

Do laboratory salinity tolerances of freshwater animals correspond with their field salinity?

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“Capsule”: *Acute laboratory salinity tolerances relate to maximum salinity where organisms occur in nature.*

Abstract

The degree to which laboratory derived measures of salinity tolerance reflect the field distributions of freshwater biota is uncertain. In this paper we compare laboratory-derived acute salinity tolerance (LC₅₀ values) of freshwater macroinvertebrates (range 5.5–76 mS/cm) and fish (range 2.7–82 mS/cm) from southeastern Australia with the salinity from which they have been collected in the field. Only 4% of the macroinvertebrates were collected at salinity levels substantially higher than their 72-h LC₅₀ obtained from directly transferring animals from low salinity water to the water they were tested (direct transfer LC₅₀). This LC₅₀ value was correlated with the maximum salinity at which a species had been collected. For common macroinvertebrates, the maximum field salinity was approximated by the direct transfer 72-h LC₅₀. For adult freshwater fish, 21% of species were collected at salinities substantially greater than their acute direct transfer LC₅₀ and there was a weak relationship between these two variables. Although there was a weak correlation between the direct transfer LC₅₀ of early life stages of freshwater fish and the maximum field salinity, 58% of the field distribution were in higher than their LC₅₀ values. In contrast, LC₅₀ determined from experiments that acclimated adult fish to higher salinity (slow acclimation) provided a better indication of the field distribution: with only one fish species (7%) being in conflict with their maximum field salinity and a strong positive relationship between these variables. This study shows that laboratory measures of acute salinity tolerance can reflect the maximum salinity that macroinvertebrate and fish species inhabit and are consistent with some anecdotal observations from other studies.

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1. Introduction

Increasing salinity in rivers and wetlands is recognized as a serious environmental problem in all inhabited continents (Williams, 1987). In order to consider the likely effects of salinity increases the maximum salinity that freshwater organisms can withstand (their salinity tolerance) must be measured. Such measures of salinity tolerance should indicate the maximum salinity

at which a species can sustain itself in nature. Determining this is, however, difficult and is rarely done.

1.1. Salinity tolerances—the issues

A number of studies report the maximum field salinity at which particular taxa have been collected (Ettershank et al., 1966; Scudder and Mann, 1968; Knowles and Williams, 1973; Aladin, 1991; Kefford, 1998a; Williams and Williams, 1998; Bailey and James, 2000; Bailey et al., 2002). Such observations indicate that a species survives in the field at those salinities. There are, however, several reasons why they may not necessarily be the maximum salinities that can be tolerated: there may be

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limited survey effort or lack of suitable habitat at higher salinity sites; the geographic distribution of the species might be restricted to areas that currently have lower salinities; or, high salinity may co-occur with changes in other factors (that might restrict a species' distribution). Additionally, the presence of a species at a particular salinity does not necessarily indicate that it can complete its life-cycle at that salinity. Due to their observational nature, field observations do not provide a causal link with the salinity tolerances of organisms (see Underwood et al., 2000).

The most common laboratory measure of salinity tolerance is the concentration that is lethal to 50% of individuals (the LC_{50} value) over an arbitrary period (usually 48–96 h). Such determinations are experimental and thus establish a causal link between salinity and mortality. They do not, however, take into account other changes in the natural environment that may co-occur with increased salinity (Kefford, 1998b, 2000a,b) including ionic proportions. They are usually conducted at constant temperatures and without other stressors, which is clearly not the case in natural ecosystems. Hence, survival in laboratory experiments may not predict survival in nature, let alone the maximum salinity that could support a self-sustaining population. Although laboratory measurements of sub-lethal effects (e.g. physiological measures, growth and reproduction) are also possible, they are more resource-intensive and can be difficult to relate to the salinity at which a species can maintain a self-sustaining population (Brinkhurst et al., 1983). Inter-specific interactions can also be measured (Galat et al., 1988) but they are often unstable (Graney et al., 1995; Connell et al., 1999) and controversial (Carpenter, 1996, 1999; Drenner and Mazumder, 1999; Huston, 1999).

While there is room for criticism of particular approaches, all have advantages and there is a need for multiple approaches (Kefford et al., 2002). In this paper we compare the laboratory measured lethal salinity tolerance of freshwater macroinvertebrates and fish from southeastern Australia to the maximum salinity at which they have been recorded in nature or maximum field distribution (MFD). This was done to assess the validity of these alternative measures of salinity tolerance. If these two measures are related, then either method can be reliably used to estimate salinity tolerance.

