

# Appendix E: Salinity and stream macroinvertebrate community structure – the case of the Hunter River Catchment, eastern Australia

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# Salinity and stream macroinvertebrate community structure – the case of the Hunter River catchment, eastern Australia

Ben J. Kefford<sup>#</sup>, Jan Miller<sup>\*</sup>, Martin Krogh<sup>\*</sup> and Ralf B Schäfer<sup>^</sup>

<sup>#</sup> Centre for Environmental Sustainability, School of the Environment, University of Technology, Sydney (UTS), Sydney, Australia.

<sup>\*</sup> Office of Environment and Heritage, Department of Premier and Cabinet, New South Wales, Sydney, Australia

<sup>^</sup> Institute for Environmental Sciences, University Koblenz-Landau, Landau, Germany

## Summary

Salinisation is an important and increasing threat to freshwater biodiversity of streams and rivers. However, determining the specific threat that salinity poses can be complicated due to salinity being confounded with other changes in the environment, variation in the ionic proportions of salinity and temporal variation in salinity levels (i.e. pulse, press or ramp).

Here we use a weight-of-evidence approach to evaluate the role of salinity on stream macroinvertebrate community structure in the Hunter River and adjoining catchments (Karuah River, Lake Macquarie & Tuggerah Lakes and Manning River) on the Central Coast of New South Wales, Australia. In terms of investigating the ecological effect of salinity, the Hunter River is a complicated catchment. The Hunter has varied geology, extensive land clearing in some areas, salinity originating from discharges of waste water from coal mines and electrical generation and seepage of saline groundwater often exacerbated by agricultural practices. The ionic composition of salinity differs between the differing salinity sources. Pulses of increased salinity as stream discharges rise are considered to be more common in the Hunter than in other Australian catchments.

SPEAR<sub>salinity</sub> is a macroinvertebrate trait-based index designed to detect the effects of salinity by using information about the salinity sensitivity of macroinvertebrate families, mostly from laboratory toxicity tests. SPEAR<sub>salinity</sub> was found to decline with increasing electrical conductivity (EC). SPEAR<sub>salinity-pulse</sub>, a novel index that combines salinity sensitivity information and traits which indicate a population's resilience following a salinity pulse, also declined with increasing EC. There were stronger relationships between SPEAR<sub>salinity-pulse</sub> and EC than with SPEAR<sub>salinity</sub> and EC, especially in the riffle habitat. EC was not the only environmental factor included in the best linear models to describe both SPEAR<sub>salinity</sub> and SPEAR<sub>salinity-pulse</sub>. However, for SPEAR<sub>salinity-pulse</sub> EC was the most important factor identified in both the edge and riffle habitats. These results suggest that salinity pulses are ecologically important in the Hunter River catchment.

Large-scale changes in macroinvertebrate community structure (by pooling samples within predefined EC categories) were observed with relatively small changes in EC, including changes below 600 microsiemens per centimetre ( $\mu\text{S}/\text{cm}$ ) and  $900 \mu\text{S}/\text{cm}^1$ , which are the current targets for salt levels in the upper and mid/lower Hunter River, respectively. For example, as EC increases from  $<100 \mu\text{S}/\text{cm}$  to  $100\text{--}199 \mu\text{S}/\text{cm}$  there was a turnover of

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<sup>1</sup> That is the salinity from saline water disposal in these sections of the Hunter River is managed with the aim that it does not rise above these targets.

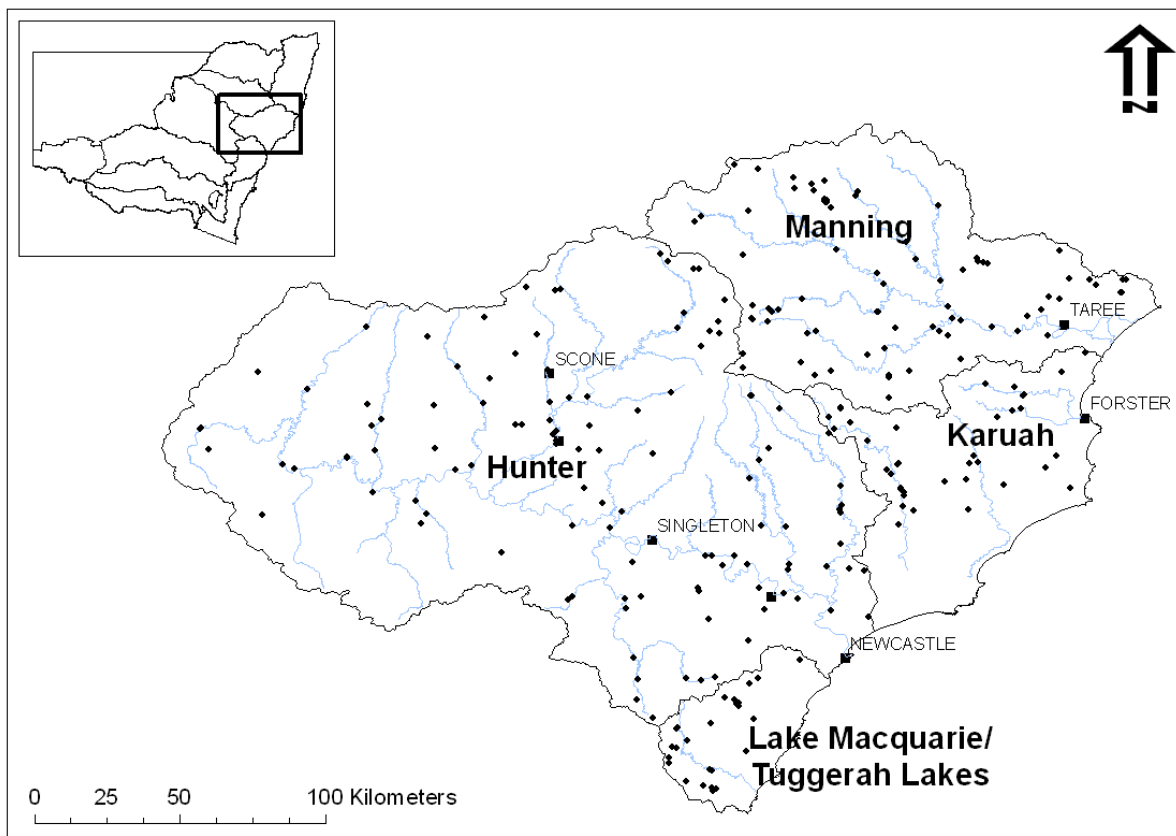
approximately 4 per cent of families across 16 samples, from 100  $\mu\text{S}/\text{cm}$  to 200–599  $\mu\text{S}/\text{cm}$  a 10 per cent turnover, from 100  $\mu\text{S}/\text{cm}$  to 600–899  $\mu\text{S}/\text{cm}$  a 16 per cent turnover and from 100  $\mu\text{S}/\text{cm}$  to 900–8130  $\mu\text{S}/\text{cm}$  a 19 per cent turnover. Multivariate analysis of individual samples shows that EC was included in the best set of environmental variables to describe the macroinvertebrate community structure in both the riffle and edge habitat.

Although the current study is correlative and thus cannot prove causality, we make the interim conclusion that salinity changes are likely (at least partly) to be causing the changes in macroinvertebrate community structure. This interim conclusion is made after considering that macroinvertebrate community structure changes have occurred at similar salinity levels elsewhere in Australia and overseas. Across these other locations salinity increases have a variety of causes and potentially a range of differing confounding factors. It would, thus, appear unlikely that salinity plays no causal role. Additionally, laboratory studies have shown that the magnitude of salinity changes observed in the Hunter do cause changes in the growth rates of some macroinvertebrate species. This interim conclusion should be reviewed in light of further studies, which we recommend, designed to establish causal relationships between salinity and changes in macroinvertebrate community structure in the Hunter catchment.

## Introduction

Salinisation is the process of increasing salinity of land and inland waters and can be either the result of natural process (primary salinisation) or the activity of humans (secondary salinisation). Secondary salinisation of rivers and streams is a major and growing problem in many regions of the world and threatens freshwater organisms, their population and communities and the ecological functions and services they produce (Cañedo Argüelles *et al.* 2013).

Determining the specific ecological impact of salinisation can be complicated by confounding factors, variations in ionic composition and the temporal pattern of salinity increase. Salinity does not occur in isolation and can co-occur with other environmental stressors (Kefford 1998, Szöcs *et al.* 2012). These other stressors can have their own effects on freshwater biodiversity and increase or decrease the environmental effects of salinity (Hall and Anderson 1995). Salinity is itself made up of component major ions (Williams and Sherwood 1994) typically chiefly: sodium ( $\text{Na}^+$ ), Calcium ( $\text{Ca}^{2+}$ ), magnesium ( $\text{Mg}^{2+}$ ), potassium ( $\text{K}^+$ ), chloride ( $\text{Cl}^-$ ), carbonate ( $\text{CO}_2^{3-}$ ) bicarbonate ( $\text{HCO}_3^{2-}$ ) and sulphate ( $\text{SO}_4^-$ ). The proportions of these and other ions in saline waters can have a greater effect on toxicity than total salinity (Mount *et al.* 1997, Farag and Harper 2012, Cañedo Argüelles *et al.* 2013). Salinity is typically a press or a ramp (Lake 2000) disturbance (Schäfer *et al.* 2011) but it can in some regions, e.g. the Hunter River catchment, be a short-term pulse disturbance (DEC 2006) and these different types of disturbances will most likely affect different groups of organisms (Schäfer *et al.* 2011). Consequently, determining the effect of salinity change in a catchment with a variety of environmental stressors, variable ionic proportions of saline water and the temporal pattern of the delivery of this saline water is a challenge.



