from 3.5 to 16 mS cm^{-1} in the Coal River valley (Finnigan 1995). In the last 10 years within the NAP region there has been a rapid expansion in cropping and irrigation (Hocking et al. 2005a) and continued growth is predicted (Bastick and Walker 2000). This is of immediate concern, and will undoubtedly pose major threats to water balance and contribute to an increase in both the extent and severity of secondary salinisation.

In a preliminary land system approach to the assessment of dryland salinity in Tasmania, Bastick and Walker (2000) estimated 53 000 hectares or 3% of cleared agriculture land to be salt-affected. This land is located in the Midlands, northern Tasmania and King and Flinders Islands (Bastick and Walker 2000). This figure is predicted to reach 93 600 hectares by 2050 and cost 9.3 million dollars in lost agricultural production (Bastick and Walker 2000). The assessment identified that the greatest risk to biodiversity from secondary salinisation is the potential impact on wetlands and freshwater systems (including 13 threatened species) (Bastick and Walker 2000). Finnigan (1995) identified approximately 14% of land within the irrigation scheme of the Coal River valley to be salt-affected. A GIS-based assessment of relative risk to ecological assets from secondary salinisation in the

Midlands, identified 148 wetlands (15% of the total within the study area) and approximately 1100 km or 7.5% of all stream sections studied (at low and medium base flows) to be at high risk. This increased to approximately 8850 km (60% of all stream sections studied) when both high and medium-risk areas were considered (Davies and Barker 2005).

To date the focus of most aquatic assessments and river health monitoring by Department of Primary Industry Water and Environment (DPIWE) in Tasmania has been outside the NAP region (Davies and Barker 2005). However a survey of 58 high-risk stream sites from within the NAP region, at base flows, revealed 25 stream sites with recorded conductivities greater than 1.5 mS cm^{-1} (≈ 1100 mg L^{-1}) and 39 stream sites above 1 mS cm^{-1} (≈ 800 mg L^{-1}) (Davies and Barker 2005). The highest conductivity recordings were in several small headwater streams in the Macquarie, Blackman and Coal River valleys, and the highest reading was 16.1 mS cm^{-1} (≈ 12 100 mg L^{-1}) (Davies and Barker 2005).

The development of regional thresholds for surface and sub-surface water salinity in areas at high risk of secondary salinisation in Tasmania is clearly required (Davies and Barker 2005). However to date, there has been no field or laboratory-based research examining the biological effects of increasing salinity on freshwater taxa or ecosystems.

1.3 Project aims

The aim of this short, preliminary study is to increase understanding of the biological effects of secondary salinisation on aquatic biota in Tasmania. Acute toxicity tests are undertaken to determine the comparative salinity tolerance of seven common macroinvertebrates found to be associated with a preference for lower and higher salinity sites, and to determine if acclimation to higher salinities can potentially increase salt tolerance. Aquatic macroinvertebrates are used because they display great variation in salt tolerance among and between taxonomic groups and thus changes in community structure are a useful ecological indicator of salinity effects (Clunie et al. 2002). In addition, they are abundant, diverse, have short life cycles and are easy to collect and handle.

This study investigates whether: (1) results from these tests are comparable to the acute salinity tolerance of macroinvertebrates from others parts of south-eastern Australia; (2) taxa that have been found to have a significant positive correlation between relative abundance and conductivity (EC) have a higher acute salinity tolerance than those identified to have a significant negative correlation; (3) any relationships exists between laboratory acute salinity tolerance and the maximum field salinity currently recorded for each taxa in south-eastern Australia; and (4) slow acclimation in the laboratory results in a higher acute salinity tolerance for *Nousia* sp. AV7.

2. METHODS

2.1 Macroinvertebrate taxa studied

Potential species for testing were identified from existing data of the Tasmanian component of the National Monitoring River Health Initiative (MRHI) and the routine stream monitoring program of DPIWE. A combined data set was collated for sites falling broadly across the Tasmanian NAP region. This data set included 207 stream sites that were sampled between 1994 and 2005. It collated information on site, season (autumn and spring), habitat (edgewater and riffle), EC and abundance of taxa identified to family level. The MRHI sites are representative of reference or 'least disturbed' sites (Davies and McKenny 1997) and the DPIWE monitoring program does not cover all high-risk sites in the NAP region. Therefore, the recorded conductivities in the data set do not reflect the full range of salinities within the NAP region. Conductivity levels in the data set ranged from below 50 to 2950 $\mu S\ cm^{-1}$ (MRHI data, Krasnicki et al. 2001).

The data set was screened for infrequently occurring taxa and all taxa with less than 10 total occurrences were removed, leaving 87 taxa. Abundance figures for each taxon at each site were then converted into four abundance categories. Spearman rank correlations were undertaken to identify any significant positive and negative relationships between relative abundance of taxa and EC for each season x habitat combination. Family groups for which these relationships were positively or negatively significant across all four season x habitat combinations were then identified (Table 1, 2 & 3). The most common species within each of these family groups were then determined from data available from approximately 50 reference sites falling within the study region.

Table 1. Summary of Spearman rank correlation coefficients for significant negative relationships between relative abundance of taxa and EC for each season x habitat combination.

	Spearman rho (r_s) > 0.19 are significant at 0.05			
Taxa (family level)	Autumn Edge	Spring Edge	Autumn Riffle	Spring Riffle
Eustheniidae	-0.212	-0.227	-0.487	-0.586
Gripopterygidae	-0.413	-0.569	-0.304	-0.193
Hydrobiosidae	-0.388	-0.368	-0.31	-0.288
Leptophlebiidae	-0.31	-0.329	-0.352	-0.323
Philorheithridae	-0.553	-0.577	-0.319	-0.436
Scirtidae	-0.531	-0.409	-0.33	-0.227
Tipulidae	-0.223	-0.311	-0.283	-0.221

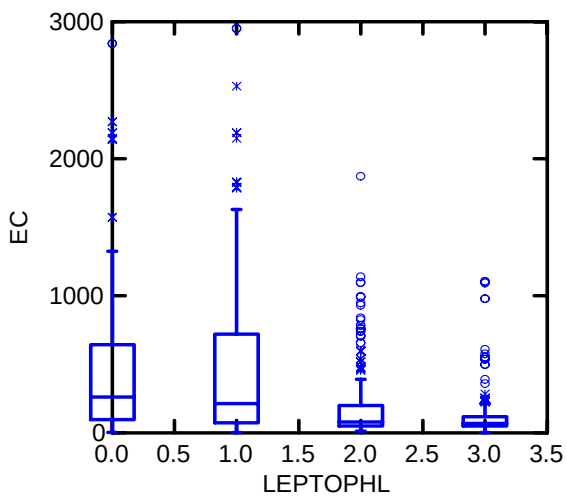
Table 2. Summary of Spearman rank correlation coefficients for significant positive relationships between relative abundance of taxa and EC for each season x habitat combination.

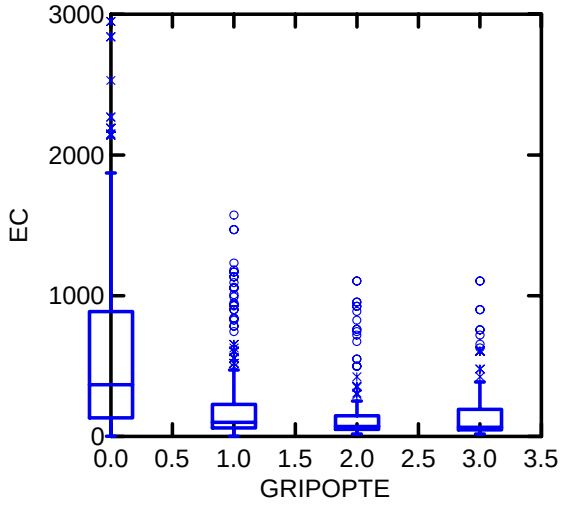
	Spearman rho (r_s) > 0.19 are significant at 0.05			
Taxa (family level)	Autumn Edge	Spring Edge	Autumn Riffle	Spring Riffle
Atyidae	0.511	0.513	0.435	0.322
Caenidae	0.461	0.512	0.379	0.325
Ceinidae	0.599	0.649	0.573	0.65
Eusiridae	0.538	0.553	0.45	0.403
Hirudinea	0.337	0.304	0.452	0.254
Hydrobiidae	0.329	0.367	0.32	0.572
Physidae	0.334	0.336	0.286	0.237
Planorbidae	0.43	0.6	0.52	0.361

Table 3. Abundance of animals in each family group currently recorded in MRHI data Tasmania.

Family (tot no of animals)	Number of individuals (% of total number of animals)			
	< 1 mS cm^{-1}	> 0.1 - < 1 mS cm^{-1}	< 0.5 mS cm^{-1}	< 0.1 mS cm^{-1}
Leptophlebiidae (15989)	15600 (98%)	6192 (39%)	14098 (88%)	9385 (59%)
Gripopterygidae (12019)	11853 (98%)	4887 (41%)	10420 (87%)	6964 (58%)
Eustheniidae (1747)	1746 (99%)	203 (12%)	1742 (99%)	1530 (88%)
Planorbidae (2743)	2076 (76%)	1936 (71%)	1094 (40%)	140 (5%)
Physidae (1281)	785 (61%)	635 (50%)	546 (43%)	150 (12%)
Ceinidae (13997)	10218 (73%)	8920 (64%)	5876 (42%)	1298 (9%)
Atyidae (2062)	1603 (78%)	1494 (73%)	906 (44%)	109 (5%)

When selecting which of the positively and negatively correlated species would be tested, consideration was also given to the ease of maintenance in the laboratory, ready availability throughout the testing period and opportunity to compare with other studies from south-eastern Australia. Seven species in total were studied. Three taxa: *Nousia* sp. AV7 (Ephemeroptera: Leptophlebiidae), *Dinotoperla serricauda* Kimmins (Plecoptera: Gripopterygidae) and *Eusthenia* spp. (Plecoptera: Eustheniidae) (*Eusthenia costalis* Banks and *Eusthenia spectablis* Gray) were negatively correlated with salinity. The remaining four species were positively correlated with salinity: *Austrochiltonia australis* Sayce (Amphipoda: Ceinidae), *Paratya australiensis* Kemp (Decapoda: Atyidae), *Physa acuta* Draparnaud (Gastropoda: Physidae) and *Glytophysa* sp. (Gastropoda: Planorbidae) (Figure 1a & b).





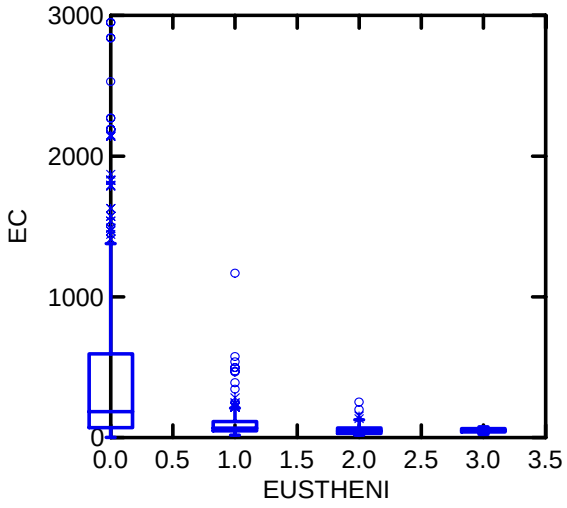


Figure 1a: Taxa (Leptophlebiidae, Gripopterygidae and Eustheniidae) with significant negative rank correlations between relative abundance and EC across all habitat (riffle and edge) x season (Autumn and Spring) combinations. 1 = low relative abundance, 2 = moderate relative abundance, 3 = high relative abundance.

