Risk assessment of salinity and turbidity in Victoria (Australia) to stream insects' community structure does not always protect functional traits

Ben J. Kefford [a,*], Ralf B. Schäfer [b], Leon Metzeling [c]

[a] Centre for Environmental Sustainability, School of the Environment, University of Technology Sydney, New South Wales, Australia
[b] Institute for Environmental Science, University Koblenz-Landau, Landau, Germany
[c] EPA Victoria, Macleod, Victoria, Australia

ABSTRACT

Ecological risk assessments mostly consider measures of community composition (structure) across large spatial scales. These assessments, using species sensitivity distributions (SSDs) or the relative species retention (RSR), may not be protective of ecosystem functions and services at smaller spatial scales. Here we examine how changes in biological traits, as proxy for ecosystem functions/services, at a fine spatial scale relate to larger scale assessment of structure. We use functional traits of stream insect species in south-east Australia in two habitats (riffle and edge/pool). We find that the protection of community structure in terms of 95% of species over multiple sites against adverse effects of salinity (as electrical conductivity) and turbidity will mostly, but not always, protect traits at smaller scales. Considering different combinations of trait modalities, contaminants and habitat, a mean of 17.5% (range 0%–36.8) of cases would result in under-protection of trait modalities despite protecting species composition (in terms of Jaccard's Index). This under-protection of trait modalities is only because of the different spatial scales that community structure and the traits were considered. We recommend that where the protection of biological traits, ecosystem functions or ecosystem services from stressors is a management goal, protective targets should not be solely set using measures of community structure such as SSDs or RSR. To protect both structural and functional attributes separate risk assessments should be done.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Assessment of the ecological condition is largely based on indicators of structure and not functions (ecosystem processes) or ecosystem services (what ecosystems provide for humans). While the direct assessment of the structure of biotic communities is an improvement over traditional methods relying on measurements of physicochemical variables alone (e.g. water quality), there is a growing recognition that ecosystem functions and services should be assessed and preserved (Millenium Ecosystem Assessment, 2005).

There are four conceptual models on whether the protection of community structure will also protect ecosystem functions and vice versa in the literature (Cairns and Pratt, 1986; Forbes and Calow, 2002a). 1. The removal of species from an ecosystem has been likened to the removal of rivets from an airplane. As each rivet is removed the structure is weakened and risk of functional failure increases (Pratt and Cairns, 1996; Forbes and Calow, 2002a). Under this view, there should be a correlation between the protection of function and structure. Protecting either should provide a similar degree of protection to the other (Forbes and Calow, 2002a). 2. Some observations, however, show that the removal of some, perhaps many, species from an ecosystem can occur without apparent changes in functions, i.e. there is structural redundancy (Calow, 1996). This suggests that protecting structure will be over-protective of function. 3. Certain species may have a much greater effect on functions because they are keystone species (Paine, 1966) or ecosystem engineers (Jones et al., 1994). Under this conceptual model, little (or no) change in function would occur from the loss of (many) species which are neither keystone species nor ecosystem engineers. But the loss of only one keystone species or ecosystem engineer would have a major effect on function and structure (Forbes and Calow, 2002a). In this conceptual model protecting all species will protect function (Forbes and Calow, 2002a) but if some species are lost, protecting a given amount of structure (e.g. 95% of species) may (or may not) result in a much greater impact on function depending on the identity of the species lost. 4. It is also possible that a chemical stressor alters the functional capacity of species, without eliminating them from the system (Cairns and Pratt, 1986). In which case, protecting structure will be under-protective of function. With these

four conceptual models, it is thus not obvious what effect a certain loss of structure will have on ecosystem functions, and vice versa and Gessner and Chauvet (2002) advocate that both need to be considered.

Assessments of the risk of chemicals to the structure of ecological communities are mostly based on the species sensitivity distribution (SSD) concept (Posthuma et al., 2001). Given certain assumptions (Forbes and Calow, 2002b), from a SSD the proportion of species at hazard (or protected) from a particular concentration of a chemical can be estimated. The particular species potentially occurring at a site will vary widely due to a range of factors unconnected with any chemical. So SSDs do not estimate the proportion of species at hazard at a particular site, rather such estimations apply across a wider spatial area (Kefford et al., 2010a, 2011). That is if a similar level of contamination were to occur across many sites (not necessary contiguous), SSDs can estimate the proportion of species at hazard across these multiple sites. This is because at a particular site at which a SSD predicts that 5% of species will be lost due to contamination, some (perhaps all) of these species (expected to be lost) may never have occurred at this site. Species might not be at an uncontaminated site because the habitat is unsuitable, past disturbances, barriers to migration, too high levels of competition or predation, lack of suitable food, etc. If species expected to be lost from a site are already absent then <5% of species would be lost. Alternatively, if a site is dominated by the species expected to be lost, then >5% of species would be lost. So the loss of species at any site will usually not be 5% and at some sites no species will be lost. However, when many sites are considered the expected loss of species will approach 5%. Thus although estimates from SSDs could result in a given degree of protection across a large spatial scale (e.g. 95% of species), at individual sites there may be smaller or larger changes in structure (Kefford et al., 2011).

Protecting community structure from chemical contamination across large spatial scales, in our view, is a reasonable value judgement. Attempting to ensure that all species are protected at all sites is an impossible goal. This is because (as previously stated) many species that might potentially be present will be absent for a range of reasons unconnected with contamination. Indeed systems for comparing taxa observed and those expected to be present at healthy sites, such as RIVPACS (Furse et al., 1984; Wright et al., 1989) and AUSRIVAS (Marchant et al., 1999; Smith et al., 1999; Turak et al., 1999), recognise that not all expected species will be present even at reference sites.

Protection of ecosystem functions and services at scales above the site would seem unjustified. Ecosystem functions depend on each other and cannot be separated spatially (or temporally). It makes no sense, for example, to protect primary consumption at one site and secondary consumption at another site because both functions are needed together to maintain complex ecosystem. Regardless of the relationship between protecting structure and function, as SSDs and RSR may poorly estimate the change in structure at individual sites, there is potential for differences in the change in functions than either method would suggest.