**Figure E1: Map showing the region studied**

Sites examined are marked by small black diamonds, major towns with larger squares.

The Hunter catchment (Figure E1) is a very important coal mining and associated electricity generation region and both of these activities generate saline water which is often disposed of to the Hunter River (DEC 2006). Since 1994 the disposal of saline water in some places of the Hunter catchment is limited to periods of elevated flow to help dilute the saline discharge. Furthermore there are now upper targets on salinity in the Hunter River set in terms of EC: in the mid and lower Hunter River (affected by saline mine water discharges) EC of 900  $\mu\text{S}/\text{cm}$  standardised to 25 °C (hereafter  $\mu\text{S}/\text{cm}$ ) and in the upper Hunter River 600  $\mu\text{S}/\text{cm}$ . That is, the salinity from saline water disposal in these sections of the Hunter River is managed with the aim that it does not rise above 600 or 900  $\mu\text{S}/\text{cm}$ . These targets are now generally not exceeded (see <http://www.epa.nsw.gov.au/licensing/hrsts/success.htm>). Prior to 1994 the lower Hunter River would at times have monthly mean EC up to 1800  $\mu\text{S}/\text{cm}$  (DEC 2006); such high salinities are now only recorded in tributaries. It is important to recognise that the Hunter River Salinity Trading Scheme targets apply only to the main stem of the Hunter River between Glenbawn Dam and Singleton and not within any of the tributaries. The targets also apply only during high or flood flow periods. As a result, the Scheme may actually have limited influence over stream salinity levels for the majority of the time and the catchment and limited ability to control any ecological effects of saline water in the broader Hunter River catchment.

Within the Hunter catchment there are also uncontrolled inputs of salt associated with agriculture (Chessman *et al.* 1997) and natural inputs of saline water from the underlying geology (Kellet *et al.* 1989). The catchment has at least four different geologies – Permian sediments, Triassic sandstones, erosion-resistant Devonian & Carboniferous rocks and

tertiary basalt flows & igneous intrusions (Chessman *et al.* 1997) – all of which have different levels of salinity in their run-off.

The ionic proportions of salinity associated with agriculture, mining and natural inputs are likely to be different. In Australian inland waters the salinity associated with agriculture typically has an ionic proportions similar to sea water (Herczeg *et al.* 2001), which is approximately 85 per cent sodium chloride (NaCl). Saline effluents from coal extractions are not similar to sea water and are also highly variable in terms of ionic proportions (Lincoln-Smith 2010, Dahm *et al.* 2011, Dunlop *et al.* 2011). Salinity guidelines in Australia have been developed assuming ionic proportions similar to sea water and there are currently no Australian guidelines to protect aquatic life related to individual major ions (ANZECC and ARMCANZ 2000).

Salinity in the Hunter can occur as a series of short-term pulses (DEC 2006) unlike other Australian regions where it is generally considered a press or ramp disturbance (Schäfer *et al.* 2011). When water flow increases in the Hunter River, salinity often increases for a few hours before declining to low levels (DEC 2006). This is thought to be because the rising water level dissolves salts that have accumulated on the dry banks of river and on the soil surface but these salts are soon transported downstream and the increased volume of water dilutes the salinity, resulting in lower salinity than immediately before and after the rise in water level. If salinity pulses are a common stressor, then the types of organisms in the Hunter catchment most at risk from salinity will likely be different from regions where salinity is a press or ramp disturbance (Schäfer *et al.* 2011).

The aim of this report is to examine patterns in stream macroinvertebrate community in the Hunter River and adjoining catchments (Figure E1) and relate them to complex patterns of salinity in the region using a method that has been suggested can detect effects of salinity on macroinvertebrates and not from other factors. This method is the SPEcies At Risk from salinity (SPEAR<sub>salinity</sub>) biomonitoring index (Schäfer *et al.* 2011). We also aimed to determine if there was any evidence that salinity targets such as 600 and 900  $\mu\text{S}/\text{cm}$  were more generally protective of large-scale community structure in the Hunter and adjoining catchments.

## Methods

### The data set

The Hunter River catchment (32–33°S, 150–152°E) is located near Newcastle in coastal New South Wales (NSW), Australia. It occupies 22,000 km<sup>2</sup> with an elevation range from sea level to 1600 m, and the climate is mostly warm temperate with rainfall ranges 600–1200 mm/year (Chessman *et al.* 1997). Stream macroinvertebrate and associated environmental data was obtained from the NSW Office of Environment and Heritage, from the Hunter River catchment and also from sites in the following adjoining catchments: Karuah River, Lake Macquarie & Tuggerah Lakes and Manning River (Figure E1) all of which fall within the area managed by the Hunter–Central Rivers Catchment Management Authority. All catchments are coastal in that they drain east to the Pacific Ocean and not into the Murray–Darling Basin.

Macroinvertebrate sampling followed the Australian River Assessment System (AUSRIVAS) protocols (see <http://ausrivas.ewater.com.au/>). These protocols define the reach of river to be sampled, the method of sampling, sorting and identification of macroinvertebrates, and the

environmental data that is collected at each site. Data includes samples collected during the 1990s for the national Monitoring River Health Initiative, and more recent samples collected since 2006 for the Monitoring Evaluating and Reporting program. Associated with the macroinvertebrate samples are a range of measurements on the environmental characteristics of the site.

Using a data set that had been collected according to AUSRIVAS protocols meant that outputs from the AUSRIVAS model could be applied to our analyses. The AUSRIVAS model compares the assemblage collected from a site to those assemblages that would be expected if the site were in reference condition, and gives observed over expected (O/E) scores for each sample (Turak *et al.* 1999). Macroinvertebrate samples were collected from the edge habitat from all sites sampled, and, where present, riffle habitat.

Most sites were sampled on only one occasion (69 per cent and 65 per cent for edge and riffle, respectively) but some were sampled on multiple occasions, of which twice was the most common (18 per cent and 24 per cent for edge and riffle habitats respectively). A few sites were sampled up to 14 and 9 occasions for edge and riffle habitats respectively. To avoid issues of pseudoreplication, for sites which were sampled on multiple occasions, only one sampling event was randomly picked for analysis (and the data from the other sampling events was not examined). The exception to this was for relative family retention, where all samples were analysed (see below).

For some sites there were two replicate edge habitat samples taken from the same site on the same date; for analysis the mean abundance of each taxa recorded was calculated to avoid any issue of pseudoreplication. However, for the indices  $SPEAR_{\text{salinity}}$  and  $SPEAR_{\text{salinity-pulse}}$  (see below) the absolute difference between these two replicate samples was calculated to provide information on the repeatability (precision) of these indices.

### **Trait-based stressor-specific biomonitoring indices: SPEAR**

The approach we used here to determine the ecological effect of salinity while reducing the impact of confounding variables was to look at changes in the distribution of traits, or attributes, of the organisms present rather than changes in their taxonomic identity. Traits of organisms are more stable than the identity of organisms in the absence of human disturbances and specific (combinations of) traits can identify particular anthropogenic stressors (Statzner and Bêche 2010). The SPECies At Risk (SPEAR) is a stream macroinvertebrate index based on traits selected to be specific to particular stressors: pulse exposure to pesticides (Liess and Von der Ohe 2005), press exposure to organic toxicants (Beketov and Liess 2008) and press and ramp exposure to salinity (Schäfer *et al.* 2011). The term 'pulse' refers to episodic or short-term stress, 'press' refers to a stress of relatively constant intensity and 'ramp' as a slowly increasing intensity, relative to the lifetime of the organisms (Lake 2000).

The premise of SPEAR is that a key trait is physiological sensitivity (Kefford *et al.* 2012b) to the general class of contaminant under consideration e.g. organic toxicants in the case of pesticides and salinity in the case of salinisation (Schäfer *et al.* 2011). For contaminants that tend to have pulse exposure traits that indicate the ability of organisms to avoid the stress and for their populations to recover following the cessation of the stress, avoidance and resilience traits, respectively, are used.

$SPEAR_{\text{salinity}}$  was developed in southern Victoria and South Australia, where salinity appears to be mostly a press or ramp disturbance. The only trait that it uses is tolerance to elevated salinities of stream macroinvertebrate families (Schäfer *et al.* 2011), mostly as derived from

acute salinity tolerance experiments using lethality as the end-point or response variable (Kefford *et al.* 2003, Kefford *et al.* 2006a, Dunlop *et al.* 2008, Kefford *et al.* 2012c). That is, taxa are regarded as at risk or not at risk of salinity based on experimental determination of their salinity tolerance. This is unlike other commonly used macroinvertebrate indices e.g. Stream Invertebrate Grade Number Average Level (SIGNAL) (Chessman 1995) where the sensitivity/tolerance is assigned to taxa based on observations of their occurrence in the field along a variety of gradients of human disturbances.

Here we calculated SPEAR<sub>salinity</sub> as per Schäfer *et al.* (2011). Briefly, all families observed in the current study were associated with the families listed in Schäfer *et al.* (2011); in the case of chironomid sub-families these were combined to the family level (i.e. Chironomidae). In the cases of any families not listed in Schäfer *et al.* (2011), these were assigned a risk (or not) at the order level. SPEAR<sub>salinity</sub> was then calculated as per:

$$\text{SPEAR} = \frac{\sum_{i=1}^n \log_{10}(x_i) * y_i}{\sum_{i=1}^n \log_{10}(x_i)} \quad (1)$$

where n is the number of taxa observed in a sample, x<sub>i</sub> is the abundance of the i<sup>th</sup> taxa and y<sub>i</sub> is 1 if the i<sup>th</sup> taxa at risk of salinity (defined as taxa with medium tolerance or with 72 h LC50 < 35 mS/cm) as listed in Schäfer *et al.* (2011), else 0 for taxa at risk of salinity.