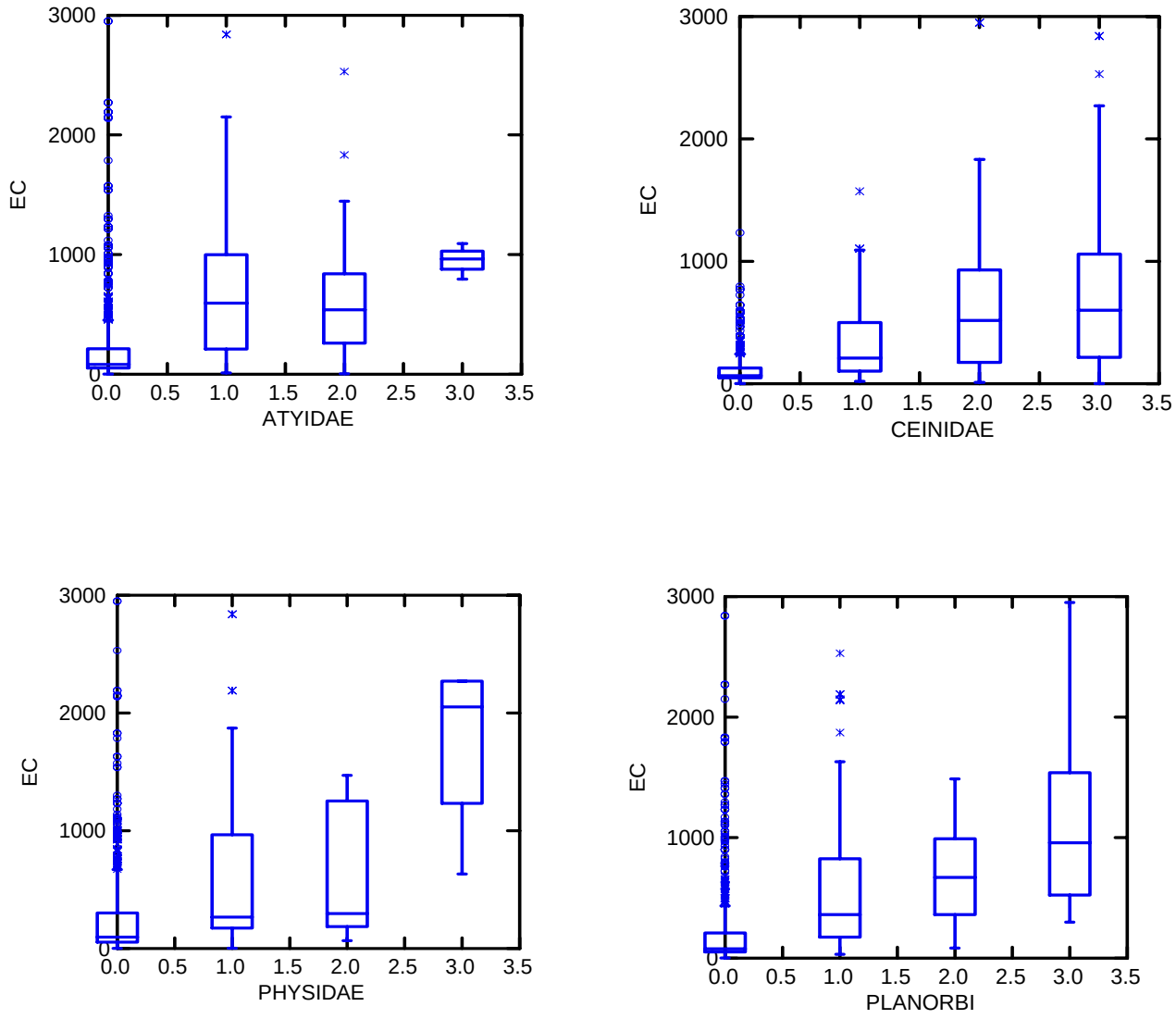


Figure 1b: Taxa (Atyidae, Ceinidae, Physidae and Planorbidae) with significant positive rank correlations between relative abundance and EC across all habitat (riffle and edge) x season (Autumn and Spring) combinations. 1 = low relative abundance, 2 = moderate relative abundance, 3 = high relative abundance.

All seven macroinvertebrates taxa tested in this study occur broadly across Tasmania, including to varying extents within the NAP region (Figure 2a & b). However, the MRHI surveys from which these field distributions were collated only sampled macroinvertebrates from riffle habitats. Hence the distribution does not fully represent the taxa with a preference for nutrient enriched waters, and/or, slow-moving or still waters (i.e. *P. acuta, Glytophysa* sp., *A. australis* and *P. australiensis*). In addition, *P. acuta, Glytophysa* sp., *A. australis* and *P. australiensis* and *D. serricauda* are all widely distributed throughout south-eastern Australia (DEH 2006). While the Eustheniidae and *Nousia* also occur throughout south-eastern Australia, *Eusthenia* spp. and *Nousia* sp. AV7 are restricted to Tasmania.

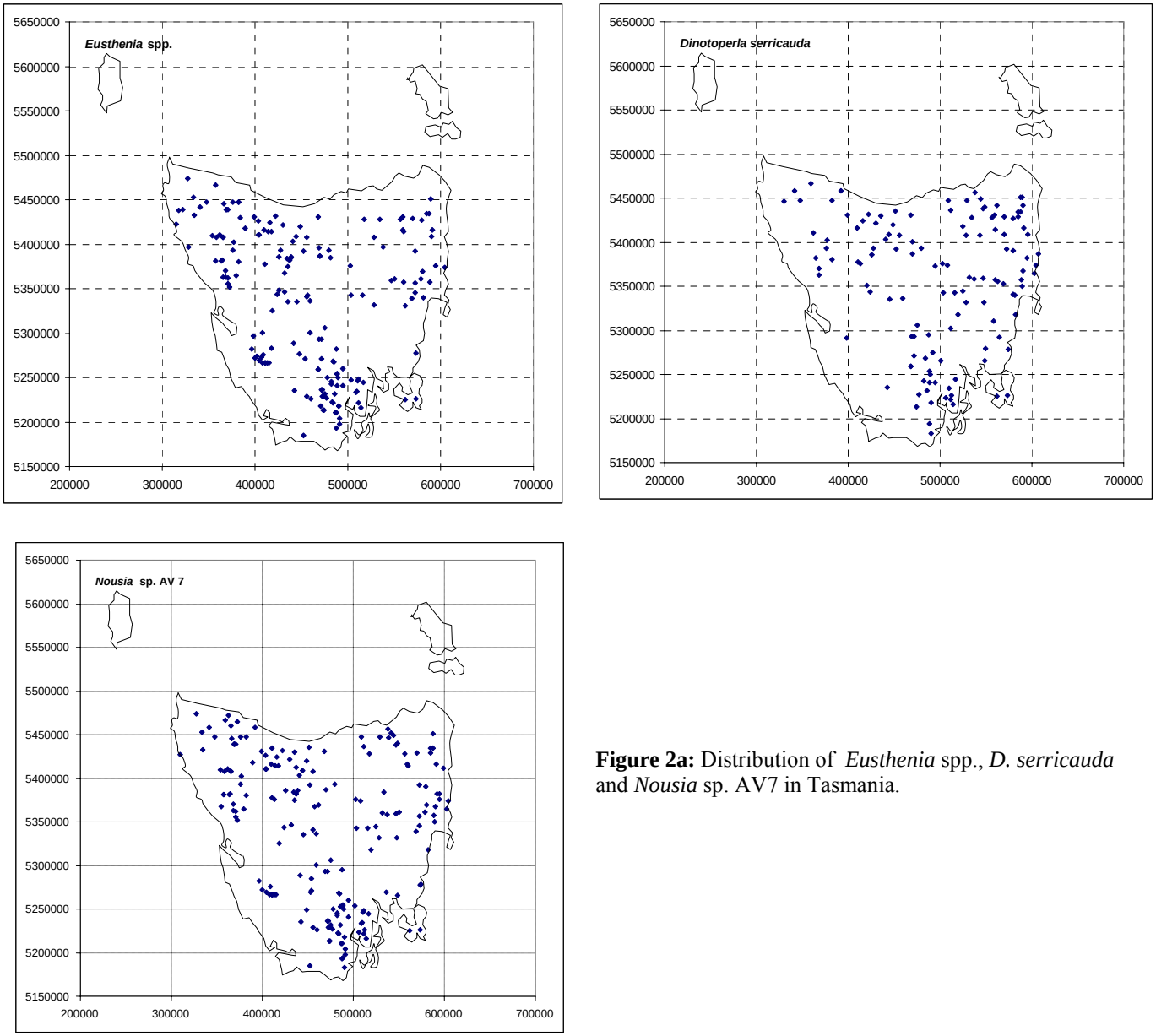
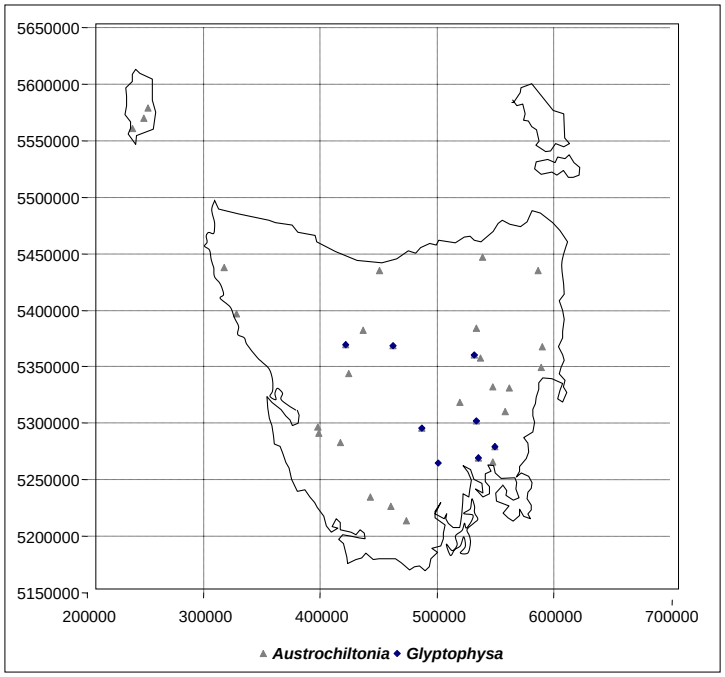


Figure 2a: Distribution of *Eusthenia* spp., *D. serricauda* and *Nousia* sp. AV7 in Tasmania.



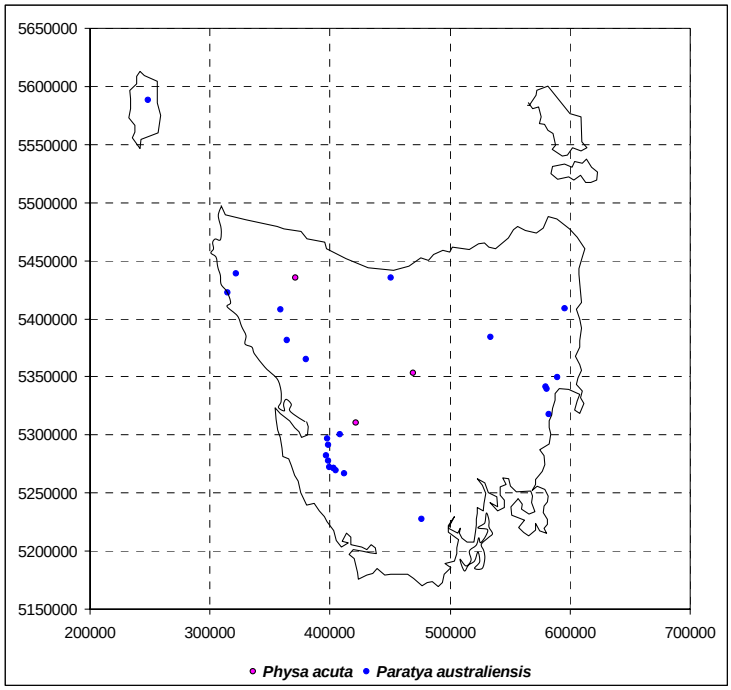


Figure 2b: Distribution of *Austrochiltonia*, *Glytophysa*, *P. acuta* and *P. australiensis* in Tasmania.

2.2 Field collection of macroinvertebrates

Macroinvertebrates were collected from three sites in southern Tasmania (Figure 3). The sites was chosen because of their close locality to Hobart, good access, abundance of animals and relatively low conductivities. The latter requirement enabled salinity tolerance testing on animals from relatively fresh waters rather than more saline sites. Salinity was measured by EC at TRef 25°C, as it is a common, reliable and accurate measure (Kefford et al 2003a).

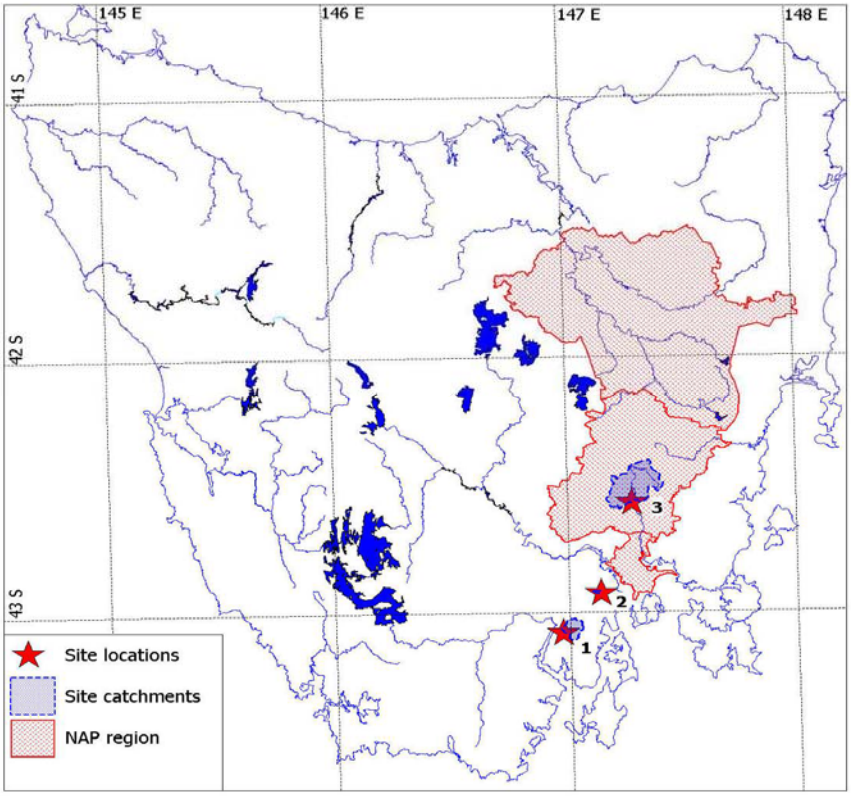


Figure 3. Map of study sites, showing Tasmanian NAP region, sites 1 (Kellaways Creek, Huon Catchment) 2 (Strickland Falls, Hobart Rivulet) and 3 (Craigbourne Dam, Coal River valley) and catchment area for each site.