Ecosystem functions and services are, however, not widely sampled and thus it is currently difficult to assess the large scale relationship between structure and functions or services. Nevertheless, across a wide range of taxa and ecosystems, including stream macroinvertebrates (Lecerf et al., 2006), there is growing evidence that the traits of organisms (e.g. in animals life-history, behavioural and feeding habit traits) in communities are associated with the provisioning of ecosystem functions and services, (McGill et al., 2006, de Bello et al., 2010). Changes in traits, while not direct measures ecosystem functions, can be associated with changes in functions.

The aim of this paper is to determine if the protection of stream insect species composition (structure) across multiple samples (Kefford et al., 2010a, 2011) with similar salinity or turbidity, will protect the mean levels of traits at the site level. We follow the

assumption (Lecerf et al., 2006; de Bello et al., 2010) that traits are linked to ecosystem functions. Salinity and turbidity were selected as both are major environmental contaminants in rivers in Victoria, south east Australia. Elevated levels of turbidity in the study region mostly originated from the input of suspended sediments into rivers, which is in turn caused by a range of human actives, including: agriculture, grazing and construction of roads and buildings. Elevated levels of salinity in southern Australia are mostly the result of dryland agriculture (Wood, 1924) where the ionic composition is similar to sea water (Bayly and Williams, 1973). Turbidity as sedimentation can affect ecosystem functions (see Apitz, this issue) but there is only a weak relationship between sedimentation/turbidity and community structure in Victoria (Marchant et al., 1997, 1999, 2006). By contrast, salinity is strongly associated with changes in community structure in Victoria at a number of spatial scales (Marchant et al., 1997, 1999, 2006; Kefford et al., 2010a).

2. Methods

2.1. Synopsis of methods

We estimated relative species retention (RSR) (Kefford et al., 2010a) for stream insects from Victoria, Australia between different salinity, as electrical conductivity (EC), and turbidity categories (Table 1). We also determined if the mean value of various trait modalities changed between the same EC and turbidity categories. We then determined whether changes in EC and turbidity which result in <5% change in community structure across multiple sites (RSR>0.95), would always result in change in mean trait modalities ≥5%.

2.2. Data set

A large dataset (Table 1) of stream macroinvertebrates from Victoria, in south-east Australia (Kefford et al., 2006a) was examined. Only insect species were considered due to uncertainty as to the functional feeding groups of some members of other taxa. Separate samples were taken during autumn and spring from the edge/pool (hereafter pool) and where present riffle habitats. At each sampling episode and habitat, samples were collected with a net (mesh size 0.25 mm) across ≈10 m of stream and sorted on-site for at least 30 person minutes and subsequently identified to species level were practical in the laboratory (Tiller and Metzeling, 2002).

Table 1
Electrical conductivity (EC) and Turbidity categories with number of samples (n) from pool and riffle habitats.

	EC categories (mS/cm) and n			Turbidity categories (NTU) and n		
		Riffles	Pool		Riffles	Pool
1	<0.050	350	309	0–0.5	39	37
2	0.050–0.099	449	382	0.5–0.7	48	40
3	0.10–0.19	265	190	0.7–1	52	43
4	0.20–0.29	134	60	1–1.25	141	121
5	0.30–0.49	121	66	1.25–1.5	76	57
6	0.50–0.99	139	73	1.5–2	168	130
7	1.0–1.49	87	40	2–3	294	218
8	1.5–1.9	79	19	3–4	211	166
9	2.0–2.9	74	22	4–5	104	73
10	3.0–3.9	30	13[a]	5–6	96	54
11	4.0–4.9	26	13[a]	6–8	141	74
12	5.0–6.9	32	9[a]	8–10	128	72
13	7.0–9.9	24	2[b]	10–13	77	37
14	10–14.9	8[a]	2[b]	13–20	101	49
15	>15 (35.6)	1[b]		20–30	54	20
16				30–80	69	19
17				80–591	38	5[a]
Total (n)		1819	1200		1837	1215

[a] Not included estimation of relative species retention (RSR).
[b] Not included in statistical analysis of traits and RSR.

Concurrent with the macroinvertebrate sampling salinity as electrical conductivity (EC) in mS/cm standardised to 25 °C (hereafter mS/cm) and turbidity as Nephelometric Turbidity Units (NTU) were measured. In order to conduct the analysis of change in species composition across multiple samples it was necessary to categorise all samples into discrete EC and turbidity categories (Table 1). The EC categories were chosen as described in Kefford et al. (2011; 2006a; 2010a). The rational for these categories is that they represent a balance between a reasonable number of samples in each category and categories in which biological responses are likely (Kefford et al., 2003; Kefford and Nugegoda, 2005; Hassell et al., 2006; Kefford et al., 2006b). The turbidity categories were chosen to reflect the same rational.

2.3. Traits used in the analysis

The traits used represent several aspects of the biology of stream insects, including: feeding type, sensitivity to contaminants (and thus their response to pollution), size and life-cycle (Table 2). In general, the chance that changes in functions are reflected by changes in traits increases with the number of traits used and with the diversity of traits used in terms of representing different aspects of the biology of stream insects. Modalities of the traits fell into four types. (a) Traits with binary categorical modality were classified as the proportion of one of the modalities (e.g. proportion of species with limited dispersal). (b) For traits with >2 nominal modalities (e.g. functional feeding) the proportion of each modality was calculated. (c) For ordinal variables with >2 modalities we chose to calculate a score for the trait similar to Vandewalle et al. (2010). Each modality was assigned a number (e.g. for generation length <1 a (year) = score 1, 1 a = score 2, >1 a = score 3) and the appropriate number assigned to each taxa in a sample and the mean value of these score in each sample was calculated (Table 2). (d) One trait, sensitivity to organic toxicants or S_{org} (Wogram and Liess, 2001; Von der Ohe and Liess, 2004) consisted of a continuous variable. This variable is defined as:

$$S_{org}(t) = \frac{\sum_{i=1}^{n} \log \frac{LC50(Daphnia\ magna)_i}{LC50(t)_i}}{n}$$

Table 2
Definitions of traits.

