



Is there an interaction of the effects of salinity and pesticides on the community structure of macroinvertebrates?

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HIGHLIGHTS

- We investigated effects of pesticides and salinity on macroinvertebrate communities.
- Both salinity and pesticides influenced community structure.
- Salinity discriminated on a higher taxonomic level than pesticides
- We found no interactions between salinity and pesticides.

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ABSTRACT

Salinization of freshwater ecosystems is a global problem affecting many regions worldwide and can co-occur with pesticides in agricultural regions. Given that both stressors are potent to affect macroinvertebrate communities, their effects could interact.

We investigated the effects of salinity and pesticides at 24 sites in an agricultural region of southern Victoria, South-East Australia. We used distance-based redundancy analysis to determine the influence of pesticides, salinity and other environmental variables on the composition of macroinvertebrate communities.

Salinity and pesticide toxicity had a statistically significant effect on communities as had the substrate composition and the percentage of pool and riffle sections in the sampled stream reaches. We did not find evidence for interactive effects between salinity and pesticides, i.e. the effect of one of these variables did not depend on the level of the other.

Nevertheless, our results show that salinization and exposure to pesticides can be major factors for the structure of macroinvertebrate communities in agricultural regions. Pesticide toxicity acted on a lower taxonomic level compared to salinity, potentially indicating evolutionary adaptation to salinity stress.

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1. Introduction

Macroinvertebrates play an important role in the functioning of freshwater ecosystems, for example they regulate rates of leaf litter decomposition (Graça, 2001) and nutrient cycling (Vanni, 2002). In addition, they are an important food source for fish (Wallace and Webster, 1996) and other animals living in or around the streams (Baxter et al., 2005). Hence they represent an important link in food webs.

In Australia salinization due to rising saline watertables is considered as one of the most serious environmental problems (Lovett et al., 2007) and increasing stream salinity may have adverse effects on macroinvertebrate communities (Metzeling, 1993). The problem of salinization is not restricted to Australia but occurs globally, including the Iberian Peninsula (Gallardo-Mayenco, 1994), the USA (Griffith et al., 2001) and Central Mexico (Sarma et al., 2002).

In agriculture, pesticides are used to increase agricultural productivity (Wilson and Tisdell, 2001), but can have adverse effects on non-agricultural systems, including macroinvertebrates in streams (Liess and von der Ohe, 2005; Schulz, 2004). Runoff during rainfall events and spray-drift are the main entry routes of pesticides from fields into surface waters (Schäfer et al., 2011c).

In agricultural landscapes, e.g. around Melbourne, Australia, salinization (Williams, 2001) and pesticide-exposure (Wightwick and Allinson, 2007) may occur concurrently and lead to interactive non-additive (i.e. antagonistic or synergistic) effects (Davies et al., 2004). A synergistic effect arises between two stressors when their joint effect is greater than the sum of the individual effects, whereas an antagonistic effect means that the joint effect is smaller than the sum of the individual effects.

Analyzing ecological communities is clearly a multivariate problem and complicated by simultaneously acting and correlated explanatory variables (Graham, 2003). Schäfer et al. (2011b) analyzed the effects of pesticides on macroinvertebrate communities using a trait-based

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indicator (SPEAR indicator for pesticides (Liess and von der Ohe, 2005)). They demonstrated that pesticides can lead to shifts in the proportion of sensitive species in communities. However, no study has examined how pesticides and salinity in combination affect stream macroinvertebrate communities. This is despite the likely common occurrence of these stressors and laboratory studies showing non-additive effects of organo-phosphate pesticides and salinity (Hall and Anderson, 1995).

Hence in this study we used multivariate techniques, to address the following questions:

- ▲ Both pesticide toxicity and salinity are expected to affect invertebrate communities, but is there an interaction between both stresses?
- ▲ How important are salinity and pesticide toxicity in comparison to other environmental variables in shaping the macroinvertebrate community composition?

2. Material and methods

2.1. General description of the dataset

The data from 24 sites investigated by Schäfer et al. (2011b, 2012) situated in a 120 km radius around Melbourne, Victoria, Australia were used here (see Supplement for coordinates). The sites covered gradients of both pesticide exposure and salinization. Pesticide toxicity was expressed in terms of Toxic Units (TU) with respect to *Daphnia magna* (concentration/EC₅₀, (Sprague, 1970)) and salinity in terms of electrical conductivity ($\mu\text{S}/\text{cm}$ at 25 °C, hereafter $\mu\text{S}/\text{cm}$).

