Relative salinity tolerance of freshwater macroinvertebrates, from the south-east of the Eastern Cape, South Africa compared to the Barwon Catchment, Victoria, Australia

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Abstract

Salinity is rising in many southern African and Australian rivers with unknown effects on aquatic organisms. The extent of spatial variation, at any scale, in salt tolerances is unknown and therefore whether data from one location should be used elsewhere. The acute tolerances (72-hour LC_{50}) to sea salt of 49 macroinvertebrate taxa from the south-east of the Eastern Cape (SEEC), South Africa were compared to 57 species from the Barwon Catchment, Victoria, Australia. The mean LC_{50} values from both locations were similar (Barwon: 31 and SEEC: 32 mS cm^{-1}) and less abundant (rare) taxa tended to be more tolerant than more abundant (common) taxa. There was, however, a greater range of LC_{50} values (5.5-76 mS cm^{-1}) in the Barwon Catchment than in the SEEC (11-47 mS cm^{-1}). The species sensitivity distribution (SSD) for SEEC taxa was bi-modal while the Barwon Catchment's SSD had a single peak. With few exceptions, members of an order had similar tolerances in both locations. The differences in SSD between the locations were related to crustacean, odonat, and non-arthropod relative richness. Although it is not ideal to extrapolate SSDs from one location to another, it may be reasonable to assume similar salinity tolerances among related taxa.

Running heading: Relative salinity tolerance: SEEC & Barwon

Additional Keywords: acute salinity tolerance, stream invertebrates, ecotoxicity, rarity

Introduction

Agricultural practices are causing increases in salinity of rivers and wetlands in semi-arid and arid parts of the world, including Australia and southern Africa (Williams 1987). There are up to 6-fold increases in mean flow-weighted salinity levels of rivers forecast in Australia's Murray-Darling Basin over the next 100 years (MDBMC 1999). South African rivers currently have a wide salinity range, from freshwater to a maximum-recorded value of 77 mS cm^{-1} in the Sak River (Dallas and Day 1993). The salinity of some South African water bodies is rising. For example, the mean annual salinity in the Vaal Dam is increasing by 2.5 mg L^{-1} $year^{-1}$ (DWA 1986) and in the Lower Vaal River (upstream of its confluence with the Orange River) by 18 ± 11 mg L^{-1} $year^{-1}$ (S. Jooste¸ pers. comm., Department of Water Affairs and Forestry, Pretoria). Especially in southern hemisphere locations, it is not known to what level salinity can increase before aquatic organisms are affected (Hart *et al*. 1991; Dallas and Day 1993). Salinity tolerance information is therefore needed for developing protective ecological guidelines, assessing ecological risk, biomonitoring studies and biodiversity conservation.

Based on Australia's aridity and commonness of inland saline waters, some have suggested that Australian freshwater organisms are more salt tolerant than organisms from other continents (see MDBMC 1987; Williams *et al*. 1991; Nielsen and Hillman 2000). Kefford *et al*. (2003) concluded that most Australian freshwater macroinvertebrates had similar salinity tolerance to related species from elsewhere. They, however, acknowledged that much of the salinity tolerance data available relates to species that are considered likely to be salt tolerant (Hart *et al*. 1991). No study has ascertained the extent to which salinity tolerance varies spatially, at any scale, with non-biased data collected by the same methods. Therefore, the applicability of using salinity tolerance data from one location to predict salinity tolerance at other locations is unknown.

In this paper, we measure the relative salinity tolerance of macroinvertebrates from the south-east of the Eastern Cape Province (SEEC) of South Africa and their tolerances to existing data (Kefford *et al*. 2003) from the Barwon Catchment, Victoria, Australia. Every effort was taken to use the same method, which is of field relevance (Kefford *et al*. 2004a), in both locations so that comparisons were valid. Southern Africa and Australia have highly variable runoff and stream flow (Gordon *et al*. 1992). By making comparisons from locations in southern Africa and Australia, the variation in tolerance from similar hydrological settings at this very large spatial scale can be considered.

The objective of this paper is to compare the salinity tolerance of freshwater macroinvertebrates from the SEEC and the Barwon Catchment. (Naturally we are not attempting to compare the salinity tolerance of macroinvertebrates from all locations within Australia and South Africa.) We investigate: whether related taxa (at the order or family level) have similar tolerances at the two locations; whether the increased salinity tolerance of rare taxa observed in the Barwon Catchment (Kefford *et al*. 2003) is repeated in the SEEC; whether the species sensitivity distributions (SSDs) at the two locations are similar.

Materials and methods

The methods used in the SEEC were identical to those used in the Barwon Catchment (Kefford *et al* 2003) and are thus only briefly described.