We also derived a novel index in the SPEAR family called SPEAR<sub>salinity-pulse</sub>. This index used the physiological tolerance of families to salinity as per Schäfer *et al.* (2011) and also the resilience traits of life-cycle length and dispersal ability and the avoidance trait (spending > 8 weeks out of the water) of macroinvertebrate families as given in Schäfer *et al.* (2011). The logic of SPEAR<sub>salinity-pulse</sub> followed that of SPEAR<sub>pesticides</sub> (pesticide contamination typically occurs in pulses). That is, for a taxa to be considered sensitive to salinity-pulses (as for pesticides) it had to be sensitive to salinity (as for organic toxicants), had to have low ability to avoid the pollution by being out of the water and had to have low population resilience to quickly recover following the spike in salinity (as for pesticides).

SPEAR<sub>salinity-pulse</sub> was calculated as per equation 1, except for y<sub>i</sub> to be 1 a taxa had to meet ALL of the following requirements as listed in Schäfer *et al.* (2011):

- not at risk of salinity (as with SPEAR<sub>salinity</sub>)
- number of generations ≤2 and time to first reproduction ≥0.5 years
- dispersal ability given as “low” or “some strong drifting or flying taxa”, and
- duration of life out of water <8 weeks or “fully aquatic” or “short” or “few weeks”.

So taxa which were at risk of salinity pulses were salinity sensitive, had low population resilience to quickly recover from a pulse disturbance and limited ability to avoid a pulse by being out of the water.

For comparative purposes with the two SPEAR indices, data on AUSRIVAS's observed to expected ratio (at 50 per cent probability; O/E50); AUSRIVAS's O/E50 SIGNAL and the SIGNAL2 index (Chessman 2003) were calculated at each site. AUSRIVAS is a predictive model very similar to the UK RIVPACS (Marchant *et al.* 1999, Turak *et al.* 1999). These three indices are widely used in Australia to assess the environmental health of macroinvertebrate stream communities.

## Relationship between macroinvertebrates index and environmental variables

To examine the relationship between the two SPEARs and the other indices and environmental variables, automatic model building using linear regression with forward selection of environmental variables was conducted. The null model from which all other models were evaluated included only the intercept term. The modelling was undertaken as per Schäfer *et al.* (2011), except that model selection was based on the Bayesian information criterion (BIC) instead of Akaike's Information Criterion. Hierarchical partitioning (Chevan and M. 1991) was used to determine the independent explanatory power of the physicochemical variables selected for the best-fitting model.

The geographic variables latitude, longitude and altitude were omitted from analysis as we were interested in the response of the macroinvertebrates to environmental variables and not to variables which indirectly affect the biota by acting on other variables (e.g. temperature). We also removed variables which had > 25 per cent missing values. This left the following variables: EC ( $\mu\text{S/cm}$ ), pH, dissolved oxygen (mg/L), turbidity (NTU), alkalinity (mg/L) and water temperature ( $^{\circ}\text{C}$ ), distance from source (m) (DFSM), rainfall (mm), slope (m/km), mode stream width (m), season (autumn or spring) and flow (none, low, moderate). Additionally the percentage cover of substrates (bedrock, boulder, cobble, pebble, gravel, sand, silt and clay) in the edge and riffle habitats was used in the analysis of edge and riffle macroinvertebrate samples, respectively. Sites with one or more missing value for these variables were excluded from the analysis.

## Relative family retention

To determine if the salinity targets for the Hunter River of 600 and 900  $\mu\text{S/cm}$  have any ecological basis in the region, we determined the relative family retention (RFR) rates using the method described in Kefford *et al.* (2010). This method looks at large-scale patterns in the change of taxonomical composition along a gradient of a stressor (salinity in this case). It is able to look at large scales by pooling (or amalgamating) multiple samples with similar levels of the stressor; these pooled sample sets (PSS) with similar levels of the stressor give an approximation of the complete set of taxa present at this level of contamination.

**Table E1: Electrical conductivity categories and the number of pooled sample sets (PSS) used**  
Each PSS consists of 16 randomly chosen edge samples.

EC category ( $\mu\text{S/cm}$ )	no of PSS	Notes
<100	6	
100–199	5	
200–599	6	
600–899	4	600 $\mu\text{S/cm}$ is the EC limit for upper Hunter
900–8130	4	900 $\mu\text{S/cm}$ is the EC limit for lower and mid Hunter, 8130 $\mu\text{S/cm}$ is the maximum salinity at any site examined.

Briefly, five EC categories were defined (Table E1) to encompass the EC limits in the current Hunter River (600 and 900  $\mu\text{S/cm}$ ) with respect to changes in macroinvertebrate relative species retention (RSR) previously observed in South Australia and Victoria (Kefford *et al.* 2010, Kefford *et al.* 2012a) and to maintain similar numbers of samples across all EC categories. Then, within each EC category samples are pooled; in the current study each PSS consisted of 16 samples from the edge habitat. (Note RFR was not calculated for the riffle habitat as the method requires a relatively large number of samples.) To form the PSS,



samples were randomly selected, without replacement, from those available within the EC category until 16 samples were selected. The process was repeated for the next PSS until < 16 samples remained from an EC category.

Jaccard's Index (JI; or the proportion of taxa in common) was calculated between all pairs of PSS. From the mean Jaccard's Index between the PSS the relative family retention was calculated. If we have the ordinal contamination categories  $i$  ranging from 1 to  $n$ , referring to least (1) and most ( $n$ ) contaminated, then  $j_{x,y}$  with  $x \neq y$  is the mean JI between categories  $x$  and  $y$ , and  $j_{x,x}$  and  $j_{y,y}$  are the mean JI's within categories  $x$  and  $y$ , respectively. The RFR between contamination categories  $x$  and  $y$  is  $j_{x,y}/j_{x,x}$  (Kefford *et al.* 2010). So, a RFR of 0.9, for example, would indicate that across 16 samples 90 per cent of families are common to both EC categories but 10 per cent are only found in one or the other EC category and thus there is a 10 per cent turnover of families.

Note that unlike the other analyses performed for this report, RFR was calculated based on all edge samples available including when multiple samples had been taken from the same site on different dates. This was because: (a) the method requires a large number of samples; and (b) no consideration was made as to the causal relationship between EC and RFR; and (c) the aim was to document changes in RFR between the EC categories.

### **Traditional Primer analysis**

Some standard methods of multivariate analysis of stream macroinvertebrate community data were also conducted using the software package Primer (Clarke and Gorley 2006) for both edge and riffle habitat data separately. In particular, the macroinvertebrate abundance data was converted to presence/absence data and the Bray-Curtis index was calculated between samples. From the Bray-Curtis index, non-metric multi-dimensional scaling (MDS) ordination was conducted in order to visualise the multivariate data. Differences in the community composition were then examined between EC categories (Table E1), AUSRIVAS bands and the catchments examined in the study using Analysis of Similarity (ANOSIM) (Clarke and Warwick 2001).

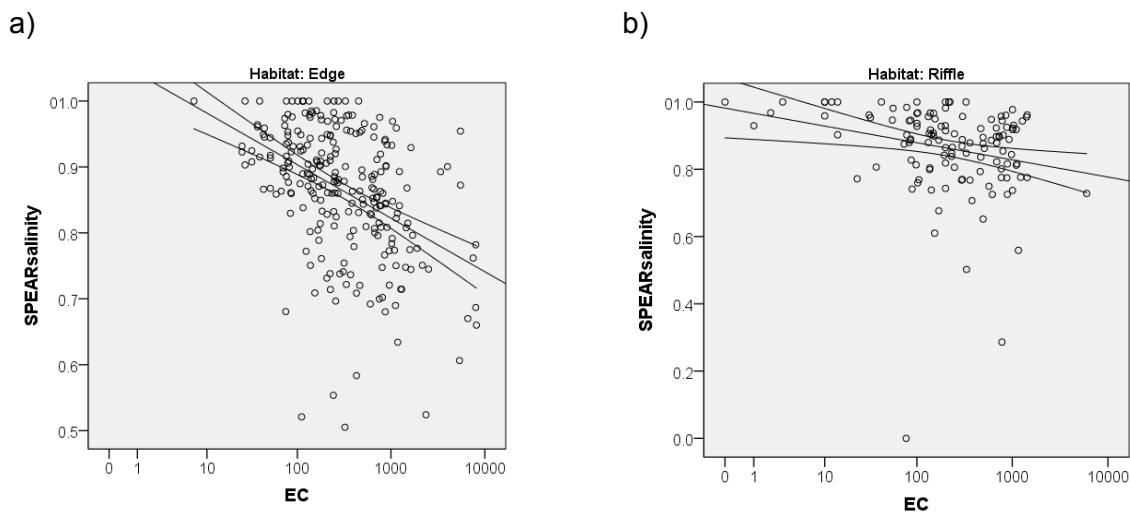
Stepwise searches for the best combination of environmental variables (BVSTEP routine in Primer) were also conducted with both the edge and riffle habitat, separately. This analysis searches for the best set of environmental variables, summarised by the Euclidean distance between the different environmental variable, to explain the Spearman Rank correlation of the environmental variables with the Bray-Curtis similarity of the macroinvertebrate community. Environmental variables considered were:  $\log_{10}$  DFSM, rainfall, slope, stream width mode, temperature,  $\log_{10}$  EC, square root turbidity, pH,  $\log_{10}$  alkalinity, and percentage cover of bedrock, boulder, cobble, pebble, gravel, sand, silt and clay. Note the percentage cover of the substrates used were from the relevant habitat (edge or riffle). Sites with one or more missing value for these variables were excluded from the analysis.