Trait	Modality	Definition
Functional feeding group	Predator	Proportion of
	Detritivore	Proportion of
	Grazer (=scraper)	Proportion of
	Shredder	Proportion of
	Filterer	Proportion of
Sensitive to organic toxicants	Continuous variable	Mean S_{org}
Salinity sensitivity[a]	Sensitive or tolerant	Proportion of salinity sensitive species
Dispersal capacity	Limited or not limited	Proportion of species with limited dispersal
Generation length in years (a)	<1, 1, >1	Score calculated as mean value of <1a = 1, 1a = 2, >1a = 3
Maximum size (mm)	<5, >5–10, >10–20, >20–40, >40–80	Score calculated as mean value of <5 = 1, >5–10 = 2, >10–20 = 3, >20–40 = 4, >40–80 = 5,
Breathers (Br) (during aquatic phases of life-cycle except egg)	Air (including plastron)	Proportion of
	Water	Proportion of
	Mixed (air breathing during part of aquatic life-cycle)	Proportion of
Aquatic stages	Eggs	Proportion of
	Larva	Proportion of
	Adults	Proportion of

[a] Not considered for salinity.

where LC50 is the median lethal concentration for substance i and n is the total number of substances for which pairs of LC50's for *Daphnia magna* and taxon t are available. Where toxicity data were not available for a taxon, its S_{org} value was taken from the related taxa as given in Schäfer et al. (2011a). Taxa equally sensitive to organic toxicants as *D. magna* have a S_{org} value of 0, those more sensitive have a S_{org} value > 1 and those more tolerant an S_{org} value < 0.

Functional feeding groups were assigned to insect species based on a database compiled by R. Marchant (Museum of Victoria, Melbourne, Australia). The database relied primarily on Chessman (1986) but also on Merritt et al. (2008) and expert opinion where no published information was available for a taxa. Other traits were classified at the family level from Schäfer et al. (2011a) but assigned to each species.

The mean value of the each trait modality was compared between each EC and turbidity categories using ANOVA with Tamhame test for multiple comparisons (because of difference in variance between categories for some modalities). Patterns in the mean values of the traits between the EC and the turbidity categories were then described. Modalities that showed an irregular pattern in a trait with increasing EC or turbidity category were considered to show no relationship with EC/turbidity, regardless of statistical significance. The analysis was conducted separately for pool and riffle samples.

2.4. Estimating relative species retention (RSR)

The so called, relative species retention (RSR) (Kefford et al., 2010a) was estimated between the EC and turbidity categories for each habitat separately. RSR is the relative proportion of species retained across multiple samples between (in this study) EC or turbidity categories. RSR is not calculated across single sites or samples, because many species will be absent in sample for reasons unconnected with EC or turbidity. As species are pooled from multiple samples with similar levels of EC or turbidity, an increasingly complete list of species present at this level of EC or turbidity is obtained.

RSR is calculated by first randomly selecting pooled sample sets (PSS), or collections of pooled samples without replacement, within the EC/turbidity categories. In this paper each PSS consisted of 19 samples. This number was selected as it was the largest number which allowed for at least one pooled sample set in most EC and turbidity categories and in both habitats (Table 1).

Then Jaccard's Index (JI) was calculated between all pairs of the pooled sample set.

$$JI = j / (a + b - j),$$

where JI is Jaccard's Index, j is the number of (joint) species recorded in both pooled sample set A and B, a is the total number of species in pooled sample set A, and b is the total number of species in pooled sample set B. JI is thus the proportion of species occurring in two pooled sample sets. Statistical differences in JI between the categories were determined by analysis of similarity (ANOSIM) using Primer 6.16 (www.primer-e.com). Next, the mean value of JI was estimated between all categories.

RSR is calculated by arranging 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 $j_{x,x}$ is the mean JIs within category x. The RSR between contamination categories x and y is $j_{x,y}/j_{x,x}$. RSR does not indicate whether a species is lost or gained as contamination increases, it only indicates the relative change in the community. Further details on this method are given in Kefford et al. (2010a).

2.5. Comparing traits and RSR

Given the common management target in risk assessment of protecting 95% of species from effects of chemical contamination

(Kefford et al., 2005), a loss of RSR≤0.95 (i.e. a ≥5% change in species composition) was used as threshold. We examined whether a scenario with RSR≤0.95 would lead to changes in the trait composition i.e. change in the mean values of trait modalities. Similar to the rationale for species loss, we regarded a change in the mean value of a trait (hereafter trait retention) by ≥5% as unacceptable. We chose to compare RSR and trait retention in categories as this is common for the management of chemicals in the environment (e.g. chemical concentrations passing or failing guideline values).

For each modality where its mean value had a systematic pattern between the EC or turbidity categories triangle matrixes of the proportion change in the trait between each category were constructed. These matrixes where compared to their corresponding triangle matrix of RSR. All category pairs in the matrixes were classified into four outcomes. (1) Where both RSR>0.95 and trait retention was<5% they were designated 'no unacceptable effect' as both the trait and community structure are protected. (2) Where RSR≤0.95 and trait retention≥5% and thus would be identified by the RSR cases were thus designated 'unacceptable effect detected'. (3) Where RSR≤0.95 and trait retention<5%, then the trait modality would be over-protected and thus these cases were designated 'over-protected'. (4) Finally where RSR>0.95 and trait retention≥5%, RSR would not protect the trait modality and such cases were designated 'under-protected'.

3. Results

3.1. Patterns in traits between EC categories

Several traits differed between the EC categories in either or both habitats (Table 3, Supplementary Tables S1–S2). The most notable was the proportion of predator species in the pool habitat samples which increased from 17% of species at salinities <0.05 mS/cm to 57% of species at 35.5 mS/cm. There was a corresponding decrease in the proportions of grazer and shredder species with increasing salinity in the pool habitat.

In contrast in the riffle habitat over the range <0.05–6.9 mS/cm (see Table 1), there was no statistically significant difference in the proportion of predator species per riffle sample. For riffle samples the variation was relatively small (11 to 18%) compared to the pool habitat (17%–39% over the same salinity range). Although not amenable to statistical analysis the two riffle samples at 10–14.9 mS/cm had more predator species (60%) than riffle samples at lower salinity (see Supplementary Table S2). This means that if the proportion of predator species increases with increasing salinity in the riffle habitat, this increase occurs only after reaching a much higher salinity than in the pool habitat.