Collection of invertebrates

In both locations, macroinvertebrates were only collected from rivers that were essentially fresh, or almost so, in order to determine the salinity tolerance of macroinvertebrates inhabiting freshwater and not the tolerance of macroinvertebrates inhabiting more saline environments. Macroinvertebrates were collected from three sites in the SEEC, an area with an average rainfall of about 400 mm year^{-1} falling mostly in spring and summer. These sites were: the Kat River (a tributary of the Great Fish River) at Amherst (S 32 ^{o} 38' 30"; E 26 ^{o} 41' 20'); Palmiet River (a tributary of Kariega River) at the N2 Highway (S 33 ^{o} 22' 10"; E 26 ^{o} 28' 30"); the Botha River (also a tributary of the Great Fish River) at Visgat Pool (S 33^{o} 13'; E 26^{o} 30'). Electrical conductivity (EC) in the Kat River during collections ranged from 0.212 to 0.320 mS cm^{-1} (all EC readings were corrected to 25^{o}C) and the Palmiet River from 0.166 to 0.177 mS cm^{-1}. There is no regular water quality monitoring on the Palmiet River, but past readings have been similar (L. Pakhomova, IWR, pers. comm.). Salinity has been monitored on the Kat River at the Kat Dam (≈ 30 km upstream) since 1977 and at Fort Beaufort (≈ 30 km downstream) since 1992. Readings from these sites ranged from 0.083 to 1.08 mS cm^{-1} (SA hydrological information system data-base, unpublished). One species (*Burnupia stenochorias* [Ancylidae]) was collected from Botha River, which had an EC of 1.98 mS cm^{-1}. There is no water quality monitoring on this river but Haigh and Davies-Coleman (1999) report single readings of 0.59 and 0.73 mS cm^{-1}.

The Barwon Catchment rainfall spans 500 to 1 400 mm year^{-1} mostly between late autumn and spring. Salinities in the Barwon Catchment during collection ranged from 0.138 to 2.45 mS cm^{-1} (Kefford *et al*. 2003).

Laboratory tolerance testing

Effective development of guidelines for environmentally safe salinity levels (and other pollutants) depends on salinity tolerance data from a wide range of taxa (Aldenberg and Slob 1993) and species selected to be representative of natural communities (Forbes and Calow 2002). Experiments were therefore designed so that approximate estimates of the salinity tolerance could be made from many species and from the range of higher taxa present at the collection locations quickly (Kefford *et al*. 2003). Relative salinity tolerance is defined as the salinity lethal to 50 % of individuals (LC_{50}) over 72 hours, which is a good indicator of the maximum salinity at which macroinvertebrates have been collected in nature (Kefford *et al*. 2004a). We acknowledge that lethal salinity tolerances may be lower with longer exposures and in some life-stages such as eggs or hatchlings (Kefford *et al*. 2004b). Sub-lethal effects are also likely (Kefford and Nugegoda in press; T. Paradise, RMIT, pers. comm.). It was not feasible to consider such exposures or effects with a large number of species.

The South African experiments were extended to 96 hours so as to allow comparisons with other data from South Africa (including, Goetsch and Palmer 1997) and elsewhere. Observations were also made at 72 hours and in this paper comparisons are made at 72 hours to be comparable with the Barwon Catchment experiments.

Taxa from both locations were tested with the identical salt source: the artificial sea salt, Ocean Nature (Aquasonic, Wauchope, NSW). This salt was chosen for the Barwon Catchment experiments because in Australia most inland saline waters have an ionic proportion similar to sea water (Bayly and Williams 1973), which is predominately sodium chloride (NaCl). NaCl is also the dominant salt in waters of the

SEEC and agriculture induced salinisation in South Africa can increase NaCl concentration (Dallas and Day 1993). For Ocean Nature, the EC (mS cm^{-1}) - total dissolved solids (TDS, in g L^{-1}) and EC - osmolatity (Osmol kg^{-1} H_2O) relationships over the range of salinity used here are described by: TDS = 0.754 * EC and osmolatity = 0.0184 * EC, respectively (Kefford *et al*. 2003).

Controls comprised river water and dechlorinated tap water (aquarium water) (0.625 ± 0.1 mS cm^{-1}). The remaining treatments had various concentrations of Ocean Nature dissolved in aquarium water depending on the assumed tolerance (from the tolerance of related species [Hart *et al*. 1991; Kefford *et al*. 2003]) of the species being tested. There were minimal differences in the ionic composition of the aquarium waters used in both locations (Kefford *et al*. 2004b) and these differences would result in negligible differences in ionic proportions in the experimental treatments.

Tests were conducted at 20 ± 2° C. On arriving in the laboratory the invertebrates were placed next to the aquariums and the tests started as soon as the water temperature of the collection water approximated 20 °C. Tests were conducted in aquariums with approximately 6.6 L of water; similar salinity tolerances have been observed in these aquariums and small artificial streams (Kefford *et al*. 2004c). Individual taxa were housed in separate containers that allowed aerated water (> 80 % oxygen saturation) to circulate but prevented different taxa interfering with each other. As the aquariums provided a non-flowing environment, all taxa tested were non-rheophilic.

Species for which $\geq$ 50 individuals could be collected on one occasion were designated common taxa; other taxa were designated as rare. To ensure comparability, collection effort was similar for all species at both locations. If only common species are tested, the salinity tolerance of rare species, which form the majority of the species in most communities (Gray 1981; Gaston 1994), would not be represented and the SSD would be biased.

Common taxa were exposed to $\geq$ 8 salinity treatments concurrently in at least one trial. In order to assess the temporal variation (or repeatability) of test results, 2 or 5 repeat trials (with individuals collected on different occasions over four months) were conducted with 4 of the 8 common taxa (Table 1).

For rare taxa we used a modified up-down test protocol (Bruce 1985, 1987; Kefford *et al*. 2003) to obtain an approximate LC_{50} value. As many individuals as could be obtained at one time were exposed to 1-3 salinity treatments. Survivorship in these treatments was used to select treatments in subsequent trials. If, for example, > 50 % survived at the highest salinity, on the next occasion, the salinity was increased.