Individuals of varying sizes were collected and tested. *Nousia* sp. AV7 and *Eusthenia* spp. were collected from Strickland Falls (42^0 55'S, 147^0 16'E) between November 2005 and March 2006. Electrical conductivity and water temperature ranged from 53 to 79 µS cm^{-1} and 8.1 to 12.8°C respectively during collections. *D. serricauda* were collected from Kellaways Creek (43^0 4'S, 147^0 5'E) between November 2005 and January 2006. Electrical conductivity and water temperature during collections ranged from 87 to 251 µS cm^{-1} and 12.7 to 19.9°C respectively. *P. acuta, Glytophysa*

sp., *A. australis* and *P. australiensis* were collected from Craigbourne Dam (42^0 33'S, 147^0 24'E) in January and February 2006 when electrical conductivity and water temperature ranged from 488 to 519 μS cm^{-1} and 17.8 to 20.3^{o}C respectively.

Individuals of *Nousia* sp. AV7 and *Eusthenia* spp. were found on the subsurface of rocks within riffles and *D. serricauda* were found just below the water line on rocks in riffles. Animals were carefully removed by hand or by washing rocks with water. Individuals of *A. australis, P. australiensis, P. acuta and Glyphysa* sp. were collected by sweeping a net (250 μm mesh) through submerged and emergent macrophytes. All animals were sorted in the field and placed in plastic containers filled with river water. These containers were then transported within eskies back to the laboratory where they were immediately aerated and the animals allowed to acclimate.

Strickland Falls is situated within Wellington Park and lies within the Hobart Rivulet catchment. It is a minimally impacted, permanent stream (~320m a.s.l.) with a boulder/cobble substrate and good water quality and riparian vegetation. The catchment area upstream of the site is approximately 2.5 km^2 and consists of mostly wet forest, approximately 10% alpine and sub-alpine heath and scrub, and 1 - 2 % scree slope.

Kellaways Creek is located in the Huon Catchment, approximately 50 km south-west of Hobart. It is a lowland stream (~70m a.s.l.) and approximately 5 - 10% of the valley has been cleared for pasture. The headwaters are mostly forested, and include the Pelverata Falls reserve. The catchment area upstream of the site is approximately 46 km^2 and consists of mostly (~75%) wet scrub and forest, approximately 15% dry scrub and forest and a very small area of plantation. Riparian vegetation upstream and at the study site is patchy. Monitoring by DPIWE of six river sites in the Huon Catchment between October 1996 and November 1997 found median conductivity for most rivers to be below 200 μS cm^{-1}. However Agnes Rivulet, the closest monitoring site (though situated within a different sub-catchment) has a median conductivity of almost 400 μS cm^{-1} (Bobbi 1998).

Craigbourne Dam (~165m a.s.l.) impounds approximately 12 400 ML of water (Foley and Bobbi 2003) and is located within the Coal River catchment. The catchment area for the dam is approximately 250 km^2, most of which been cleared for farmland,

however approximately 10% dry forest and 1 - 2% wet forest remains. While the maximum dam depth is approximately 25m, the majority (> 95%) is < 15m and approximately 80% is ≤ 10m. The dam substrate consists predominately of sand, silt and clay.

The catchment lies within the driest region of Tasmania with an average annual rainfall of between 500 - 600mm, falling mostly in winter (Foley and Bobbi 2003). It is a naturally saline environment (Foley and Bobbi 2003), and extensive land clearing and intensive agriculture (including grazing and irrigated cropping) have resulted in secondary salinisation (Foley and Bobbi 2003). Indeed, the sustainability of agriculture in the area has been questioned (Foley and Bobbi 2003). Surface water conductivity at many locations within the catchment are well in excess of 1.5 mS cm^{-1} (Foley and Bobbi 2003). The nearest water quality monitoring site (to the collection site) is situated just below the Craigbourne Dam, and the median conductivity for this site (based on limited data) between 1990 - 1997 and in 2002 was 481 μS cm^{-1}. The Coal River flows into the Pitt Water-Orielton Lagoon which is a Ramsar wetland.

2.3 Laboratory testing

Acute toxicity tests were implemented in accordance with OECD guidelines (OECD 1996) to evaluate the effects of salinity on survival over a 72 hour period and to determine the lethal concentration estimated to kill 10, 25 and 50% of the test population ($LC_{10,}$ LC_{25} and LC_{50}) (Adams and Rowland 2003). While a 96 h test period is often used in toxicity testing, Kefford et al. (2003a) found no major difference in the survival response for common macroinvertebrates over a 72 h or 96 h period. Confidence in LC values decreases as distance from 50% effects increases, however, Tasmanian freshwater systems are at the lower end of the range of salinities experienced across Australia. As such, lower salinity and sublethal thresholds, reflected in LC_{10} and LC_{25} values may be more indicative of expected rises in salinity and ecological risk. Furthermore, no-observed effect concentration (NOEC) is the highest concentration in which there is no significant difference in mortality from the control treatment within the test period (OECD 1996). LC_{10} values can be used as a substitute for NOEC for acute tests (Sebaugh et al. 1991 in Adams and Rowland 2003).

Both the saline and fresh waters of Tasmania have an ionic composition similar to sea water (Buckney and Tyler 1973; Vanhoutte et al. 2006). Thus the artificial sea salt Ocean Nature (Aquasonic, Port Macquarie, NSW) which has a similar ionic proportion to seawater was the salt used in the salinity treatments. For Ocean Nature (ON) the relationship between EC and total dissolved solids (TDS) is described by: TDS (g L^{-1}) = 0.754 x EC mS cm^{-1} (Kefford et al. 2003a). ON was dissolved in 'mountain water' (low salinity, unchlorinated water sourced from Mount Wellington) to make water to the required test concentrations.

Animals were randomly allocated to a range of salinity treatments. The aim was to achieve a range of mortality responses, from less than 20% mortality up to 100% mortality (OECD 1996). An initial salinity regime of roughly 0.5, 1, 5, 10 and 15 mS cm^{-1} was implemented for each species. Concentrations used in subsequent treatments depended on the response to the initial regime and each round of testing thereafter. Where sufficient animals and time were available a more accurate estimation of LC_{50} was permitted by implementing a finer range of treatments (intervals of 1 to 2 mS cm^{-1}) within the 30 to 80% mortality range. Most often, two to four replicates were undertaken for each species at each salinity (Table 4).

Tests were conducted at 14±1^{o}C which was indicative of the average water temperature (13.7 ^{o}C) experienced at the three field sites on collection occasions. Experiments commenced once the temperature of the river water in which the animals were collected reached laboratory temperature. This acclimation time varied from between 24 to 48 h after the animals reached the laboratory. Tests were undertaken in 600ml plastic containers (approximate dimensions: 17 x 12 x 4 cm) secured with perforated lids to minimise evaporation and to prevent animals from moving out of the test solution. Water was aerated (> 90% oxygen saturation) and it was not changed during the experiment. A 12:12 hour light-dark photoperiod was simulated to reflect natural conditions.

Table 4. Number of replicates and animals tested for each salinity treatment.

	Number of replicates (total number of animals)						
Treatment (mS cm^{-1})	*N.* sp. AV7	*D. serricauda*	*Eusthenia* spp.	*Glyptophysa* sp.	*P. acuta*	*A. australis*	*P. australiensis*
'Mountain water' (control A)[a]	4 (40)	4 (40)	6 (6)	2 (20)	2 (20)	4 (40)	2 (20)
'River water' (control B)[b]		4 (40)		2 (20)	3 (40)	4 (40)	2 (20)
'Mountain river' (control C)[c]		3 (30)		2 (30)	2 (30)	4 (40)	2 (20)
0.5	4 (40)	4 (40)	6 (6)				2 (20)
1		2 (20)		2 (20)	1 (20)	4 (40)	2 (20)
5	4 (40)	4 (40)	4 (4)	2 (20)	1 (20)	4 (40)	2 (20)
7	4 (40)	2 (20)					
10	4 (40)	4 (40)	6 (6)	2 (20)	1 (20)	4 (40)	2 (20)
12	4 (40)	2 (20)		2 (17)			
13	2 (20)	4 (40)					
14	2 (20)	2 (20)					
15	4 (40)	4 (40)	6 (6)	2 (19)	1 (20)	4 (40)	2 (20)
16	2 (20)	1 (10)					
17	4 (40)	4 (40)		1 (9)	2 (30)		
19	4 (40)	4 (40)			1 (20)		
20		1 (10)	4 (4)	2 (18)	1 (20)	4 (40)	2 (20)
21	2 (20)	4 (40)					
22	4 (40)	2 (20)					
23							
24							
25	2 (20)	4 (40)	6 (6)		1 (20)	4 (40)	2 (20)
27		3 (30)					
30			6 (6)		1 (20)	4 (40)	2 (20)
35			6 (6)	1 (10)		4 (40)	2 (20)
40			6 (6)			4 (40)	2 (20)
42						4 (40)	
45			6 (6)			4 (40)	2 (20)
47						4 (40)	
50						4 (40)	
52						4 (40)	
55						4 (40)	
Total number of replicates (animals)	50 (500)	62 (620)	62 (62)	20 (203)	17 (280)	72 (720)	26 (260)

[a] EC of 'mountain water' ranged from approximately 50 to 110 µS cm^{-1}.
[b] EC of 'river water' ranged from approximately 95 to 150 µS cm^{-1} for *D. serricauda* and ranged from approximately 480 to 535 µS cm^{-1} for *Glyptophysa sp., P acuta, A. australis and P. australiensis.*
[c] EC of 'mountain river' ranged from approximately 95 to 150 µS cm^{-1} for *D. serricauda* and ranged from approximately 485 to 555 µS cm^{-1} for *Glyptophysa sp., P acuta, A. australis and P. australiensis.*

Up to 10 individuals were randomly placed in each test container, except for *Eusthenia* spp., where individuals were housed separately due to their aggressive behaviour. In addition, one opaque tile (7 x 7 cm) was placed on top of an embryological watch glass in each container to provide the animals with flow and light shelter. Animal survival, EC and temperature were recorded at 24, 48 and 72 hours. Where necessary 'mountain water' was added to maintain treatment conductivity within 10% of the nominal concentration. Animals were not fed during the experiments and animals that emerged or were cannibalised were excluded from subsequent analysis. Death was identified by a lack of movement and the failure of an animal to respond to gentle probing. Retracted snails were placed in river water collected from Craigbourne dam for 30 minutes and were then considered dead if they had not responded (Kefford et al 2003a). Dead animals were removed from the treatment container and were preserved in 70 % alcohol. At the conclusion of the experiment all remaining animals were retained and preserved in labelled vials of alcohol.

All species, except for *Nousia* sp. AV7 and *Eusthenia* spp. were tested with three controls. These comprised of 'mountain water' (control A) collected from Strickland Falls (approximately 50 to 110 µS cm^{-1}), 'river water' (control B) from the collection sites and 'mountain water' with ON added to achieve a conductivity similar to that at the collection site 'mountain river' (control C). *Nousia* sp. AV7 and *Eusthenia* spp. which were collected at Strickland Falls only required control A. Control A, a low salinity treatment, also provided the opportunity to determine if low salinity had a detrimental effect on survival for any of the other species tested. Control A (for *Nousia* sp. AV7 and *Eusthenia* spp) and Control B (for *D. serricauda, A. australis, P. australiensis, P. acuta and Glyphysa* sp.) gave an indication of the death rate resulting from laboratory conditions. Control C enabled the impact of potentially differing water quality variables (e.g. nutrients, pH and ionic composition) between 'mountain water' and 'river water' to be considered.