2. Materials and methods

2.1. Macroinvertebrates

Laboratory toxicity data for macroinvertebrates were obtained from Kefford et al. (2003). This data set contains LC_{50} values (which ranged from 5.5 to 76 mS/cm) for a range of macroinvertebrate taxa collected from the

Barwon Catchment in southwestern Victoria. LC_{50} values were measured at 72-h with the exception of *Cricotopus* (Diptera: Chironomidae) which was determined at 48 h. The toxicity experiments were conducted by directly transferring the macroinvertebrates to various salinity concentrations prepared with an artificial sea salt, Ocean Nature, which has the same ionic proportions as sea water. These ionic proportions are common in Australian inland waters (Bayly and Williams, 1973: 1). All experiments were conducted at 20°C. Four of the taxa tested by Kefford et al. (2003) were composites of a number of species and, with the exception of *Cricotopus*, were not considered here due to uncertainty of their identity. *Cricotopus* was the dominant taxon present in a mix of Chironomidae tested. For taxa identified to levels other than species, they were compared to field data from the same taxonomic level.

The LC_{50} values were compared to MFDs obtained from a number of sources: the Victorian Environmental Protection Authority (EPA) macroinvertebrate database, the Arthur Rylah Institute (ARI) Aquatic Fauna Information System (DSE, 2002), Ryan and Davies (1996) and the salt sensitive database (Bailey et al., 2002). The EPA database consists of standardized qualitative macroinvertebrate samples (Davies, 2000) and associated environmental data, including electrical conductivity (EC) collected from pool/edge and riffle habitats in flowing water from about 4000 samples from 1050 sites throughout Victoria and identified to lowest practical level (Tiller and Metzeling, 1998). Samples were collected in the spring (October–December) and autumn (March–May). Data from the EPA database has been previously published (Marchant et al., 1997, 1999). The DSE (2002) database contains macroinvertebrate records from Victoria and southeastern New South Wales (NSW) mostly collected using the same methods as those from the EPA database but at fewer sites (a total of 894 samples). Ryan and Davies (1996) and Bailey et al. (2002) are reviews and contain values from various published and unpublished Australian studies. The maximum salinity that each taxon was recorded at was defined as its MFD. A total of 49 macroinvertebrate taxa were available with laboratory LC_{50} and MFD (of which Coleoptera accounted for 12%, Diptera 2%, Gastropods 12%, Ephemeroptera 6%, Hemiptera 12%, macrocrustaceans 8%, Odonata 12%, other non-arthropods 6%, Plecoptera 6% and Trichoptera 24%).

2.2. Freshwater fish

Clunie et al. (2002) recently reviewed the salinity tolerance of native and introduced freshwater fish in Australia and their summary of laboratory derived LC_{50} values was used in this paper. This information comes from studies conducted mostly in the Murray-Darling Basin with fewer studies elsewhere in mainland

southeastern Australia. There were three broad methods from which the LC_{50} of fish were determined. (1) Direct transfer of adults from their control water to the salinity in which they are tested (referred to as direct transfer or direct LC_{50}) and conducted over a period that considers acute toxicity (96 h in most cases). (2) Slowly increasing the salinity (over a period of days) and allowing the adult fish time to acclimatize to the increase before they were subject to potentially lethal levels (referred to as slow acclimation or slow LC_{50}). (3) Direct transfer of the early life-stages (eggs, sperm or larva) from the control salinity to their test salinity (referred to as early life-stage or early LC_{50}). Due to the large differences in LC_{50} values between these methods (ranges of 9.8–78, 21–82 and 2.7–26 mS/cm, respectively), they were investigated separately (see Hart et al., 1990; Ryan and Davies 1996; Bailey and James, 2000; Clunie et al., 2002 for discussion about differences in tolerance from these test methods). Where multiple early life-stage LC_{50} values for a species were available, the minimum was used because we wished to consider the most sensitive stage. Where a species had several direct transfer or slow acclimation LC_{50} values, the maximum was used to give an upper estimate of its laboratory tolerance.

The MFD for each species was obtained from Ryan and Davies (1996), Clunie et al. (2002), DSE (2002), Bailey et al. (2002) and NSW Fisheries database. Records for fish on DSE (2002) were spread throughout Victoria and produced 876 samples with fish and EC records. The NSW Fisheries database contained similar data with a total of 768 suitable samples. The maximum value at which any life stage had been recorded alive in nature was defined as the MFD.

2.3. Measures of salinity and data comparability

While MFD values are based on one off spot reading, such reading can be reflective of the past salinity at a site (Kefford, 2001). In this paper, salinity was expressed in terms of EC (mS/cm) at 25 °C, as most of the available data used this measure of salinity. EC is the most common measure of salinity and is accurately, rapidly and easily measured. Records expressed in EC at ambient temperature (°C) were adjusted to 25 °C with the formula: $EC_{25} = EC_{Amb} / [(1 + 0.025)(Ambient\ temperature - 25)]$ (Williams and Sherwood, 1996). Records expressed in total dissolved solids (TDS, in g/L) were converted using the formula $EC = 0.754\ TDS$ (Kefford et al., 2003).