Importantly, this dataset contained sites with both low salinity (<0.2 mS/cm) and pesticide toxicity (<0.001 TU_{D.magna}), low salinity but high pesticide toxicity (>0.01 TU_{D.magna}), high salinity (>1 mS/cm) and high pesticide toxicity and high salinity but low pesticide toxicity. Since no industrial facilities or waste-water treatment plants were located upstream of the sampling sites, any organic toxicants in surface waters most likely originated from agricultural pesticide use.

Macroinvertebrates and environmental variables other than pesticides (see below) were collected three times in: September (2008), November (2008) and February (2009), but in February three sites were not sampled (i.e. n = 21) due to inaccessibility and stream drying. Macroinvertebrates were sampled according to the rapid bioassessment method (EPA Victoria, 2003) which gives a semi-quantitative measure of the community: samples were taken from edge/pool (sweep sampling, 250 μm mesh size) and where present (= 6 sites) in riffle habitats (kick sampling, 500 μm mesh) over a reach of at least 10 m (per habitat), live picked on site (minimum 30 person-minutes per habitat) and identified in the laboratory. The abundance of each taxon was estimated as suggested for the German sampling protocol which is compliant with the Water Framework Directive (WFD) (Meier et al., 2006). If applicable, the samples from both pool and riffle habitats from each site were pooled for data analysis.

Twenty-four water quality and hydrological characteristics were measured in the field (see Table 1). The data consisted of 4 datasets: toxicity, water chemistry, habitat and macroinvertebrate abundance data (all raw data available in Supplement).

Exposure to 97 pesticides was assessed using three methods: grab water samples, sediment samples, and 2,2,4-trimethylpentane passive samplers (TRIMPS). All three methods were used on 4 to 6 occasions between September (2008) and February (2009). The maximum toxicity (in terms of TU) derived from all three sampling methods and sampling periods was used as measure for pesticide stress. Standard physico-chemical parameters were measured: nitrate, nitrite, ammonium, phosphate and dissolved oxygen concentrations, as well as temperature, pH, electric conductivity and turbidity. Measured and estimated stream characteristics included maximum and minimum widths, sampling reach wide current velocity, depth, proportion of pools and riffle and substrate composition as outlined in detail in the rapid bioassessment

Table 1
Summary of environmental variables.

	Variable	Unit	Range
Water variables	Temperature	°C	8.2–22.8
	pH	–	6.21–8.94
	Salinity ^a	$\mu\text{S}/\text{cm}$ (25 °C)	39.7–5530.0
	Dissolved oxygen	% sat.	4.0–141.2
	Ammonia ^a	mg/L	0–8.0
	Nitrite ^a	mg/L	0–2.7
	Nitrate ^a	mg/L	0–9.4
	Phosphate ^a	mg/L	0–40.0
	Turbidity ^a	NTU	1.2–33.1
	Depth ^b	m	0.05–1.00
Habitat variables	Width _{max} ^b	m	2–30
	Width _{min} ^b	m	1–17
	Pool	%	20–100
	Riffle ^b	%	0–80
	Bedrock	%	0–30
	Boulder (>25.6 cm)	%	0–30
	Cobble (6.4–26.5 cm) ^b	%	0–40
	Pebble (1.6–6.4 cm) ^b	%	0–30
	Gravel (0.2–1.6 cm) ^b	%	0–60
	Sand (0.06–0.2 cm)	%	0–70
	Clay (<0.06 cm) ^b	%	5–100
	Velocity ^a	cm/s	0.02–50.00
	Discharge ^a	L/s	0.2–2000.0
Toxicity variables	TU max	log TU _{D.magna}	–5.14 to –0.95

^a Variables log₁₀ transformed prior to analysis.

^b Variables excluded from analysis due to high correlation with other variables.

protocol (EPA Victoria, 2003). Discharge was estimated by multiplying depth with mean width and the spatially-weighted average current velocity (see Supplement).

2.2. Data analysis

Where concentrations of water chemistry variables were less than the limit of detection (LOD) the values were set to LOD/2 (Clarke, 1998). Skewed and wide spread environmental variables were log₁₀(x) transformed before analysis (Table 1).

For statistical analyses invertebrate data was aggregated (from mostly genus) to family level in order to have a consistent taxonomic resolution. Previous studies showed that similar results are found for family and lower level ordinations (Jones, 2008; Metzeling et al., 2006).