A seven day acclimation trial was also undertaken with *Nousia* sp. AV7 to test whether laboratory acclimation resulted in a significantly different survival response. This trial (T1) involved a stepwise increase (slow acclimation) in EC over the first four days (96 h). Conductivities started at 0.5 mS cm^{-1} and were increased to 1, 1.5 and 2 mS cm^{-1} at 24, 48 and 72 h respectively. A conductivity of 0.5 mS cm^{-1} was chosen as the initial

concentration for the acclimation regime because survival for *Nousia* sp. AV7 over 72 hours in the 0.5 mS cm^{-1} treatments was found to be very high (100% for 75% of the replicates). At each stepped increase in salinity, animals were transferred to fresh containers with water at the higher conductivity. At 96 h, surviving animals were then tested over a 72 hour period with a range of salinity treatments (0.5 – 25 mS cm^{-1}). This involved randomly transferring the acclimated animals to treatment salinities where they remained for 72 hours. A control was also implemented (T2), whereby animals remained for the first 96 h in 'mountain water' (EC of approximately 82 to 92 µS cm^{-1}) and then tested with a similar range of salinity treatments over 72 h. Three or four replicates were undertaken at each salinity (Table 5).

Table 5. Number of replicates and animals tested for acclimation trials with *Nousia* sp. AV7.

	N. sp. AV7 Number of replicates (total number of animals)	
Treatment EC (mS cm^{-1}) for 72hrs post acclimation period.	*Acclimation regime 1* Animals acclimated with a stepwise increase in EC (approx 0.5 to 2 ms cm^{-1}) over 96 h.	*Acclimation Control* Animals acclimated in 'mountain water' (approx 82 to 92 µs cm^{-1}) for 92 h.
0.5	4 (35)	3 (29)
5	4 (38)	3 (29)
7	3 (27)	3 (29)
10	4 (38)	3 (30)
12	4 (35)	3 (29)
13	3 (27)	
15	4 (38)	3 (29)
17	4 (35)	3 (30)
19	4 (36)	3 (29)
21	4 (35)	3 (28)
22	4 (36)	
25	4 (38)	
Total number of replicates (animals tested)	46 (418)	27 (262)

2.4 Relationship between LC_{50} values and field distributions

For macroinvertebrates, a statistically significant positive correlation between laboratory LC_{50} values and the maximum salinity at which animals have been recorded in the field (MFD) has been reported (Kefford et al 2004a). Because taxonomic information is currently only readily available for river sites with salinities up to 2.95 mS cm^{-1} and the taxa have only been identified to family level, it was therefore not possible to gain a realistic indication of the MFD in Tasmania for the taxa tested in this study. This information was therefore complemented with data from the Australian Biodiversity Salt Sensitivity Database by Bailey et al. (2002) and other relevant field research. The Salt Sensitivity Database contains information on the known field distribution and tolerance with respect to salinity of over 1200 species of Australian taxa. This enabled a comparison of the LC_{50} values derived for each taxa, at least to family level, to be made with their known field distribution in south-eastern Australia.

2.5 Statistical analysis

To determine the relationship between salinity and survival for each taxon, generalized linear modelling was used to fit the proportion dead (y-variable) to EC (x-variable) using binomial errors and the complementary log-log link. Inspection of residuals and normal probability plots showed that the complementary log-log link was superior to the conventional logit or probit links for these data. $LC_{10,\ 25,\ 50}$ values were then calculated from the best fit model for each taxa. Where survival response apparently differed between the control treatments for any taxa, generalized linear modelling was again used to compare proportion dead between replicated controls using binomial errors and the complementary log-log link. Control B 'river water' was considered the standard control against which Control A ('mountain water') and Control C ('mountain river') were compared. To determine if the stepwise increase in EC (slow acclimation) resulted in a significantly different survival response for *Nousia* sp. AV7 than the control, the generalised linear models were compared between T1 (slow acclimation) and T2 (control, without slow acclimation). A model for probability of death based on EC + trial + EC x trial was developed and a likelihood ratio test was then applied to analyse whether the probability of death was significantly different when the interaction between Trial and EC was omitted from the model. All analyses were run on R software (MA, USA).

3. RESULTS

Six taxa (excluding *P. australiensis*) were collected in sufficient numbers to be tested with a range of salinity treatments associated with less than 20% to 100% mortality. A mortality rate of only 60% was reached for *P. australiensis*. For three other taxa (*P. acuta*, *Glyptophysa* sp. and *Eusthenia* spp.), numbers were not adequate to achieve the most optimal scale of testing and replication around LC_{50} levels (Table 4). The conductivities of all treatment salinities were maintained within 7.5% of the nominal concentration during the test period.

Residuals and normal probability plots were inspected for each dataset. For *Nousia* sp. AV7, *D. serricauda* and *A. australis,* where there was good replication, implementation of a fine gradation of salinity treatments around LC_{50} levels and a gradual change in survival response to increasing EC, residuals appeared close to normal and the model fitted well (Figures 4, 5 & 6). For the remaining taxa (*P. australiensis, P. acuta*, *Glyptophysa* sp. and *Eusthenia* spp.) this was not the case and the problem was not resolved with alternative transformation (Figures 7, 8, 9 and 10). A greater level of replication and finer scale testing around LC_{50} would help to resolve this problem. Nonetheless, a generalised linear model with the complementary log-log link was fitted to the dose-response curve for each taxa (Table 6). All data collected were used in the analyses, with the exception of the data from the low salinity treatments (70 – 82 µS cm^{-1}) for *A. australis.* They were removed from the analysis due to the uncharacteristically high mortality rates, see results below.

None of the species were collected in sufficient numbers on any one occasion to be exposed to the full range of test salinities in one experimental period. Taxa were collected on different occasions over 2 to 4 months and the longest collection period was for *Nousia* sp. AV7 which was collected over 5 months, from spring to autumn (early November to late March). Thus any possible temporal variation in test results could not be evaluated. Conductivities at the collection sites over the collection period remained fairly constant, with only a 26 and 31 µS cm^{-1} increase in salinity at Strickland Falls and Craigbourne Dam respectively. Conductivities at Kellaways Creek (where *D. serricauda* was collected) were more variable in response to rainfall events and ranged from 85 to 250 µS cm^{-1}. However, only 10 of the 620 individuals

tested were collected when the conductivity reading was highest (250 µS cm^{-1}). The remainder were collected when conductivities varied between 87 and 157 µS cm^{-1}.

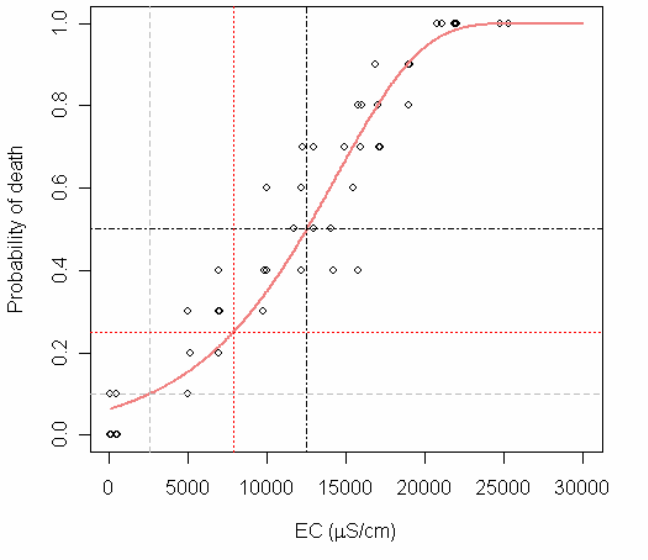


Figure 4. Generalised linear modelling line for the 72 h salinity tolerance experiments with *Nousia* sp. AV7. Dashed vertical lines indicates the $LC_{50,\ 25\ \&\ 10}$ values, black = $LC_{50,}$ red = $LC_{25,}$ grey = $LC_{10.}$

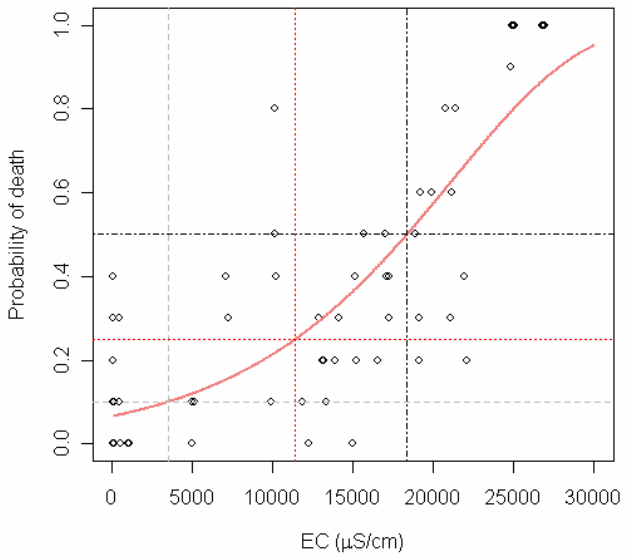


Figure 5. Generalised linear modelling line for the 72 h salinity tolerance experiments with *D. serricauda.* Dashed vertical lines indicates the $LC_{50,\ 25\ \&\ 10}$ values, black = $LC_{50,}$ red = $LC_{25,}$ grey = $LC_{10.}$

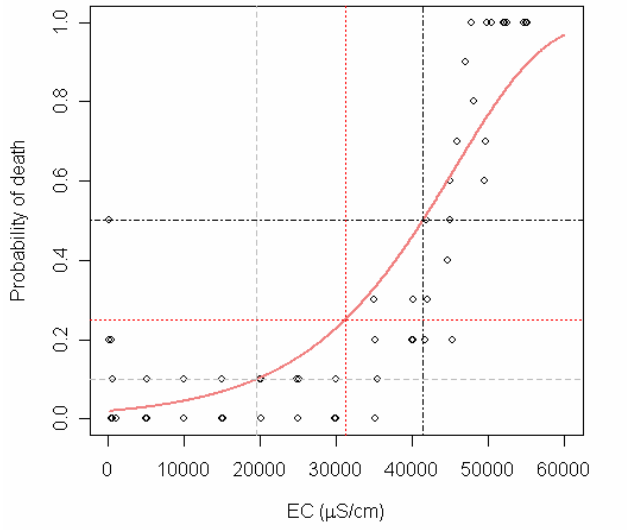


Figure 6. Generalised linear modelling line for the 72 h salinity tolerance experiments with *A. australis.* Dashed vertical lines indicates the $LC_{50,\ 25\ \&\ 10}$ values, black = $LC_{50,}$ red = $LC_{25,}$ grey = $LC_{10.}$

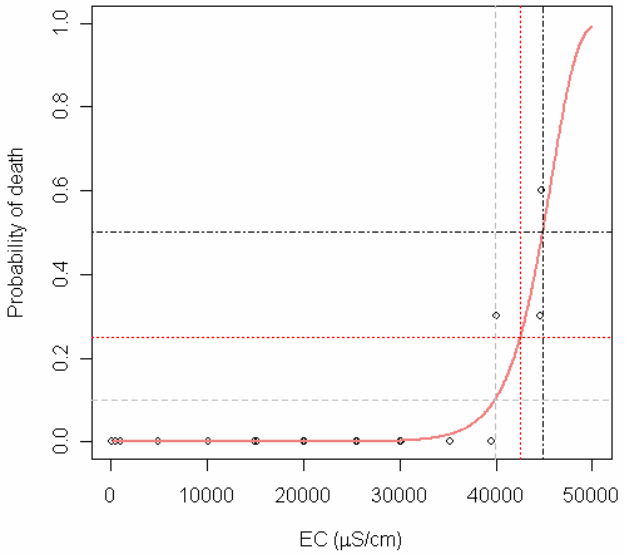


Figure 7. Generalised linear modelling line for the 72 h salinity tolerance experiments with *P. australiensis*. Dashed vertical lines indicates the $LC_{50,\ 25\ \&\ 10}$ values, black = $LC_{50,}$ red = $LC_{25,}$ grey = $LC_{10.}$

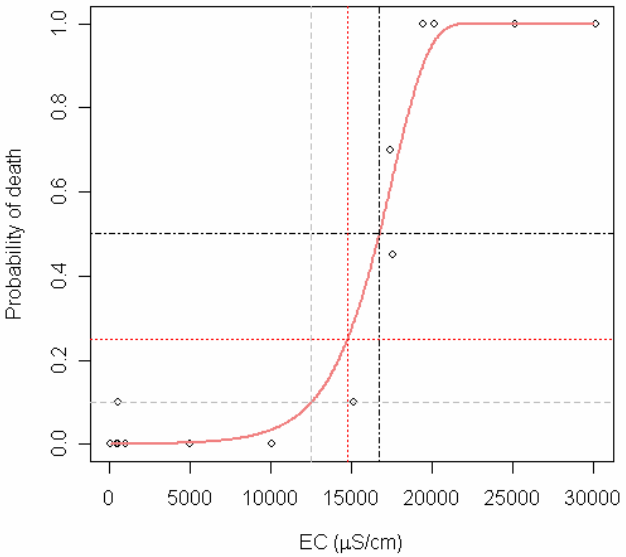


Figure 8. Generalised linear modelling line for the 72 h salinity tolerance experiments with *P. acuta*. Dashed vertical lines indicates the $LC_{50,\ 25\ \&\ 10}$ values, black = $LC_{50,}$ red = $LC_{25,}$ grey = $LC_{10.}$