# Results

## SPEAR<sub>salinity</sub>

Where two replicate samples were taken from the same site from the habitat on the same occasion (date), the mean absolute difference in SPEAR<sub>salinity</sub> was 0.070 (stdev 0.049, range = 0.004–0.168, n=10). This generally indicated a small difference in SPEAR<sub>salinity</sub> for replicate samples collected from the same site at the same occasion.

In both the riffle and the edge habitat there were significant negative linear correlations between SPEAR<sub>salinity</sub> (Schäfer *et al.* 2011) and log<sub>10</sub> transformed electrical conductivity (EC) – see Figure E2 (P<0.001 & P = 0.012; r = -0.435 & r = -0.226; n = 250 & n = 124, for edge and riffle habitats, respectively). However, r<sup>2</sup> values were low with EC only explaining 19 per cent and 6 per cent, for edge and riffle habitats respectively, of the variation in SPEAR<sub>salinity</sub>.

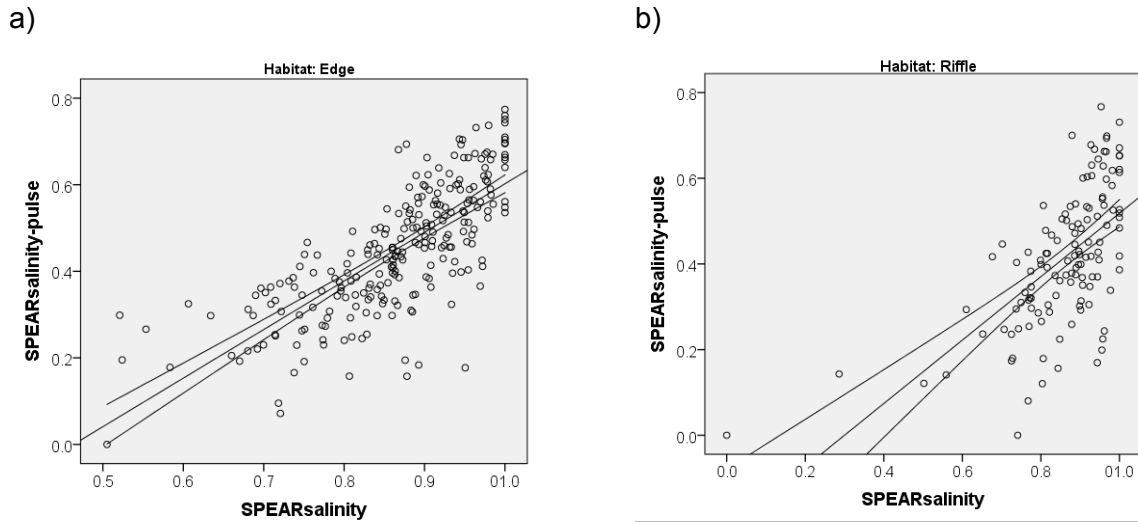


**Figure E2: The relationship between SPEAR<sub>salinity</sub> and electrical conductivity in  $\mu\text{S}/\text{cm}$  at 25 °C from (a) edge habitat and (b) riffle habitat**  
Note the different minimum values on the y-axis.

## SPEAR<sub>salinity-pulse</sub>

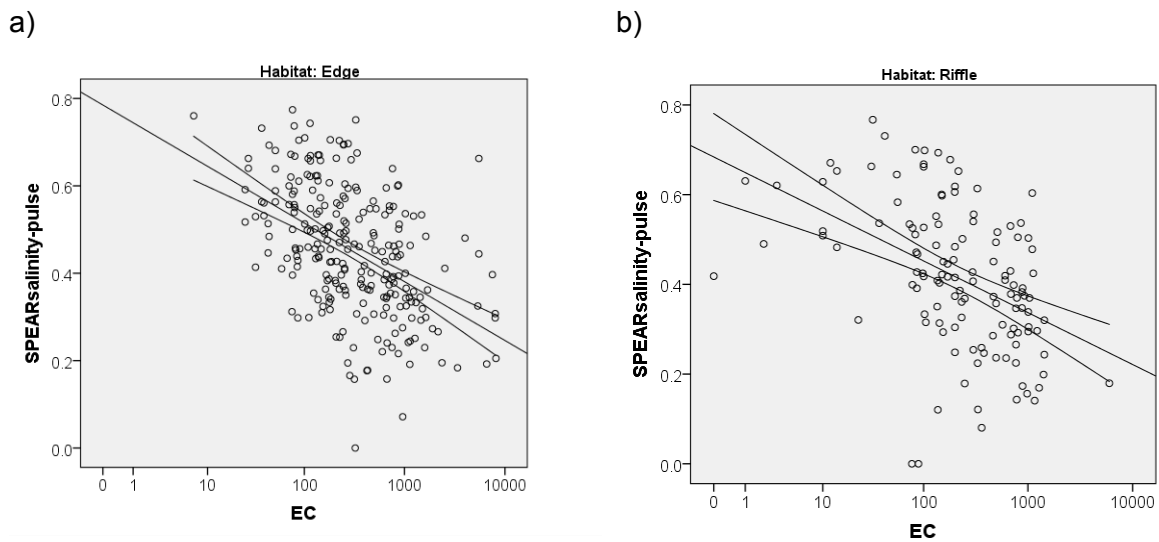
Where two replicate samples were taken from the same site from the edge habitat on the same occasion, the mean absolute difference in SPEAR<sub>salinity-pulse</sub> was 0.080 (stdev 0.073, range = 0.013–0.256, n=10). Again this indicates a small difference in SPEAR<sub>salinity-pulse</sub> for replicate samples collected from the same site at the same occasion.

The values of the newly derived SPEAR<sub>salinity-pulse</sub> index were approximately 50 per cent less than the existing SPEAR<sub>salinity</sub> index. The former index requires a taxon to be both salinity sensitive and its population to have traits that indicate low resilience, while the latter index only that a taxon was salinity sensitive. Furthermore, both SPEAR<sub>salinity-pulse</sub> and SPEAR<sub>salinity</sub> were correlated (P <0.001; r = 0.752 & r=0.623; n = 251 & 147, for edge and riffle habitats, respectively, see Figure E3).



**Figure E3: The relationship between  $SPEAR_{salinity}$  and  $SPEAR_{salinity-pulse}$  from (a) edge habitat and (b) riffle habitat**

$SPEAR_{salinity-pulse}$  was significantly negatively correlated with  $\log_{10}$  transformed EC in both habitats ( $P < 0.001$ ;  $r = -0.487$  &  $r = -0.479$ ;  $n = 250$  &  $n = 124$ , for edge and riffle habitats, respectively) and these correlations were stronger than for  $SPEAR_{salinity}$  and EC (Figure E4), with  $r^2$  values of 24 per cent and 23 per cent, respectively, for the edge and riffle habitats.



**Figure E4: The relationship between  $SPEAR_{salinity-pulse}$  and electrical conductivity in  $\mu S/cm$  at 25 °C from (a) edge habitat and (b) riffle habitat**

## What environmental variables best predict macroinvertebrate indices?

Environmental variables, like EC, are generally correlated with other environmental variables and showing that EC is correlated with  $SPEAR_{\text{salinity}}$  and  $SPEAR_{\text{salinity-pulse}}$  does not imply causality. In fact, establishing definitive causality between EC and  $SPEAR_{\text{salinity}}$  as well as  $SPEAR_{\text{salinity-pulse}}$  would require experimentation. However, we determined what environmental variables were generally accepted to influence stream macroinvertebrate communities and best described  $SPEAR_{\text{salinity}}$ ,  $SPEAR_{\text{salinity-pulse}}$  and three other commonly used macroinvertebrate indices (Table E2), as this increases the weight of evidence that a particular environmental variable, such as salinity, causes the change in the macroinvertebrate index.

EC was selected in the set of environmental variables best explaining  $SPEAR_{\text{salinity}}$ ,  $SPEAR_{\text{salinity-pulse}}$  in both habitats (Table E2), however, unlike in Schäfer *et al.* (2011), EC was never the only variable selected.

**Table E2. Explanatory power of environmental variables (and transformations) for the macroinvertebrate indices, as determined in hierarchical partitioning, and goodness of fit measures:  $r^2$  and Bayesian information criterion (BIC)**

(Presented for the (a) edge and (b) riffle habitats. Variables with no percentage given for a particular macroinvertebrate index were not selected in describing that index and variables not displayed were not selected in describing any of the macroinvertebrate indices. A full list of environmental variables considered is listed in the 'Methods' section of this report.)

