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SEVENTH FRAMEWORK PROGRAMME

WISER

Water bodies in Europe: Integrative Systems to assess Ecological status and Recovery

DELIVERABLE

Deliverable D3.3-2: The importance of invertebrate spatial and temporal variation for ecological status classification for European lakes

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Content

Non-technical summary

European lakes are affected by many human induced disturbances. In principle, ecological theories predict that the structure and functioning of benthic invertebrate assemblage, one of the Biological Quality Elements following the Water Framework Directive (WFD) terminology, change according to the level of disturbances, making this biological element suitable to assess the status and manage lake ecosystems. In practice, to set up assessment systems based on invertebrates, we need to distiguish community changes that are related to human pressures from those that are inherent natural variability. This task is complicated by the fact that invertebrate communities hinhabiting the littoral and the profundal zones of lakes are costrained by different factors and respond unevenly to distinct human disturbances. For example it is not clear yet how the invertebrates assemblages respond to watershed and shoreline alterations, the relative importance of spatial and temporal factors on assemblage dynamics and relative bioindicative values of taxa, the habitat constraints on species traits and other taxonomic and methodological limitations.

The current lack of knowledge on basic features of invertebrate temporal and spatial variations is limiting the fulfillment of the EU-wide intercalibration of the lake ecological quality assessment systems in Europe, and thus compromising the basis for setting the environmental objectives as required by the WFD. The aim of this deliverable is to provide a contribution towards the understanding of basic sources of spatial and temporal variation of lake invertebrate assemblages. The report is structured around selected case studies, manly involving the analysis of existing datasets collected within Wiser. The case studies come from different European lake types in the Northern, Central, Alpine and Mediterranean regions. All chapters have an obvious applied objective and our aim is to provide to those dealing with WFD implementation at various levels hopefully useful information to account for when designed monitoring programs and or invertebrate based classification systems.

Chapter 1. Introduction and aim of the deliverable

Angelo Solimini

1.1 Why we need to assess invertebrate spatial and temporal variation in lakes

Abiotic factors in lake systems are highly variable in time and space and often account for a significant proportion of the variation of community patterns in terms of species diversity, abundance, biomass, and production. Differences between seasons and years are strongly correlated with relative changes of abundances of many invertebrate taxa. Changes in temperature, oxygen and concentration of ions, food supply through the year are takled by life history traits of benthic species in the different lake zones. Benthic invertebrates show also considerable spatial variation at multiple scales. At local scale (site level) proportional distribution of different habitats (like different macrophyte species and morpho types), near shore vegetation, sediment texture and patchy distribution of trophic resources largely account for invertebrate spatial variability. At lake level the morphological differences and the uneven distribution of habitats (e.g. macrophyte beds, woods, sandy and stony substrate areas etc.), form and wind exposure of shorelines, wave action, riparian vegetation structure, fish predation etc. determine the between site variability. One single factor (sampling depth) resemble most of the differences in abiotic and biotic variables affecting invertebrate assemblages. Typically, the depth profile of lakes can be divided into the littoral and the profundal zones based on the light penetration. Light can reach the bottom in the littoral (euphotic zone) but not in the profundal, making possible for macrophytes and periphytic algae the colonization of the littoral zone only. Sometimes the nearshore lake bottom area where emerged macrophytes grow is referred to as upper littoral, the zone colonised by the submerged macrophytes as infralittoral and the zone, still well oxygenated, just below the lowest depth colonised by macrophytes is called sublittoral. Within and between regions, sources of variation for invertebrate fauna include climatic and geological factors, chemical and thermal regimes, biogeographic factors landscape and antropogenic pressure levels.

European lakes are affected by many human induced disturbances that derive from human activities in lake basins. The most often reported man-made threats for lake ecosystems include: nutrient enrichment, modification of water level dynamics (e.g. water depletion, level regulations etc.), navigation and boating, artificial shoreline stabilisation, shoreline deforestation, recretional intensive uses (bathing, angling), acidification, introduction of alien species and many others. In principle, ecological theories predict that the structure and functioning of benthic invertebrate assemblge change according to the level of disturbances, making this biological element suitable to assess the status and manage lake ecosystems. In practice, to set up assessment systems based on invertebrates, we need to distiguish community changes that are related to human pressures from those that are inherent natural variability. This task is complicated by the fact that littoral and profundal invertebrate communities are driven by different governing factors and respond unevenly to distinct human disturbances. Open questions include: unclear response to watershed and shoreline alterations, relative importance of spatial and temporal factors on assemblage dynamics and relative bioindicative values of taxa, habitat constraints on species traits and other taxonomic and methodological limitations.

The current lack of knowledge on basic features of invertebrate temporal and spatial variations is limiting the fulfillment of the EU-wide intercalibration of the lake ecological quality assessment systems in Europe, and thus compromising the basis for setting the environmental objectives as required by the Water Framework Directive (WFD).

The aim of this deliverable is to provide a contribution towards the understanding of basic sources of spatial and temporal variation of lake invertebrate assemblages. The report is structured around selected case studies from different European lake types. All chapters have an obvious applied objective and our aim is to provide to those dealing with WFD implementation at various levels hopefully useful information to account for when designed monitoring programs and or invertebrate based classification systems.