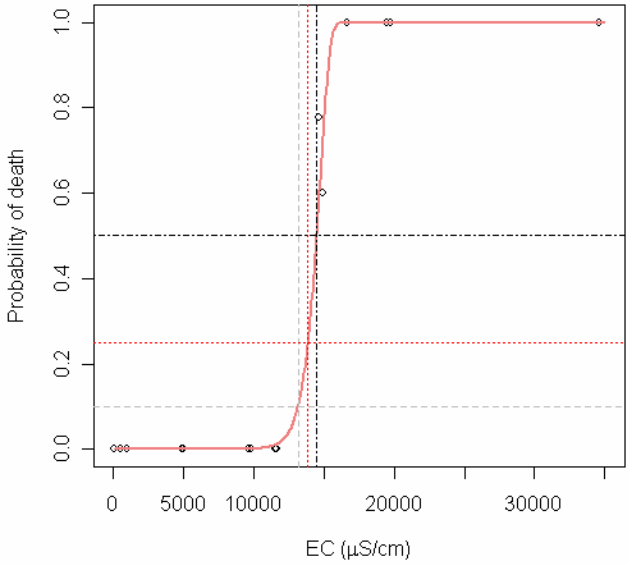


Figure 9. Generalised linear modelling line for the 72 h salinity tolerance experiments with *Glyptophysa* sp. Dashed vertical lines indicates the $LC_{50,\ 25\ \&\ 10}$ values, black = $LC_{50,}$ red = $LC_{25,}$ grey = $LC_{10.}$

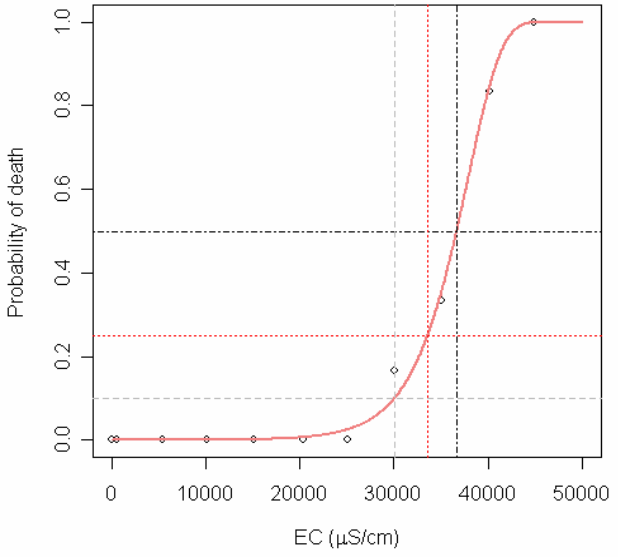


Figure 10. Generalised linear modelling line for the 72 h salinity tolerance experiments with *Eusthenia* spp. Dashed vertical lines indicates the $LC_{50,\ 25\ \&\ 10}$ values, black = $LC_{50,}$ red = $LC_{25,}$ grey = $LC_{10.}$

Table 6. Logarithms for each taxa of the proportion dead (y-variable) to EC (x-variable) using binomial errors and the complementary log-log link.

Taxa	Intercept	***Slope***	n	Change in deviance	p
N. sp. AV7	-2.739 (± 0.2332)	1.885×10^{-4} ($\pm 1.507 \times 10^{-5}$)	51	269.78	< 0.001
D. serricauda	-2.691 (± 0.1986)	1.268×10^{-4} ($\pm 1.043 \times 10^{-5}$)	62	179.15	< 0.001
Eusthenia spp.	-10.77 (± 3.37)	2.835×10^{-4} ($\pm 8.944 \times 10^{-5}$)	10	47.14	< 0.001
Glyptophysa sp.	-20.86 (± 8.981)	1.414×10^{-3} ($\pm 5.082 \times 10^{-4}$)	20	200.35	< 0.001
P. acuta	-7.832 (± 1.326)	4.464×10^{-4} ($\pm 7.293 \times 10^{-5}$)	17	266.74	< 0.001
A. australis	-3.927 (± 0.2769))	8.614×10^{-5} ($\pm 6.217 \times 10^{-6}$)	72	318.09	< 0.001
P. australiensis	-17.28 (± 4.934)	3.77×10^{-4} ($\pm 1.133 \times 10^{-4}$)	26	52.158	< 0.001

3.1 Controls

For all taxa, survival over 72 h in control A ('mountain water') or B ('river water') containing water from the collection sites was high (Table 7). Only two replicates for one taxon, *D. serricauda* fell below 80% and most fell within the 90 to 100% survival range. Survival was very high (90 - 100%) for *D. serricauda, A. australis, P. australiensis, P. acuta and Glyphysa* sp. in control C, 'mountain river'. Thus over 72 h when correcting for salinity, other potentially differing water quality variables (e.g. nutrients, pH and ionic composition) between 'mountain water' and water from the collection sites did not appear to affect the survival of any taxa. This was important to determine as 'mountain water' was used as the water base to which ON was added to make up the salinity treatments. Survival was also high (90 – 100%) for *D. serricauda, P. australiensis, P. acuta and Glyphysa* sp. in the low salinity treatment ('mountain water').

Table 7. Summary of control survivorship (%) for each taxa at 72-hr.

Taxa	Survivorship @ 72hr (%)					
	Control A[a] 'mountain water'	Total number of individuals tested	Control B[b] 'river water'	Total number of individuals tested	Control C[c] 'mountain river'	Total number of individuals tested
N. sp. AV7	90 – 100	40	na	na	na	na
D. serricauda	80 - 100	40	60 - 100	40	90 - 100	30
Eusthenia spp.	100	6	na	na	na	na
Glyptophysa sp.	100	20	100	20	100	30
P. acuta	100	20	100	40	100	30
A. australis	50 - 80	40	80 - 100	40	90 - 100	40
P. australiensis	100	20	100	20	100	20

[a] control A, 'mountain water' from Strickland Falls (minimally impacted site, approximately 50 to 110 µS cm^{-1}).
[b] control B, 'river water' was water from the sites where animals were collected: Strickland Falls (approximately 50 to 110 µS cm^{-1}) for *N.* sp. AV7 and *Eusthenia* spp; Kellaways Creek (approximately 87 to 251 µS cm^{-1}) for D. *serricauda;* Craigbourne Dam (approximately 488 to 519 µS cm^{-1}) for *Glyptophysa* sp., *P. acuta, A. australis and P. australiensis.*
[c] control C, 'mountain river' was 'mountain water' with Ocean Nature added to achieve a conductivity similar to that at the collection site.
na not applicable.

Survival however, for *A. australis* at 50 to 80% in 'mountain water' was found to be significantly different from survival in 'river water' (80 – 100%) ($z_{(1)} = 2.812$, $p = 0.005$) (Figure 11). Sublethal (e.g. reduced growth and reproduction) and lethal effects for macroinvertebrates at low salinities are possible. Reductions in growth have been recorded for *P. acuta* (Kefford and Nugegoda 2005) below 0.5 mS cm^{-1} and for the salt-tolerant, freshwater damselfly *Ischnura heterosticta* below 1 mS cm^{-1} (Kefford et al. 2006). Increased energy demands of osmoregulation at low salinities were among the mechanisms proposed to explain the reductions in growth. However neither study was able to conclusively demonstrate any one cause. Similarly, growth for most freshwater fish is optimal at salinities above normal freshwater (Boeuf and Payan 2001 in Kefford and Nugegoda 2005). Increased mortality at low salinities may indicate that the hyper-osmoregulatory mechanisms of *A. australis* are less well developed than other halotolerant freshwater species, e.g. *P. australiensis*, which maintained a 100% survival rate at low salinities (< 0.1 mS cm^{-1}). However in order to determine this, research on the osmoregulatory capacity of *A. australis* would be required. Studies of

the amphipod *Corophium curvispinum* (Corophiidae) reveal osmoregulatory capacities of the gills and renal organs indicative of a relatively recent brackish-water ancestry (Taylor and Harris 1986a & b).

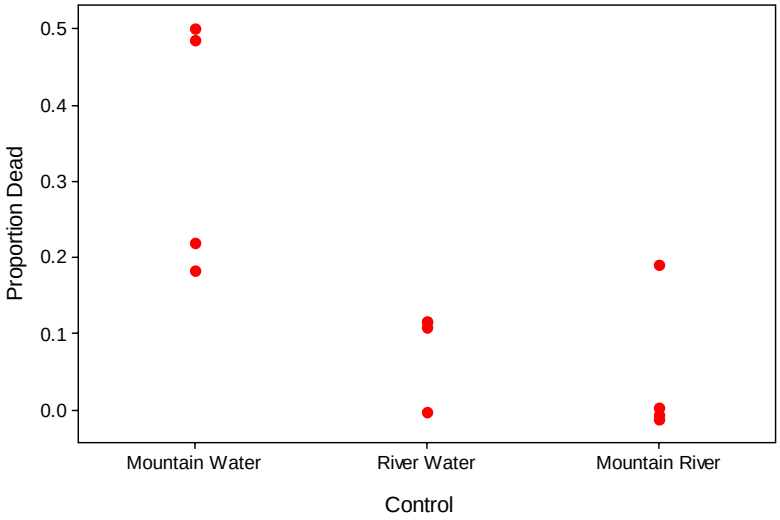


Figure 11. Proportion dead of *A. australis* at 72 h in response to the control treatments (4 replicates of each control). 'Mountain Water' = control A, approx 68 to 83 µS cm^{-1}, 'River Water' = control B, approx 488 to 517 µS cm^{-1}, 'Mountain River' = control C, approx 501 to 517 µS cm^{-1}.

3.2 Taxonomic differences

The 72 h LC_{25} and LC_{50} values for all taxa ranged from 7.9 to 42.5 mS cm^{-1} and from 12.6 to 44.9 mS cm^{-1} respectively, and the NOEC (LC_{10}) ranged from 2.6 mS cm^{-1} to 39.9 mS cm^{-1} (Table 8). As expected, *Nousia* sp. AV7, (Ephemeroptera: Leptophlebiidae) had the lowest salinity tolerance, while *P. australiensis* (Decapoda: Atyidae) had the highest tolerance. Based on LC_{50} values taxa are ranked from the least to the most salinity tolerant in the following order: *Nousia* sp. AV7, *Glyphysa* sp., *P. acuta, D. serricauda, Eusthenia* spp., *A. australis* and *P. australiensis.* Therefore taxa from the family groups that were positively correlated with salinity, generally showed a higher salinity tolerance than negatively correlated taxa. By contrast, however, the Plecoptera, *Eusthenia* spp. and *D. serricauda,* which were negatively correlated with salinity had higher LC_{50} values than the two gastropods (*P. acuta* and *Glyphysa* sp.). In addition, the gastropods had a comparatively abrupt change in survival response from minimal or no mortality to 100% mortality within a narrow salinity range (5 mS cm^{-1}) (Figures 8 & 9). For the other taxa, this transition in mortality occurred over a wider range of salinities, approximately 10 to 20 mS cm^{-1}.

Table 8. Summary of 72-hr salinity tolerance (mS cm^{-1}) of taxa.

Taxa	LC_{50}	95% Cl of LC_{50}	LC_{25}	LC_{10}	Control survivorship @ 72 h (%) in water from collection site	Total number of individuals tested
N. sp. AV7	12.6	11.7 - 13.4	7.9	2.6	90 - 100	500
D. serricauda	18.3	17.2 - 19.5	11.4	3.5	60 - 100	620
Eusthenia spp.	36.7	34 - 39.3	33.6	30	100	62
Glyptophysa sp.	14.5	14 - 15	13.9	13.2	100	203
P. acuta	16.7	15.9 - 17.5	14.8	12.5	100	280
A. australis	41.3	39.7 - 43	31.1	19.5	80 - 100	720
P. australiensis	44.9	43.1 - 46.6	42.5	39.9	100	260

For *P. australiensis, Eusthenia* spp., *P. acuta* and *Glyptophysa sp.* the LC_{10} values are associated with a mortality response which is higher than that of the control treatment (Figures 7, 8, 9 & 10), and the LC_{10} values derived for *P. australiensis, Eusthenia* spp., *P. acuta* and *Glytophysa* sp. coincided with conductivities associated with signs of *obvious* physiological stress, such as reduced movement, retraction into the shell or coloration (*P. australiensis*). Thus adverse physiological effects (sublethal responses) were being realised for these species at salinities likely to be well below this level.