Variable relevance (%)	$SPEAR_{\text{salinity}}$	$SPEAR_{\text{salinity-pulse}}$	AUSRIVAS's O/E50	AUSRIVAS's O/E50 SIGNAL	SIGNAL2
<b>(a) Edge habitat</b>					
EC ( $\log_{10}$ )	30%	46%			48%
Dissolved oxygen	20%	10%	20%		
Turbidity (Sqrt)		10%		50%	9.3%
pH		6.1%			
Alkalinity log				50%	
Temperature					17%
Season					5.6%
DFSM ( $\log_{10}$ )	8.9%		9.1%		
Stream width mode	28%	9.3%	38%		
Edge silt %		11%	33%		11%
Edge sand %	13%	7.8%			8.3%
$r^2$	0.327	0.394	0.214	0.095	0.533
BIC	-1059	-981	-629	-988	-254

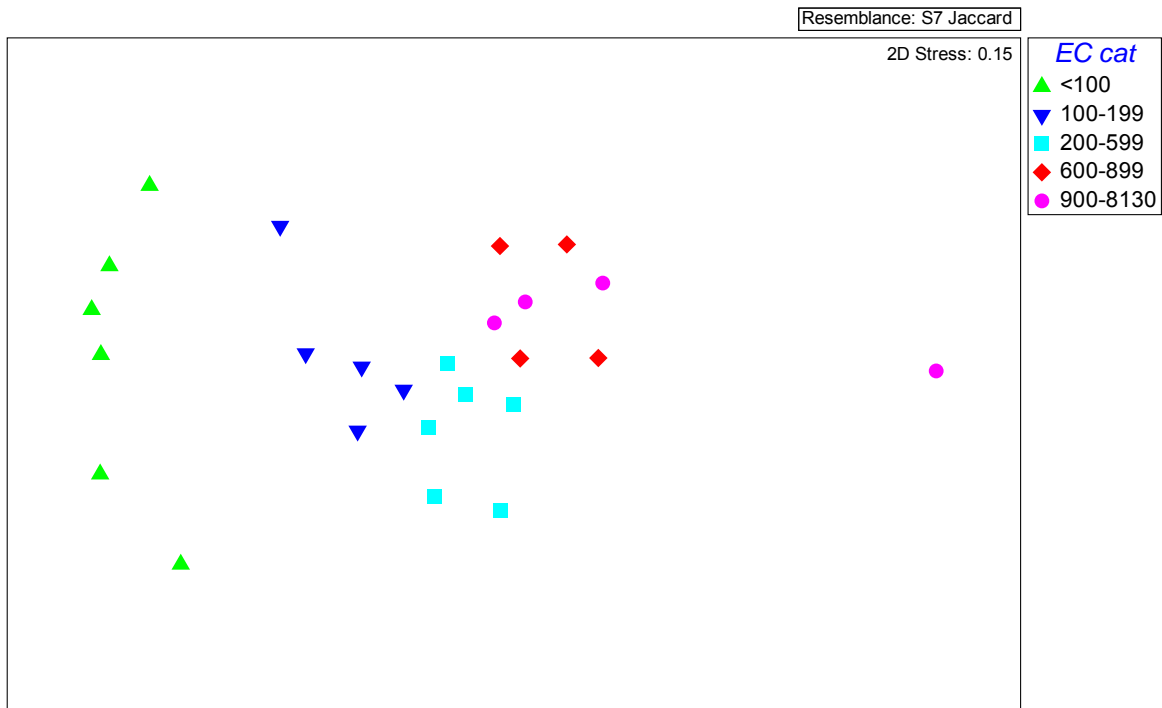
Variable relevance (%)	SPEAR <sub>salinity</sub>	SPEAR <sub>salinity-pulse</sub>	AUSRIVAS's O/E50	AUSRIVAS's O/E50 SIGNAL	SIGNAL2
<b>(b) Riffle habitat</b>					
EC (log <sub>10</sub> )	25%	56%	50%		62%
Turbidity	14%	9.2%			
Temperature					15%
Season		18%		37%	6.7%
Slope		16%			12%
Riffle clay %	60%				4.3%
Riffle silt %			50%		
Riffle sand %				19%	
Riffle pebble %				44%	
Riffle cobble %					1.1%
r <sup>2</sup>	0.329	0.556	0.264	0.217	0.715
BIC	-645	-465	-364	-601	-167

In both habitats the best model explaining SPEAR<sub>salinity-pulse</sub> had higher r<sup>2</sup> values than SPEAR<sub>salinity</sub>. For SPEAR<sub>salinity-pulse</sub> EC was always clearly the most important environmental variable in the model (Table E2). For SPEAR<sub>salinity</sub>, although EC was the most important variable in the edge habitat, the next most important variable (stream width mode) explained only 2 per cent less variation than EC. In the riffle habitat, the most important variable in explaining SPEAR<sub>salinity</sub> was the percentage of clay in the riffle (60 per cent) followed by EC (25 per cent). Collectively these results suggest that salinity pulses may be ecologically significant in the Hunter catchment.

EC was generally not selected as explaining the AUSRIVAS observed/expected ratio with at a ≥ 50 per cent probability (O/E50) or the AUSRIVAS observed SIGNAL/expected SIGNAL (O/E50 SIGNAL) indices; the exception being O/E50 in the riffle habitat where EC and percentage of silt in the riffle were equally important (50 per cent). EC was the most important variable in both habitats in explaining the SIGNAL2 index (Chessman 2003), accounting for 48 per cent and 62 per cent of the variance explained by the model for edge and riffle habitats, respectively. The r<sup>2</sup> of the best models in explaining the SIGNAL2 index were higher than any of the other macroinvertebrate indices calculated.

### Relative family retention

All samples from the edge habitat were classified into one of five EC categories (Table E1). The EC category boundaries were set to have similar number of pooled sample sets (PSS) in each category and to have category boundaries at the current EC targets for the Hunter River Salinity Trading Scheme of 600 µS/cm in the upper Hunter and 900 µS/cm in the lower and mid Hunter. Each PSS consisted of 16 randomly selected (without replacement) samples from the edge habitat within the relevant EC category (Table E1).



**Figure E5: Non-metric multi-dimensional scaling plot of the pooled sample sets**  
 Each point represents 16 randomly selected edge samples from the indicated EC categories.

Analysis of Similarity (ANOSIM) revealed a significant difference in similarity (as defined by Jaccard's Index) between the EC categories (Global  $R = 0.517$ ,  $P < 0.00001$ ). Pair-wise comparisons show significant differences ( $R = 0.276-1$ ,  $P = 0.019-0.002$ , see upper triangle in Table E3) between all categories, except between the two highest categories (600–899  $\mu\text{S/cm}$  and 900–8130  $\mu\text{S/cm}$ ,  $R = 0$ ,  $P = 0.543$ ). This indicates that the community across multiple (16) samples is different between each of the EC categories, except between 600–899  $\mu\text{S/cm}$  and 900–8130  $\mu\text{S/cm}$ . This is shown graphically in Figure E5.

In this analysis relative family retention (RFR) rates were calculated because invertebrates were generally identified to family level and not to species level as previously (Kefford *et al.* 2010, Kefford *et al.* 2012a) with the previous studies using relative species retention (RSR) rates. The RFR between the EC categories  $<100 \mu\text{S/cm}$  and  $100-199 \mu\text{S/cm}$  was 0.96 (lower triangle in Table E3) indicating that across 16 samples, 96 per cent of families were present in both of these categories and the remaining 4 per cent were present in only one of these categories. Between the EC  $<100 \mu\text{S/cm}$  and  $200-599 \mu\text{S/cm}$  the RFR was 0.90 so changes in salinity below 600  $\mu\text{S/cm}$  do appear to result in regional changes in the pool of families present (Table E3).

**Table E3: Results of relative family retention**

(The top right triangle gives the mean Jaccard's Index (JI) within and between the EC categories ( $\mu\text{S/cm}$ ), and in brackets are analysis of similarity (ANOSIM) pair-wise R statistics. The bottom left triangle gives relative family retention (RFR) across 16 samples between EC categories.)

EC category	<100	100–199	200–599	600–899	900–8130
<100	0.66	0.63 (0.62) <sup>a</sup>	0.59 (0.92) <sup>a</sup>	0.55 (1) <sup>a</sup>	0.53 (0.90) <sup>a</sup>
100–199	0.96	0.70	0.68 (0.35) <sup>a</sup>	0.65 (0.65) <sup>a</sup>	0.61 (0.57) <sup>a</sup>
200–599	0.90	0.98	0.71	0.69 (0.28) <sup>b</sup>	0.65 (0.36) <sup>b</sup>
600–899	0.84	0.94	0.97	0.71	0.69 (0)
900–8130	0.81	0.88	0.92	0.97	0.65

