



Bachelor Thesis

Effects of salinity and pesticides on community structure of macroinvertebrates in Australian streams

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Declaration of authorship

I, Eduard Szöcs, hereby declare that this Bachelor thesis is entirely my own. Where I have consulted the work of others, this is always clearly stated. It has not been submitted for a degree at this or any other University.

Landau, 19.10.2011

Eduard Szöcs

Abstract

Salinisation of freshwater ecosystems is a global problem mainly affecting arid and semi-arid regions. Land-clearing associated with agriculture is a leading cause of salinisation in Australia. Furthermore, pesticides are potent to affect macroinvertebrate communities in agricultural streams.

We investigated the effect of both salinity and pesticides at 24 sites in an agricultural region of South-East Australia over a period of 5 months, also with respect to possible interactions between the stressors. Grab water samples, sediment samples, and 2,2,4-trimethylpentane passive samplers (TRIMPS) were used to determine the exposure to 97 pesticides. Macroinvertebrates and additional environmental variables were recorded thrice during the study period. The analysis of the effects of salinity and pesticides on the community structure of macroinvertebrates represents a multivariate problem. We used distance-based redundancy analysis to determine the influence of environmental variables on the composition of macroinvertebrate communities.

Salinity and pesticide toxicity had a statistically significant effect on communities. Moreover, the substrate of aquatic habitats and the percentage of pool and riffle sections in the sampled reach were identified as relevant variables for the community structure. Temporal variation in assemblages played a minor role.

Crustaceans and molluscs were tolerant to high salinity. Baetidae and Simuliidae were the families most sensitive to pesticides. We did not find evidence for interactive effects between salinity and pesticides.

Our results show that salinisation and exposure to pesticides can be major factors for the structure of macroinvertebrate communities in agricultural regions.

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Introduction

Macroinvertebrates play an important role in the function of freshwater ecosystems, for example they regulate rates of leaf litter decomposition (Graça, 2001) and nutrient cycling (Vanni, 2002). They are important food for running-water fish (Wallace and Webster, 1996) and other animals living in or around the water (Baxter et al., 2005; Burdon and Harding, 2008). Hence they represent an important link in food webs and between ecosystems.

The influence of various factors like hydrology (Chinnayakanahalli and Merrill-Cazier, 2010; Statzner and Higler, 1986), temperature (Haidekker and Hering, 2008), pesticides (Colville et al., 2008; Liess et al., 2005; Schulz, 2004) and nutrients (Gafner and Robinson, 2007) on macroinvertebrate communities have already been investigated.

In Australia salinisation due to rising saline watertables is considered as one of the most serious environmental problems (Kefford et al., 2002). There are two different types of salinity: *dryland salinity* and *irrigation salinity*. Dryland salinity is caused by removal of deep rooted plants, perennial trees, shrubs and grasses and their replacement by annual crops and pastures for agricultural purposes with a reduced plant water use which leads to a rise of saline groundwater tables. Irrigation salinity occurs when irrigation water leads to a rise of underground water table, bringing salt to the surface (Hart et al., 1991). Increasing salinity in streams may have adverse effects on macroinvertebrate communities (Kefford, 1998; Metzeling, 1993).

In agriculture pesticides are used to increase productivity (Wilson and Tisdell, 2001). Runoff during rainfall events or spray-drift are the main entry routes of pesticides from fields into surface waters (Schäfer et al., 2011c). It is widely accepted that pesticides can have adverse effects on macroinvertebrate communities (Colville et al., 2008; Liess et al., 2005; Schulz, 2004).

In arid and semi-arid agricultural landscapes, e.g. around Melbourne, Australia (Fig. 1.1), salinisation (Williams, 2001) and pesticide-exposure (Wightwick and Allinson, 2007) may occur together and lead to non-additive effects.

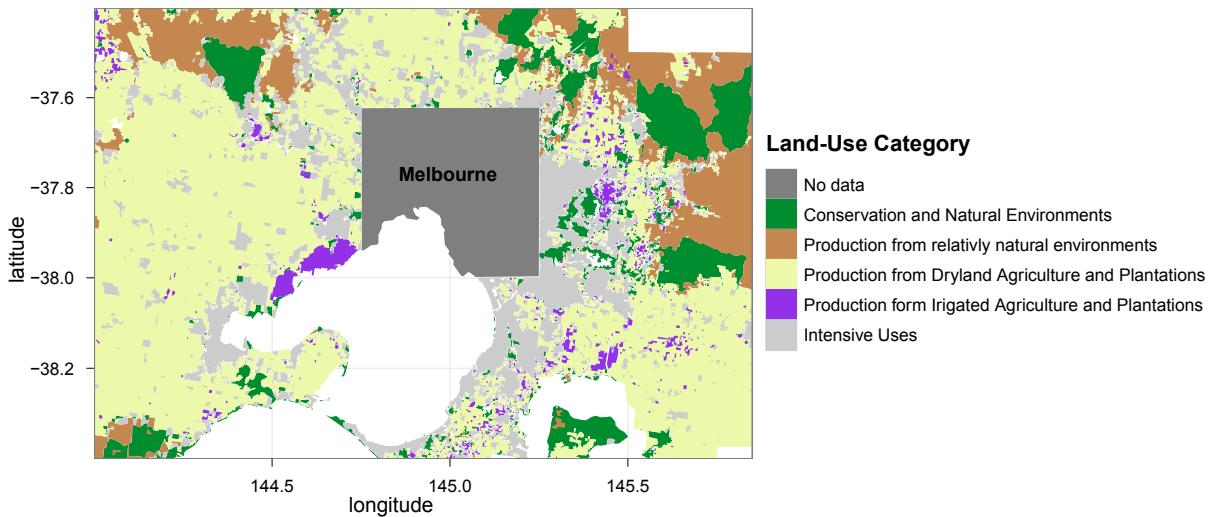


Figure 1.1: Land-use around Port Phillip, Victoria, Australia. Data from ARABES (2011).

Analyzing ecological communities is clearly a multivariate problem (McCune et al., 2002) and not an easy task, because of simultaneously acting and correlated variables (Graham, 2003).

Schäfer et al. (2011b) analyzed the effects of pesticides on macroinvertebrate communities using a trait-based indicator (SPECies At Risk indicator for pesticides (Liess and von der Ohe, 2005)).

In my thesis I will analyze the same dataset using multivariate techniques. The questions addressed by this thesis are:

- How important is temporal variation (between sampling times) compared to spatial variation (between sites)?
- What are important environmental variables determining the macroinvertebrate community composition?
- We expect that toxicity and salinity have an effect on community structure, but is there also an interaction between these two stressors?

Methods

2.1 General description of the dataset

Schäfer et al. (2011b) investigated 24 sites situated in a 120 km radius around Melbourne, Victoria, Australia (Fig. 2.1). Sites were chosen to cover a broad range of pesticide exposure. Since no industrial facilities or waste-water treatment plants were located upstream of the sampling sites, organic toxicants in surface waters most likely originate from agriculture pesticide use.