While not quantified, the mortality for *Nousia* sp. AV7 in salinities approaching and beyond the LC_{50} value (12.6 mS cm^{-1}) appeared to be particularly high in the later instar larval stages, just before emergence. If correct, this may be due to the body surface (cuticle) being more permeable to ions at this stage of the moult cycle (Sutcliffe 1974). Vulnerability at this stage of the life cycle may be a particularly critical factor determining the viability of populations of this species if subjected to repeated fluctuations in salinities around their tolerance levels.

While only 66 individuals of the stonefly *Eusthenia* spp. were tested they were surprisingly salt-tolerant, with a survival rate of 100% until conductivity levels reached 30 mS cm^{-1} (Figure 10). *D. serricauda* displayed the most variable mortality response across the salinity treatments (Figure 5) and individuals were collected from the site (Kellaways Creek) that displayed the greatest variability in conductivity over the collection period. However this variation of 87 to 250 µS cm^{-1} was trivial compared

with the range of treatment salinities against which animals were tested and examination of the results indicate that it is unlikely to have played a significant role.

The variable response of *D. serricauda* may reflect differences in such factors as body size, stage of moult cycle and development, nutritional status and degree of acclimation. All of these factors can affect the ability of an individual to maintain body solute concentrations (Hart et al. 1991). Alternatively, the variability observed may be peculiar to this particular species.

3.3 Acclimation

No significant difference in the survival response of *Nousia* sp. AV7 was found between T1 (slow acclimation) and T2 (control, without slow acclimation) (likelihood ratio test: change in deviance due to deletion of interaction: $\chi^2_{(1)} = 0.760$, $p = 0.383$) (Figure 12). LC_{50} for T1 and T2 were 11.7 and 10.8 mS cm^{-1} respectively (Table 9). While acclimation appears to have resulted in a small increase in salinity tolerance, the 95% confidence limits for the means overlapped (10.6 – 12.8 mS cm^{-1} for T1 and 9.4 – 12.2 mS cm^{-1} for T2) and overall the survival response was not significantly different to the control.

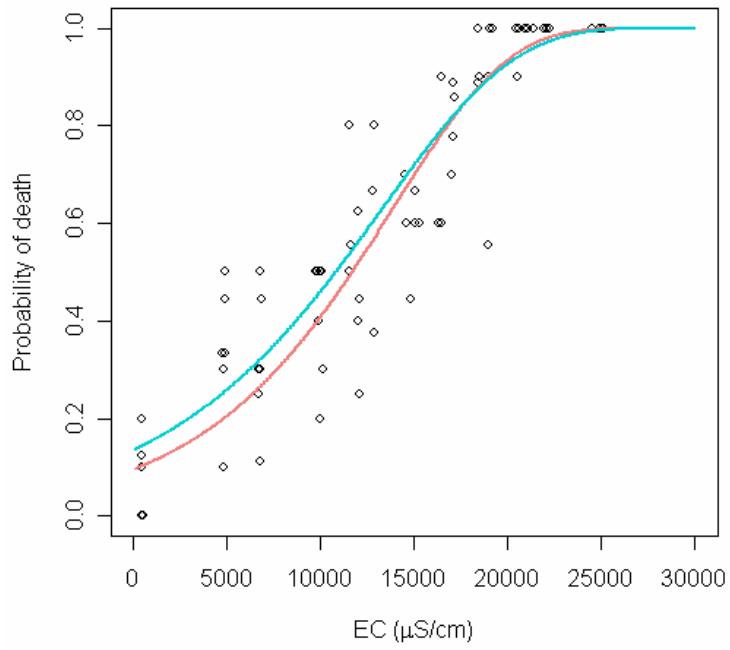


Figure 12. Generalised linear modelling lines for the acclimation trials with Nousia sp. AV7. Red line = T1 (slow acclimation) and blue line = T2 (control).

Table 9. Summary of 72-hr salinity tolerance (mS cm^{-1}) of *Nousia* sp. AV7 after laboratory acclimation.

Taxa	LC_{50}	95% Cl of LC_{50}	LC_{25}	Control survivorship @ 168hr (%)	Total number of individuals tested
Acclimation regime 1[a]	11.7	10.6 – 12.8	6.4	na	418
Acclimation Control[b]	10.8	9.4 – 12.2	4.7	80[c]	262

[a] Animals acclimated with a stepwise increase in EC (approximately 0.5 to 2 ms cm^{-1}) over 96 h and then tested with a range of salinities (approximately 0.5 – 25 mS cm^{-1}) for 72 hrs.
[b] Animals acclimated in 'mountain water' (approximately 82 to 92 µs cm^{-1}) for 92 h and then tested with a range of salinities (0.5 – 21 mS cm^{-1}) for 72 h.
[c] 3 replicates @ 10 animals each - animals remained in 'mountain water' (approximately 65 – 140 µs cm^{-1}) for 168 h (96 h acclimation period + 72 h test period).
na not applicable.

3.4 Relationship between laboratory salinity tolerance and MFD

Refer to Tables 10 and 11 for the comparison of MFD and 72 h LC_{50} values for each taxa. Abundance of taxa with respect to salinity however, provides a better indication of the sustainability of a population at a particular salinity than MFD, or the presence or absence of a taxon at a particular site (Bailey and James 2000). Analysis of abundance figures from the Tasmanian MRHI data (Table 3) highlights, for example, that while Leptophlebiidae has been recorded at a maximum salinity of 2.95 mS cm^{-1} (Table 10), the vast majority of individuals are located at sites below 0.5 mS cm^{-1}. In addition, finer scale taxonomic resolution is likely to find that the individuals recorded at 2.95 mS cm^{-1} are from a genus other than *Nousia.* Likewise, the vast majority of individuals of Eustheniidae and Gripopterygidae are located at sites below 0.5 mS cm^{-1}, and individuals of all the family groups tested have a preference for sites with conductivities of less than 1 mS cm^{-1}.

Pulmonate gastropods have been found to be among the macroinvertebrates most sensitive to increases in salinity in nature (Hart et al. 1991) and to laboratory tests of acute salinity toxicity (Kefford et al. 2003a). Given this and the relatively low LC_{50} values found for *Glytophysa* sp and *P. acuta* in this study, it is interesting that a positive correlation between abundance and salinity tolerance was found for the two gastropods. It is likely however that a different pattern, for example, an inverted U-shaped relationship between EC and abundance would be found over a greater salinity

range, as was found by Kefford and Nugegoda (2005) for sublethal effects with *P. acuta*. As further laboratory research and aquatic assessment within the NAP region is undertaken, a more complete assessment of the relationship between laboratory tolerance thresholds and recorded field salinities for invertebrate taxa in Tasmania will be possible.

Table 10. Maximum field salinity (mS cm^{-1}) currently recorded in MRHI data Tasmania and in south- eastern Australia by the Salt Sensitivity Database (Bailey et al. 2002).

Family	Tasmania data MRHI Other (taxonomic group; location)	Salt Sensitivity Database and other studies (species / taxonomic group, location)
Leptophlebiidae	2.95	4.2 (*Atalophlebia australis*, western district, VIC)
Gripopterygidae	1.57	2.49 (*D. serricauda,* Wimmera, Werribee, Maribrynong Rivers, VIC) 7.42 (*D. serricauda*, Hopkins River, western VIC[a])
Eustheniidae	1.17	0.22 (unidentified sp., Jervis Bay, south-east Australia)
Planorbidae	2.95 10.70 (*Glyptophysa* sp, Brents Lagoon, Midlands TAS[b])	5.17 (*Glyptophysa aliciae,* Glenelg River, VIC) 6.5 (*Glyptophysa* sp., south-west VIC[c])
Physidae	2.84	7.69 (*P. acuta,* Hopkins River, VIC)
Ceinidae	2.95 60.00 (*A. australis*, Bar Lagoon, Midlands TAS[b])	26.53 (*A. australis*, Hopkins River, western VIC[a]) 79.31 (*A. australis*, western district, VIC)
Atyidae	2.84	10.74 (*P. australiensis*, Glenelg River, VIC) 28.30 (*P. australiensis*, estuaries, south- west VIC[d])

[a] Mitchell and Richards 1992.
[b] Tassell 2004.
[c] Kefford 1998a.
[d] Walsh and Mitchell 1995.

Table 11. Comparison of 72-hr LC_{50} (mS cm^{-1}) between the Tasmanian and south west Victorian (SWV) study (Kefford et al. 2003a) & other studies as listed in Accessory Publication of Kefford et al. (2003a).

	LC_{50}		
Family	Tasmania (72 h, ON*)	SWV (72 h, ON*)	Other studies (h, salt source)
Leptophlebiidae	12.6 (*N.* sp. AV7)	15 (mixture of *Austrophlebioides, Atalophlebia, Koorrnonga, Ulmerophlebia* and *Nousia*)	20 (96 h, food salt) (Leptophlebiidae and Caenidae) Australia
Gripopterygidae	18.3 (*D. serricauda*)	18 (*D. thwaitesi*)	NA
Planorbidae	14.5 (*Glyptophysa* sp.)	9 – 12.6 (*G. aliciae* & *G. gibbosa*)	NA
Physidae	16.7 (*P. acuta*)	14 (*P. acuta*)	7.1 – 8.5 (96 h, NaCl & oil brine) (*Physa*) USA
Ceinidae	41.3 (*A. australis*)	52 (*Austrochiltonia*)	39 (96 h, salt water) (*A. australis*) Australia
Atyidae	44.9 (*P. australiensis*)	38 (*P. australiensis*)	6 - 35 (96 h, salt water & ON*) (*P. australiensis*) Australia

* ON = Ocean Nature was used as the salt source.

4. DISCUSSION

4.1 Comparison with other south-east Australian studies

The seven taxa tested were taxa commonly found within the NAP region (MRHI data, Krasnicki et al. 2001). While they were chosen from family groups found to be among the least and most salt-tolerant, the range of LC_{50} values obtained does not reflect the full range of salinity tolerances expected among naturally occurring freshwater macroinvertebrate communities (Kefford et al. 2005a). Nonetheless, the 72 h LC_{50} values ranged from between 12.6 to 44.9 mS cm^{-1}, with a mean of 26.4 mS cm^{-1}(n = 7), which is nearly 60% of the conductivity of seawater (≈ 46 mS cm^{-1}). This indicates that a broad range and relatively high *potential* salt tolerance exists among Tasmanian freshwater invertebrates. However testing macroinvertebrates representative of a broader range of taxonomic and functional groups, rarities, life stages and geographic locations would achieve a more accurate indication of the true range of acute lethal salinity tolerance (Kefford et al. 2004b and 2005a).

In a comprehensive, south-west Victorian (SWV) study, Kefford et al. (2003a) tested animals from a broad range of taxonomic groups, including rare taxa and found 72 h LC_{50} values ranging from 5.5 to 76 mS cm^{-1} (mean 31 mS cm^{-1}, n = 57). While fewer individuals of each taxon were tested in the SWV study, other important aspects of the research methodology were consistent (including the same salt source, ON, being used) and so the studies are comparable (Kefford et al. 2004b). In the SWV study taxa with the highest salinity tolerances, above that found for *Austrochiltonia* were rarely found in the field, while the most salt-sensitive taxa (e.g. Baetidae, Oligochaeta, Chironomidae) (Kefford et al. 2003a) were not included in this study. Kefford et al. (2003a) also found from examining the frequency distributions of the LC_{50} values that few species had LC_{50} values < 10 mS cm^{-1}, about 50% had LC_{50} values < 20 mS cm^{-1} and the remainder had tolerances above this level. These patterns were generally consistent with the results of the present study. Taxon specific results were also consistent with the findings of Kefford et al. (2003a) (Table 11).

Kefford et al. (2003a) also summarised the published research available on acute, laboratory based, salinity tolerance tests for the macroinvertebrates relevant to their study (Table 11). Comparison between the studies was used to broadly assess spatial

variation in tolerance (Kefford et al. 2004b). Accounting for any differences in research methodologies (e.g. test time frames and salt sources used) once again, the findings of these studies were broadly consistent with the results of this study. The most salt-sensitive taxa tend to be simple multicellular organisms, (e.g. *Hydra spp.* Hirudinea and Nematomorpha), soft bodied insect larvae (e.g. Baetidae and Chironomidae) and non-arthropods (e.g. Oligochaeta and pulmonate gastropods) (Kefford et al. 2003a; Hart et al. 1991).