EC values that were considered unlikely for their geographic location (too high or low) were queried with the manager of the respective database. Where possible, the collectors of the data were contacted or original records checked and corrected when errors were detected.

Statistical tests were considered significantly different with a P level < 0.05 and marginally significant if P was between 0.05 and 0.1.

3. Results

3.1. Macroinvertebrates

Eight out of 49 macroinvertebrate taxa (16%) had an MFD $>$ their LC_{50} (Fig. 1), 3 of these 8 had censored (see Smith, 2002) LC_{50} values so their ‘real’ LC_{50} values are higher than indicated and may not be in conflict with their MFD. These taxa were thus excluded in the following analysis. Of the 5 remaining taxa with MFD values $>$ their LC_{50} , 3 (*Cricotopus*, Baetid Genus 1, sp 5 [Ephemeroptera: Baetidae] and *Cloeon/Centropitulum* [Baetidae]) had LC_{50} values not greatly lower than their MFD (< 2.8 mS/cm). Given statistical and other potential errors in estimation of LC_{50} , their LC_{50} values are not in serious conflict with their MFD. Thus only 2 of 46 taxa (4.3%) had a MFD substantially $>$ their LC_{50} . MFD values of both, *Austrochiltonia* (Amphipoda: Ceinidae) and *Agraptocorixa eurynome* (Hemiptera: Corixidae), come from saline lakes (Bailey et al., 2002). Additionally, *A. eurynome* has aerial adults, it thus might accidentally be found out of its usual salinity range. If distribution records are restricted to those from flowing water the MFD values are not in conflict with the LC_{50} .

Excluding censored LC_{50} , there was a statistically significant correlation between LC_{50} and MFD ($r = 0.37$, $P = 0.033$, $n = 32$) for all taxa. Considering common taxa, defined as those where ≥ 50 individuals collected on at least one occasion, (Fig. 2) this correlation was considerably stronger despite a reduced sample size ($r = 0.87$, $P = 0.003$, $n = 11$). The line of best fit was similar to the one to one relationship (Fig. 2). The removal of the outlier caused by *Austrochiltonia* did not alter the significance of this relationship ($r = 0.78$, $P = 0.009$, $n = 10$) and its removal makes the relationship closer to the one to one. In contrast, there was no evidence of any relationship for the rare taxa, defined as

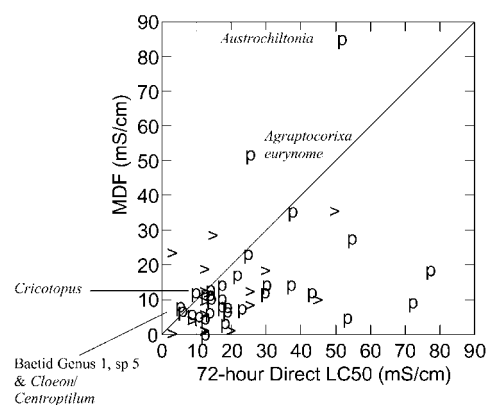


Fig. 1. Relationship between the direct transfer LC_{50} values of the macroinvertebrate tested by Kefford et al. (2003) and their MFD. Where “p” refers to point estimates of the LC_{50} and “>” indicated the LC_{50} not reached in the laboratory test; thus EC at which 50% mortality occurs is greater than specified (i.e. censored data [Smith, 2002]). The straight diagonal line is the one to one relationships.

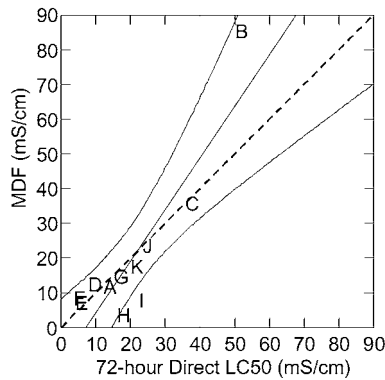


Fig. 2. Relationship between the MFD and 72-h direct transfer LC_{50} values of the common macroinvertebrate taxa tested by Kefford et al. (2003). Where A = *Physa acuta*, B = *Austrochiltonia*, C = *Paratya australiensis*, D = *Cricotopus*, E = Baetid Genus 1, sp 5, F = *Cloeon/Centropitulum*, G = *Micronecta annae*, H = *Dinotoperla thwaitesi*, I = *Anisocentropus*, J = *Notalina spira* and K = *Triplectides australicus*. The dashed diagonal line is the one to one relationship and the solid lines are the least squares regression line \pm its 95% confidence intervals (CI).

those taxa where all collections were < 50 individuals ($r = 0.27$, $P = 0.26$, $n = 21$).