Shredders declined from 14% of species in the riffle habitat at <0.05 mS/cm to 7–11% at >0.3 mS/cm (Table 3). The proportion of filterers increased from ≈8% of species in riffle samples at <0.09 mS/cm to 10–12% at higher salinities.

In both habitats the mean value of a generic sensitivity to organic chemicals, S_{org} (Von der Ohe and Liess, 2004), declined with increasing EC category indicating that species present at higher salinities tend to be less sensitive to organic chemicals than those species at lower salinities (Table 3).

In both habitats the proportion of species with limited dispersal ability declined (from 87% to 81%, for pool and riffle habitats, respectively, of species at <0.05 mS/cm) to an asymptote at ≈0.5 mS/cm (where 63–73% and 59–62%, respectively of species had limited dispersal). There was no evidence of any systematic differences in the score based on generation time between the EC categories in either habitat.

The size class distribution in the pool habitat followed an approximately U-shape with respect to salinity with large species being more common at both low (<0.2 mS/cm) and high (>7 mS/cm) salinity relative to intermediate salinities (Table 3). In the riffle there was a similar trend with fewer large species at salinity <0.2 than at

Table 3

Summary of patterns in mean values of trait modality with increasing electrical conductivity (EC) and turbidity categories. See Table 2 for definitions of the trait modalities. Mean trait values, standard errors and statistical differences between all categories are given in Supplementary information Tables S1–S4.

Modality	EC categories		Turbidity categories	
	Pool	Riffle	Pool	Riffle
Predator	Increase[a]	None but see text	Threshold Increase	None
Detritivore	Irregular	Outlier	None	Threshold increase
Grazer	Threshold decline	Outlier	Threshold decline	Outlier
Shredder	Irregular	Decline to asymptote	None	None
Filterer	Irregular	Increase to asymptote	Threshold decline	None
S_{org}	Decline	Decline	Irregular	None
Salinity assess	NA	NA	Decline	None
Dispersal assess	Decline to asymptote	Decline to asymptote	Decline	Threshold decline
Gen scores	None	None	None	Outlier
Size class	U-shaped	Decline to asymptote	None	Outlier
Air Breathers (Br)	Increase	None	Increase	Outlier
Water Br	Decline	None	Threshold decline	Outlier
Mixed Br	∩-shaped	Increase to asymptote	Outlier	Outlier
Eggs aquatic	None	None	None	None
Larva aquatic	None	None	None	None
Adults aquatic	Increase	Increase to asymptote	Threshold increase	Outlier

[a] Increase = an increase in the modality with increasing EC/turbidity category over the entire range of EC/turbidity; decline = a decline in the modality over the entire range of EC/turbidity; threshold = increase/decrease only occurs after some threshold is reached; asymptote = an initial increase/decrease then a maximum/minimum is reached; U-shaped indicate maximum values of trait occur at low and high EC/Turbidity categories; ∩-shaped = maximum values of trait occur at intermediate EC/turbidity values; none = no statistical significant difference between any pairs of categories; irregular = while statistical differences between categories no discernable pattern with EC/turbidity; and outlier = statistical significant difference between categories but caused by outlying categories.

0.2–6.9 mS/cm. A lack of riffle samples precluded an analysis at higher salinities.

In the pools there was an increase in the proportion of species that are air breathers (during all of their aquatic life-cycle) from 13% at <0.05 mS/cm to 43% at 10–14.9 mS/cm (Table 3). By contrast, no statistically significant differences were detected in the riffle habitat. In the pools there was a corresponding decline in the proportion of water breathers with increasing salinity (82% to 40%). The proportion of species in pools with mixed breathing (air in one stage of their aquatic life-cycle but water in another stage) increased from 5% at <0.1 mS/cm to mostly ≈10% across 0.2 to 1.0 mS/cm before falling to 6–7% of species at salinities >1.0 mS/cm. In the riffles a similar trend was observed, with <0.05 mS/cm having about 8% of species with mixed breathing and 12–15% of species having mixed breathing at higher salinities.

In both habitats there was no difference in the proportion of species with aquatic eggs or larvae between the EC categories. The proportion of species with aquatic adults was ≈9% in both habitats at <0.05 in both habitats. This proportion increased to >43% in the pools at >7 mS/cm and 15–30% in the riffles at salinities >0.3 mS/cm.

3.2. Patterns in traits between turbidity categories

In the pools proportion of predator species was mostly less than 25% at turbidities <6 NTU, whereas at higher turbidity categories from 6 to 80

NTU 26% to 43% of species were predators (Table 3, Supplementary Tables S3–S4). There was no change in the proportion of predators in the riffle habitat. In contrast, in riffles there was a tendency for a greater proportion of detritivores <5.9 NTU, but no change in pools. At turbidities <13 NTU grazers represented 13–17% of species in the pool habitat but at higher turbidity categories only 7–9% of species were grazers. There was no clear change in riffles. In neither habitat were there any changes in the proportions of shredders and filter feeding species.

In pools the proportion of salinity sensitive species tended to decline with increasing turbidity. There were, however, no systematic changes in S_{org} in either habitat or the proportion of salinity sensitive species in the riffle habitat.

The proportion of species with limited dispersal ability declined with increasing turbidity in both habitats. There were no systematic differences in the scores based on the generation time or size classes between the turbidity categories in either habitat.

None of the breathing modalities changed systematically between the turbidity categories in the riffle habitat. In contrast, in pools the proportion of air breathers increased with increasing turbidity from 14% at <0.05 NTU to 36% at 30–80 NTU. The proportion of species with water breathing declined, correspondingly. There were no systematic differences in the proportion of species with mixed breathing between the turbidity categories in either habitat.

In neither of the habitats did the proportions of species with aquatic eggs or larvae differ systematically between the turbidity categories. Nor were there any systematic differences in the proportion of species with aquatic adults in the riffle habitat. The proportion of species with aquatic adults was lower at turbidity <3 NTU (< 16%) than >10 NTU (>20%) in pools.

3.3. Changes in Jaccard's Index (JI) between the EC and turbidity categories

There were significant differences in JI between both EC and turbidity categories for both pool (EC: Global R = 0.690, P<0.0001; Turbidity: Global R = 0.114, P = 0.0011) and riffle (EC: Global R = 0.666, P<0.0001; Turbidity: Global R = 0.261, P = 0.0002) habitats. The higher R values for comparisons between EC categories than turbidity categories indicate relatively more consistent differences in the species composition in the PSS between EC categories than between turbidity categories.