Variables measured at the same time and site may be collinear. Hierarchical variable clustering was used to identify and eliminate redundant variables from the dataset (Khattree and Naik, 2000). Variables with a strong correlation to other variables (Spearman's Rho > 0.7) were removed from the dataset, based on expert judgment.

We used distance-based redundancy analysis (db-RDA) (Legendre and Anderson, 1999; McArdle and Anderson, 2001) to examine the effects of environmental variables on macroinvertebrate communities after confirming a monotone or linear univariate response to salinity and pesticides for the majority of the 20 most abundant taxa. Db-RDA is a constrained ordination method (showing only the variation that can be explained by constraining variables), which allows the usage of every distance measure. Since 82% of the abundance data were zero entries, we used the Bray–Curtis dissimilarity. Abundance data was 4th root transformed prior to calculating dissimilarity to focus on composition rather than on abundance (Anderson et al., 2011). Forward selection of the explanatory variables was performed to find a parsimonious model and determine the most influential environmental variables. Two stopping criteria were used in forward selection: (1) permutation p-values (1000 permutations per step) and (2) adjusted R-squared of the global model as proposed by Blanchet et al. (2008).

For investigating the effects of salinity (as electrical conductivity), pesticide toxicity (as TU_{D. magna}) and their interaction we used manual model building with salinity, pesticide toxicity and their interaction as predictors. Permutation p-values were calculated for every sampling

event separately, because missing data in February ($n = 21$) did not allow for a permutation design taking temporal autocorrelation into account. Ordinations were made pooling all three sampling events and removing a temporal effect in order to show the effects.

R-Code and data, which were used for computations and graphics (R, version 2.14.2 on Linux, 64bit (R Development Core Team, 2012) and “vegan, version 2.0–3” (Oksanen et al., 2012)) are supplied in the Supplemental material, in order to reproduce our analysis (Barnes, 2010).

3. Results

3.1. Influential environmental variables

Forward selection revealed that salinity, pesticide toxicity, substratum and flow conditions were important factors shaping the invertebrate assemblages. Of the selected variables, velocity, discharge and %pool in the reach explained most of the variance. Water chemistry parameters other than salinity and pesticide toxicity showed no correlation to macroinvertebrate communities (Table 2).

Corixidae spp. were found only at sites with a high amount of pools, whereas Leptophlebiidae spp. were associated with fast flowing habitats. Hydropsychidae spp. were found at sites with riffles but medium velocity (Fig. 1).

3.2. Effects of salinity, toxicity and their interaction

Both salinity and pesticide toxicity had a statistically significant relationship with macroinvertebrate community structure. However, the interaction between both stressors was not statistically significant (Table 2).

Ceinidea spp. and Lymnaeidae spp. were most abundant at saline sites, whereas mayflies of the family Leptophlebiidae were sensitive to increasing salinity. Baetidae spp. and Simuliidae spp. reacted sensitive to pesticide pollution, whereas the snail species *Physa acuta* (Family Physidae) was not affected by increasing pesticide toxicity. Taxonomic groups like molluscs and crustaceans were salt tolerant, whereas mayflies, caddisflies and stoneflies were sensitive. Such discrimination between taxonomic groups was not apparent for pesticide toxicity because within a taxonomic group there were different tolerances towards pesticides: For example within the mayflies, Caenidae spp. were relatively tolerant to pesticides in contrast to Baetidae spp. but both were relatively sensitive to salinity (Fig. 2).

Table 2

Results of marginal permutation tests of db-RDA (1000 permutations). Bold values indicate statistically significant effects ($p < 0.05$).

Forward selection	F	p	cum ^a			
Pool %	4.49	0.001	0.039			
Discharge	3.58	0.001	0.095			
Sand	3.24	0.001	0.135			
Conductivity	3.17	0.001	0.179			
Velocity	3.05	0.001	0.216			
Boulder	1.86	0.015	0.239			
TU max	1.59	0.038	0.259			
Interaction	September		November	February		
	F	p	F	p	F	p
Conductivity	3.02	0.002	2.36	0.005	2.43	0.008
TU max	1.83	0.043	2.70	0.005	0.63	0.868
Conductivity x TU max	0.54	0.917	1.21	0.249	0.99	0.436

^a Cumulative proportion of explained variance.

4. Discussion

4.1. Salinity

Salinity is a major factor shaping macroinvertebrate communities and increasing salinity due to agriculture may adversely affect these communities. Db-RDA showed that salinity explained a high amount of variation in the community data (Table 2). Stream invertebrate communities have been shown in a number of other studies to respond to salinity (Kefford et al., 2010, 2011; Kefford, 1998; Metzeling et al., 2006).