Species that are rare at one site are often also found in low abundances elsewhere, and such species may tend to have restricted geographical and habitat distribution (Gaston, 1994; Gaston and Blackburn, 2000). This was the case with macroinvertebrates from the Barwon Catchment. Thus the MFD for rare taxa may be seriously underestimated compared with common taxa. We can also define rarity in terms of the number of occurrences of a taxon in EPA samples. A stronger relationship between MFD and LC_{50} values exist for taxa with about 100 or more occurrences in EPA samples (Fig. 3).

If LC_{50} values represent at least relative salinity tolerance then they should be rank-correlated with MFD. This was the case for all taxa ($r = 0.55$, $n = 32$,

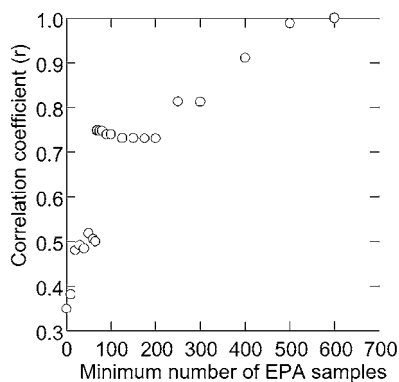


Fig. 3. The strength of the correlation between the MFD and 72-hour direct transfer LC_{50} values for macroinvertebrate taxa at various levels of rarity. The x-axis is the cut-off for inclusion of taxa in the correlation. An x-value of 100, for example, indicates that for taxa to be included they have to be recorded in ≥ 100 EPA samples.

$P = 0.001$), common taxa ($r = 0.61$, $n = 11$, $P = 0.0458$) and rare taxa ($r = 0.59$, $n = 21$, $P = 0.005$).

As previously stated, the MFD may not indicate the maximum salinity at which a population can maintain itself in the field. So we considered only sites where a particular taxon occurred regularly. A list of EPA sites where each taxon had been recorded on ≥ 3 occasions was compiled. As most of the rare taxa were recorded in few EPA samples, only the common taxa and non-censored rare taxa recorded in > 99 EPA samples were used. For each taxon, the mean EC for each site was calculated and from this the maximum mean EC values were determined. These give an indication of the maximum EC that can regularly support taxa. There was a significant relationship between the maximum mean EC and the LC_{50} ($r = 0.67$, $n = 17$, $P = 0.003$). This relationship was significantly different ($P < 0.05$) from the one to one relationship and in all cases the maximum mean EC was \leq the LC_{50} indicating that the maximum mean EC of taxa may approach their LC_{50} values but not exceed them.

3.2. Freshwater fish

Direct transfer LC_{50} values for freshwater fish were substantially (> 2.1 mS/cm) lower than their MFD in 4 out of 19 (21%) of species (Fig. 4a). Early life-stage LC_{50} were in conflict with their MFD in 7 of 12 species (58%) (Fig. 4b). In contrast, out of 14 species, only the slow acclimation LC_{50} for *Macquaria ambigua* (Golden perch) was in conflict with its MFD (7.1%) (Fig. 4c) and only by a relative small amount: 3.1 mS/cm.

The relationship between MFDs and laboratory data for the fish was a marginally significant correlation with direct transfer LC_{50} ($r = 0.43$, $P = 0.063$, $n = 19$, Fig. 4a) and early life-stages LC_{50} ($r = 0.51$, $P = 0.086$, $n = 12$, Fig. 4b). There was, however, a stronger and highly significant correlation between MFD and the slow acclimation LC_{50} ($r = 0.69$, $P = 0.006$, $n = 14$, Fig. 4c). There was also a strong correlation between the direct transfer and slow acclimation LC_{50} ($r = 0.93$, $P < 0.001$, $n = 13$) but not the direct transfer and early life-stage ($r = -0.36$, $P = 0.34$, $n = 9$) or slow acclimation and early life-stage ($r = -0.40$, $P = 0.29$, $n = 9$) LC_{50} values.

4. Discussion

Although lethal laboratory toxicity testing is commonly conducted, and many standard methods have been widely accepted (OECD, 1996; ASTM, 1998), there has been controversy as to its value in predicting field occurrences of taxa. Furthermore, there have been few field validations of laboratory toxicity data (Connell et al., 1999: 98). There are many criticisms that can be leveled at acute tolerance testing, for example: unrealistic