As is standard in acute toxicity tests, animals were not fed during the experiments (OECD 1996; ASTM 1998). Survivorship was assessed, and dead individuals removed, at 1, 24, 48, 72 and 96 hours. Immobility after probing was taken as death. Where cased caddisfly larva had retracted deeply, the case was removed and the immobility criterion applied. Retracted gastropods were placed in freshwater and if they failed to respond within 30 minutes were regarded as dead. Retracted individuals were seldom alive but were excluded at subsequent time periods.

Data analysis

For common taxa, standard logistic regressions were fitted and LCx values were calculated. LC_{50} values for rare taxa were estimated directly from experimental results. Depending on the data available, LC_{50} values for rare taxa were assigned either a point estimate, a range (and assumed to be the mid-point of this range in analysis and graphics) or as greater than a certain value (censored LC_{50}). Due to the presence of censored LC_{50} values it was not possible to use standard methods of generating SSDs, instead survival analysis was used to generate the SSD(s) using the Kaplan-Meier method (Smith 2002). This is a conservative empirical method where censored LC_{50} values are only included in the calculation of proportions at $\leq$ the EC at which they are censored. The Kaplan-Meier method calculates the proportion of taxa with higher LC_{50} values, higher censored LC_{50} values are included in the proportion but all lower censored LC_{50} values are excluded (Kefford *et al*. 2003).

Results

For the common taxa there was high survivorship in the control treatments over 72 and 96 hours (Table 1). *Micronecta piccanina* was, however, an exception with considerable mortality between 72 and 96 hours (in all treatments including controls) and the tolerance of this species is best considered only at 72 hours. There were no appreciable differences in the tolerances between repeat trials with the same species or for two taxa (*M. piccanina* and *Leptocerina*) collected from different sites, as shown in the Barwon Catchment, despite a wide range in EC from which species were collected (Kefford *et al*. 2003). All data for each taxa was therefore analysed together. LC_{50} values calculated over 96 hours of exposure were 0 to 31 % lower than over 72 hours but the 95 % confidence intervals for 72-h and 96-h LC_{50} values overlapped

(Table 1), and as with Kefford *et al*. (2004c), is suggesting that there is little difference in the interpretation of results obtained from either exposure period.

There was 100 % survivorship of *Dineutus grossus* at $\leq$ 6.4 mS cm^{-1} but at higher EC survivorship was variable and its LC$_{50}$ was conservatively recorded as > 6.4 mS cm^{-1} (Table 1). Tricladida had 100 % survival at 5.7 mS cm^{-1} and no survival at the next treatment (16 mS cm^{-1}); for subsequent analyses its LC$_{50}$ was assumed to be the midpoint between these values.

Forty-one rare taxa - mostly comprising Odonata, Hemiptera and Coleoptera - were tested (Table 2). There were only two differences between 72-hour and 96-hour LC$_{50}$ values, which extended from > 1.6 to 47 mS cm^{-1}.

Salinity tolerances of the taxa tested from the Barwon Catchment and the SEEC were similar especially in terms of mean LC$_{50}$ values (Table 3). The range of LC$_{50}$ values was, however, wider in the Barwon Catchment (Table 4). First, in the SEEC fewer taxa had very low LC$_{50}$ compared to the Barwon Catchment (Fig. 1a). Resulting in lower 5th percentile of LC$_{50}$ values from the Barwon Catchment (6.1 mS cm^{-1}), than the SEEC (11 mS cm^{-1}). Second, there were proportionally more taxa from the SEEC (53 %) with censored LC$_{50}$ values compared to the Barwon Catchment (34 %). These taxa only contribute to the Kaplan-Meier function to the point where their LC$_{50}$ values are censored. Therefore the upper end of the SEEC tolerances may be an under-estimate. Third, macrocrustaceans (a tolerant group in the Barwon Catchment) were poorly represented in the SEEC and this could also account for the few taxa in this region with very high tolerance. Given the similarity of the LC_{50} values between the two locations, the combined dataset is plotted (Fig. 1b).

While there were some rare species that were relatively salt sensitive, as with species from the Barwon Catchment, the common species tended to have lower salinity tolerance in the SEEC (Table 3, Fig. 2). Despite a few exceptions, the tolerances of most members of particular taxonomic groups were similar between the two locations (Table 3). The two non-arthropods tested, without censored LC_{50} values, had substantially lower salinity tolerance than most of the insects from the SEEC. It was not possible to assess the tolerance of crustaceans from SEEC as only one taxon was tested ($LC_{50} > 25.6$ mS cm^{-1}); but it is clearly not sensitive.

As in the Barwon Catchment, the most sensitive species tested from the SEEC was a baetid, *Cheleocloeon* sp.; its tolerance was, however, slightly higher than the two baetids from the Barwon Catchment (72-h LC_{50} of 5.5 and 6.2 mS cm^{-1}). The tolerances of major insect orders in the SEEC (Fig. 3a) overlapped more than in the Barwon Catchment, yet their patterns of tolerance were similar at both locations. Coleoptera and Odonata tended to be more tolerant than Hemiptera in the SEEC (Fig. 3a). This pattern was repeated when data from both locations were considered (Fig. 3b).