This consistency may indicate that the aquatic systems from which the animals were collected were not sufficiently different in terms of historic salinity regimes to result in a significant variation in, *at least*, acute salinity tolerance due to local adaptation. However, local adaptation may also result in differences in sub-lethal responses (such as egg production) between populations, which are not reflected in lethal responses (Kefford et al. 2003b). Conductivities at the study sites within the Barwon River catchment (Victoria) were moderately higher than the sites in this study; most animals in the Barwon were collected at conductivities ranging from 0.74 to 2.45 mS cm^{-1}, and fewer individuals were collected from sites with conductivities ranging from 0.84 to 1.75 mS cm^{-1} and 0.14 to 0.19 mS cm^{-1} (Kefford et al. 2003a). In the present study, all individuals were collected from sites with conductivities below 0.52 mS cm^{-1}. Details of historic salinity differences between the Tasmanian and Barwon sites are not known, however the late Miocene and Pliocene periods were associated with arid climates across the entire Australian region (Jackson 1999a; Nix 1982). In addition, major climatic fluctuations in south-eastern Australia occurred between glacial and interglacial periods during the last 2 million years, including very dry periods (and thus high salinities) in eastern Tasmania during the last glaciation (30 - 13 ka) (Jackson 1999b).

It therefore appears that the osmoregulatory capacity for the macroinvertebrates tested (at least at genus and family level and *reflected* in 72 h LC_{50}) is largely determined by inherent physiological adaptations (Hart et al. 1991) that have been shaped by evolutionary and biogeographical processes operating over large spatial and temporal scales. It is very likely that genetic differences between populations of the "same species" accounts for differences at the species level.

Species that spend their entire life cycle in streams have limited capacity for dispersal, and genetic structuring of populations is often determined by geomorphology and physical in-stream barriers (Baker et al. 2004). Thus genetic divergence between geographically isolated populations within Tasmania and between populations in south-eastern Australia and Tasmania is likely, at least for *P. australiensis*, *A. australis*, *P. acuta* and *Glyptophysa* sp. In addition, recent phylogenetic research on *P. australiensis* which has amphidromous (migration of freshwater species to salt water at non-breeding, usually larval, life history stage) ancestors supports the likelihood that multiple, independent invasions and freshwater transitions have resulted in numerous divergent genetic lineages, including both widespread and geographically restricted species (Cook et al. 2006). Thus potential differences in life histories, dispersal characteristics (Baker et al. 2004) and salinity tolerance may occur between lineages. Furthermore, population genetic studies of *P. australiensis* have found significant genetic variation among local populations, even between streams within the same subcatchment (Baker et al. 2004; Hurwood et al. 2003). The authors proposed this to be associated with dramatic geographical barriers to gene flow, within and between mountain streams.

It is therefore likely that many invertebrate "species" are composed of multiple cryptic species, which potentially accounts for differences in salinity tolerance between populations. For example, the LC_{50} value for *P. australiensis* found in this study is higher than has been reported elsewhere and conversely the LC_{50} value for *A. australis* is higher in the SWV study. Furthermore, genetic differences between populations may also be expressed in sublethal rather than lethal responses.

4.2 Relationship between LC_{50} and maximum recorded field salinity (MFD)

Kefford et al (2004a) found that for *commonly* collected macroinvertebrates which have accurate estimates of their MFD, laboratory LC_{50} values are a good reflection of the maximum mean salinity at which animals had been recorded in the field. Maximum mean salinity for these taxa was $\leq$ the LC_{50} values and they therefore concluded that for common taxa, LC_{50} values may provide a useful indicator of the limits to salinity tolerance in the field. However, marked discrepancies often occur between the experimental capabilities of an animal and their distribution in nature. Viable populations are constrained by a complex mix of resources (e.g. food, habitat),

species interactions (e.g. competition, predation, parasitism), metapopulation dynamics, dispersal abilities, abiotic conditions (e.g. temperature, flow regime, depth, substrate, dissolved oxygen, turbidity, nutrients, pH, ionic composition), biogeographical factors (e.g. climate, catchment boundaries, topography and geology) and chance. Furthermore, indirect, sublethal and long term effects of salinity, along with fluctuating salinity regimes and the composite effects of altered land use, mean that in nature adverse effects impact on population viability at salinity concentrations much lower than that tolerated in the laboratory (Kefford et al. 2004a; Clunie et al. 2002; Hart et al. 1991).

4.2.1 *Taxa whose LC_{50} values are lower than their MFD (*Nousia *sp. AV7,* Eusthenia *spp.,* D. serricauda, P. acuta *and* Glytophysa *sp.)*

It is not uncommon for high salinity tolerances to be found under laboratory conditions, despite a species being restricted to freshwater in nature (e.g. *Cherax destructor*, Mills and Geddes 1980; *Syncaris pacifica,* Bayley 1972). Generally the solute concentration of a freshwater organism's haemolymph roughly determines the upper limit of salinity tolerance in the laboratory (e.g. *Pomacea bridgesi*, Jordan and Deaton 1999, *Viviparus viviparus*, Little 1965; *Callibaetis coloradensis*, Wichard et al. 1973; *Paragnetina media*, Kapoor 1978, Kapoor 1979). Ionic haemolymph concentrations in freshwater invertebrates vary from between 1 000 to 15 000 mg L^{-1} and generally they are unable to adequately maintain osmotic regulation in waters with salts in excess of 9 000 mg L^{-1} (Hart et al. 1991).

During toxicity tests freshwater invertebrates commonly tolerate, for short periods, salinities more concentrated than their haemolymph, but this tolerance cannot be maintained and death is inevitable as osmoregulatory processes break down (e.g. *Syncaris pacifica,* Bayley 1972; *Hexagenia Limbata*, Chadwick et al. 2002; *Pomacea bridgesi*, Jordan and Deaton 1999). Thus long term salinity tolerances are most often lower than acute tolerances and are a limiting factor in the distribution of freshwater invertebrates in nature (Little 1965). For example, despite having a 72 h LC_{50} value of approximately 50 mS cm^{-1}, the mortality of the salt-tolerant damselfly *Ischnura heterosticta* at 35 mS cm^{-1} over 44 days was 100%. Its maximum chronic lethal salinity tolerance was estimated to be between 20 and 30 mS cm^{-1} (Kefford et al. 2006).

Furthermore, for many taxa it may take many generations for sublethal effects to manifest at the community level (Neilsen et al. 2003).

Long term viability of any population is dependent on sustaining reproductive and recruitment processes and ensuring optimal survival and development across all life history stages (James et al. 2003). Sublethal effects can impact on freshwater organisms at salinities much lower than acute and chronic LC_{50} values. In a study of two species (including *P. acuta*), the effects of salinity on growth and reproduction were found to occur at between 3 to 40% of the species' short term lethal tolerance (Kefford et al. 2003b). Optimal growth and reproduction for *P. acuta* occurred at salinities of 0.1 to 1 mS cm^{-1} (Kefford and Nugegoda 2005), and a 50% reduction in egg production for *P. acuta* was found at salinities of 2.5 mS cm^{-1} (Kefford et al. 2003b).

The salinity tolerance of the eggs and hatchlings of 12 macroinvertebrates from south-eastern Australia and South Africa were found to have 72 h LC_{50} values of between 5 to 100% of the older life stages (Kefford et al. 2004c). Eggs of *P. acuta* and the stonefly *Dinotoperla thwaitesi,* respectively, had tolerances between 12 to 55 % and 65 to 96% lower than that of older life stages (Kefford et al. 2004c). According to the findings of the current experiment, the lower end of these tolerance ranges, equates to a 72 h LC_{50} value of 7.5 mS cm^{-1} for the eggs of *P. acuta* and 0.8 mS $cm^{-1}$for the eggs of *D. serricauda* (which had the same LC_{50} as *D. thwaitesi*). Longer term, sub-lethal and indirect impacts are likely however to further reduce these thresholds. In addition, while the larvae and adults of *P. australiensis* were found to have similar 72 h LC_{50} values, salinity tolerance of the larvae was reduced (to 27.1 mS cm^{-1}) with extended exposure, compared with that of adults (38 mS cm^{-1}) (Kefford et al. 2003b).

Stoneflies (Plecoptera) are generally restricted to well-oxygenated, fast-flowing streams and rivers (Hart et al. 1991) and have a preference for low salinity waters (Hynes and Hynes 1975). There is little available data on the field distribution of Eustheniidae (Plecoptera) (Bailey et al. 2002), however they are particularly known for their sensitivity to most forms of water pollution (Chessman 2003). In addition, their requirement for cool waters means they are often restricted to higher altitude, fast flowing streams with cobble or boulder substrate (Gooderham and Tsyrlin 2002). The relatively high LC_{50} value derived for *Eusthenia* spp. is a good example of the lack of

ecological relevance that laboratory-based acute tolerance testing can have. Clearly other abiotic factors (e.g. temperature, flow, dissolved oxygen, substrate), are more important determinants of the field distribution of *Eusthenia* spp. rather than salinity alone. In addition, the long term, indirect or sublethal effects of salinity may be important factors limiting the field distribution of *Eusthenia* spp.

Glytophysa sp. has also been recorded at Brents Lagoon in the Tasmanian Midlands at a conductivity reading of 10.5 mS cm^{-1} (Tassell 2004). However *Glyptophysa* is a diverse genus and the limited dispersal capacity of gastropods favours the establishment of discrete populations (Kangas and Skoog 1978). Thus the animals found at Brents Lagoon are likely to be a different species or sub-species than that found at Craigbourne Dam and used in this research. The preservation of distinct evolutionary lineages of the same "species" should be an important consideration in management (Hughes et al. 2003), and thus in the determination of appropriate salinity thresholds.

4.2.2 *Taxa whose LC_{50} values are roughly consistent with their MFD* (P. australiensis *and* A. australis)

Based on the 72 h LC_{50} value and the field distribution of *A. australi*s (Tables 10 & 11), this species appears to be tolerant of a wide range of salinities, from subsaline (< 3 ‰ or 3 g L^{-1}, ≈ 4 mS cm^{-1}) to moderately hypersaline waters (> 50 ‰ or 50 g L^{-1}, ≈ 66 mS cm^{-1}), for at least limited periods. *A. australis* is one of the most widespread amphipods in southern Australian lowland rivers and wetlands (Williams 1962) and it is also likely that cryptic species of *A. australis* exist.

The osmoregulatory behaviour of *A. australis* has not been studied (Hart et al. 1991). However the Ceinidae clearly had marine ancestors (A. Richardson, pers. comm., April 2006) and based on the known field distributions at the time, Hart et al. (1991) concluded that *A. australis* was likely to tolerate fresh and moderately saline waters, but not capable of tolerating hypersaline waters (> 50 ‰ or 50 g L^{-1}, ≈ 66 mS cm^{-1}). However, the LC_{50} value of 41.3 mS cm^{-1} found for *A. australis* in this experiment is potentially compatible with the long term maintenance of populations in the salt lakes of the Midlands (e.g. Bar Lagoon) and macroinvertebrates have been recorded at salinities above their LC_{50} value (Kefford et al. 2004a). It is suggested that the potential for adaptation to high salinities is greater in lentic waters than lotic systems,

where salinities can fluctuate widely (Kefford et al. 2004a). In addition, ratios of other ions, such as bicarbonate, calcium and magnesium have also been found to influence salinity tolerance of some invertebrates in salt lakes (Halse et al. 1998; William 1998), however the vast majority of salt lakes in the Midlands are dominated by sodium and chloride ions, although magnesium is also present in relatively large concentrations (De Deckker and Williams 1982; Buckney and Tyler 1973). Regardless of the ionic composition, given the physical isolation of the salt lakes of the Midlands, it is highly probable that there is genetic divergence between individuals occurring in the lakes and those from the Coal River valley tested in this study. In spite of this, the results of this experiment support the conclusion of Hart et al. (1991), that it is unlikely that changes in salinity will have any deleterious effect on this "species".

Ancestors of the *Paratya* shrimps are known to have been tolerant of a wide range of salinities (Carpenter 1977 in Cook et al. 2006) and they inhabit a diverse range of environments, from lowland rivers and lentic freshwaters to brackish waters and estuaries (Walsh and Mitchell 1995; Williams 1977). Based on recorded field distributions (Table 10), *P. australiensis* appears to tolerate hyposaline (3 – 20 g L^{-1} ≈ 4 – 26 mS cm^{-1}) and mesosaline (20 – 50 g L^{-1} ≈ 26 – 66 mS cm^{-1}) waters. As discussed, differences in genetic structure between populations help to explain differences that have been found in acute salinity tolerance. Diverse life history strategies have also been documented in populations of this species, including omnivorous and filter-feeding capabilities and distribution across a range of flow types, including fast-flowing stream sections during breeding and recruitment (Richardson et al. 2004). These are important adaptations, because hydrological regime (important to the survival of the planktonic larval stage) and the presence of suitable aquatic vegetation have been thought to be important determinants of the distribution of *P. australiensis* (Walsh and Mitchell 1995; Williams 1977). Thus populations of *P. australiensis* may also be fairly resilient to indirect and composite effects, such as altered habitat and hydrology which are associated with salinisation and land use changes.