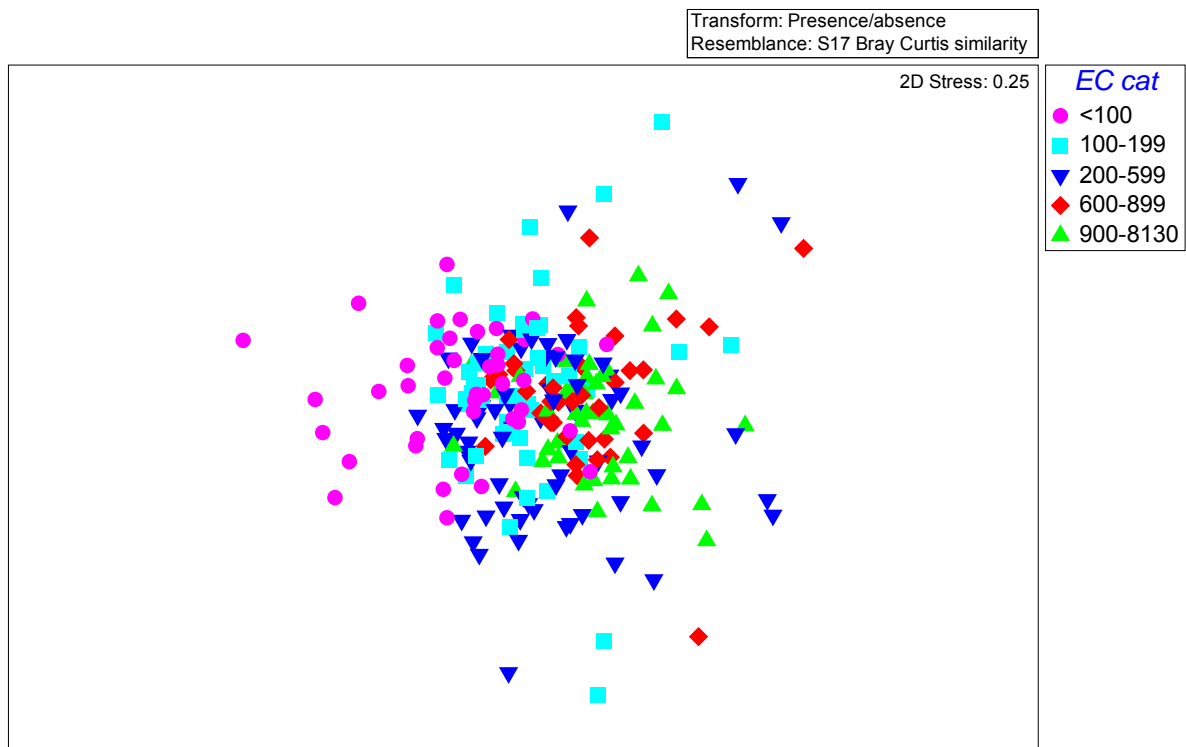
<sup>a</sup> P <0.05

<sup>b</sup> P <0.01

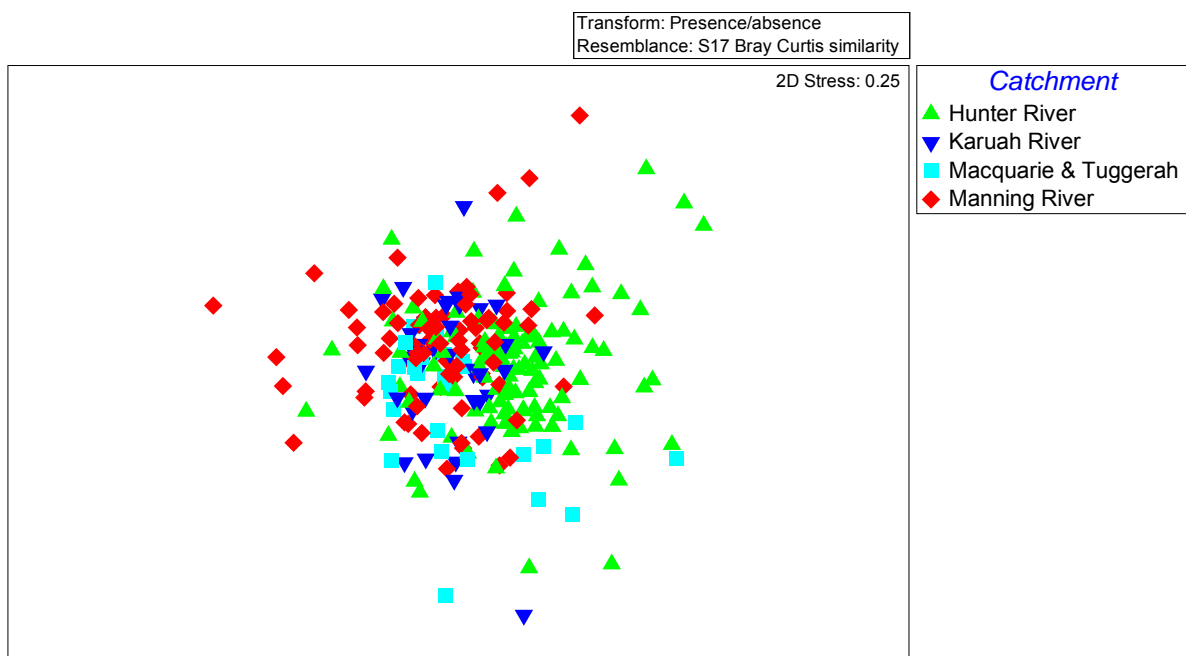
## Traditional Primer analysis

### *Edge habitat*

In the edge habitat ANOSIM indicated significant differences ( $P < 0.0001$ ) in the similarity (as defined by the Bray-Curtis index applied to presence/absence data) between the EC categories (Table E1); catchments the sites were located in; and the AUSRIVAS O/E Bands. However these differences were not large with Global R's of 0.090 and 0.100 for the EC categories and catchments, respectively. This indicates that despite the statistical significance, the practical difference in the invertebrate community between sites between EC categories and catchments is low, and ordinations illustrate this graphically (Figures E6 and E7). Pair-wise comparisons of the EC categories did indicate some greater differences when the changes in EC, e.g. <100 versus 900–8130  $\mu\text{S/cm}$ ,  $R = 0.354$  ( $P < 0.0001$ ) and <100 vs. 600–899  $\mu\text{S/cm}$ ,  $R = 0.213$  ( $P < 0.0001$ ). However, there was no evidence of a difference between 200–599  $\mu\text{S/cm}$  and 600–899  $\mu\text{S/cm}$ ,  $R = -0.024$  ( $P = 0.754$ ) or between 200–599  $\mu\text{S/cm}$  and 900–8130  $\mu\text{S/cm}$ ,  $R = 0.029$  ( $P = 0.119$ ) (Figure E6). So while increases in EC from a low base (<100  $\mu\text{S/cm}$ ) to levels greater than current targets in the Hunter (600 and 900  $\mu\text{S/cm}$ ) were associated with changes in community structure, more modest changes in EC were not.



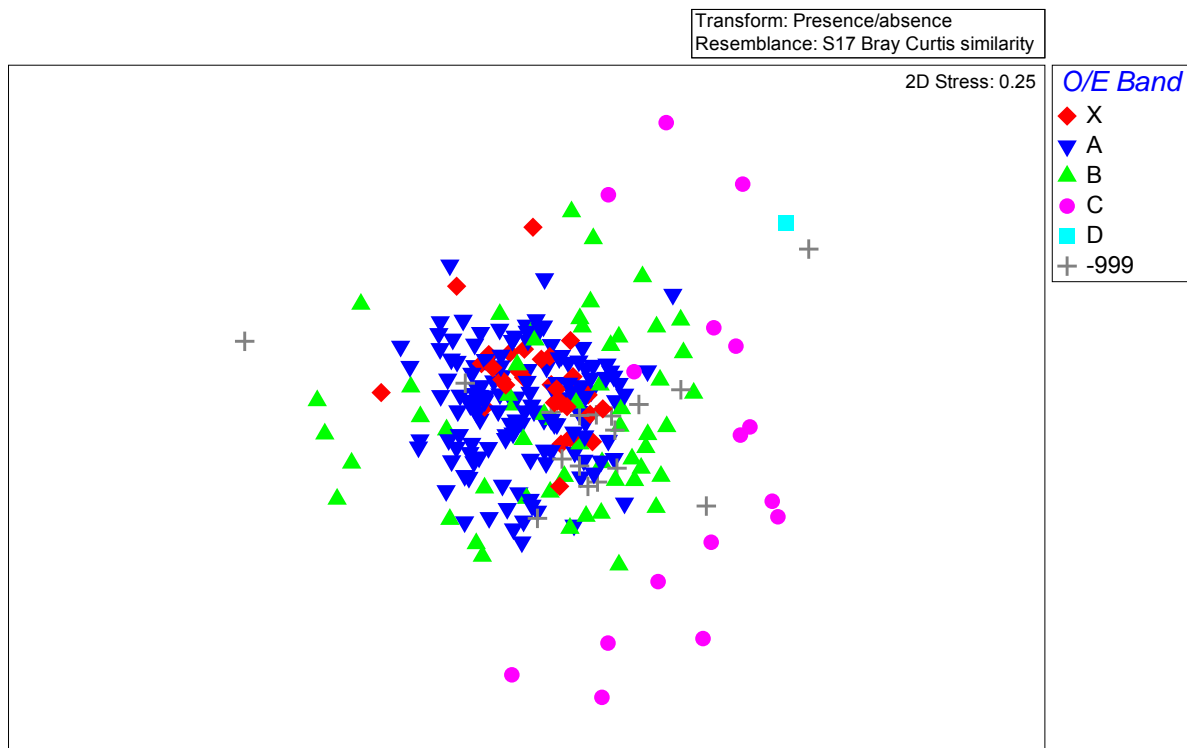
**Figure E6: Non-metric multi-dimensional scaling plot of edge samples showing differences between the EC categories**  
Each point represents a site sampled once from the edge habitat.



**Figure E7: Non-metric multi-dimensional scaling plot of edge samples showing differences between the catchments**  
Each point represents a site sampled once from the edge habitat.



The difference between the AUSRIVAS O/E bands was greater with a Global R of 0.263 and was largely driven by differences between bands X and A with band C (Figure E8).