The SSD from the SEEC is bi-modal with peaks around 10 - 15 mS cm^{-1} and 35 - 40 mS cm^{-1} (Fig. 4a). This is evident in Fig. 1a where the Kaplan-Meier function is steepest at these ranges. This contrasts with the Barwon Catchment where there was a single peak around 10 –15 mS cm^{-1} (Kefford *et al*. 2003). Most Odonata had LC_{50}

values around the second peak and a greater number of Odonata taxa were tested in the SEEC than the Barwon Catchment (Table 3). Exclusion of Odonata eliminated the second peak.

Discussion

The macroinvertebrates tested in both locations had a similar mean LC_{50} value, rare taxa in both locations tended to be more tolerant than common taxa and most species from specific higher taxa (orders and families) had similar LC_{50} values. However, the SSDs differed between the two locations, with a greater range of LC_{50} values in the Barwon Catchment than the SEEC. There was also a bi-modal SSD in the SEEC but only one mode in the Barwon Catchment's SSD. The range of LC_{50} values recorded in both the Barwon Catchment and the SEEC (Table 3) extends over the EC range currently recorded in south-east Australian (Kefford *et al*. 2004a) and South African (Dallas and Day 1993) rivers and even the most tolerant taxa in both locations have LC_{50} values considerably less than EC values recorded in south-west Australian rivers (Kay *et al*. 2001).

Spatial differences in salinity tolerance

The LC_{50} values of macroinvertebrates from the Barwon Catchment spanned a larger range than in the SEEC, which might result from fewer non-arthropods (which in the Barwon Catchment were sensitive) and crustaceans (which in the Barwon Catchment were tolerant) being tested in the SEEC. The higher number of Odonata tested in the SEEC caused the second mode in its SSD (Fig. 4). There are relatively few freshwater macrocrustaceans in the SEEC (Day *et al*. 2001) and this is confirmed by comparing the taxa lists from the Barwon Catchment and SEEC (Table 4). Additionally, more non-arthropods (mainly gastropods) have been collected from the Barwon Catchment than the SEEC (Table 4). Studies from the SEEC do not report Odonata below sub-order or only list common taxa (Table 4), making it difficult to compare Odonata diversity. The difference in the SSDs between locations appears to be mostly due to the number of taxa tested from different taxonomical groups at each location (and not due to differences in the tolerance of these groups).

Are the SSDs from the two locations similar enough to suggest that it is valid to use a SSD from a location elsewhere? The mean LC_{50} values are very similar but the means may be of little importance in many applications, such as risk assessment. Risk assessment typically determines the risk to the majority of species (Solomon *et al*. 2000), often around 95 %. There was a greater proportion of taxa with relatively low LC_{50} values in the Barwon Catchment than in the SEEC and 5th percentile of LC_{50} values from the Barwon Catchment was 45 % of that from the SEEC. If one did not want to exceed 5 % of species LC_{50} values, there would be important differences depending on whether the SSD used Barwon or SEEC data.

We acknowledge that there will be situations were little data exists in a region and management needs dictate that a SSD must be examined before new data can be collected. The differences in ranges of LC_{50} values between the SSDs for each location and the difference in distribution (bi- and uni- modal) suggest that this is not ideal. The broad similarity in salinity tolerances within most orders at both locations suggests that it may be acceptable, in the absence of other information, to assume similar salinity tolerance in different geographic locations within families and orders but not for entire SSDs.

Kefford *et al*. (2003) concluded that there was no indication that the Australian macroinvertebrate fauna as a whole differed in salinity tolerance to that from elsewhere. The literature does not use consistent methods to measure salinity tolerance and is also potentially biased in favour of species with particular tolerances. This bias and inconsistent methods have been removed in the current study. Thus, the finding that salinity tolerance within higher taxa is similar at both locations does not support the hypothesis (Williams *et al*. 1991; Nielsen and Hillman 2000) that the Australian freshwater macroinvertebrate fauna is more tolerant than that of other continents. Differences in the relative diversity of specific higher taxa between geographic regions may, however, result in different SSDs between regions.

It is not known whether the similarity between these two locations extends to other continents. There are naturally salinised rivers in southern Africa (Forbes and Allanson 1970b; O'Keeffe and DeMoor 1988) and Australia (Williams *et al*. 1991; Kefford 1998) and both continents have substantial arid and semi-arid areas. Both locations have, by global standards, extremely variable and unpredictable rainfall and thus stream-flow (Gordon *et al*. 1992). This variation has led, on occasions, to extended periods of low stream flow, high evaporation and thus elevated salinity. Elements of the macroinvertebrate fauna of both locations may, therefore, be salt adapted.

Few macroinvertebrate taxa are naturally found at salinities substantially above their 72-hour LC_{50} (Kefford *et al*. 2004a). Several macroinvertebrate families have, however, been collected from rivers in southwest Australia (SWA) at salinity levels considerably higher than the LC_{50} values measured from the Barwon Catchment and the SEEC (Kay *et al*. 2001). Baetids, for example, were collected up to 19.6 mS cm^{-1} in SWA (maximum LC_{50} in Barwon and SEEC was 11 mS cm^{-1}) and members of Ceratopogonidae, Chironomidae, Culicidae, Dolichopodidae, Dytiscidae, Hydrophilidae and Tipulidae have been recorded in rivers up to 192 mS cm^{-1} in SWA. Rivers in SWA have probably experienced high salinity levels for hundreds of thousands of years and, although not well documented, evolutionary processes may have led to higher salt tolerances of aquatic macroinvertebrates (Kay *et al*. 2001). Thus, some regional differences in salinity tolerance appear likely.