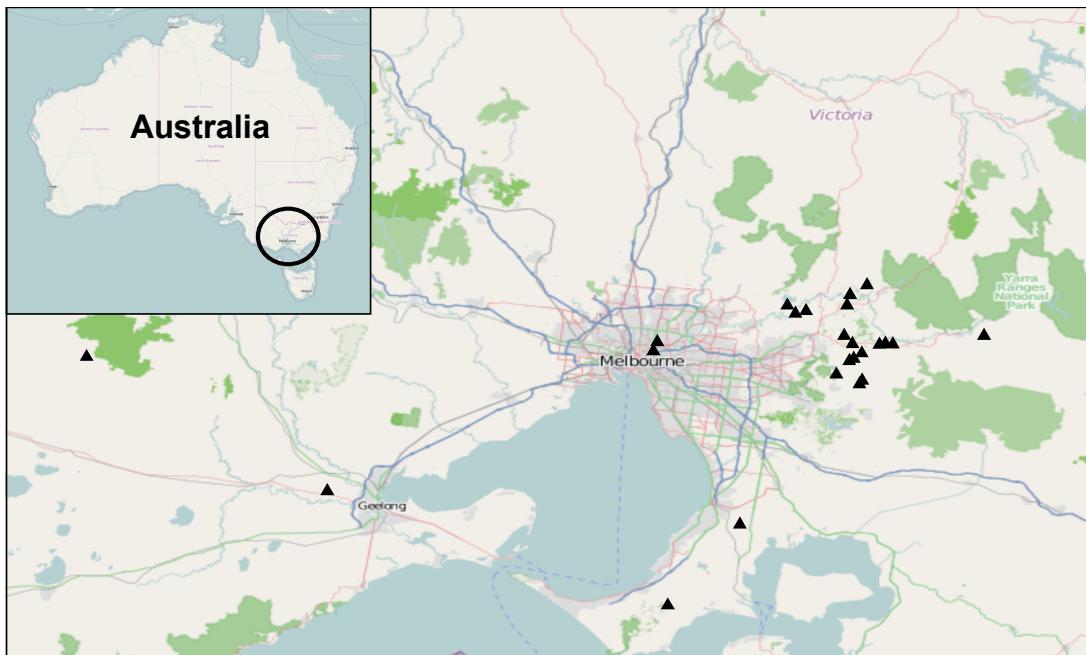


Figure 2.1: Location of the sampling sites (black triangles) around Melbourne in Victoria, Australia. Data from <http://www.openstreetmap.org/>, published under Creative Commons BY-SA 2.0

Several water quality and hydrological characteristics were measured in the field (see Tab. 2.2 and Appendix A.1). The whole available data consisted of 4 datasets: toxicity (Tab. A.1), water chemistry (Tab. A.2), habitat (Tab. A.3) and macroinvertebrate abundance data.

Exposure to 97 pesticides was assessed using three methods: Grab water samples, sediment samples, and 2,2,4-trimethylpentane passive samplers (TRIMPS). Toxicity

was expressed in terms of Toxic Units with reference to *Daphnia magna* (concentration / EC50, Sprague (1970)). The maximum toxicity per sampling was derived from all three sampling methods.

Standard physico-chemical parameters were measured: nitrate, nitrite, ammonium, phosphate and oxygen concentrations, as well as temperature, pH, electric conductivity, turbidity and alkalinity. Measured stream characteristics were: maximum and minimum width, depth, proportion of pools and riffles, and sediment constitution (7 groups: Bedrock, Boulder(>20 cm), Cobble (5 - 20 cm), Gravel (1 - 5 cm), Grit (0.1 - 1 cm), Sand (0.01 - 0.1 cm), Clay and Silt (<0.01 cm)).

Macroinvertebrates were collected using surber sampling. Invertebrates were sorted live in the field and identified to lowest possible taxonomic level. Data was collected three times: in September, November and February, but in February there was data missing for some sites ($n = 21$) due to inaccessibility and drying out of some sites. For further details see Schäfer et al. (2011b).

2.2 Data analysis

2.2.1 Data Manipulations

Missing values

The water chemistry dataset contained a few missing values (Tab. A.2). I did not discard the observations with incomplete data, because of information loss and shorter gradients. Therefore we imputed the missing values by predictions from multiple regression (Enders, 2010). For every variable containing missing values we fitted a regression model by R^2 and AIC-based backward model-selection (Faraway, 2006) and used the predicted values in further analysis.

Nearly 37 % of the alkalinity data was missing (Tab. A.2), thus we excluded this variable from analysis (Gelman and Hill, 2009). Because concentrations less than the limit of detection (LOD) were reported as zero, the data was left censored. I substituted the zero-values by LOD/2 prior to analysis (Clarke, 1998), enabling $\log_{10}(x)$ transformations and counteracting overestimation of zero values. Positively skewed and wide spread environmental variables were $\log_{10}(x)$ transformed (Tab. 2.2) (Sparks, 2000).

Invertebrate Data

For my analyses invertebrate data was aggregated to family level in order to have uniform level of taxonomic resolution. This reduced resolution (from genus to family) has only little effect on ordinations (Jones, 2008; Metzeling et al., 2006). Often rare taxa are discarded from analysis, but since this means a loss in information all taxa were retained (Faith and Norris, 1989).

Abundance data was recorded in 7 abundance classes, in a first step data was transformed into the mean of the abundance class, class 7 (>1000 individuals) was set to 1000 (Tab. 2.1). Then abundance data was aggregated to family level.

Table 2.1: Transformation of the raw abundance data

Abundance class	1	2	3	4	5	6	7
No. individuals	1 - 3	4 - 10	11 - 30	31 - 100	101 - 300	301 - 1000	>1000
Mean abundance class	2	7	21	66	201	606	1000

Variable selection

Variables measured at the same time and site may be redundant and 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 by expert judgement from the dataset. For example the proportion of pools and riffles added to 100% and therefore one variable can be left out without any information loss. The final variables used in my analysis are shown in table 2.2.

Distance measure

A crucial step in multivariate analysis is the choice of an appropriate distance measure. Since the species abundance data contained only 18 % non-zero entries, the Euclidean distance measure is inappropriate in this case (Legendre and Gallagher, 2001; Legendre and Legendre, 1998). The Bray-Curtis dissimilarity (Bray and Curtis, 1957) provides one of the ecologically most meaningful measures in community data (Clarke and Ainsworth, 1993) and was used in this study.

Table 2.2: Summary of environmental variables after variable selection included in the analysis.

	Variable name	Variable	Unit	Range
Water variables	T	Temperature	°C	8.2 - 22.8
	pH	pH	-	6.21 - 8.94
	Cond	Electric conductivity *	µS/cm	39.7 - 5530.0
	oxygen	Dissolved oxygen	% sat.	4.0 - 141.2
	NH4	Ammonia *	mg / L	0 - 8.0
	NO2	Nitrite *	mg / L	0 - 2.7
	NO3	Nitrate *	mg / L	0 - 9.4
	PO4	Phosphate *	mg / L	0 - 40.0
Habitat variables	Turb	Turbidity *	NTU	1.2 - 33.1
	Depth	Depth	m	0.05 - 1.00
	pool_perc	Pool	%	20 - 100
	Bedrock	Bedrock	%	0 - 30
	Boulder	Boulder (> 25.6 cm)	%	0 - 30
	Sand	Sand (0.06 - 0.2 cm)	%	0 - 70
Toxicity variables	Clay.silt	Clay/silt (< 0.06 cm)	%	5 - 100
	maxTU	Maximum Toxicity *	TU _{D. Magna}	-0.95 to -5.14

* variables log₁₀ - transformed prior to analysis

Abundance data was 4th root transformed prior calculating Bray-Curtis dissimilarity emphasizing more on composition than on abundance (Anderson et al., 2011) by reducing the influence of abundant taxa on the calculation of similarity (Clarke, 1993; Quinn and Keough, 2009).

The Bray-Curtis dissimilarity matrix on 4th root transformed abundance data was the basis for all statistical computations.

2.2.2 Multivariate Analysis

Relative importance of spatial and temporal effects

The relative importance of the 2 factors "time" (3 levels) and "site" (24 levels) was assessed by partitioning the total variation in the macroinvertebrate data using "Permutational Multivariate Analysis of Variance" (PERMANOVA, Anderson (2001a)). To visualize the relative sizes of variation the average abundances were calculated for (i) each time point and (ii) each site and the dissimilarities were visualized using non-metric multidimensional scaling (NMDS) (Kruskal, 1964; Shepard, 1962). Since NMDS makes fewer assumptions than many other multivariate ordination techniques (Faith et al., 1987)

and can handle any distance measure (Legendre and Legendre, 1998), it is considered to be one of the most robust ordination techniques (Clarke and Warwick, 2001; Fasham, 1977; Minchin, 1987). Ordinations with a stress value less than 0.2 were considered to be acceptable (Clarke and Warwick, 2001; Kruskal, 1964).