Tolerance differences between major taxonomic groups were observed (Fig. 2): crustaceans and molluscs were tolerant and ephemeropterans were sensitive to increasing salinity. These results are partly supported by other studies showing that crustaceans are the most salt tolerant order (Berezina, 2003; Kefford et al., 2003; Piscart et al., 2005) and ephemeropterans the most sensitive order (Dunlop et al., 2008; Kefford et al., 2003, 2005, 2006, 2012; Short et al., 1991).

Hart et al. (1990) expected molluscs, especially pulmonate gastropods (Hart et al., 1991), like Lymnaeidae spp., to be sensitive to increasing salinities. In contrast to laboratory studies supporting this conjecture (Kefford et al., 2003), our results suggest that in the field this family reacts less sensitively than the other families. A possible explanation is the problem of extrapolation from laboratory tests to community effects in the field due to indirect effects (Seitz and Ratte, 1991).

As Kefford et al. (2004) pointed out laboratory tests of salinity tolerance reflect the maximum salinity a species can inhabit, which is in agreement with our findings. In the current study Baetidae spp. were one of the most sensitive families with none observed above 1000 $\mu\text{S}/\text{cm}$. This reflects the relatively low maximum field distribution (Kefford et al., 2004) and results of laboratory tests of Baetidae spp. reported elsewhere (Dunlop et al., 2008; Hassell et al., 2006; Kefford et al., 2003, 2005, 2006).

4.2. Pesticides

Pesticides affected macroinvertebrate communities, which has been shown in several other studies (Liess and von der Ohe, 2005; Schäfer et al., 2007; Schäfer et al., 2011d). But compared to salinity pesticide toxicity explained less of the observed variation (Table 2) and may therefore have less importance for shaping the communities, at least in the region studied. However it must be noted that the study took place during a drought in 2008/2009, with no precipitation in January and February 2009 (BOM, 2009). This could also influence our findings, since lower precipitation would likely reduce pesticide input due to reduced run-off from fields and in turn increase salinity due to evaporation.

In a mesocosm study Beketov et al. (2008) found that the baetid mayfly *Cloeon dipterum* and *Simulium latigonium* were the most affected species by the insecticide thiacloprid. Baetidae spp. and Simuliidae spp. were also among the most pesticide sensitive families in our study (Fig. 2). In a field study in Germany Berenzen et al. (2005) found that the abundance of *Radix ovata* (Family: Lymnaeidae) was positively correlated with increasing pesticide toxicity. We made similar observations in Australian streams.

Laboratory data (as compiled by von der Ohe and Liess (2004)) also suggest that molluscs are among the most tolerant taxa towards pesticides. Plecoptera spp. and Trichoptera spp. are considered being the most pesticide sensitive insects, which was also the case in the current study. Fig. 2 suggests that Calamoceratidae spp. and Austroperlidae spp. being exceptions, but these two families were found only occasionally. In laboratory acute toxicity tests Corixidae spp. and Baetidae spp. had a similar sensitivity to pesticides (von der Ohe and Liess, 2004). Daam et al. (2009) found Corixidae spp. being the most sensitive family using outdoor microcosms, but as they remark this may be a result of emigration rather than toxicity. Our field data suggests a higher tolerance of Corixidae spp. then predicted from this experimental data.