3.4. Will the protection of community structure via RSR protect trait modalities?

The triangle matrixes of relative species retention (RSR) are given in supplementary Tables S5–S8. From these matrixes we determined whether preventing RSR≤0.95 would protect the mean value of trait modalities which changed systematically with EC or Turbidity (Table 3). As an example, we look at the proportion of predators in the pool habitat and the stress EC (Table 4). In 5 of a total of 77 comparisons (or 9.1%) preventing RSR from being ≤0.95 would fail to protect mean proportion of predators changing by ≥5% and thus the modality is under-protected. For another 8 comparisons (10.4%) the mean proportion of predator species would be over-protected. In 5 comparisons (6.5%) there is <5% change in the mean value of the modality and RSR>0.95 and thus there is no unacceptable effect (in terms of both RSR and the modality). In the remaining 57 comparisons (74.0%), although the modality changes by ≥5%, RSR is also ≤0.95 and thus unacceptable effects were detected.

Considering modalities, habitat and stressor combinations (where the mean value of a trait changed systematically with EC or turbidity (Table 3), a mean of 17.5% of comparisons resulted in a trait modality being under-protected (Table 5). However, for particular trait modalities up to 36.8% of comparisons could be under-protecting trait modality. All cases where >20% of comparisons indicated under-protection of trait modalities were for the stressor turbidity (Table 5). Considering only EC, then a mean of 9.5% of comparisons (range 0–15.6%) resulted in under-protection of the modality, which was less than that for turbidity with 23.4%. There were also lower proportions of under-protection of modality in the riffle habitat (means 5.7% and 15.5% for EC and turbidity, respectively) than the pool habitat (means 11.1 and 25.6 for EC and turbidity, respectively).

4. Discussion

The protection of stream insect community structure from effects of salinity and turbidity would lead to under-protection of a trait modality in 17.5% (range 0%–36.8%) of cases. This under-protection is a consequence of the different scales at which change in community structure and traits was assessed in this study. In both species sensitivity distributions (SSDs) and the relative species retention (RSR) method (Kefford et al., 2010a) used here, community structure is protected not at sites but across larger spatial scales (Kefford et al., 2011; Kefford et al., 2010a). In contrast, ecosystem functions are typically assessed at the site scale. If the assumption holds that traits represent a link to ecosystem functions and services (de Bello et al., 2010), our findings suggest that SSDs and the RSR method will result in the under-protection of ecosystem functions and services in a minority of cases. Given that up to 36.8% of cases can be under-protected, we suggest that the potential level of under-protection is of practical significance. It is thus possible to have no, or less, change in community structure (e.g. as indicated by the RSR method), yet still have changes in the mean value of trait modalities, as occurred in a

Table 4
Level of protection of proportion of predator species in pool habitat between electrical conductivity (EC, in mS/cm) offered by relative species retention (RSR) with a 5% threshold on RSR and proportion of predators. Where NUE (no unacceptable effect) = less than a 5% trait (in this table predators) retention and RSR>0.95; UED (unacceptable effect detected) = although trait retention is >5%, RSR is also ≤95 and thus protecting community structure would protect the modality (proportion of predators); over = protection of the community structure (via RSR>0.95) would be over-protective of trait retention; and under = protection of the community structure (via RSR>0.95) would not be protective trait retention and it would be under-protective.

	<0.05	0.050–0.099	0.10–0.19	0.20–0.29	0.30–0.49	0.50–0.99	1.0–1.49	1.5–1.9	2.0–2.9	3.0–3.9	4.0–4.9
0.050–0.099	Under										
0.10–0.19	UED	Over									
0.20–0.29	UED	UED	Under								
0.30–0.49	UED	UED	Under	NUE							
0.50–0.99	UED	UED	UED	Under	UED						
1.0–1.49	UED	UED	UED	UED	UED	NUE					
1.5–1.9	UED	UED	UED	UED	UED	Over	Over				
2.0–2.9	UED	UED	UED	UED	UED	Over	Over	NUE			
3.0–3.9	UED	UED	UED	UED	UED	Over	Over	Over	NUE		
4.0–4.9	UED	UED	UED	UED	UED	UED	UED	UED	UED	UED	
5.0–6.9	UED	UED	UED	UED	UED	UED	UED	UED	UED	UED	NUE
7.0–9.9	UED	UED	UED	UED	UED	UED	UED	UED	Under	Under	Under

Table 5
Summary of the outcome for trait retention by preventing relative species retention (RSR) from being ≤0.95. EC = electrical conductivity, Turb = turbidity. Trait modalities are defined in Table 2, NUE = no unacceptable effect, UED = unacceptable effect detected, over-protective and under-protective are as per Table 4.