Determine important environmental variables influencing community structure

Distance-based redundancy analysis (db-RDA) (Legendre and Anderson, 1999; McAr-
dle and Anderson, 2001) combined with a forward variable selection was used to ex-
tract important variables shaping community composition. The method of db-RDA in-
cludes the following steps (Fig. 2.2) (Borcard et al., 2011; Legendre and Gallagher,
2001):

- Compute a dissimilarity matrix of the community data
- Run a Principle Coordinate Analysis (PCoA also known as "multidimensional
scaling" (MDS)) of the dissimilarity matrix
- Use RDA to analyze the relationship between the principle coordinates (repre-
senting species data) and explanatory variables

One drawback of this method is that species cannot directly be drawn into a bi- or triplot, since they were replaced during analysis by principle coordinates (Legendre and Gallagher, 2001). However species scores can be added as "weighted sums of the community matrix" (Oksanen et al., 2011).

A forward selection of explanatory variables was performed in order to find a parsimo-
nious model and determine the most influencing environmental variables. Two stopping
criteria were used in forward selection: (1) permutation p-values and (2) adjusted R-
squared of the global model as proposed by Blanchet et al. (2008).

Permutation p-values were calculated taking temporal autocorrelation into account.
This was done using restricted permutations: Since sites were measured repeatedly 3
times (September, November and February), samples within a site are exchangeable,
but not the sites themselves(Fig. 2.3) (Anderson, 2001b). Significance tests of con-
straining variables were performed using restricted permutations as described above.

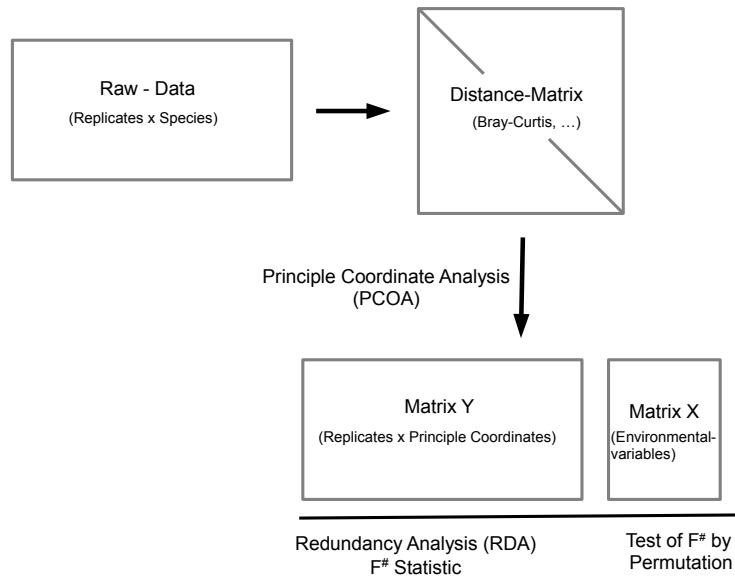


Figure 2.2: Principle of the distance-based Redundancy Analysis (db-RDA). Db-RDA allows to use every distance-measure and relates environmental variables linearly to principle coordinates. The "significance" of these relationships can be tested via permutations. Modified from (Legendre and Anderson, 1999).

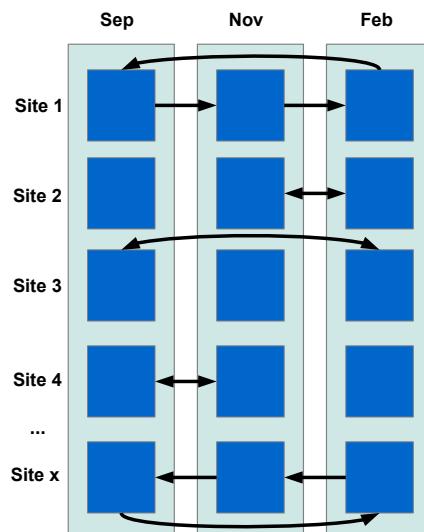


Figure 2.3: Permutation-scheme for restricted permutations (within sites) taking temporal autocorrelation into account.

Effects of salinity, toxicity and their interaction

For investigating the effects of salinity, toxicity and their interaction we used a more hypothesis driven approach than the forward-selection. We used manual model building with salinity, pesticide toxicity and their interaction. Significance tests of constraining variables were performed using restricted permutations as described above.

2.2.3 Software

All computations, graphics and maps were created with the open source software R, version 2.13.2 (on Windows 7, 64bit) (R Development Core Team, 2011) and supplemental packages (VEGAN (Oksanen et al., 2011), GGPLOT2 (Wickham, 2009), RESHAPE (Wickham, 2007), PLYR (Wickham, 2011), XTABLE (Dahl, 2009), MAPTOOLS (Lewin-Koh et al., 2011)) , RGOOGLEMAPS (Loecher, 2011) and a few additional functions defined by the author.

Results

3.1 Relative importance of spatial and temporal effects

Is spatial or temporal variation more important? Partitioning the variance clearly shows that the variation between sites was greater than the variation between the three sampling times (Tab. 3.1). The NMDS-plot of the main effect centroids reflects the results of PERMANOVA (Fig. 3.1). The most important factor is the spatial variation, whereby the centroids for different sampling times are much closer together, forming a cluster in the middle of the ordination diagram.

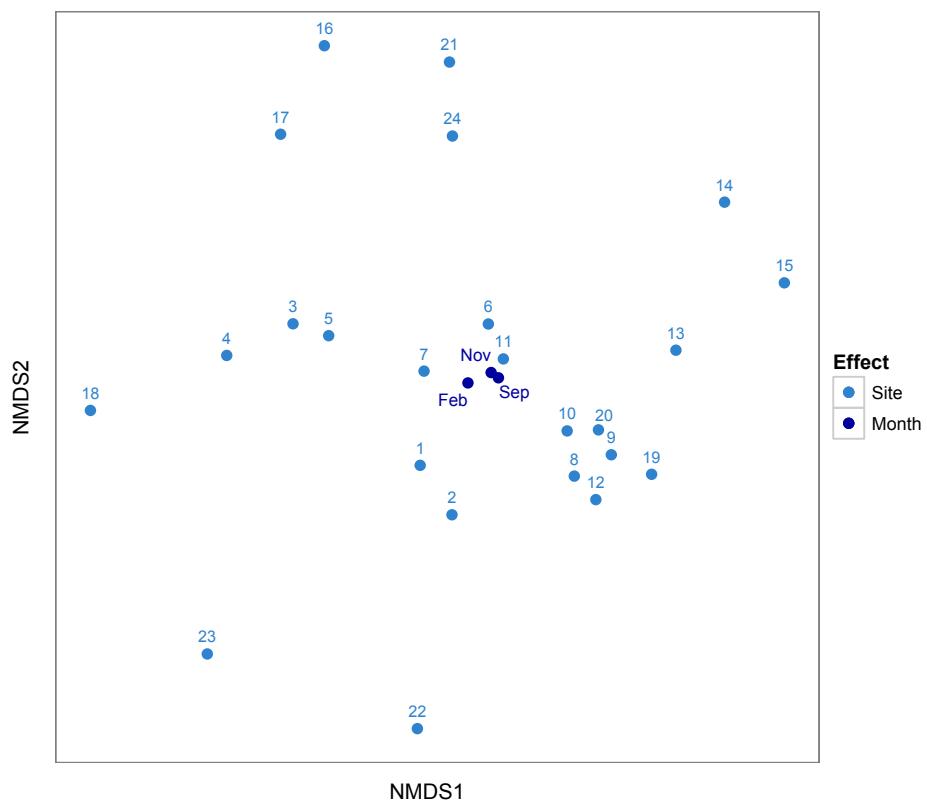


Figure 3.1: NMDS-Plot of effect centroids, based on all taxa. Bray-Curtis dissimilarity, fourth-root transformed abundance data. Stress = 0.15

Table 3.1: Partitioning spatial and temporal multivariate variation in macroinvertebrates by PER-MANOVA.

	df	SS	MS	F	p
Site	23	9.87	0.43	5.45	0.001
Month	2	0.41	0.20	2.59	0.002
Residuals	43	3.39	0.08		
Total	68	13.67			

3.2 Environmental variables determining the macroinvertebrate community

Forward selection revealed that salinity, pesticide toxicity, substratum as well as flow conditions are important factors shaping invertebrate assemblages (Tab. 3.2). The first axis of the db-RDA biplot (Fig. 3.2) can be interpreted as a "stressor gradient" of salinity and pesticides. Whereas the second axis describes a flow gradient (from pools to riffles and with it a change of substrate composition).