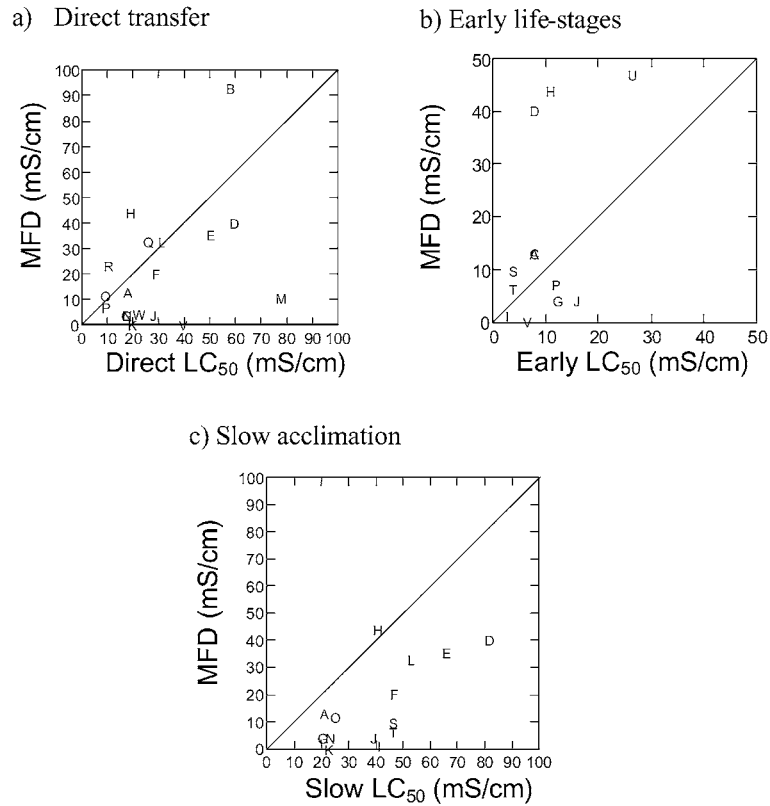


Fig. 4. Relationship between laboratory LC₅₀ and MFD in Australian freshwater fish (a) direct transfer, (b) early life-stages and (c) slow acclimation. Where A = *Bidyanus bidanus* (Silver perch), B = *Craterocephalus fluviatilis* (Unspecked hardyhead), C = *Gadopsis marmoratus* (River blackfish), D = *Galaxias maculatus* (Common galaxias), E = *Hypseleotris klunzingeri* (Western carp gudgeon), F = *Leiopotherapon unicolor* (Spangled perch), G = *Maccullochella peelii peelii* (Murray cod), H = *Macquaria ambigua* (Golden perch), I = *Macquaria australasica* (Macquarie perch), J = *Melanotaenia fluviatilis* (Crimson-spotted rainbow fish), K = *Mogurnda adspersa* (Purple-spotted gudgeon), L = *Philiynodan grandiceps* (Flat-headed gudgeon), M = *Retropinna semoni* (Australian smelt), N = *Tandanus tandanus* (Freshwater catfish), O = *Carassium auratus* (Gold fish), P = *Cyprinus carpio* (European carp), Q = *Gambusia holbrooki* (Mosquito fish), R = *Perca fluviatilis* (Redfin), S = *Salmo gairdneri* (Rainbow trout), T = *Salmo trutta* (Brown trout), U = *Macquaria novemaculeata* (Australian bass), V = *Prototroctes maraena* (Australian grayling) and W = *Pseudaphiritus vrvilli* (Tupon/Congoli). The straight diagonal lines are one to one relationships.

laboratory conditions and no consideration of sub-lethal effects or interactions between species.

The current study has shown that for salinity the MFD of freshwater macroinvertebrate taxa and their acute (72-h) LC₅₀ values were correlated. For common macroinvertebrates, which should have relatively good estimates of their MFD (because they tend to be frequently recorded in the field) their LC₅₀ values were similar to their MFD. The lack of relationship between LC₅₀ and MFD for rare taxa may be an artifact caused either by poor estimates of the actual maximum or restricted geographic distributions. The relationship with the ranked data, however, suggests 72-h LC₅₀ values are useful for assessing the relative salinity tolerance of macroinvertebrates regardless of their rarity. MFD are, however, only useful indicators of salinity tolerance of commonly collected taxa. The positive relationship between the maximum mean EC and LC₅₀ values suggest that LC₅₀ values are useful in assessing upper salinity levels for sites that typically support a species.

Our results are in accordance with Scudder and Mann (1968) who recorded an LC₅₀ between 1.232 and 1.25 mS/cm for the leech *Nepheleopsis obscura* and found it in lakes up to 1.13 mS/cm. Similarly, in a large estuary the amphipods *Melita zeylanica* and *Paracorophium* sp. were generally found at salinities less than 70 mS/cm but were sporadically recorded up to 83 mS/cm which is similar to their LC₅₀ at optimum temperature of 78 and 79 mS/cm, respectively (Kangas and Geddes, 1984).

There may be changes in other water quality variables, including ionic proportions, and other stresses at particular sites that when combined with elevated salinity result in salinity tolerances different to those indicated by LC₅₀ values. Likewise, at certain sites, salt tolerant taxa may still be affected by salinity indirectly by salinity affecting their habitat or more sensitive prey, predators, competitors or parasites. This study suggests that when a broad range of sites are considered, such phenomena are not important in determining the relationship between LC₅₀ value for commonly occurring macroinvertebrates and their MFD.