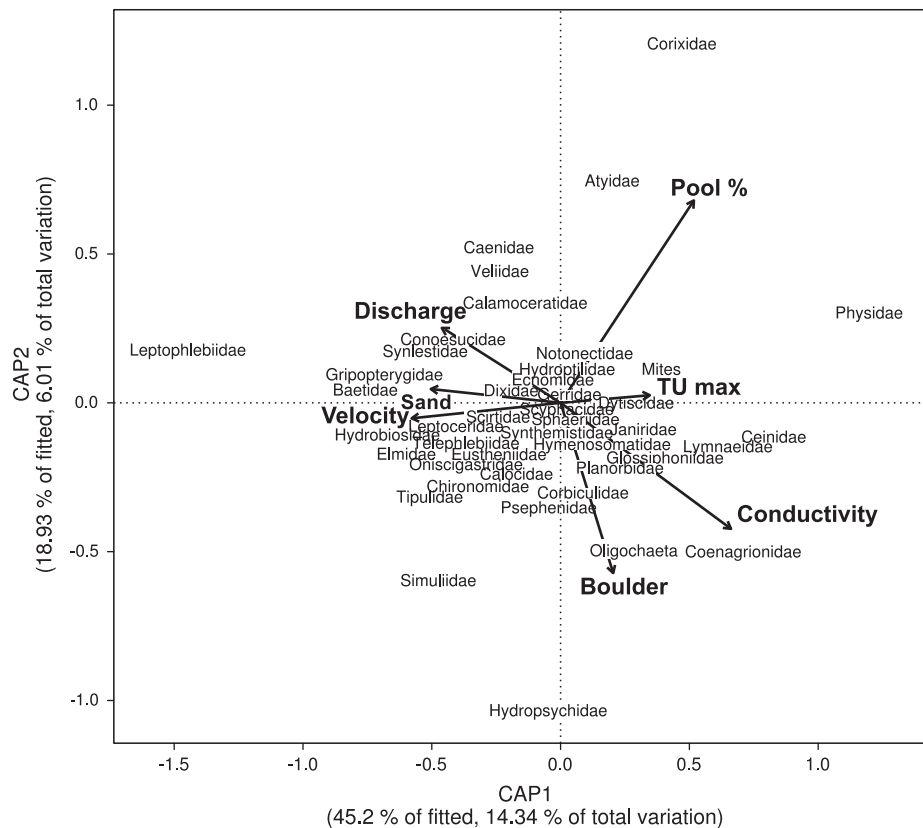


Fig. 1. Forward selection of environmental variables using distance-based redundancy analysis. Only the first two axes of the correlation biplot are shown, site scores not displayed for clarity, species scores displayed as weighted sums. Only families with a minimum abundance greater than 10 individuals are displayed. Ordination based on 4th root transformed abundance data and Bray–Curtis dissimilarity.

Corixidae spp. as air-breathing organisms (a) have a reduced toxicant uptake (Buchwalter et al., 2003) and (b) could avoid pesticide peaks on land, which may explain the observed differences between experimental and field data.

In contrast to salinity, pesticide toxicity acted on a lower taxonomic level. For example Ephemeroptera contained a broad range of pesticide sensitive and tolerant families but all were salinity sensitive. Likewise toxicity tests of stream macroinvertebrates with pesticides (von der Ohe and Liess, 2004) and salinity, showed that the sensitivity to salinity is less variable within orders than to pesticides (Kefford et al., 2012). Some authors argued that taxa with a more recent divergence from marine ancestors may be more tolerant to salinity (Hart et al., 1991; James et al., 2003). The fact that salinity discriminates on a high taxonomic level might be a legacy of these marine ancestors, with crustaceans and molluscs having near marine ancestors (Vermeij and Dudley, 2000). Salinity has been also naturally occurring in Australia and been acting on organism for a long time relative to organic pesticides which have only been used intensively after World War II (Schäfer et al., 2011d). The much shorter exposure period of communities can be an explanation why no adaption could evolve.

4.3. Other factors

Besides pesticides and salinity, factors describing the physical habitat (substratum, presence of pools, discharge and velocity) were identified as influential for macroinvertebrate communities. These local factors are partially interrelated and well known to shape the distribution of species (Costa and Melo, 2008; Minshall, 1984; Rempel et al., 2000). For example filter feeders like Hydropsychidae spp. and Simuliidae spp. were found at sites with riffles, rough substrate and medium velocity (Fig. 1), since they need some flow to gather their food. However the distinction of the taxa between riffles and pools was not

prominent. This may be due to riffles only being present in six sites and therefore data pooling of pool and riffle habitats. Hence, differences between pools and riffles were probably masked in this analysis. There was no correlation to other water chemistry variables. This can be explained as the study was designed to cover primarily a broad range of salinities and pesticide exposure with other gradients being as small as possible.

In other parts of the world such as Northern Europe lotic communities vary seasonally because of life histories of various species (Šporka et al., 2006). In Victoria, Australia, we did not find major differences between September, November and February – all common families were present in all samplings. This lack of seasonality may be attributed to the taxonomic resolution at family level and a higher taxonomic level could reveal a seasonal pattern (Marchant, 1990).

4.4. Salinity and pesticides combined

Our results show that salinization and exposure to pesticides can be important factors for the structure of macroinvertebrate communities in agricultural regions. We did not, however, find evidence for non-additive effects between salinity and pesticides on macroinvertebrate communities. That is the effect of salinity and pesticide combined is equal to the sum of both stressors.