Fine substrate was correlated with slow flowing waters and communities at these sites where characterized by Veliidae and Corixidae (Subordo: Heteroptera). By contrast Hydropsychidae were found at sites with a coarser substratum and flowing water (Fig. 3.2). Water chemical parameters, except salinity, showed no correlation to macroinvertebrate communities. Salinity and pesticides have an influence on the communities, their effects will be described in the next section.

Table 3.2: Results of forward selection using permutations restricted to month. Displayed are the results of marginal permutation tests (1000 permutations). cum = Cumulative proportion of explained variance. Variables are described in Table 2.2

Variable	F	p	cum
Cond	5.61	0.001	0.07
pool perc	3.07	0.001	0.11
Clay.silt	2.87	0.001	0.15
Depth	1.95	0.006	0.17
Boulder	1.92	0.010	0.19
Bedrock	1.72	0.030	0.22
maxTU	1.57	0.046	0.24

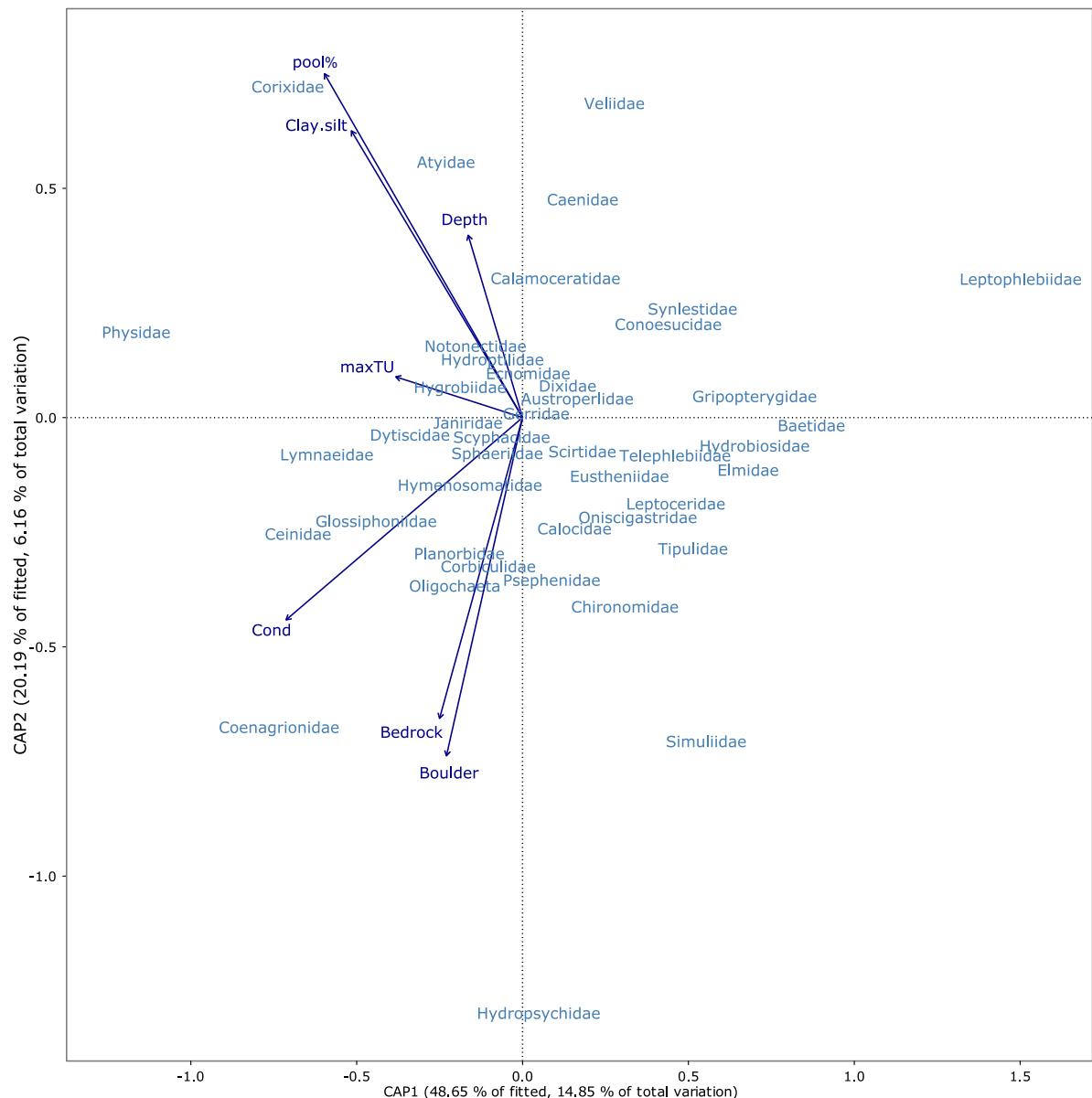


Figure 3.2: Forward selection of environmental variables using distance-based redundancy analysis on pooled data by restricted permutations. Only the first two axes of the correlation biplot are shown, site scores not displayed for clarity, species scores displayed as weighted sums. Only families with a minimum abundance greater than 10 individuals are displayed. Ordination based on 4th root transformed abundance data and Bray-Curtis-Dissimilarity.

3.3 Effects of salinity, toxicity and their interaction

Salinity and pesticide toxicity had a statistically significant relationship with macroinvertebrate community structure. The interaction between both stressors was statistically not significant (Tab. 3.3).

Ceinidea and Lymnaeidae were most abundant at saline sites, whereas mayflies of the family Leptophlebiidae were sensitive to increasing salinity. Baetidae and Simuliidae reacted sensitive to pesticide pollution, whereas the snails of the family Physidae were not affected by increasing pesticide toxicity.

Taxonomic groups like molluscs and crustaceans were salt tolerant, whereas mayflies and caddisflies were sensitive. Such a discrimination between taxonomic groups was not apparent for pesticide toxicity - within a taxonomic group there were different tolerances towards pesticides: For example within the mayflies, Ceinidae were relatively tolerant to pesticides in contrast to Baetidae, but both were relatively sensitive to salinity (Fig. 3.3).

Table 3.3: Results of permutation tests (1000 permutations) for the effects of salinity and pesticed toxicity on macroinvertebrate communities. The interaction between salinity and pesticide toxicity was statistically not significant (marginal test, $F = 1.021$, $p = 0.41$, 1000 permutations)

Variable	df	Var	F	p
Cond	1	1.356	6.519	0.001
maxTU	1	0.523	2.512	0.006
Residual	66	13.728		
interaction	1	0.212	1.021	0.41