Stressor	Habitat	Modality	# of comparisons	% NUE	% UED	% over-protective	% under-protective
EC	Edge	Predator	77	6.5	74.0	10.4	9.1
EC	Edge	Grazer	77	3.9	77.9	6.5	11.7
EC	Edge	S$_{org}$	77	0.0	70.1	14.3	15.6
EC	Edge	Dispersal	77	6.5	62.3	22.1	9.1
EC	Edge	Size class	77	11.7	10.4	74.0	3.9
EC	Edge	Air Br	77	2.6	77.9	6.5	13.0
EC	Edge	Water Br	77	3.9	74.0	10.4	11.7
EC	Edge	Mixed Br	77	2.6	79.2	5.2	13.0
EC	Edge	Adult aq	77	2.6	77.9	6.5	13.0
EC	Riffle	Shredder	35	0.0	85.7	5.7	8.6
EC	Riffle	Filterer	35	0.0	88.6	2.9	8.6
EC	Riffle	S$_{org}$	35	2.9	74.3	17.1	5.7
EC	Riffle	Shredder	35	0.0	85.7	5.7	8.6
EC	Riffle	Filter	35	0.0	88.6	2.9	8.6
EC	Riffle	S$_{org}$	35	2.9	74.3	17.1	5.7
EC	Riffle	Dispersal	35	5.7	77.1	14.3	2.9
EC	Riffle	Size class	35	8.6	34.3	57.1	0.0
EC	Riffle	Mixed Br	35	0.0	85.7	5.7	8.6
EC	Riffle	Adult aq	35	2.9	88.6	2.9	5.7
Turb	Edge	Preditor	136	13.2	52.9	4.4	29.4
Turb	Edge	Grazer	136	11.0	48.5	8.8	31.6
Turb	Edge	Filter	136	5.9	54.4	2.9	36.8
Turb	Edge	Salinity	136	39.7	33.8	23.5	2.9
Turb	Edge	Dispersal	136	26.5	52.9	4.4	16.2
Turb	Edge	Air Br	136	8.1	56.6	0.7	34.6
Turb	Edge	Water Br	136	23.5	53.7	3.7	19.1
Turb	Edge	Adult aq	136	8.1	56.6	0.7	34.6
Turb	Riffle	Detritivore	119	17.6	52.1	14.3	16.0
Turb	Riffle	Dispersal	119	18.5	50.4	16.0	15.1
EC	Edge	Overall	693	4.5	67.1	17.3	11.1
EC	Riffle	Overall	350	2.3	78.3	13.1	6.3
Turb	Edge	Overall	1088	17.0	51.2	6.2	25.6
Turb	Riffle	Overall	238	18.1	51.3	15.1	15.5
EC	Overall	Overall	1043	3.7	70.9	15.9	9.5
Turb	Overall	Overall	1462	17.8	50.8	8.1	23.4
Overall	Edge	Overall	1781	12.1	57.4	10.5	20.0
Overall	Riffle	Overall	588	8.7	67.3	13.9	10.0
Overall	Overall	Overall	2369	11.3	59.9	11.4	17.5

mean of 17.5% of comparisons. The implication of this study is that while SSDs and RSR might protect community structure at large spatial scales from environmental contaminants, they do not necessary protect trait modality (and thus ecosystem functions and services) at smaller scales. However, the results depend on the type and number of traits and their modalities used in the analysis. Here we used 8 traits with 24 modalities (Table 2), which is comparable to other studies e.g. 8 traits and 34 modalities (Piscart et al., 2006).

There was a greater discrepancy between the protection of trait modalities and RSR for the stressor turbidity relative to salinity (EC). Furthermore, turbidity had a poorer relationship to community structure in comparison to salinity (ANOSIM global R of 0.11–0.26 and 0.67–0.69, respectively). Previous studies (Marchant et al., 1997; 1999; 2006) in the region studied (Victoria) also found that EC better explained stream invertebrate community structure than did turbidity. There were also fewer trait modalities which were associated with the turbidity categories than the EC categories (Table 3). The poor relationship between turbidity and direct stress on aquatic insect species may explain the relatively poor relationships between turbidity with both community structure and trait modalities, relative to EC. Direct effects of sedimentation on invertebrates are probably the result of burial by sediment (Kefford et al., 2010b) and the infilling of spaces between rocks (Waters, 1995) and not from suspended sediments in the water column. To sum up, the discrepancy between the two protection goals for turbidity and salinity is probably a

consequence of a weaker ecological link between turbidity and community structure compared to salinity.

We had previously formulated hypotheses regarding changes in trait modalities with increasing salinity. Table 1 of Schäfer et al. (2011a) suggested that predators may have more energy available for osmoregulation. Yet we observed that the proportion of predators increased only in the pool habitat. Coleopterans found in streams are predominantly predators. In Victoria the number of species of slow water Coleoptera (defined as Dystiscidae, Hydrophilidae, Gyrinidae, Hydraenidae, Staphylinidae, Haliplidae, Hydrochidae and Hygrobiidae) increases with increasing salinity in the pool habitat and not the riffle habitat (Marchant et al., 2006). While running water Coleoptera (Elmidae, Scirtidae, Psephenidae, Ptilodactylidae) species richness was uncorrelated with salinity in both habitats (Marchant et al., 2006). Experimental studies on the salinity tolerance of Australian macroinvertebrates have species collected from the pool habitat (Kefford et al., 2003; 2006a; Dunlop et al., 2008), and there is a need to study the salinity tolerance of riffle species too.

Eggs and hatchings of most stream macroinvertebrates are more salt sensitive than their older aquatic life-stages (Kefford et al., 2004; Kefford et al., 2007) and thus we hypothesised that the proportion of species with terrestrial eggs may increase with increasing salinity (Schäfer et al., 2011a). But we observed no difference in the proportion of species with aquatic eggs over the salinity categories in either habitat. Most species of stream insects had aquatic eggs (96% and 98% in pools and riffles, respectively) and there may not have been enough variation in this trait for it to respond to salinity.

Respiratory organs in an aquatic environment may place higher osmoregulatory stress and thus the proportion of species air breathing may increase with increasing salinity (Schäfer et al., 2011a). Again, we only observed an increase of air breathers in the pool habitat. It is noteworthy that the proportion of air breathing species also increased with increasing turbidity in the pool habitat but not in the riffle habitat. This difference between habitats most likely reflects that returning to the surface to obtain air is difficult in the faster and/or more turbulent water of riffles thus restricting the proportion of air breathing species in this habitat.

Although our previous hypotheses were either not supported by this study or were only supported in one habitat, Schäfer et al. (2011a) found that species which met all three of the following criteria (a) predators, (b) laid eggs out of the water and (c) air breathers tend to be more at risk by salinity than other species. These traits, better predicted species at risk of salinity in the pool habitat than the riffle habitat (see Supplementary Table S3 of Schäfer et al. (2011a)). This is consistent with the current study where the proportion of predators and air breathers only increased in the pool habitat.

The proportion of filter feeding species increased to an asymptote with increasing EC in riffles but not in pools. This is likely due to the slower flowing water of the pool habitat not being well suited for this feeding modality. However, the reasons for the different responses of other (Table 3) trait modalities between habitats are unclear.