Rarity and tolerance

Contrary to the usual assumption (Cain 1940; Brown 1995), in both the Barwon Catchment and the SEEC, locally rare macroinvertebrates tended to be more tolerant than locally common macroinvertebrates. Indeed, the published evidence, across a range of taxa, stressors and pollutants, supports our conclusion. Rare species of mosses (Cleavitt 2002), terrestrial macrophytes (Hodgson 1986; Baskauf and Eickmeier 1994), freshwater macrophytes (Greulich *et al*. 2000), butterflies (Hodgson 1993) and freshwater fish (Hamilton 1995; Sappington, *et al*. 2001) have been shown to be more stress tolerant than common species.

Cao and Williams (1999) claim that rare freshwater macroinvertebrates are more sensitive to disturbances than abundant species. Marchant (1999, 2002), however, contends that there is no objective evidence to evaluate this claim. Our study shows the reverse of what Cao and Williams (1999) expected: rare species were more tolerant, which paradoxically supports Cao and Williams' (1999) overall argument (that rare and common species will behave differently). Metzeling's (1993) observations that rare macroinvertebrate species were more restricted than common species to particular salinity ranges, also supports Cao and Williams (1999)'s argument. A better understanding of the relationship between rarity and tolerance to other stressors is needed before Cao and Williams (1999)'s argument can be fully evaluated.

It is possible that some of the species we found to be locally rare, are in fact common more widely. It would seem unlikely, however, that most of the species we found to be rare are generally common elsewhere (Gaston 1994). It is curious that an arbitrary but consistent definition of rarity, in both locations, was related to salinity tolerance. Rare species did tend to belong to particular taxa, for example, Coleoptera and Odonata. The relationship between rarity and salt tolerance may be due to chance that these taxa tend to be salt tolerant and rare (Kefford *et al*. 2003). Alternatively, if we assume that rare and common species tend to be K- and r- selected, respectively, then the inability for K-selected species to rapidly recover following disturbances should be a strong selection pressure to develop resistance to environmental extremes (McMahon 2002). This hypothesis would predict that in localities where salinity levels are intermittently elevated (as in parts of the Barwon Catchment and the SEEC), rare species would tend to be more salt tolerant than common species. In localities where salinity is relatively constant (low or high) over evolutionary time this hypothesis would predict that rarity would not affect salinity tolerance.

Variations in tolerances within a taxon

Despite most members of particular orders from both locations having similar salinity tolerance there were some notable exceptions. *Paragomphus* (Odonata: Gomphidae) from the SEEC had a LC_{50} that was about half of that of the least tolerant odonatan from the Barwon Catchment. While some Odonata species are salt sensitive (Clemens and Jones 1954; Shirgur and Kewalramani 1973; Berezina 2003), most Odonata from the SEEC and Barwon (Kefford *et al.* 2003) were relatively tolerant and thus they exhibit considerable variation in salinity tolerance.

Two species in the beetle family Gyrinidae, *Aulonogyrus sharpi* and *A. marginatus,* were sensitive, compared to other freshwater Coleoptera (Shirgur and Kewalramani 1973; Kefford *et al*. 2003; this study), suggesting variation in this diverse order.

Despite the regular occurrence of corixids in saline waters outside Australia, there are very few records of them in Australian saline water (Knowles and Williams 1973). The two SEEC corixids species tested were not more tolerant than those from the Barwon Catchment. It is therefore apparent that not all non-Australian corixids are more tolerant than Australian corixids.

In the current study one baetid from the SEEC was more tolerant than two tested from the Barwon Catchment. Forbes and Allanson (1970a) found a SEEC baetid, *Cloeon crassi*, had a similar tolerance to the baetid we tested from the SEEC (between 9.3 and 14 mS cm^{-1}). They also found another, *C. africanum*, had a tolerance (between 4.6 and 9.3 mS cm^{-1}) similar to those from the Barwon Catchment. Only one plecopteran was tested from the SEEC and it was more sensitive than the three tested from the Barwon Catchment. In contrast, the one trichopteran tested in the SEEC, without a censored LC_{50}, was more tolerant than those tested from the Barwon Catchment. Further testing of these groups will be needed to assess the importance of these differences.

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Table 1. Salinity tolerance (mS cm^{-1}) of common taxa. h = hour, id = insufficient data. A = adult