There have been many laboratory studies investigating the combined effect of pesticides and salinity (Hall and Anderson, 1995). However there was no clear trend identifiable from these single species and single substance tests. Schäfer et al. (2011a) correlated biomonitoring data from the Australian River Assessment System (AUSRIVAS) program with pesticide-exposure estimated by a runoff-model (Burgert et al., 2011; Schriever and Liess, 2007). They likewise observed no interaction effect of salinity and estimated pesticide risk. Moreover, the sampling method could be another possible explanation for not detecting

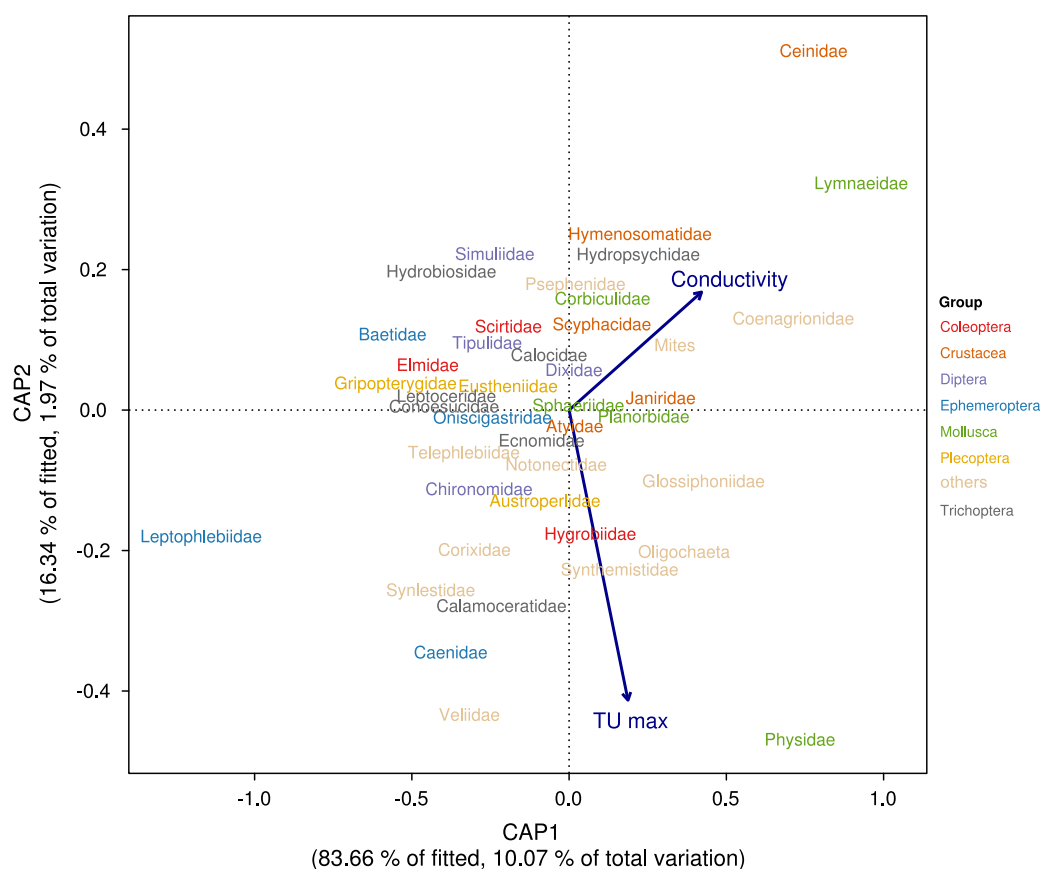


Fig. 2. Distance based redundancy analysis of salinity and pesticide toxicity, see Fig. 1 for details.

interactions between pesticides and salinity. The sampling was conducted only semi-quantitatively and the taxonomic resolution was homogenized at the family level. A quantitative sampling (i.e. surber sampling) with species level taxonomic resolution could reveal interaction effects.

Overall, controlled experiments like stream mesocosms isolating the two factors salinity and pesticides would be required for a deeper understanding of the underlying mechanisms.

5. Conclusions

Salinization and exposure to pesticides can be important factors for the structure of macroinvertebrate communities in agricultural regions. In the region and year studied, salinity was more important than pesticide toxicity for community composition. No interaction between salinity and pesticide toxicity was apparent: therefore we suggest no stronger effects of pesticides when used in salinization-prone regions.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2012.07.066>.

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