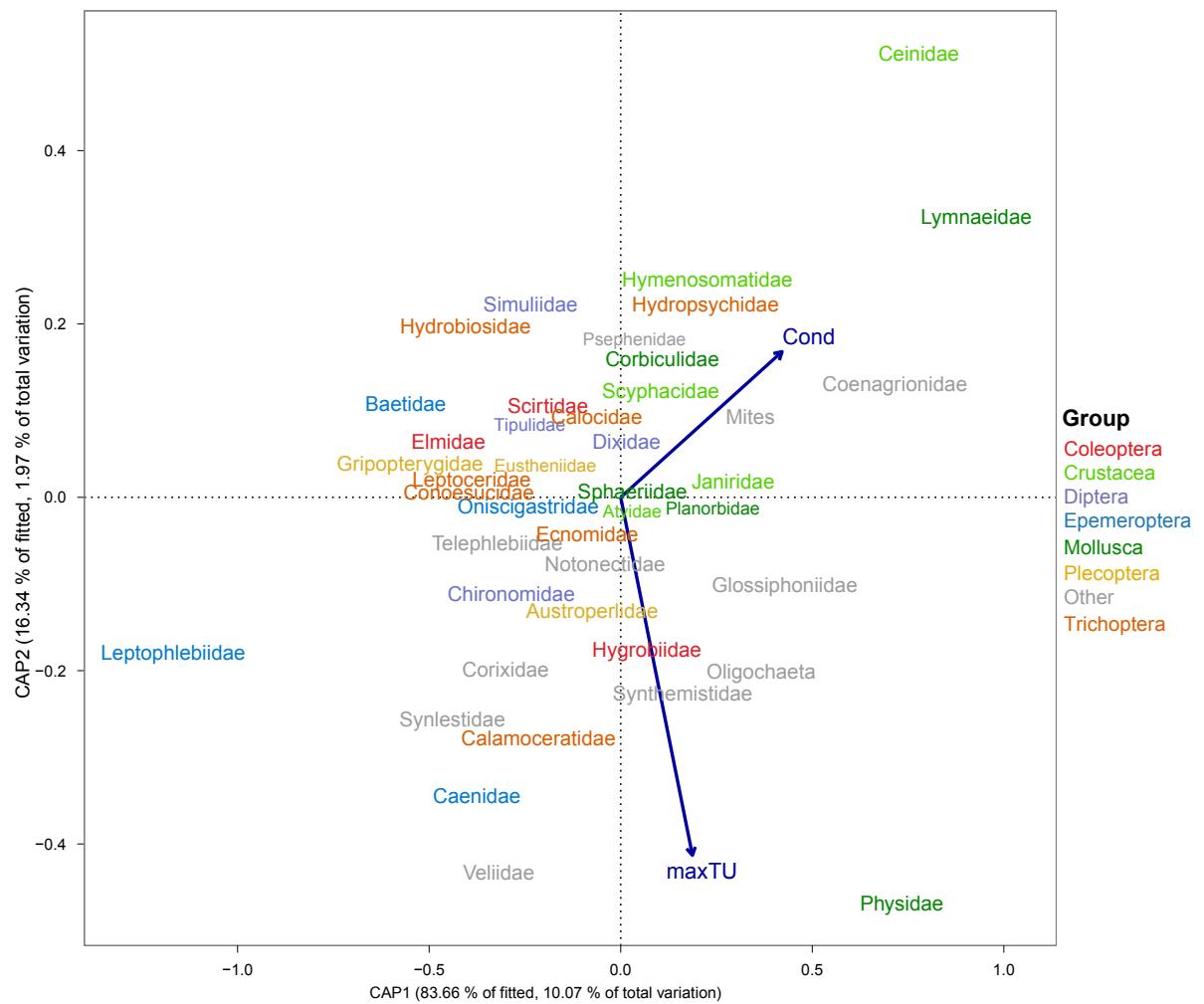


Figure 3.3: Distance based redundancy analysis of salinity and pesticide toxicity on pooled data using restricted permutations. Shown are only the first two axes of the correlation biplots, site scores not displayed for clarity, species scores displayed as weighted sums. Only families with a minimum abundance greater than 10 individuals are displayed. Ordination based on 4th root transformed abundance data and Bray-Curtis-Dissimilarity.

Discussion

4.1 Justification of db-RDA

As mentioned in the methods section, the euclidean distance is an inappropriate distance measure for zero-inflated abundance data. Therefore ordination techniques preserving euclidean distance like Principle Components Analysis (PCA) and Redundancy Analysis (RDA) are also inappropriate.

An alternative to PCA and RDA is Correspondence Analysis and its direct form Canonical Correspondence Analysis (CCA) (Ter Braak, 1986) which can handle data containing double-zeros and unimodal responses (Legendre and Legendre, 1998). But as Faith et al. (1987) pointed out the χ^2 - distance (which is preserved by CCA) is "one of the worst distances for community composition data" (Legendre and Gallagher, 2001). Constrained Gaussian ordination (CQO) (Yee, 2004) fits bell-shaped curves to species data and might be superior to CCA. There is no clear evidence for unimodal, symmetric responses to environmental gradients and their validity is open to debate (Oksanen and Minchin, 2002). Anyway, an unimodal response to pesticides is implausible.

The Mantel test (Mantel and Valand, 1970) could also be used to test correlation between two distance matrices, but db-RDA has a greater power (Legendre and Fortin, 2010). Another alternative represents to do a NMDS on the abundance data and afterwards fit an environmental vector onto the ordination plot. However this is an "exploratory, descriptive approach" (Borcard et al., 2011), since explanatory variables are not taken into account to produce the ordination (Legendre and Legendre, 1998). Pre-transformation of species data and afterwards running a RDA (transformation-based RDA) is also a possibility to analyze such data. As Legendre and Gallagher (2001) showed in their study "the horseshoe [using Chord or Hellinger transformations in combination with RDA] is not stronger than in the ordination obtained using the Bray-Curtis distance [in combination with PCoA]". Therefore db-RDA was chosen and used with the Bray-Curtis-Distance calculated on 4th root transformed abundance data to relate species data to environmental conditions.

Temporal variation is less important than spatial variation in shaping community structure (Tab. 3.1), which justifies the pooling of data from the three sampling dates and analyzing this data employing restricted permutations. The used permutation scheme (Fig. 2.3) is strictly speaking not assessing the right null-hypothesis, since we have three consecutive sampling dates. Therefore permutations must be done in the same temporal order (September - November - February), but by doing this there would be only 3 possible permutations left and the lowest possible p-value would be $p = 0.33$. Assumptions were relaxed in order to enable permutation tests: by restricting permutations to sites temporal autocorrelation within sites was respected, but not across sites. Given that the obtained p-values are very low (Tab. 3.3, much lower than the commonly used threshold of 0.05, it is reasonable to assume that we have statistically significant effects of pesticides and salinity.

4.2 Factors shaping macroinvertebrate communities

Salinity

Db-RDA showed that salinity explains the highest amount of variation in the community data (Tab. 3.2). Stream invertebrates have been shown in a number of other studies to be sensitive to salinity (Hart et al., 1991; Kefford, 1998; Metzeling, 1993; Metzeling et al., 2006; Nielsen et al., 2003).

Tolerance differences between major taxonomic groups were observed (Fig. 3.3): crustaceans and molluscs are tolerant and ephemeroptera are 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 ephemeroptera the most sensitive order (Kefford et al., 2003; Short et al., 1991).

Hart et al. (1990) expected molluscs, especially pulmonate gastropods (Hart et al., 1991), like Lymnaeidae, to be sensitive to increasing salinities. Kefford et al. (2003) reported experimental LC_{50} - Salinity values, where *Austropelea lessonii* (Family: Lymnaeidae) (LC_{50} - Salinity = 9 mS / cm) and *Austropelea tomentosa* (Family: Lymnaeidae) (LC_{50} - Salinity = 12.6 mS / cm) were within the most sensitive tested species. However our field data shows that they do not react very sensitive: Lymnaeidae and Physidae were found in high numbers at sites with highest measured salinities (Fig. 3.3).

Highest measured salinities were lower than these LC₅₀-Salinity values, but as Kefford et al. (2004) pointed out "laboratory measures of acute salinity tolerance reflect the maximum salinity that macroinvertebrates inhabit" which is not in conflict with our findings.

In the field Baetidae were one of the most sensitive families observed. At salinity levels above 1000 $\mu\text{S}/\text{cm}$ no Baetidae were found. This reflects the reported observed maximum field distribution (Kefford et al., 2004) and results of laboratory tests (Kefford et al., 2003).

Our results show that salinity is a major factor shaping macroinvertebrate communities and increasing salinity due to agriculture may adversely affect these communities.

Pesticides

It is widely accepted that pesticides can affect invertebrate communities (Berenzen et al., 2005; Liess et al., 2005; Liess and von der Ohe, 2005; Schäfer et al., 2007).

In a mesocosm study (Beketov et al., 2008) the baetid mayfly *Cloeon dipteron* (Family: Ephemeroptera) and *Simulium latigonum* (Family: Simuliidae) were the most affected species by the insecticide thiacloprid. Baetidae and Simuliidae were among the most pesticide sensitive families in our study (Fig. 3.3). 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 supports that molluscs are among the most tolerant taxa towards pesticides. Plecoptera and Trichoptera are considered being the most pesticide sensitive insects, which was also the case in this field study. Figure 3.3 suggests that Calamoceratidae and Austroperlidae being exceptions from this rule, but these two families were found only occasionally in the field. In laboratory acute toxicity tests Corixidae and Baetidae have a similar sensitivity to pesticides (von der Ohe and Liess, 2004). Daam et al. (2009) found Corixidae being the most sensitive family using outdoor microcosms, but as they remark this may be a result of emigration from lower dosed microcosms. Our field data suggests a higher tolerance than predicted from this experimental data. Corixidae as air-breathing organisms (a) have a reduced toxicant uptake (Buchwalter et al., 2003) and

(b) could avoid pesticides peaks on land, which would explain these differences between laboratory and field data.