There were a few trait modalities which changed with salinity or turbidity consistently in both habitats (Table 3). For example, the generic measure of sensitivity to organic toxicants S$_{org}$ (Von der Ohe and Liess, 2004) and the proportion of species with limited dispersal declined with increasing salinity in both habitats. These changes are possibly because salinity in Victoria is associated with historical vegetation clearing for agriculture and grazing. Thus sites with high salinity will be in regions with intensive agriculture or grazing and thus pesticide use. So sites with high salinity may tend to have a history of pesticide exposure (Burgert et al., in press; Schäfer et al., 2011a). Moreover, toxicologically significant pesticide exposures in streams tend to occur in pulses and the dispersal ability of species may affect the recovery of species' populations following such pulses (Liess and Von der Ohe, 2005). Studies in both Europe (Schäfer et al., 2007) and Victoria (Schäfer et al., 2011a; 2011b; Burgert et al., in press) have

shown that the populations of species sensitive to organic toxicants, with limited dispersal tend to be more sensitive to pesticides. However, other explanations are possible; including salinity tolerant taxa tend to have particular trait modalities that correlate with tolerance to organic toxicants (Poff et al., 2006).

Piscart et al. (2006) is the only study to compare traits at sites of different salinities. They compared the mean proportion of trait modalities in the Meurthe River, France (in composite samples from across various habitats) at four sites along a salinity gradient (0.28–3.4 mS/cm). Differences in the definition of traits make comparisons of the current study and that of Piscart et al. (2006) problematic, except for feeding groups. In the Meurthe River, there were no statistically detectable changes in the proportion of predators between four sites (Piscart et al., 2006). However, we found in the pool habitat that only the proportion of predator species increased. In the Meurthe River (Piscart et al., 2006) and the pool habitat in our study, the proportion of grazing species declined with increasing salinity. Shredders declined to an asymptote in the riffle habitat but not in the pool or in the Meurthe River. In the current study, the proportion of filters declined in only the riffle habitat, while in the Meurthe River their proportion was the greatest at an intermediate salinity (1.8 mS/cm). In summary, changes in proportion of feeding groups with salinity in our study differed between the two habitats and between Victoria and the Meurthe River. Given these differences, further comparisons of relationships between trait modalities and salinity from other regions are needed before general statements of their relationships can be made.

5. Conclusion

If protection of trait modalities, ecosystem functions or ecosystem services from adverse effects of stressors is a management goal, SSDs and/or the RSR method used here are not necessarily protective for all sites. In order to protect both community structure and trait modalities, separate risk assessments would be needed.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.scitotenv.2011.05.056.

References

Apitz SE. Conceptualizing the role of sediment in sustaining ecosystem services: Sediment-Ecosystem Regional Assessment (SEcoRA). Science of the Total Environment (this issue).

Bayly IAE, Williams WD. Inland waters and their ecology. Camberwell: Longman; 1973.

Burgert S, Schäfer RB, Foit K, Kattwinkel M, Metzeling L, McEwan R, Kefford BJ, Liess M. Modelling Aquatic Exposure and Effects of Pesticides – Application to Southeastern Australia. Science of the Total Environment 2011;409:2807–14.

Cairns Jr J, Pratt JR. On the relation between structural and functional analyses of ecosystems. Environmental Toxicology and Chemistry 1986;5:785–6.

Calow P. Ecology in ecotoxicology: some possible 'rules of thumb'. In: Baird DJ, Maltby L, Greig-Smith PW, Douben PET, editors. ECOtoxicology: ecological dimensions. London: Chapman & Hall; 1996. p. 5–12.

Chessman BC. Dietary studies of aquatic insects from two Victorian rivers. Australian Journal of Marine and Freshwater Research 1986;37:129–46.

de Bello F, Lavorel S, Dı̍az S, Harrington R, Cornelissen JHC, Bardgett RD, et al. Towards an assessment of multiple ecosystem processes and services via functional traits. Biodiversity and Conservation 2010;19:2873–93.

Dunlop JE, Horrigan N, McGregor G, Kefford BJ, Choy S, Prasad R. Effect of spatial variation on macroinvertebrate salinity tolerance in Eastern Australia: implications for derivation of ecosystem protection trigger values. Environmental Pollution 2008;151:621–30.

Forbes VE, Calow P. Extrapolation in ecological risk assessment: balancing pragmatism and precaution in chemical controls legislation. BioScience 2002a;52:249–57.

Forbes VE, Calow P. Species sensitivity distributions revisited: a critical appraisal. Human and Ecological Risk Assessment 2002b;8:473–92.

Furse MT, Moss D, Wright JF, Armitage PD. The influence of seasonal and taxonomic factors on the ordination and classification of running-water sites in Great Britain and on the prediction of their macro-invertebrate communities. Freshwater Biology 1984;14:257–80.

Gessner MO, Chauvet E. A case for using litter breakdown to assess functional stream integrity. Ecological Applications 2002;12:498–510.

Hassell KL, Kefford BJ, D. N.. Sub-lethal and chronic lethal salinity tolerance of three freshwater insects: Cloeon sp. and Centroptilum sp. (Ephemeroptera: Baetidae) and Chironomus sp. (Diptera: Chironomidae). Journal of Experimental Biology 2006;209: 4024–32.

Jones CG, Lawton JH, Shachak M. Organisms as ecosystem engineers. Oikos 1994;69: 373–86.

Kefford BJ, Nugegoda D. No evidence for a critical salinity thresholds for growth and reproduction of the freshwater snail Physa acuta. Environmental Pollution 2005;54: 755–65.

Kefford BJ, Papas PJ, Nugegoda D. Relative salinity tolerance of macroinvertebrates from the Barwon River, Victoria, Australia. Marine and Freshwater Research 2003;54: 755–65.

Kefford BJ, Dalton A, Palmer CG, Nugegoda D. The salinity tolerance of eggs and hatchlings of selected aquatic macroinvertebrates in south-east Australia and South Africa. Hydrobiologia 2004;517:179–92.

Kefford BJ, Palmer CG, Jooste S, Warne MSJ, Nugegoda D. What is it meant by '95% of species'? An argument for the inclusion of rapid tolerance testing. Human and Ecological Risk Assessment 2005;11:1025–46.

Kefford BJ, Nugegoda D, Metzeling L, Fields EJ. Validating species sensitivity distributions using salinity tolerance of riverine macroinvertebrates in the southern Murray–Darling Basin (Victoria, Australia). Canada Journal of Fisheries and Aquatic Science 2006a;63:1865–77.