Taxa	**72-h LC_{50}**	**95% CI of 72-h LC_{50}**	**72-h LC_{25}**	**72-h LC_{10}**	**72-h LC_{5}**	**96-h LC_{50}**	**95% CI of 96-h LC_{50}**	**Control survivorship @ 72 h**	**Control survivorship @ 96 h**	**Number of repeat trials**	**Total number of individuals**
Dineutus grossus (Coleoptera: Gyrinidae) A	> 6.4	id	id	id	id	> 6.4	id	100 %	100 %	2	98
Tricladida	5.7 - 16	id	id	id	id	5.7 - 16	id	100 %	100 %	1	59
Cheleocloeon sp. (Ephemeroptera; Baetidae)	11	8.3 - 13	7.6	id	id	8.6	6 - 11	80 %	80 %	1	75
Burnupia stenochorias (Gastropoda: Ancylidae)	11	9.6 - 13	8.9	6.9	5.0	11	9.6 - 13	83 - 86 %	83 - 86 %	1	181
Euthraulus elegans (Ephemeroptera: Leptophlebiidae)	16	15 - 17	13	12	11	15	13 - 16	100 %	100 %	1	169
Micronecta piccanina (Hemiptera; Corixidae) A	19	17 - 20	13	7.9	4.2	id	id	90 - 100 %	40 – 100 %	5	386
Aulonogyrus sharpi (Coleoptera; Gyrinidae) A	20	17 - 23	16	11	8.7	14	11 - 17	100 %	100 %	2	124
Leptocerina (Trichoptera; Leptoceridae)	32	28 - 36	25	19	15	25	22 - 28	100 %	100 %	5	153

Table 2. Assigned 72-hour LC$_{50}$ (mS cm^{-1}) from rare species tests. LC$_{50}$ values at 96 hours were identical except where noted. A = adult, L = larva.

Taxa	**Assigned 72-h LC$_{50}$**	**Total no. of individuals**
Corbicula fluminalis (Bivalve; Corbiculidae)	> 1.6	8
Nychia sp a (Hemiptera; Notonectidae) A	> 3.2	3
Nychia sp b (Hemiptera; Notonectidae) A	> 3.2	2
Gyraulus connollyi (Gastropoda; Planorbidae)	> 6.4	3
Dyschimus (Trichoptera; Lepidostomatidae)	> 6.4	2
Paragomphus (Odonata; Gomphidae)	12.6	12
Aphanicercella (Plecoptera; Notonemouridae)	12.6	15
Aulonogyrus marginatus (Coleoptera; Gyrinidae) A	12.6 - 15	10
Anax (Odonata; Aeshnidae)	> 12.6	1
Chlorolestes (Odonata; Chlopocyphidae)	> 12.6	2
Pseudagrion (Odonata; Coenagrionidae)	> 12.6	23
Philonomon (Odonata; Libellulidae)	> 12.6	1
Tetrathemis (Odonata; Libellulidae)	> 12.6	1
Micronecta gorogaiqua (Hemiptera; Corixidae) A	14	16
Anisops sp a (Hemiptera; Notonectidae) A	15 – 35	4
Helodidae (Coleoptera) L	15 – 40	8
Hydrocoptus (Coleoptera; Dytiscidae) A	> 15	1
Hemiosus (Coleoptera; Hydrophilidae) A	> 15	1
Nychia sp c (Hemiptera; Notonectidae) A	> 15	7
Zygonyx (Odonata; Libellulidae)	25.6 – 47	10
Synlestidae (Odonata)	25.6 – 47	4

Laccophilus (Coleoptera; Dytiscidae) A	> 25.6	2
Rhanthus capensis (Coleoptera; Dytiscidae) A	> 25.6	2
Berosus sp (Coleoptera; Hydrophilidae) A	> 25.6	20
Potomanautes sidneyi (Decapoda; Potamonautidae)	> 25.6	2
Allocnemis sp (Odonata; Platycnemididae)	> 25.6	1
Laccocoris (Hemiptera; Naucoridae) A	30	2
Canthydrus cooperae (Coleoptera; Dytiscidae) A	30 – 47	2
Platycypha (Odonata; Chlorocyphidae)	30	2
Onychogomphus (Odonata; Gomphidae)	35	25
Acisoma (Odonata; Libellulidae)	35[#]	10
Lestes sp a (Odonata; Lestidae)	35 – 47	15
Lestes sp b (Odonata; Lestidae)	35 – 47	14
Anisops sp c (Hemiptera; Notonectidae) A	> 35	1
Lestes sp c (Odonata; Lestidae)	> 35	1
Sympentrum (Odonata; Libellulidae)	> 35*	1
Protoneuridae sp b (Odonata)	> 35	3
Protoneuridae sp a (Odonata)	40	8
Helobata (Coleoptera; Hydrophilidae) A	> 40	1
Phyllomacromla (Odonata; Corduliidae)	> 40	1
Philaccolus (Coleoptera; Dytiscidae) A	47	13

[#] 26.6 – 35 at 96 h

* insufficient data at 96 h

Table 3. Mean (and range) of LC$_{50}$ (mS cm^{-1}) and number of taxa tested (n) for various sub divisions of the different datasets. Note apparent contradictions in the sample size of some entries in the combined column are due to the inclusion of additional censored LC$_{50}$ values.