In contrast to salinity, pesticide toxicity acts on a lower taxonomic level, for example Ephemeroptera containing a broad range of sensitive and tolerant species. Some authors argued that taxa with more recent divergence 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 this marine ancestors, crustacea and molluscs having near marine ancestors. Pesticides in contrast are acting on communities for a much shorter time period with changing composition, therefore no adaption could evolve.

Pesticides affect macroinvertebrate communities, but compared to salinity they explain less of the observed variation (Tab. 3.2 and 3.3) and may therefore have less importance.

Other factors

Besides pesticides and salinity, forward-selection identified stream depth as a factor influencing the communities. Depth was correlated with width, which was removed during variable selection and is an indicator for stream size. Larger streams harbor greater habitat diversity which facilitates a higher species diversity (Allan and Castillo, 2007).

Conditions in micro-habitats like pools and riffles support different invertebrate assemblages (Costa and Melo, 2008). For example in this study Hydropsychidae, like in other studies (McCulloch, 1986; Subramanian and Sivaramakrishnan, 2005), were correlated with a low amount of pools, since they are adapted to flowing waters. In contrast the lifestyle of semi-aquatic waterbugs like the Veliidae on the watersurface (Covich and Thorp, 2001), explains why they were found only in slow flowing sites.

It is well known that substratum is a primary factor influencing the occurrence of aquatic insects (Minshall, 1984): Taxa richness and abundance decrease with smaller particle sizes (Erman and Erman, 1984).

There was no correlation to other water chemistry variables. The sampling design was set up to cover a broad range of salinities and pesticide exposure and therefore other gradients were relatively small (Tab. 2.2).

It is well known that in Europe lotic communities vary seasonally because of life histories of various species (Šporka et al., 2006). We did not find big differences between September, November and February - all common families were present all the time. This indicates that seasonal variation plays a minor role in macroinvertebrate communities of Victoria, Australia.

Salinity and Pesticides combined

Our results show that salinisation and exposure to pesticides can be major factors for the structure of macroinvertebrate communities in agricultural regions. We did not find evidence for non-additive effects between salinity and pesticides on macroinvertebrate communities. Also Schäfer et al. (2011a) did not find an interaction of effects. Therefore we assume no strengthened effects of pesticides used in salinisation-prone regions. Salinity explains most of variation in communities followed by habitat variables, pesticide stress explains least variance in this study area. However controlled experiments like stream mesocosms isolating the two factors salinity and pesticides would be required for a deeper understanding of the underlying mechanisms.

Conclusions

Our results show that salinisation and exposure to pesticides can be major factors for the structure of macroinvertebrate communities in agricultural regions and may adversely affect these communities. Salinity was more important than pesticide toxicity for community composition. No interaction between salinity and pesticide toxicity was apparent, therefore we assume no strengthened effects of pesticides used in salinisation-prone regions. Further experimental studies are required to answer this question.

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Appendix

A.1 Raw data

Table A.1: Maximum pesticide concentrations displayed as log Toxic Units for *Daphnia magna*.

"-" : value less than LOD

Site	Month	Water	Sediment	TRIMPS	TRIMPSeq
1	2	-6.745	-6.745	-	-
1	9	-5.893	-5.893	-	-
1	11	-2.633	-2.633	-3.824	-3.463
2	2	-	-	-	-
2	9	-2.607	-2.607	-	-
2	11	-3.021	-3.021	-3.678	-5.069
3	2	-4.854	-4.854	-	-
3	9	-6.018	-6.018	-	-
3	11	-3.610	-3.610	-4.143	-
4	2	-1.577	-1.577	-	-
4	9	-5.371	-5.371	-	-
4	11	-2.977	-2.977	-	-
5	2	-	-	-	-
5	9	-	-	-	-
5	11	-3.608	-3.608	-3.891	-4.938
6	2	-3.453	-3.453	-	-
6	9	-6.071	-6.071	-	-
6	11	-3.231	-3.231	-4.507	-
7	2	-6.060	-6.060	-	-
7	9	-6.678	-6.678	-	-
7	11	-2.745	-2.745	-2.493	-3.443
8	9	-6.959	-6.959	-	-
8	11	-2.675	-2.675	-	-
9	2	-2.561	-2.561	-	-
9	9	-	-	-	-
9	11	-4.383	-4.383	-4.084	-
10	2	-4.534	-4.534	-	-
10	9	-4.321	-4.321	-	-
10	11	-4.893	-4.893	-4.128	-
11	2	-6.854	-6.854	-	-
11	9	-	-	-	-
11	11	-	-	-	-
12	2	-2.211	-2.211	-	-
12	9	-6.071	-6.071	-	-

Table A.1: Maximum pesticide concentrations displayed as log Toxic Units for *D. magna*. (cont.)

Site	Month	Water	Sediment	TRIMPS	TRIMPSeq
12	11	-2.233	-2.233	-3.706	-
13	2	-3.930	-3.930	-	-
13	9	-2.365	-2.365	-	-
13	11	-3.328	-3.328	-2.628	-5.200
14	2	-	-	-	-
14	9	-4.190	-4.190	-	-
14	11	-4.793	-4.793	-4.491	-
15	9	-	-	-	-
15	11	-	-	-	-
16	2	-5.975	-5.975	-	-
16	9	-5.510	-5.510	-	-
16	11	-4.100	-4.100	-4.606	-
17	2	-5.652	-5.652	-	-
17	9	-5.860	-5.860	-	-
17	11	-4.449	-4.449	-4.400	-
18	9	-6.495	-6.495	-	-
18	11	-1.833	-1.833	-1.045	-2.324
19	2	-2.896	-2.896	-	-
19	9	-	-	-	-
19	11	-3.753	-3.753	-4.335	-
20	2	-6.456	-6.456	-	-
20	9	-4.884	-4.884	-	-
20	11	-4.863	-4.863	-3.661	-
21	2	-3.220	-3.220	-	-
21	9	-5.479	-5.479	-	-
21	11	-3.034	-3.034	-	-
22	2	-5.824	-5.824	-	-
22	9	-	-	-	-
22	11	-4.616	-4.616	-	-
23	2	-	-	-	-
23	9	-2.627	-2.627	-	-
23	11	-1.889	-1.889	-5.137	-2.417
24	2	-5.383	-5.383	-	-
24	9	-5.656	-5.656	-	-
24	11	-4.139	-4.139	-	-7.097

Table A.2: Waterchemistry variables per site and month. Missing data is marked with "-".