4.3 Slow acclimation and salinity tolerance of *Nousia* sp. AV7

The slow acclimation regime undertaken in this study did not result in a higher acute salinity tolerance or an altered survival response for *Nousia* sp. AV7. It is possible that an alternative acclimation regime may have resulted in an increased salinity tolerance. While salinity tolerance for any organism is determined by inherent physiological adaptations (Hart et al. 1991), the capacity for aquatic animals to acclimate to higher salinity levels is profoundly affected by the salinity and water regime (James et al. 2003; Nielsen et al. 2003a). For some biota slow increases of 10 to 50 % of initial salt concentration are better tolerated than rapid and dramatic increases (e.g. 100 - 200 %) (James et al. 2003). In addition, adequate incubation time before an organism is subjected to incremental increases in salinity is likely to be important (James et al. 2003). Survival for *P. australiensis* was 30%, with constant exposure at 38 mS cm^{-1} for 96 hours. Survival increased to 100% when salinity was gradually increased from the control level, 0.1 mS cm^{-1} to 38 mS cm^{-1} over 96 hours (B. Kefford, unpublished data). Furthermore, those animals did well (only 10 - 20 % mortality) when the salinity was either dropped back to the control level or gradually lowered to 10 mS cm^{-1}, over 96 hours (B. Kefford, unpublished data).

In this experiment there was a doubling of initial salinity after 24 h, followed by a 50% increase at 48 h and a 30% increase at 72 h. In addition, the maximum acclimation salinity of 2 mS cm^{-1} is below the NOEC (LC_{10}) found in this study for *Nousia* sp. AV7. A change in acclimation regime and use of a peristaltic pump may improve the acclimation capacity of *Nousia* sp. AV7. The pump would eliminate the need to physically transfer the animals between salinity gradations, and provide a continual increase in conductivity (rather than a stepwise increase) which is more representative of natural salinity fluctuations. A more appropriate acclimation regime would involve allowing a longer time (e.g. 48 hours) for initial acclimation at 0.5 mS cm^{-1}, then implementing a 50% increase in salinity progressively over a 12 hour period until a maximum acclimation salinity of at least the LC_{25} level (e.g. 8 mS cm^{-1}) is achieved. In this case, a 5 day acclimation period would be required. Alternatively, the results of this experiment may indicate that *Nousia* sp. AV7 does not have the capacity for an acute adaptive response, at least within four days in the laboratory.

Research indicates that some macroinvertebrate communities may be adapted to recover from very high salinities and poor water quality associated with natural climatic variation (e.g. Chessman and Robinson 1987). Several taxa (including members of Baetidae, Leptoceridae and Chironomidae) have been recorded in rivers of south western Australia (SWA) at salinities well above their maximum recorded field salinities elsewhere in Australia (Kay et al. 2001). The authors hypothesised that aquatic macroinvertebrates of SWA had evolved to tolerate hypersaline conditions as a result of exposure to discharge of salt from groundwaters over hundreds of thousands of years.

There is however limited published research on the laboratory tolerance of the same invertebrate species collected from sites with differing salinity regimes. An investigation of the salinity tolerance of freshwater and brackish populations of the gastropod *Theodroxus fluviatilis* found that individuals inhabiting saline habitats of approximately 6 ‰ salinity (≈ 8 mS cm^{-1}) had a higher salinity tolerance compared with individuals from a freshwater habitat of approximately 0.1 ‰ (≈ 0.13 mS cm^{-1}) (Kangas and Skoog 1978). Genetic differences between the populations were not investigated however, though recency of invasion led the authors to conclude that genetically distinct populations were unlikely (Kangas and Skoog 1978). In contrast, Kefford et al. (2003a) found no appreciable difference in the mortality response of two species (*P. acuta* and *M. annae*) collected from two sites with differing salinity profiles (0.74 to 2.45 mS cm^{-1} and 0.141 to 0.193 mS cm^{-1}). However a higher s*ublethal* salinity tolerance with respect to egg mass production was found for *P. acuta* (from the Barwon River catchment) from a higher salinity site than a lower salinity site (Kefford et al. 2003b).

4.4 Role and limitations of acute salinity toxicity testing

While acute lethal salinity thresholds provide useful information on the osmoregulatory capacity of specific species, such tests do not detect sublethal or long term effects. An animal with a high lethal tolerance (e.g. *Eusthenia* spp.) may well experience adverse sublethal impacts (e.g. detrimental metabolic or behavioural changes) at salinities well below lethal thresholds. Thus lethal tolerance values provide no indication of salinity thresholds for sublethal responses. The current study also indicates that LC_{10} values may not always provide an accurate indication of no-observed effect concentration

(NOEC) for all taxa, particularly if experimental replication is low. Substituting LC_{10} values for NOEC is therefore not recommended as a universal protocol.

Studies to date that have examined the acute salinity tolerances of invertebrates do not account for genetic differences that may exist between populations of the same "species", and whether in fact any differences in acute tolerance are due to local genetic adaptation or due to the capacity of individuals to acclimatise to changes in salinity. It is vital that differences in genetic structure between populations are considered when interpreting field distributions and comparing acute tolerance values. *Nousia* sp. AV7 is likely to be a complex of cryptic species (L. Barmuta, pers. comm., May 2006) and its widespread distribution, including within the NAP region, makes it an ideal "species" for comparing salinity tolerances and genetic structure across populations. In addition, the fact that many Australian freshwater invertebrate groups have not been well studied (Ponder et al. 1993) compounds the difficulties in establishing appropriate salinity thresholds in nature. For example, the lack of knowledge of the diverse genus *Glytophysa*, which has also been recorded in a saline Tasmanian lake, may lead to an overestimation of MFD and thus salinity tolerance for some species of this genus.

Similar acute salinity tolerances within taxonomic groups (e.g. orders) are common (Hart et al. 1991), including across large spatial scales (Kefford et al. 2005b). However variation in acute tolerance, such as found with the two plecopteran taxa in this study, reinforce that caution is required when predicting generalised acute response patterns within particular groups (Bailey and James 2000). This is particularly important for large and complex families such as Leptophlebiidae. Relating laboratory-based salinity tolerances to field distributions requires that taxa collected in the field are identified to species level.

The protection of ecosystem processes in freshwater systems (in the face of rising and fluctuating salinities) requires an understanding of tolerance at the population, community and ecosystem level (Chapman 1995). Salinity tolerance needs to be determined across taxonomic and functional groups within communities and to consider species interactions and trophic and habitat interrelationships (James et al. 2003). Laboratory toxicity testing is most useful when interpreting patterns in community structure and salinity across diverse aquatic ecosystems (Cairns and

Niederlehner 2003). Thus acute toxicity testing is only one component of a range of research required to identify ecological thresholds and environmental guidelines for salinity (Kefford et al. 2004 b & c; Cairns and Niederlehner 2003; Galat et al. 1988; Cairns 1986; Mills and Geddes 1980).

4.5 Future research

Further research is required for the development of appropriate salinity thresholds for aquatic systems at high risk of secondary salinisation in Tasmania. The first and most important priority is for long term and intensive monitoring of rivers and wetlands within the NAP region, particularly those aquatic systems rated as high risk. The aim of such monitoring would be to establish salinity levels in combination with other abiotic variables that are associated with the maintenance of sustainable populations and ecosystem processes. Calow and Forbes (2003) argue that protection of species composition should also ensure protection of ecosystem processes. The monitoring should incorporate rates and patterns of change in salinity and other abiotic variables over time, in conjunction with the associated biological effects, including structural components (e.g. community composition, keystone species and most sensitive biota). Priority sites for monitoring should include the stream sections and small floodplain or valley floor tributaries rated as high risk (see Davies and Barker 2005).

Secondly, laboratory-based research on long term, sublethal, and indirect effects across a broader range of taxonomic and functional groups, including rare species, sensitive life stages (including just before emergence for aquatic insects) and the most salt-sensitive of aquatic biota (e.g. microbes, algae and microinvertebrates) is required. The efficiency and usefulness of such research could be enhanced with the use of laboratory-based rapid tests or 'up-and-down' test protocols (see Kefford et al. 2003a; Bruce 1987) and laboratory-based and field-based mesocosm experiments simulating ecologically important characteristics (such as flow and salinity regime) (Galat et al. 1988). This would enable the relationship between, for example, sublethal and long term tolerance and field salinities to be assessed. Importantly this research would also help to determine whether salinity thresholds or models developed in other similar locations (e.g. south-eastern mainland Australia) can be adapted to predict salinity impacts in Tasmania. As the Leptophlebiidae is diverse and widespread, it would be a useful group for such future research.

Thirdly, field-based and laboratory-based studies are urgently required to quantify the capacity of macroinvertebrate communities to adapt to rising salinity levels, both in the short and longer term and to rapidly fluctuating salinity regimes. This research should include tolerance testing of individuals of the same species from different locations that vary in natural salinity, in order to evaluate whether acclimatisation history can affect salinity tolerance. Sensitive life stages, long term and sublethal effects need to also be considered in this research. Incorporating genetic markers from different population groups would assist to determine if any differences in tolerance are potentially of genetic origin. Genetic testing would be particularly useful for widely distributed, sensitive taxa or keystone species for which information on population and genetic structure is also available. Furthermore, it is vital that tolerance testing quantifies the biological impact of, for example, longer durations of higher salinities compared with short sharp peaks in salinity and the effects of repeated fluctuations around tolerance thresholds for particular species.

Finally research into the biological impacts of salinity must go hand-in-hand with ongoing research into abiotic factors that shape salinity regimes, such as hydrology, geology and groundwater flow systems. Such research is necessary to understand and quantify the processes of salt mobilisation, spatial and temporal variation in salinisation, responsiveness of systems to change, flow paths and likely areas of high discharge. The development of effective management strategies will depend on this information.

5. CONCLUSION

Results of this study on the acute lethal tolerance (72 h LC_{50}) of seven Tasmanian freshwater macroinvertebrates, suggests that 72 h acute tolerances are broadly consistent with macroinvertebrates tested in the Barwon River catchment in Victoria. Both the range and specific acute lethal tolerances of members of the same family or genus are comparable, and differences in genetic structuring between populations of the "same species" are likely to account for differences at the species level. The results therefore provide an initial indication that salinity thresholds or models developed in other similar locations (e.g. south-eastern mainland Australia) may be applicable to Tasmania. Such models may be particularly relevant for areas with a history of moderate salinity, such as the Coal River valley. However, further research is required to confirm this consistency across long term, sublethal and sensitive life stages. In addition, taxonomic differences and/or population genetics must be considered, so that we are confident that populations of the same species are being compared.

Acute toxicity testing indicates salinity levels at which lethal effects occur (e.g. death of 50% of the individuals, LC_{50}). The results of this study confirm that these levels do not indicate salinity thresholds associated with sublethal osmotic effects for an individual species and thus salinity thresholds for adverse effects on populations. Furthermore, due to the complex mix of abiotic and biotic processes that influence the distribution and viability of populations and communities in nature, acute tolerance thresholds ($LC_{50,}$ $LC_{25,}$ or LC_{10}) may not be directly relevant to, or predict field distributions or salinities at which populations can sustain themselves over time.

The acute lethal tolerances of the taxa tested in this study, in combination with other tolerance research and data on field distributions, indicates that small increases in salinity are likely to be associated with adverse affects for the mayfly (*Nousia* sp. AV7), the stoneflies (*Eusthenia* spp. and *D. serricauda*) and the snails (*P. acuta* and *Glytophysa* sp). Populations of these taxa in high-risk headwater streams are possibly particularly threatened due to a reduced potential for acclimatisation to higher salinities. In contrast, the two salt-tolerant crustaceans (*P. australiensis* and *A. australis*) are likely to be able to tolerate much higher increases in salinity. However whether a particular population of a species is viable in the long term will depend on

many factors such as the degree of change in salinity, the salinity regime (rate, magnitude, frequency and duration of fluctuations), overall ionic proportions, the availability of refuges and specific dispersal capabilities.

The capacity of macroinvertebrates to adapt to rapid changes (increases and fluctuations) in salinity resulting from secondary salinisation remains largely unknown. The acclimation regime implemented in this research failed to result in an increase in acute salinity tolerance for *Nousia* sp. AV7. This may indicate that this species does not have the capacity, at least for an acute acclimation response under laboratory conditions. However an alternative acclimation regime that allows a longer incubation period, a continuous and more gradual salinity increase and reaches a maximum salinity at least equivalent to the LC_{25} level for *Nousia* sp. AV7 may result in a higher acute lethal threshold (LC_{50}).

Finally, it is known that once secondary salinisation processes are underway, management is extremely difficult and costly. In addition, at least decades are required before strategies that contain dryland salinity result in reductions in the delivery of salt to streams, rivers and wetlands. If we are to contain the potential extent and severity of secondary salinisation in Tasmania, appropriate monitoring, land management and planning decisions are now necessary for those areas identified as high risk.

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