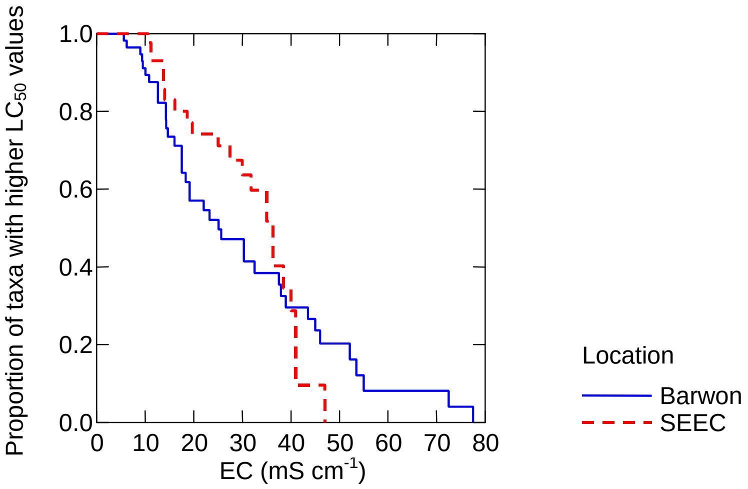
	SEEC 72-h	SEEC 96-h	Barwon 72-h	Combined 72-h
Whole data set	32 (11-47) n=43	31 (8.5 – 47) n=41	31 (5.5 – 76) n= 57	32 (5.5 – 76) n=103
Common	17 (11-32) n=7	14 (8.5-25) n=7	21 (5.5 – 52) n=15	20 (5.5 – 52) n=23
Rare	35 (13-47) n=36	35 (13-47) n=44	37 (9-76) n=42	37 (9-76) n=78
Non-arthropods	11 (10.8–11.1) n=2	11 (10.8–11.1) n=2	12 (9-14) n=11	12 (9-14) n=13
Insects and mites	33 (11-47) n=40 #	32 (8.5-47) n=38 #	30 (5.5-55) n=41	32 (5.5-55) n=83
Crustaceans	> 25.6, n=1	> 25.6, n=1	57 (38 – 76) n=5	57 (38 – 76) n=5
Ephemeroptera	13 (11-16) n=2	12 (8.5-15) n=2	10 (5.5-15) n=4	11 (5.5-16) n=6
Plecoptera	13, n=1	13, n=1	18, n=2	17 (13-18) n=5
Trichoptera	32, n=1	25, n=1	21 (9.5 – > 27) n=9	23 (9.5-32) n=12
Hemiptera	24 (14-40) n=6	23 (13-30) n=6	27 (18 – 44) n=7	21 (14 –44) n=13
Coleoptera	35 (14-47) n=11	35 (14-47) n=11	42 (27 – 54) n=5	38 (3.8-53) n=17
Odonata	37 (13-41) n=19	37 (13-41) n=19	47 (35 – 55) n=4	41 (13-55) n=26

Insects only

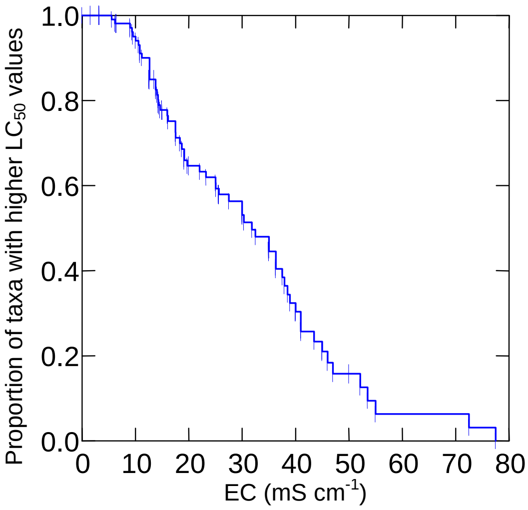
Table 4. Macrocrustaceans, Odonata and non-arthropod-invertebrates richness (number of taxa recorded) from the Barwon Catchment and the SEEC.

Location	Macro crustaceans	Odonata	Non-arthropods	Data source
Barwon River	5	3	8	Kefford (2000)
Barwon Catchment	5	8	18	Canale *et al*. (2001)
Barwon Catchment	6	7	11	Kefford *et al*. (2003)
Great Fish River, SEEC	0	No ID	2	O’Keefe & de Moor (1988)
Great Fish River, SEEC	1	No ID	5	Palmer & O’Keefe (1990)
Buffalo River, SEEC *	0	1	4	Palmer & O’Keefe (1991)
Buffalo River, SEEC	1	No ID	5	Palmer *et al*. (1994)
SEEC	1	17	4	This study

* Only 49 most common taxa (out of 103) are listed in the original publication.



a) SEEC and Barwon



b) Combined

Fig. 1. Kaplan-Meier functions for 72-hour LC_{50} from (a) the Barwon Catchment and the south-east of the Eastern Cape (SEEC) and (b) the combined data set.