Two macroinvertebrate taxa were recorded from saline lakes at salinities substantially greater than their LC_{50} value. However, the field distributions from flowing water were not in conflict with their LC_{50} values. While the salinity of a saline lake in southeastern Australia fluctuates, it tends to be high, whereas salinity in a river can be both fresh and saline. There is, therefore, more potential for adaptation and acclimation to high salinity in lentic waters. In southwestern Australia, an area with historic salinization, many macroinvertebrate families occur in rivers with salinity levels considerably higher than LC_{50} and MFD values for members of those families reported here (Kay et al., 2001). Exposure to high salinity levels over generations may lead to adaptations that increase the salt tolerance of freshwater macroinvertebrates (Kay et al., 2001). Regional differences thus appear likely and caution, therefore, should be exercised when extrapolating results from laboratory experiments to field settings that substantially differ from those where the organisms were collected.

Relative to macroinvertebrates, direct transfer LC_{50} values from freshwater fish provided a poorer estimate of the MFD values. Likewise, Clemens and Jones (1954) found several freshwater fish in chloride concentrations higher than they could withstand in direct transfer laboratory experiments. As with this paper, they found that LC_{50} values for two invertebrates approximated the maximum chloride concentrations that they were collected from. In the current study, slow transfer LC_{50} did, however, provide a better estimate of the MFD for freshwater fish. In contrast, the LC_{50} for early life-stages of freshwater fish provided a poor prediction of their MFD. Changes in the salinity at a site are often slow (Kefford, 2001). Thus for a short-lived species many individuals may not experience large changes during their life. The longer life spans and increased mobility of many fish, compared to macroinvertebrates, may allow adult fish to survive in or move into areas where salinity levels would be lethal in their early life-stages. In many natural circumstances, changes in salinity that fish experience would be gradual and this would appear to permit some fish species to tolerate higher salinity than indicated by their direct transfer LC_{50} . The relative low salinity tolerance on young life-stages of freshwater organisms is well known (Hart et al., 1991; Ryan and Davies, 1996; Bailey and James, 2000; Clunie et al., 2002; Kefford et al., in press). The current study does not indicate that fish populations are capable of self sustaining at salinity levels above the tolerance of their most sensitive life stage indefinitely. It does, however, suggest that the salinity tolerance of the young stages may not have yet limited the large scale distribution of freshwater fish with respect to salinity. The protection of freshwater fish from rises in salinity will likely require consideration of the rates of change in salinity, seasonal changes in salinity, their life-cycles and the salinity tolerance of each life-stage.

This paper suggests that populations of southeastern Australian macroinvertebrates and fish will mostly be eliminated if salinity levels exceed their direct transfer 72-h LC_{50} value and slow acclimation LC_{50} value, respectively. No assessment was made of the sustainability of populations at their MFD and thus the current study cannot suggest what salinity levels are required for the protection of species.

Macroinvertebrate community structure is often related to salinity (Short et al., 1991; Metzeling, 1993; Marchant et al., 1997, 1999; Kefford, 1998a, 2000b). Yet it is not known whether salinity causes these patterns, as increases in salinity can co-occur with changes in other factors (Kefford, 1998b, 2000a,b). It would seem unlikely that factors confounded with salinity would eliminate taxa at the same salinity as their laboratory-measured tolerance. Therefore, the similar LC_{50} and MFD values for common macroinvertebrates suggest that the MFD is the direct result of short-term mortality. The relationship between the maximum mean EC and LC_{50} acknowledges that salinity may affect the distribution of taxa at levels below their acute lethal tolerance. The current study suggests that acute lethal effects of salinity impose an upper level on the distribution of macroinvertebrate taxa. Below this upper limit other effects (such as sub-lethal, indirect and effects of low salinity [BJK unpublished]) may influence macroinvertebrate distribution further.

Laboratory measured toxicity of pollutants other than salinity (e.g. metals, pesticides, oils, etc), are usually seen as comparative rather than predictive (Graney et al., 1995). A positive relationship has been observed between copper toxicity and organism field distribution with respect to copper contaminated sediments, yet acute toxicity alone could not explain this relationship (Malueg et al., 1984). Geckler et al. (1976) concluded that laboratory derived toxicity data underestimated the effects of copper on fish in the field because of avoidance of areas with high copper concentration. These studies contrast with the one to one relationship observed here with common macroinvertebrates. Salinity and its component ions are conservative, and bioaccumulation, biotransformation and bioavailability are probably not issues, as they are with many other toxins.