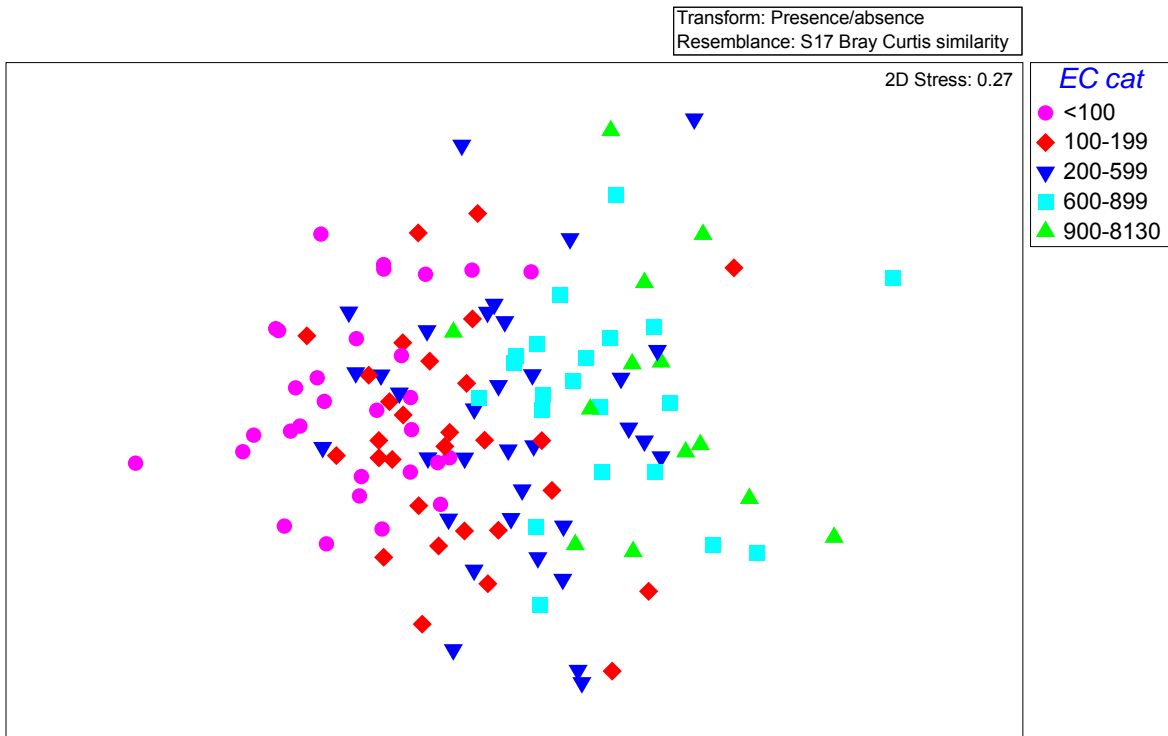
**Figure E8: Non-metric multi-dimensional scaling plot of edge samples showing differences between the AUSRIVAS O/E bands.**

Each point represents a site sampled once from the edge habitat. – 999 indicates that the band could not be calculated as the site characteristics were outside the experience of the AUSRIVAS model.

Stepwise searches for the best combination of environmental variables (BVSTEP routine in Primer) were conducted to explain the Spearman Rank correlation of the Bray-Curtis similarity of the macroinvertebrate community at the edge habitat to the Euclidean distance to the environmental variables. This analysis found a statistically significant relationship ( $Rho = 0.443$ ,  $P < 0.001$ ) with the following combination of variables (and transformations) giving the greatest explanatory power: DFSM ( $\log_{10}$ ), rainfall, EC ( $\log_{10}$ ), Turbidity (Sqrt), pH, percentage of silt in the edge and percentage of clay in the edge. The BVSTEP did not select any other models.

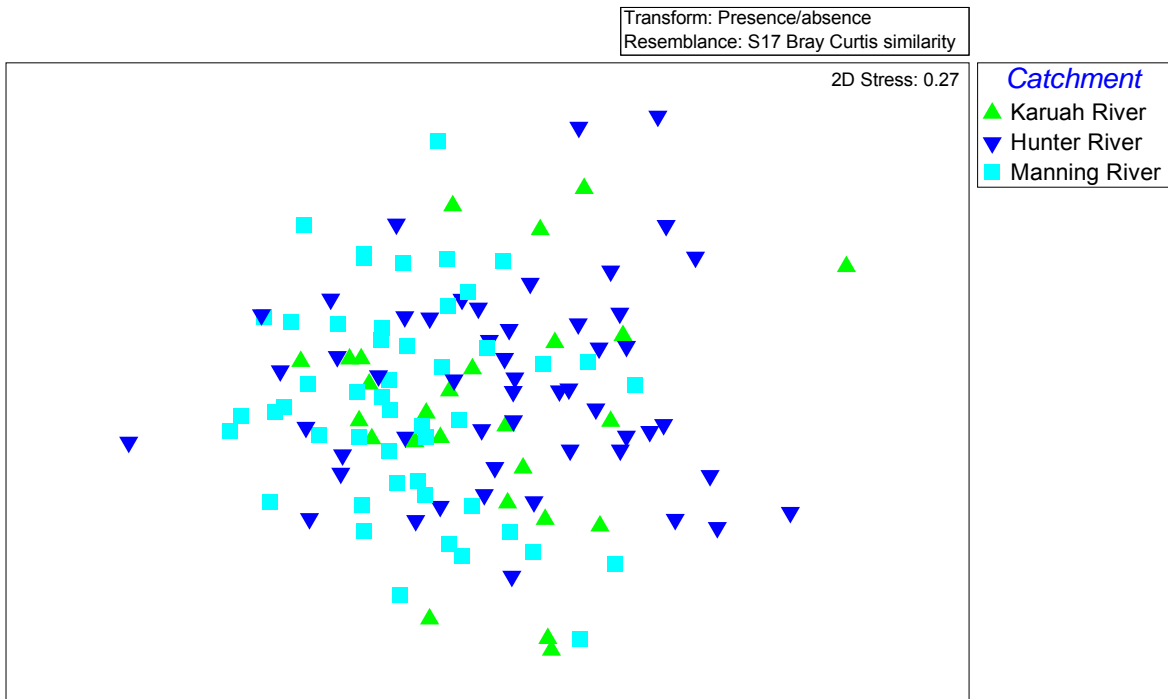
#### *Riffle habitat*

There were significant differences ( $P < 0.0001$ – $0.0006$ ) in the Bray-Curtis similarity index (applied on presence/absence data) of riffle samples between the EC categories (Table E1), catchments and the AUSRIVAS O/E bands. Unlike the edge habitat, the Global R (0.212) value for differences between the EC categories in the riffle habitat was of practical significance, with sites generally ordinated along the EC gradient (Figure E9). However, for the catchments and O/E bands, the Global R values were lower, 0.088 and 0.112, respectively, with ordinations not showing clear separation of samples between the categories (Figures E10 and E11).



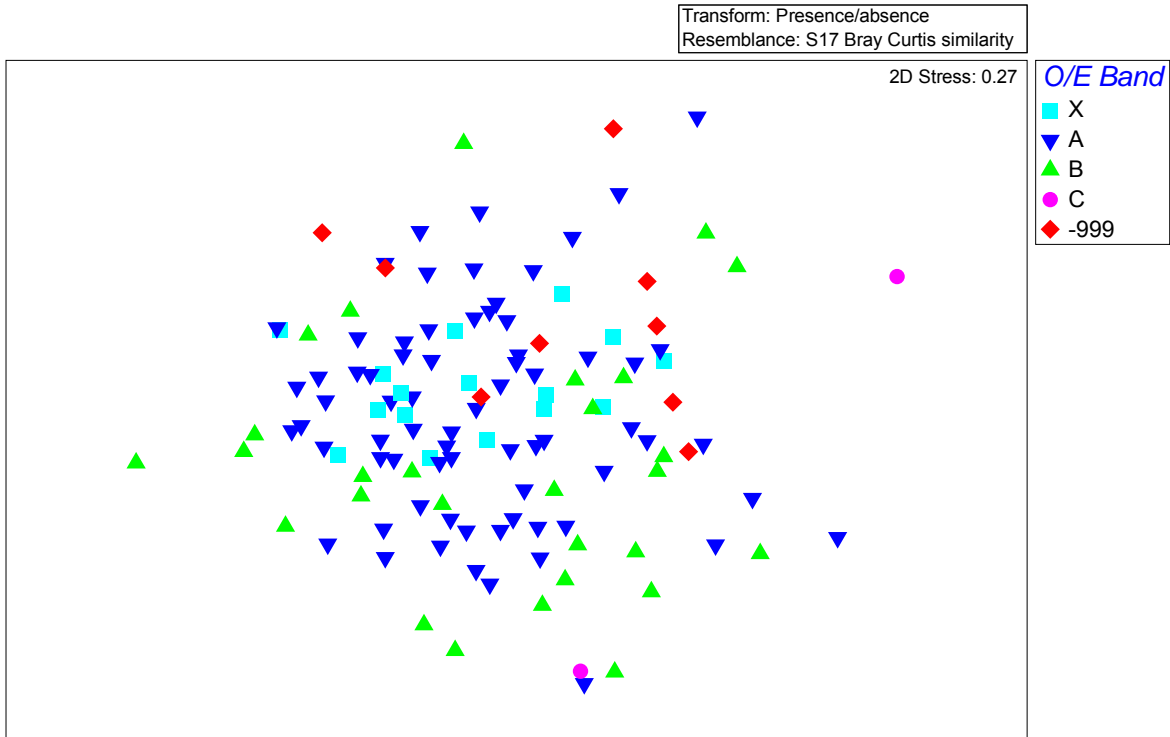
**Figure E9: Non-metric multi-dimensional scaling plot of riffle samples showing differences between the EC categories**

Each point represents a site sampled once from the riffle habitat.



**Figure E10: Non-metric multi-dimensional scaling plot of riffle samples showing differences between the catchments**

Each point represents a site sampled once from the riffle habitat.



**Figure E11: Non-metric multi-dimensional scaling plot of riffle samples showing differences between the AUSRIVAS O/E bands**

Each point represents a site sampled once from the riffle habitat. – 999 indicates that the band could not be calculated.

Stepwise searches for the best combination of environmental variables (BVSTEP routine in Primer) were conducted to explain the Spearman Rank correlation of the Bray-Curtis similarity of the macroinvertebrate community at the riffle habitat to the Euclidean distance to the environmental variables. This analysis found a statistically significant relationship (Rho = 0.383, P < 0.001) with the following combination of variables (and transformations) giving the greatest explanatory power: DFSM ( $\log_{10}$ ), EC ( $\log_{10}$ ), pH, percentage of sand in riffle and percentage of silt in riffle. It is noteworthy that in the ten best models (Rho = 0.363–0.383) that BVSTEP selected all included EC ( $\log_{10}$ ).