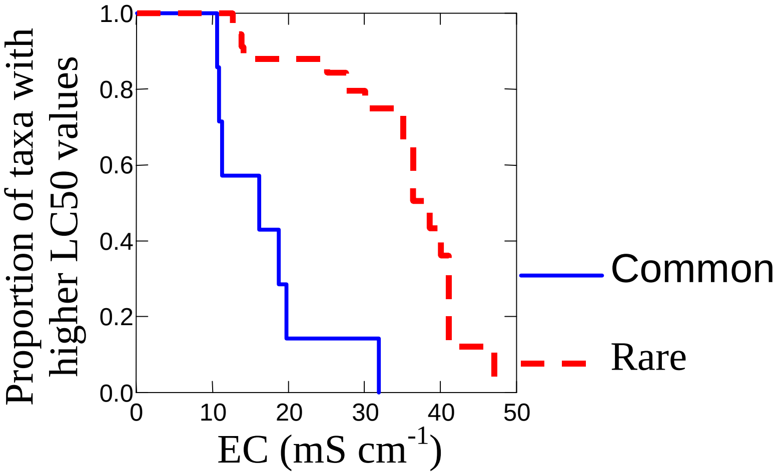
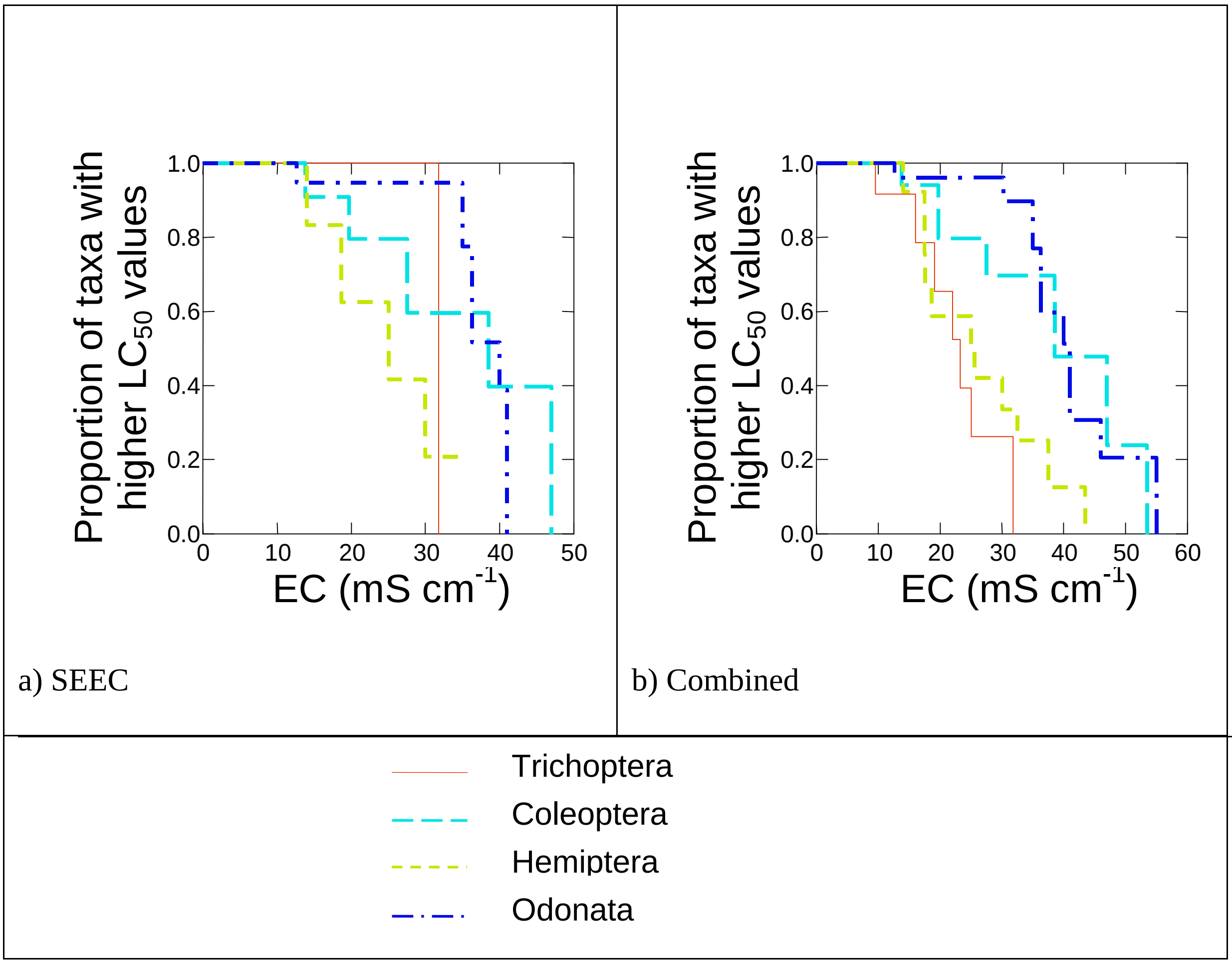


Fig. 2. Kaplan-Meier functions for 72-hour LC_{50} of rare and common taxa from the south-east Eastern Cape.



a) SEEC

b) Combined

Fig. 3. Kaplan-Meier functions for 72-hour LC_{50} of four insect orders from (a) the south-east Eastern Cape and (b) the combined data from Barwon River and south-east Eastern Cape.

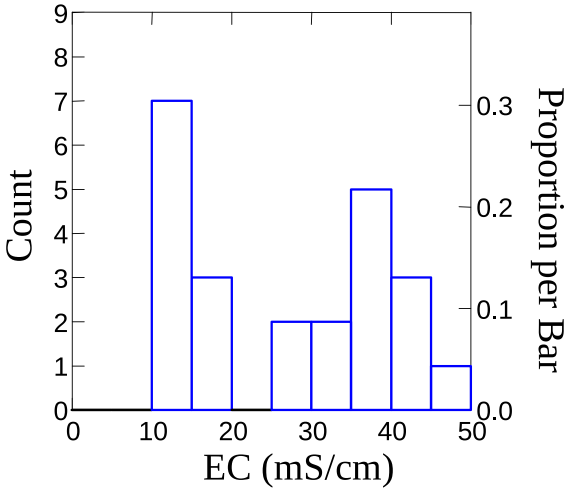


Fig. 4. Histogram of non-censored 72-hour LC_{50} values from the south-east Eastern Cape.