Predicted values by regression imputation in parentheses

Site	Month	T	pH	EC (25°C)	Dissolved oxygen	NH4	NO2	NO3	PO4	Turbidity	Alkalinity
		(°C)	(µS/cm)	(% sat.)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(NTU)	(mmol)
1	2	19.1	7.81	114.5	63.0	0.22	0.00	1.60	22.90	6.0	2.0
1	9	12.0	7.23	90.0	70.0	0.00	0.00	0.33	3.50	6.5	-
1	11	16.8	7.67	95.2	80.1	0.62	0.00	0.20	3.20	15.2	1.3
2	2	16.7	7.18	73.0	74.8	0.40	0.00	0.00	0.26	4.8	3.0
2	9	11.7	6.43	75.0	62.0	2.60	0.00	0.00	25.00	7.9	-
2	11	16.5	7.29	69.5	83.0	0.40	0.00	0.00	0.80	9.5	1.0
3	2	18.0	7.54	1073.0	93.6	0.28	0.00	0.00	4.20	8.3	10.0
3	9	12.7	6.93	488.0	97.0	0.00	0.00	0.46	2.75	10.7	-
3	11	17.3	7.20	466.0	- (77.9)	0.24	0.00	0.00	2.40	15.7	2.1
4	2	17.0	7.24	1433.0	31.6	0.48	0.00	0.00	6.80	1.3	-
4	9	12.1	8.09	537.0	77.0	0.23	0.00	0.00	1.00	9.0	-
4	11	15.6	7.84	586.0	72.0	0.84	0.00	0.00	0.80	5.5	2.3
5	2	17.2	6.97	57.5	69.9	0.40	0.00	0.00	13.20	3.0	-
5	9	10.6	6.21	39.7	61.0	0.89	0.00	3.60	5.00	4.8	-
5	11	17.2	6.97	57.5	69.9	0.40	0.00	0.00	13.20	3.0	2.0
6	2	16.5	7.17	802.0	20.0	0.20	0.00	0.00	8.50	7.8	6.0
6	9	13.2	7.59	500.0	58.2	0.61	0.00	0.70	40.00	8.0	-
6	11	15.5	7.26	615.0	80.0	0.18	0.00	0.00	0.40	4.0	1.7
7	2	15.6	7.15	152.0	102.0	0.18	0.01	0.00	11.20	11.2	1.0
7	9	12.2	7.18	139.1	63.3	2.27	0.00	0.02	15.00	14.6	1.2
7	11	20.4	7.68	177.0	77.6	0.24	0.00	0.00	7.20	12.0	-
8	9	11.6	7.07	127.5	55.0	1.00	0.00	0.00	30.00	12.7	-
8	11	16.9	7.19	159.0	80.0	0.20	0.01	1.30	3.80	2.3	1.0
9	2	- (16.8)	7.02	124.5	84.2	0.67	0.00	0.00	0.90	17.0	1.4
9	9	11.9	6.64	101.0	60.6	0.55	0.00	0.00	10.00	18.8	-
9	11	16.8	7.38	100.0	75.4	0.00	0.00	0.38	0.00	20.8	2.0
10	2	14.7	7.27	174.0	62.0	0.39	0.00	0.00	2.60	24.9	2.0
10	9	11.5	6.21	720.0	61.3	0.08	0.06	0.00	30.00	11.7	-
10	11	17.0	6.44	725.0	88.4	0.25	0.00	1.90	11.00	11.7	0.8
11	2	16.4	7.50	159.5	65.0	0.32	0.00	1.00	23.20	10.8	2.0
11	9	11.7	6.89	138.0	56.6	0.67	0.00	0.00	3.00	13.6	-
11	11	15.2	7.13	138.0	96.0	0.59	0.00	0.00	3.20	10.4	1.5
12	2	19.1	7.52	47.7	84.3	0.22	0.00	1.00	11.00	2.6	1.0
12	9	11.7	6.58	50.4	65.0	1.24	1.80	0.01	2.14	3.7	-
12	11	15.4	6.92	43.3	96.0	0.27	0.00	1.50	30.00	33.1	0.7
13	2	18.7	8.03	64.7	89.6	0.26	0.00	1.60	3.40	6.1	1.0
13	9	11.5	6.91	76.5	63.6	0.84	2.70	0.00	2.50	13.6	-
13	11	15.5	7.05	76.5	90.0	0.64	0.00	0.00	1.80	7.6	1.0
14	2	17.1	7.65	65.7	87.0	0.43	0.01	1.90	0.30	6.0	5.0
14	9	10.0	6.40	56.5	73.0	0.87	0.01	1.50	0.56	6.2	-
14	11	13.2	6.25	44.9	99.0	0.16	0.00	0.00	1.30	1.8	1.0
15	9	8.2	6.42	47.2	66.8	0.00	0.69	1.90	0.20	4.9	-

Table A.2: Waterchemistry variables per site and month. (cont.)

Site	Month	T	pH	EC (25°C)	Dissolved oxygen (% sat.)	NH4 (mg/L)	NO2 (mg/L)	NO3 (mg/L)	PO4 (mg/L)	Turbidity (NTU)	Alkalinity (mmol)
		(°C)	(μ S/cm)								
15	11	12.0	7.07	512.0	108.7	0.43	0.00	0.00	0.10	2.2	1.0
16	2	20.5	6.47	2013.0	21.3	0.15	0.02	0.00	2.50	4.8	15.0
16	9	14.5	8.70	1030.0	96.2	0.14	0.01	0.00	3.00	3.3	-
16	11	22.8	8.94	471.0	141.2	0.36	0.00	0.00	25.60	5.1	2.0
17	2	17.9	7.92	578.0	94.6	0.33	0.00	0.00	3.60	2.2	1.5
17	9	14.2	8.72	1500.0	82.0	0.08	0.01	2.10	1.00	9.2	-
17	11	20.3	6.60	1704.0	60.0	0.16	0.18	0.00	1.60	2.6	15.0
18	9	13.5	7.34	302.0	68.0	0.00	0.00	1.20	4.00	12.6	-
18	11	16.8	7.07	296.0	75.0	0.34	0.01	0.40	4.60	11.9	0.9
19	2	15.0	7.14	135.0	53.0	0.42	0.02	0.00	0.00	8.6	1.0
19	9	12.3	6.71	138.0	60.2	0.00	0.02	0.00	25.00	15.6	-
19	11	17.0	7.33	134.6	85.7	0.27	0.00	0.00	2.40	7.1	1.5
20	2	14.6	7.12	188.0	76.0	0.41	0.02	0.00	5.70	20.2	3.0
20	9	12.3	6.71	138.0	60.8	4.49	0.00	0.60	20.00	10.7	-
20	11	16.5	7.30	144.6	88.5	0.13	0.00	0.70	0.60	9.5	1.0
21	2	16.3	7.45	3220.0	40.4	0.43	0.01	0.00	30.00	5.7	2.8
21	9	11.6	7.93	2750.0	84.0	3.06	0.00	1.20	0.75	4.9	-
21	11	18.0	8.11	3500.0	26.0	0.64	0.00	0.00	32.00	1.2	24.0
22	2	18.2	7.65	5530.0	48.1	0.30	0.00	0.00	0.10	1.3	17.0
22	9	10.9	6.40	1450.0	67.2	0.25	0.00	0.00	2.00	4.9	10.0
22	11	14.2	7.24	2660.0	104.8	0.03	0.00	0.70	25.40	6.2	2.0
23	2	18.9	7.12	3350.0	22.5	2.13	0.26	2.50	20.00	7.1	10.0
23	9	10.9	6.89	3080.0	39.7	3.13	0.00	9.40	20.00	24.2	-
23	11	14.7	6.90	4260.0	4.0	8.00	0.86	0.06	3.00	16.6	2.5
24	2	20.6	7.73	3250.0	41.1	0.50	0.01	2.00	0.10	8.1	9.0
24	9	15.0	8.26	1560.0	110.0	0.00	- (0.00)	0.40	- (0.00)	2.7	-
24	11	20.0	7.98	2245.0	93.5	0.30	0.00	0.00	9.90	3.2	2.4