## Discussion

There were correlations between  $\text{SPEAR}_{\text{salinity}}$  and  $\log_{10}$  transformed EC in both the edge and riffle habitats. However, the strengths of these correlations were not great ( $r^2$  of 19 per cent and 6 per cent in edge and riffle, respectively) and certainly less than in southern Victoria (50 per cent and 44 per cent) and South Australia (45 and 38 per cent) (Schäfer *et al.* 2011). Unlike in southern Victoria (Schäfer *et al.* 2011), the best linear model to describe  $\text{SPEAR}_{\text{salinity}}$  in the Hunter included several variables other than  $\log_{10}$  transformed EC (Table E2). Furthermore  $\log_{10}$  transformed EC explained less than or about the same amount of variation in  $\text{SPEAR}_{\text{salinity}}$  than another variable in both habitats. The difference could not be due to different taxonomic resolution as the data was analysed at the family level in all regions.

Several factors could be important for explaining the differences between the Hunter and that observed by Schäfer *et al.* (2011) in southern Victoria and South Australia. While the minimum salinity were similar in all regions, the maximum salinity were markedly greater in southern Victoria (22,950  $\mu\text{S}/\text{cm}$ ) and South Australia (61,500  $\mu\text{S}/\text{cm}$ ) than in the current study in the Hunter (8,130  $\mu\text{S}/\text{cm}$ ) and the higher salinity levels might have forced a stronger relationship with  $\log_{10}$  EC. There are streams in the Hunter catchment that have had EC > 10,000 recorded but these were not sampled in the dataset examined. Future studies should specifically target such streams. Additional factors which might have been important are higher salinity levels in the Hunter often occur as short-term pulses (DEC 2006), variation in ionic proportions of waters with elevated salinity (Lincoln-Smith 2010, Dahm *et al.* 2011, Dunlop *et al.* 2011) altering the effect of a particular level of EC and the possibility of other pollution in saline water disposed from mining or electrical generation.

Although very little salinity tolerance information was available for stream macroinvertebrates from NSW for developing the SPEAR<sub>salinity</sub> index, it would seem unlikely that this is the reason for stronger relationships in Victoria and South Australia. This is because related stream macroinvertebrates generally have similar laboratory measured salinity tolerances regardless of whether collected in Victoria, Tasmania or Queensland (Allan 2006, Dunlop *et al.* 2008) and even eastern Australia, South Africa, France or Israel (Kefford *et al.* 2012c).

The novel index SPEAR<sub>salinity-pulse</sub> had stronger linear correlations with  $\log_{10}$  transformed EC than SPEAR<sub>salinity</sub> especially in the riffle habitat ( $r^2$  of 24 per cent and 23 per cent for the edge and riffle, respectively). Although  $\log_{10}$  transformed EC was not the only variable selected to explain SPEAR<sub>salinity-pulse</sub>,  $\log_{10}$  transformed EC was the most important variable in both habitats (Table E2). These results suggest that in the Hunter River catchment pulses of salinity may be ecologically relevant.

The EC measurements consisted of spot measures of salinity made while collecting the macroinvertebrate samples. In investigating relationships between SPEAR<sub>salinity-pulse</sub> and spot EC, there is an implicit assumption that there is a positive correlation between the spot EC and the maximum EC occurring during salinity pulses. While some positive correlation seems reasonable – sites with very low salinity would likely not have these salinity pulses as few salts would be expected to be deposited on the dry banks – the correlation would unlikely to be one-to-one and there could be some outlying sites. So the correlations between SPEAR<sub>salinity-pulse</sub> and spot EC are quite remarkable.

Consideration of the relative family retention rates between the EC categories (Table E1) show that changes in salinity below 600  $\mu\text{S}/\text{cm}$  do result in changes in the regional pool of families (Table E3). For example, if salinity increases from < 100  $\mu\text{S}/\text{cm}$  to the range of 600–899  $\mu\text{S}/\text{cm}$  in the edge habitat there is a 16 per cent turnover in species across 16 samples for this dataset (Table E3). This large-scale (regional) change was much less evident at the site scale (see Figure E7). This is the result of reduced ‘noise’ by pooling samples, providing a more complete list of species. That is, when single samples are considered many taxa are not recorded not because they do not live at a particular salinity but due to other environmental factors, past disturbance and even chance that they were not collected in that sample. The variability in individual macroinvertebrate family detections based on a single rapid-based assessment (RBA) sample can often be very high (Gillies *et al.* 2009). When multiple samples are pooled the chances that such species have not been sampled, if they in fact do live at a particular salinity, is reduced.

The reasons for selecting EC category boundaries at 600  $\mu\text{S}/\text{cm}$  and 900  $\mu\text{S}/\text{cm}$  is that these are the current limits of the Hunter River Salinity Trading Scheme in the upper and

middle/lower Hunter, respectively (DEC 2006). The observation that changes in salinity below 600  $\mu\text{S}/\text{cm}$  and 900  $\mu\text{S}/\text{cm}$  were associated with regional turnover of families suggesting that these limits may not fully protect the stream macroinvertebrate community from impacts of salinity. If short-term pulses of salinity are responsible for these community level changes, then the salinity levels indicated by the EC categories will almost certainly underestimate the level of salinity during the damaging pulses. This is because it is extremely unlikely that the spot measurements of EC during macroinvertebrate collection will capture the level of salinity during a pulse.

Although it is not proven, there is a reasonable case that the changes in community below 600  $\mu\text{S}/\text{cm}$  and 900  $\mu\text{S}/\text{cm}$  are caused by salinity. Changes in community structure associated with similarly low salinity have been observed in Victoria and South Australia (Kefford *et al.* 2005, Kefford *et al.* 2010), the Appalachia mountains, USA (Pond 2010, USEPA 2011, Passmore *et al.* 2012) and France (Piscart *et al.* 2005a, Piscart *et al.* 2005b, Piscart *et al.* 2006). So if confounding factors are really the cause in the community change, they need to be invoked in a number of geographically distant locations with different causes of increased salinity. In the case of the French studies, the increase in salinity resulted from discharges from soda factories, and salinity (and component ions) was the only environmental parameter which changed upstream and downstream of the discharge (Piscart *et al.* 2005a, Piscart *et al.* 2005b, Piscart *et al.* 2006). Furthermore, changes in salinity below 600 or 900  $\mu\text{S}/\text{cm}$  have been experimentally shown to affect the growth of stream macroinvertebrates (Kefford and Nuggeoda 2005, Hassell *et al.* 2006, Kefford *et al.* 2006b, Kefford *et al.* 2007b), microinvertebrates (Kefford *et al.* 2007a) and freshwater fish (Boeuf and Payan 2001).

## Research needs

From the data currently available it is impossible to definitively determine the extent to which salinity in the Hunter Catchment is a **causal** factor for changes in stream macroinvertebrate communities. This is because the current non-experimental data does not capture the fine-scale temporal salinity variation, variation in ionic composition of the saline water or other potential contaminants.

To establish the causal relationship between salinity and stream macroinvertebrate communities a research program would be required involving each of the following elements:

- experimental mesocosm studies where various salinity treatments are implemented and the response of the stream macroinvertebrate community observed. These experiments should also manipulate other factors potentially confounded with salinity in the Hunter catchment and deliver salinity as pulse, ramp and press disturbances so as to disentangle the effect of salinity and these other factors. These experimental treatments should be maintained for extended periods (e.g. 6 months to one year) so that all components of the organisms' lifecycle are considered and there is a sufficient period for long-term effects of salinity to occur. It would be useful for mesocosm experiments to not only determine the response of macroinvertebrate community structure to salinity but to also consider the effect of salinity on major food items of macroinvertebrates (e.g. algae and decay rate of leaves) in case salinity is affecting macroinvertebrates indirectly through alterations to the food chain. The purpose of these mesocosm experiments would be to demonstrate causal connection between salinity and changes in stream macroinvertebrate community.

- field studies at selected sites where, in addition to measuring standard macroinvertebrate, and associated environmental variables, major food items of macroinvertebrates, ionic composition and other potential contaminants are measured periodically, and EC, water temperature and discharge are logged continuously. Where possible sites could be co-located at existing water quality monitoring sites to reduce costs. The purpose of such a field study would be to ensure that (1) the aforementioned mesocosms are environmentally realistic and (2) the responses of the invertebrates to salinity (and other factors) are similar in the mesocosm and in real streams. It is important to make these comparisons because if mesocosm experiments are poorly designed they can underestimate the response of macroinvertebrates in real streams (Beketov *et al.* 2008, Liess and Beketov 2011, Schäfer *et al.* 2012).
- long-term laboratory experiments to determine the chronic and sub-lethal salinity sensitivity of macroinvertebrate taxa from the Hunter which appear to be salinity sensitive. Experiments should look at how salinity sensitivity is altered by other co-occurring environmental stressors and variation in ionic proportions that occur in the Hunter. Some initial steps in laboratory experiments have been undertaken by Lincoln-Smith *et al.* (2010) and PhD student R. Dowse (RMIT University) but further experimental work is still required. The purpose of these laboratory experiments would be to aid in the interpretation of results of the aforementioned mesocosm and field studies.

## Conclusion

As expected, it is clear that salinity is one of several factors affecting stream macroinvertebrate communities in the Hunter River catchment. However, salinity would appear to be a relatively important factor because it was consistently selected in the best models to explain univariate macroinvertebrate indices and multivariate community structure, and has previously been demonstrated to be associated with changes in regional family composition. Studies elsewhere have observed changes in macroinvertebrate community structure at similar salinity levels and laboratory experiments have shown that such salinity can alter the growth of stream macroinvertebrate species. It is thus reasonable to conclude in the interim, that changes in salinity below 600  $\mu\text{S}/\text{cm}$  and 900  $\mu\text{S}/\text{cm}$  can potentially impact on macroinvertebrate communities, until subsequent studies along the lines of those outlined in the 'Research needs' section of this report confirm or refute this conclusion.

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