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References

- Aladin, N.V., 1991. Salinity tolerance and morphology of the osmoregulation organs in Cladocera with special reference to Cladocera from the Aral Sea. *Hydrobiologia* 225, 291–299.
- ASTM, 1998. Annual book of ASTM Standards, Water and Environmental Technology, Biological Effects and Environmental Fate, Biotechnology; Pesticides. Volume 11.05. American Society for Testing and Materials, West Conshohocken.
- Bailey, P., Boon, P., Morris, K., 2002. Australian biodiversity salt sensitivity database. Available from: <http://www.rivers.gov.au/research/contaminants>.
- Bailey, P.C.E. and James, K. 2000. Riverine and wetland salinity impacts: assessment of R&D Needs. LWRRDC Occasional Paper No. 25/99, Land & Water Research & Development Corporation. Canberra, Australia.
- Bayly, I.A.E., Williams, W.D., 1973. *Inland Waters and Their Ecology*. Longman Australia Pty Limited, Hawthorn, Australia.
- Brinkhurst, R.O., Chapman, P.M., Farrell, M.A., 1983. A comparative study of respiration rates of some aquatic oligochaetes in relation to sublethal stress. *Internationale Revue der gesamten Hydrobiologie* 68, 683–699.
- Carpenter, S.R., 1996. Microcosm experiments have limited relevance for community and ecosystem ecology. (Special Feature: the roles of microcosm in ecological research). *Ecology* 77, 677–681.
- Carpenter, S.R., 1999. Microcosm experiments have limited relevance for community and ecosystem ecology: reply. (response to Ray W. Drenner et al this issue, p 1081). *Ecology* 80, 1085–1089.
- Clemens, H.P., Jones, W.H., 1954. Toxicity of brine water from oil wells. *Transactions of American Fisheries Society* 84, 97–109.
- Clunie, R., Ryan, T., James, K., Cant, B., 2002. Implications for Rivers from Salinity Hazards: Scoping Study. Report produced for Murray-Darling Basin Commission, Strategic Investigations and Riverine Program- Project R2003. Department of Natural Resources and Environment, Heidelberg, Australia.
- Connell, D., Lam, P., Richardson, B., Wu, R., 1999. *Introduction to Ecotoxicology*. Blackwell Science Ltd, Oxford.
- Davies, P.E., 2000. Development of a national river bioassessment system (AUSRIVAS) in Australia. In: Wright, J.F., Sutcliffe, D.W., Fuse, M.T. (Eds.), *In Assessing the Biological Quality of Freshwater: RIVPACS and Other Techniques*. Freshwater Biological Association, Ambleside, UK, pp. 113–124.
- Drenner, R.W., Mazumder, A., 1999. Microcosm experiments have limited relevance for community and ecosystem ecology comments. response to S.R. Carpenter, *Ecology*, vol. 77, p. 677, 1996). *Ecology* 80, 1081–1085.
- DSE, 2002. Aquatic fauna information system. Arthur Rylah Institute for Environmental Research. Department of Sustainability and Environment, Victoria, Australia.
- Ettershank, G., Fuller, M., Brough, E.J., 1966. Hemiptera from saline waters in inland Australia. *Australian Journal of Science* 29, 144–145.
- Galat, D.L., Coleman, M., Robinson, R., 1988. Experimental effects of elevated salinity on three benthic invertebrates in Pyramid Lake, Nevada. *Hydrobiologia* 158, 133–144.
- Gaston, K.J., 1994. *Rarity*. Chapman & Hall, London.
- Gaston, K.J., Blackburn, T.M., 2000. *Pattern and process in macroecology*. Blackwell Science, Oxford.
- Geckler, J.R., Horning, W.B., Neihsel, T.M., Pickering, Q.H., Robinson, E.L. and Stephan, C.E., 1976. Validity of laboratory tests for predicting copper toxicity in streams. Report no. EPA-600/3-76-116, U.S. Environmental Protection Agency, Duluth, USA.
- Graney, R.L., Giesy, J.P., Clark, J.R., 1995. Field studies. In: Rand, G.M. (Ed.), *Fundamentals of Aquatic Toxicology Effects, Environmental Fate and Risk Assessment*. Second Edition. Tylar & Francis, Washington, DC, pp. 257–305.
- Hart, B., Bailey, P., Edwards, P., Hortle, K., James, K., McMahon, A., Meredith, C., Swadling, K., 1991. A review of salt sensitivity of Australian freshwater biota. *Hydrobiologia* 210, 105–144.
- Huston, M.A., 1999. Microcosm experiments have limited relevance for community and ecosystem ecology. *Ecology* 80, 1088–1089.
- Kangas, M., Geddes, M.C., 1984. The effects of salinity on the distribution of amphipods in the Coorong, South Australia, in relation to their salinity tolerance. *Transaction of the Royal Society of South Australia* 108, 139–145.
- Kay, W.R., Halse, S.A., Scanlon, M.D., Smith, M.J., 2001. Distribution and environmental tolerances of aquatic macroinvertebrate families in the agricultural zone of southwestern Australia. *Journal of the North American Benthological Society* 20, 182–199.
- Kefford, B.J., 1998a. The relationship between electrical conductivity and selected macroinvertebrate communities in four river systems of south-west Victoria, Australia. *International Journal of Salt Lake Research* 7, 153–170.
- Kefford, B.J., 1998b. Is salinity the only water quality parameter affected when saline water is disposed in rivers? *International Journal of Salt Lake Research* 7, 285–300.
- Kefford, B.J., 2000a. A preliminary investigation of the toxicity of saline lakes that are disposed into the Barwon River, south-west Victoria. Department of Natural Resources and Environment, Heidelberg, Australia.
- Kefford, B.J., 2000b. The effect of saline water disposal: implications for monitoring programs and management. *Environmental Monitoring and Assessment* 63, 313–327.
- Kefford, B.J., 2001. A single measure of salinity: is this representative of a river's past salinity? *Journal of Freshwater Ecology* 16, 45–51.
- Kefford, B.J., Dalton, A., Palmer, C.G., Nuggeoda, D., In press. The salinity tolerance of eggs and hatchlings of selected aquatic macroinvertebrates in south-east Australia and South Africa. *Hydrobiologia* In press.
- Kefford, B.J., Papas, P.J., Crowther, D., Nuggeoda, D., 2002. Are salts toxicants? *Australasian Journal of Ecotoxicology* 8, 63–68.
- Kefford, B.J., Papas, P.J., Nuggeoda, D., 2003. Relative salinity tolerance of macroinvertebrates from the Barwon River, Victoria, Australia. *Marine and Freshwater Research* 54, 755–765.
- Knowles, J.N., Williams, W.D., 1973. Salinity range and osmoregulatory ability of Corixids (Hemiptera: Heteroptera) in south-east Australian inland waters. *Australian Journal of Marine and Freshwater Research* 24, 297–302.
- Malueg, K.W., Schuytema, G.S., Krawczyk, D.F., Gakstatter, H.H., 1984. Laboratory sediment toxicity, sediment chemistry and distribution of benthic macroinvertebrates in sediments from the Keweenaw waterway, Michigan. *Environmental Toxicology and Chemistry* 3, 233–242.
- Marchant, R., Hirst, A., Norris, R.H., Butcher, R., Metzeling, L., Tiller, D., 1997. Classification and prediction of macroinvertebrate assemblages from running water in Victoria, Australia. *Journal of the North American Benthological Society* 16, 664–681.
- Marchant, R.A., Hirst, A., Norris, R.H., Metzeling, L., 1999. Classification of macroinvertebrate communities across drainage basins in Victoria, Australia: consequences of sampling on a broad spatial scale for predictive modeling. *Freshwater Biology* 41, 253–268.
- Metzeling, L., 1993. Benthic macroinvertebrates community structure in streams of different salinities. *Australian Journal of Marine and Freshwater Research* 44, 335–351.
- OECD, 1996. *Guidelines for Testing of Chemicals*. Organization for Economic Cooperation and Development, Paris.