Table A.3: Habitat variables per site and month

Site	Month	Depth (m)	Width (max) (m)	Width (min) (m)	Riffle (%)	Pool (%)	Bedrock (%)	Boulder (%)	Cobble (%)	Pebble (%)	Gravel (%)	Sand (%)	Clay (%)
1	2	0.6	25	15	0.00	1.00	0.0	0.0	0.0	0.0	0.0	10.0	90.0
1	9	1.0	20	17	0.00	1.00	0.0	0.0	0.0	0.0	0.0	0.0	100.0
1	11	0.9	15	12	0.00	1.00	0.0	0.0	0.0	0.0	0.0	20.0	80.0
2	2	0.7	15	10	0.00	1.00	0.0	0.0	0.0	0.0	0.0	15.0	85.0
2	9	1.0	20	12	0.00	1.00	0.0	0.0	0.0	0.0	0.0	20.0	80.0
2	11	0.9	30	15	0.00	1.00	0.0	0.0	0.0	0.0	0.0	10.0	90.0
3	2	0.3	4	2	0.00	1.00	0.0	0.0	5.0	0.0	0.0	5.0	90.0
3	9	0.4	4	2	0.00	1.00	0.0	0.0	0.0	0.0	0.0	0.0	100.0
3	11	0.4	4	2	0.00	1.00	0.0	0.0	10.0	0.0	0.0	10.0	80.0
4	2	0.5	10	3	0.00	1.00	0.0	0.0	0.0	0.0	0.0	5.0	95.0
4	9	0.8	10	2	0.00	1.00	0.0	0.0	0.0	0.0	0.0	0.0	100.0
4	11	0.7	8	2	0.00	1.00	0.0	0.0	0.0	0.0	0.0	10.0	90.0
5	2	1.0	7	4	0.00	1.00	0.0	0.0	0.0	0.0	0.0	5.0	80.0
5	9	1.0	7	4	0.00	1.00	0.0	0.0	0.0	0.0	0.0	0.0	100.0
5	11	0.9	7	4	0.00	1.00	0.0	0.0	0.0	0.0	0.0	10.0	90.0
6	2	0.0	2	1	0.05	0.95	0.0	0.0	15.0	22.5	17.5	15.0	30.0
6	9	0.2	3	2	0.20	0.80	0.0	0.0	15.0	20.0	20.0	25.0	20.0
6	11	0.2	3	2	0.05	0.95	0.0	0.0	15.0	25.0	15.0	5.0	40.0
7	2	0.2	4	2	0.00	1.00	0.0	5.0	2.5	0.0	0.0	10.0	82.5
7	9	0.7	7	4	0.00	1.00	0.0	5.0	5.0	0.0	0.0	10.0	80.0
7	11	0.6	5	3	0.00	1.00	0.0	5.0	0.0	0.0	0.0	10.0	85.0
8	9	0.4	8	4	0.05	0.95	0.0	0.0	5.0	0.0	0.0	20.0	75.0
8	11	0.3	8	4	0.00	1.00	0.0	0.0	0.0	0.0	0.0	10.0	90.0
9	2	0.2	2	1	0.00	1.00	0.0	0.0	0.0	0.0	30.0	50.0	20.0
9	9	0.4	4	2	0.05	0.95	0.0	0.0	0.0	0.0	60.0	30.0	10.0
9	11	0.3	4	2	0.05	0.95	0.0	0.0	0.0	0.0	0.0	70.0	30.0

Table A.3: Habitat variables per site and month (cont.)

Site	Month	Depth (m)	Width (max) (m)	Width (min) (m)	Riffle (%)	Pool (%)	Bedrock (%)	Boulder (%)	Cobble (%)	Pebble (%)	Gravel (%)	Sand (%)	Clay (%)
10	2	0.2	4	2	0.05	0.95	0.0	2.5	10.0	2.5	20.0	50.0	10.0
10	9	0.4	4	2	0.05	0.95	0.0	0.0	15.0	5.0	20.0	50.0	10.0
10	11	0.3	5	2	0.00	1.00	0.0	5.0	5.0	0.0	20.0	50.0	20.0
11	2	0.1	2	2	0.05	0.95	0.0	5.0	5.0	5.0	20.0	37.5	17.5
11	9	0.2	3	2	0.10	0.90	0.0	5.0	10.0	5.0	20.0	35.0	25.0
11	11	0.1	2	2	0.05	0.95	0.0	5.0	0.0	5.0	20.0	40.0	30.0
12	2	0.5	15	10	0.00	1.00	0.0	0.0	0.0	0.0	0.0	25.0	75.0
12	9	1.0	18	12	0.00	1.00	0.0	0.0	0.0	0.0	0.0	20.0	80.0
12	11	0.9	15	10	0.00	1.00	0.0	0.0	0.0	0.0	0.0	30.0	70.0
13	2	0.2	7	4	0.50	0.50	0.0	5.0	12.5	25.0	12.5	25.0	20.0
13	9	0.4	7	3	0.70	0.30	0.0	10.0	20.0	30.0	10.0	20.0	10.0
13	11	0.4	8	4	0.70	0.30	0.0	0.0	5.0	20.0	15.0	30.0	30.0
14	2	0.2	6	2	0.40	0.60	0.0	0.0	5.0	30.0	35.0	20.0	10.0
14	9	0.5	4	2	0.50	0.50	0.0	5.0	40.0	15.0	10.0	20.0	10.0
14	11	0.4	6	2	0.50	0.50	0.0	5.0	15.0	30.0	30.0	10.0	10.0
15	9	0.4	4	3	0.80	0.20	0.0	5.0	15.0	10.0	10.0	30.0	30.0
15	11	0.4	4	2	0.80	0.20	0.0	5.0	15.0	25.0	20.0	20.0	15.0
16	2	0.1	6	2	0.20	0.80	5.0	20.0	20.0	7.5	7.5	15.0	25.0
16	9	0.4	15	5	0.40	0.60	0.0	30.0	20.0	5.0	5.0	10.0	30.0
16	11	0.2	8	3	0.20	0.80	10.0	10.0	20.0	10.0	10.0	20.0	20.0
17	2	0.1	6	1	0.25	0.75	22.5	17.5	15.0	10.0	5.0	12.5	17.5
17	9	0.3	6	1	0.25	0.75	30.0	15.0	10.0	5.0	5.0	10.0	25.0
17	11	0.3	6	3	0.15	0.85	15.0	20.0	20.0	15.0	5.0	15.0	10.0
18	9	0.3	2	1	0.05	0.95	0.0	0.0	0.0	0.0	0.0	15.0	85.0
18	11	0.2	2	1	0.05	0.95	0.0	0.0	0.0	0.0	0.0	15.0	75.0
19	2	0.4	5	2	0.00	1.00	0.0	0.0	0.0	0.0	0.0	15.0	85.0
19	9	0.3	4	2	0.00	1.00	0.0	0.0	0.0	0.0	0.0	10.0	90.0

Table A.3: Habitat variables per site and month (cont.)

Site	Month	Depth (m)	Width (max) (m)	Width (min) (m)	Riffle (%)	Pool (%)	Bedrock (%)	Boulder (%)	Cobble (%)	Pebble (%)	Gravel (%)	Sand (%)	Clay (%)
19	11	0.6	6	4	0.00	1.00	0.0	0.0	0.0	0.0	0.0	20.0	80.0
20	2	0.0	2	2	0.00	1.00	0.0	0.0	20.0	25.0	25.0	10.0	20.0
20	9	0.2	4	2	0.00	1.00	0.0	0.0	0.0	0.0	0.0	10.0	90.0
20	11	0.2	4	3	0.00	1.00	5.0	0.0	5.0	20.0	20.0	30.0	20.0
21	2	0.3	10	5	0.00	1.00	0.0	0.0	0.0	0.0	0.0	5.0	95.0
21	9	0.5	10	6	0.00	1.00	0.0	0.0	0.0	0.0	0.0	0.0	100.0
21	11	0.4	10	5	0.00	1.00	0.0	0.0	0.0	0.0	0.0	10.0	90.0
22	2	0.2	2	1	0.00	1.00	0.0	7.5	15.0	5.0	10.0	42.5	20.0
22	9	0.5	2	1	0.10	0.90	0.0	5.0	15.0	5.0	10.0	45.0	20.0
22	11	0.4	2	1	0.05	0.95	0.0	10.0	15.0	5.0	10.0	40.0	20.0
23	2	0.0	3	1	0.00	1.00	0.0	0.0	2.5	10.0	17.5	25.0	45.0
23	9	0.1	4	1	0.00	1.00	0.0	0.0	5.0	15.0	25.0	10.0	45.0
23	11	0.1	4	2	0.00	1.00	0.0	0.0	0.0	5.0	10.0	40.0	45.0
24	2	0.3	12	8	0.00	1.00	0.0	30.0	15.0	15.0	20.0	15.0	5.0
24	9	0.3	15	5	0.05	0.95	0.0	0.0	5.0	5.0	50.0	35.0	
24	11	0.4	15	10	0.10	0.90	10.0	30.0	15.0	15.0	15.0	5.0	

A.2 Code

Using only Open Source software enables everyone to reproduce the findings (Barnes, 2010). With this thesis comes a CD with a project-folder. This folder contains all data, code (R and Latex), figures, a poster-presentation as well as a literature database. A "README.txt" file can be found on the CD, explaining the structure and how to reproduce the results.

The R-code was not written for prettiness or elegance, therefore it may be hard to "read". However the code should work properly and calculate the same results (actually nearly the same, because of random permutations). This code runs on a Win7 (64bit)-Machine with R 2.13.2 and the packages mentioned in the method section without any errors - however it has not been tested on other systems.