Kefford BJ, Zalizniak L, Nugegoda D. Growth of the damselfly Ischnura heterosticta is better in saline water than freshwater. Environmental Pollution 2006b;141: 409–19.

Kefford BJ, Nugegoda D, Zalizniak L, Fields EF, Hassell KL. The salinity tolerance of freshwater macroinvertebrate eggs and hatchlings in comparison to their older life-stages. Aquatic Ecology 2007;41:335–48.

Kefford BJ, Schäfer RB, Liess M, Goonan P, Metzeling L, Nugegoda D. A similarity-index based method to estimate chemical concentration limits protective for ecological communities. Environmental Toxicology and Chemistry 2010a;29:2123–31.

Kefford BJ, Zalizniak L, Dunlop JE, Nugegoda D, Choy SC. How are macroinvertebrates of slow flowing lotic systems directly affected by suspended and deposited sediments? Environmental Pollution 2010b;158:543–50.

Kefford BJ, Marchant R, Schäfer RB, Metzeling L, Dunlop JE, Choy SC, et al. The definition of species richness used by species sensitivity distributions approximates observed effects of salinity on stream macroinvertebrates. Environmental Pollution 2011;159:302–10.

Lecerf A, Usseglio-Polatera P, Charcosset J-Y, Lambrigot D, Bracht B, Chauvet E. Assessment of functional integrity of eutrophic streams using litter breakdown and benthic macroinvertbrates. Archiv Fur Hydrobiologia 2006;165:105–26.

Liess M, Von der Ohe PC. Analyzing effects of pesticides on invertebrate communities in streams. Environmental Toxicology and Chemistry 2005;24:954–65.

Marchant R, Hirst A, Norris RH, Butcher R, Metzeling L, Tiller D. Classification and prediction of macroinvertebrate assemblages from running water in Victoria, Australia. Journal of the North American Bentholigcal Society 1997;16:664–81.

Marchant R, Hirst A, Norris R, Metzeling L. Classification of macroinvertebrate communities across drainage basins in Victoria, Australia: consequences of sampling on a broad spatial scale for predictive modelling. Freshwater Biology 1999;41:253–68.

Marchant R, Ryan D, Metzeling L. Regional and local species diversity patterns for lotic invertebrates across multiple drainage basins in Victoria. Marine and Freshwater Research 2006;57:675–84.

McGill BJ, Enquist BJ, Weiher E, Westoby M. Rebuilding community ecology from functional traits. Trends in Ecology & Evolution 2006;21:178185.

Merritt RW, Cummins KW, Berg MB. Introduction to the aquatic insects of North America fourth edition. Dubuque, IA: Kendall/Hunt Publishing Company; 2008.

Millenium Ecosystem Assessment. Ecosystems and human well-being: biodiversity synthesis. Washington, DC: World Resources Institute; 2005. p. 86.

Paine RT. Food web complexity and species diversity. The American Naturalist 1966;100:65–75.

Piscart C, Usseglio-Polatera P, Moreteau J-C, Beisel J-N. The role of salinity in the selection of biological traits of freshwater invertebrates. Archiv Fur Hydrobiologia 2006;166:185–98.

Poff NL, Olden JD, Vieira NKM, Finn DS, Simmons MP, Kondratieff BC. Functional trait niches of North American lotic insects: traits-based ecological applications in light of phylogenetic relationships. Journal of the North American Benthological Society 2006;25:730–55.

Posthuma L, Suter GW, Traas T. Species sensitivity distributions in ecotoxicology Boca Raton. CRC Press; 2001.

Pratt JR, Cairns Jr J. Ecotoxicology and the redundancy problem: understanding effects on community structure and function. In: Newman MC, Jagoe CH, editors. Ecotoxicology a hierarchical treatment. Boca Raton: Lewis Publishers; 1996. p. 347–70.

Schäfer RB, Caquet T, Siimes K, Mueller R, Lagadic L, Liess M. Effects of pesticides on community structure and ecosystem functions in agricultural streams of three

biogeographical regions in Europe. The Science of the Total Environment 2007;382: 272–85.

Schäfer RB, Kefford BJ, Metzeling L, Liess M, Burgert S, Marchant R, et al. A trait database of stream invertebrates for the ecological risk assessment of single and combined effects of salinity and pesticides in South-East Australia. Science of the Total Environment 2011a;409:2055–63.

Schäfer RB, Pettigrove V, Rose G, Allinson G, Wightwick A, von der Ohe PC, et al. Effects of pesticides monitored with three sampling methods in 24 sites on macroinvertebrates and microorganisms. Environmental Science and Technology 2011b;45: 1665–72.

Smith MJ, Kay WR, Edwards DHD, Papas PJ, et al. AusRivAs: using macroinvertebrates to assess ecological condition of rivers in Western Australia. Freshwater Biology 1999;41:269–82.

Tiller D, Metzeling L. Australia-wide assessment of river health: Victorian AusRivAS sampling and processing manual. Monitoring river heath initiative. Canberra and Melbourne: Commonwealth of Australia and Victoria Environment Protection Authority; 2002.

Turak E, Flack LK, Norris RH, Simpson J, Waddel N. Assessment of river condition at a large spatial scale using predictive models. Freshwater Biology 1999;41:283–93.

Vandewalle M, Bello Fd, Berg MP, Bolger T, Dole'dec S, Dubs F, et al. Functional traits as indicators of biodiversity response to land use changes across ecosystems and organisms. Biodiversity Conververation 2010;19:2921–47.

Von der Ohe PC, Liess M. Relative sensitivity distribution of aquatic invertebrates to organic and metal compounds. Environmental Toxicology and Chemistry 2004;23: 150–6.

Waters TF. Sediments in streams, sources, biological effects and control. Bethesda, Maryland: American Fisheries Society; 1995.

Wogram J, Liess M. Rank ordering of macroinvertebrate species sensitivity to toxic compounds by comparison with that of *Daphnia magna*. Bulletin of Environmental Contamination and Toxicology 2001;67:360–7.

Wood WE. Increasing salt in soil and streams following the destruction of native vegetation. Journal of the Royal Society of Western Australia 1924;10:35–47.

Wright JF, Armitage PD, Furse MT. Prediction of invertebrate communities using stream measurements. Regulated Rivers: Research & Management 1989;4:147–55.