- Ryan, T., Davies, P., 1996. Environmental effects of salinity and nutrients from salt disposal: approaches to the development of management criteria. Flora and Fauna Technical Report, no. 137, Department of Natural Resources and Environment, Melbourne, Australia.
- Scudder, G.G.E., Mann, K.H., 1968. The leeches of some lakes in the Southern Interior Plateau region of British Columbia. *Syesis* 1, 203–209.
- Short, T.M., Black, J.A., Birge, W.J., 1991. Ecology of a saline stream: community responses to spatial gradients of environmental conditions. *Hydrobiologia* 226, 167–178.
- Smith, P.J., 2002. *Analysis of Failure and Survival Data*. Chapman & Hall, Boca Raton.
- Tiller, D., Metzeling, L., 1998. Rapid biological assessment methods used by the Environmental Protection Authority in Victoria. Publication 604. Environmental Protection Authority, Victoria, Australia.
- Underwood, A.J., Chapman, M.G., Connel, S.D., 2000. Observations in ecology: you can't make progress on processes without understanding the patterns. *Journal of Experimental Marine Biology and Ecology* 250, 97–115.
- Williams, D.D., Williams, N.E., 1998. Aquatic insects in an estuarine environment: densities, distribution and salinity tolerance. *Freshwater Biology* 39, 411–421.
- Williams, W.D., 1987. Salinization of rivers and streams: an important environmental hazard. *Ambio* 16, 180–185.
- Williams, W.D., Sherwood, J.E., 1994. Definition and measurement of salinity in salt lakes. *International Journal of Salt Lake Research* 3, 53–63.