1.2 Brief description of case studies

The report is structured around selected case studies, manly involving the analysis of existing datasets collected within Wiser. The case studies come from different European lake types in the Northern, Central, Alpine and Mediterranean regions and offer different angles and approaches to quantify the spatial and temporal variations of invertebrates.

Helen Michels and coworkers focus on the variability of different invertebrate metrics looking at a very long time series of data coming from the shallow lake Naardermeer. Time-for-space analysis is used to disentagle the unexplained variation caused by replicate samples, sample processing, natural temporal variation and stochastic events in the data series of 23 years of macroinvertebrate data and abiotic variables.

Francesca Pilotto and Angelo Solimini use the variance partitioning approach in order to quantify the combined impact of eutophication and morphological pressures on the invertebrate assemblages inhabiting different lake zones of 57 Alpine lakes. This method allows to disentangle the problem of interactions among different groups of explaining factors including variables connected to the pure spatial pattern and to lake morphology and geology By including those sets of variables in the variance partitioning analysis the authors could isolate the pure effects of eutrophication and morphological pressures from the effects due to interactions with the other tested variables.

Despite the great importance of those water bodies in areas of water scarcity, data from Mediterranean natural lakes are particularly scarce. For this reason, Marcello Bazzanti and coworkers look at the response to eutrophication of qualitative metrics (e.g. those based on presence – absence data) in different zones of lakes belonging to the volcanic district of Italy. A list of several taxa is proposed as tolerant and sensitive to euthrophication stressors that might be used in the development of the complete WFD compliant classification system.

Elaine McGoff and Leonard Sandin apply a multivariate method in order to partition the variance of littoral invertebrates between riparian related habitat variables, littoral substrate variables and trophic status of Swedish lakes. They also look at the impact of large scale land use patterns, investigating whether invertebrate communities in impaired and natural lakes differ in their response to local habitat and nutrient descriptors, and how the different environmental descriptors affect the variation in the macroinvertebrate data set among different land use types.

Gwendolin Porst and co-workers focus on a a lake near Berlin in Germany (lake Werbellin) and aim to quantify the impact of hydromorphological shoreline alterations on the community structure and diversity of lake macroinvertebrates by comparing unmodified with soft (recreational beaches) and hard (retaining walls, ripraps) altered shorelines. They further test whether a composite macroinvertebrate sample could represent a sampling site adequately when compared with stratified habitat specific macroinvertebrate samples and can, thus, serve as a cost and time effective alternative methodology for the monitoring of lakes.

The seventh chapter is authored by Mike Dunbar and Ralph Clark and provides methodological insights on how to tackle spatial and temporal veraition when designing sampling campaigns. How to extract statistically meaningfull models of between and within lake variations in macroinvertebrate community composition and estimate lake-level values for particular metrics are takled by the authors using hierarchical variance models.

The final chapter of this deliverable authored by Martin Pusch and Gwendolin Porst describes the design of the sampling lake invertebrate campaign within WISER, which had to meet several theoretical and practical requirements. It is pointed out that the database produced based on the invertebrate field exercise enables valuable analyses e.g. on pressure-response relationships and on its potential variation with latitudes, on within-lake and among-lake sources of uncertainty, on the efficiency of low-cost sampling techniques, and on the harmonization of assessment results with those elaborated by use of other biological quality elements.

Chapter 2. Time-for Space analysis on a long term data series of macroinvertebrates of the Naardermeer

Helen Michels, Piet Verdonschot

2.1 Introduction

The assessment of the ecological status of a water body is often based on values of metrics which are based on the taxonomic composition of a sample. Any measure of ecological quality or status is of little value without some knowledge of its level of uncertainty (Clarke, et al. 2006). Therefore the EU Water Framework Directive (WFD) requires all partner countries to include estimates of the confidence and precision of the of results provided by the monitoring programs in their river basin management plan (WFD Annex V, Section 1.3 “Monitoring of ecological status of surface waters). The uncertainty of estimates of ecological class of a water body depends on unexplained variation of the metric values and hence the unexplained variation in the observed biota at a site. This variation of biota has different sources, namely: 1) sampling variation and sampling method, 2) sampling processing and taxonomic identification errors, 3) natural temporal variation and 4) effects of pollution or environmental stress on the biota (Clarke et al., 2006). Values of metrics with little unexplained variation will provide a better estimate of the ecological status of a site. In order to provide confidence estimates in ecological status assessment of a water body, it is important to identify and quantify the different sources of unexplained variation within metric values.

For rivers, already few studies investigated different sources of unexplained variation in macroinvertebrate communities (Clarke et al., 2006, Clarke et al., 2002, Hose et al., 2004)

As part of the WISER project of the European Union 7th Framework program a large replicate field campaign is taking place to estimate the different source of unexplained variation in macroinvertebrate communities in European shallow lakes. All this studies use an ‘a priori’ designed replicate field sampling program to investigate the different sources of unexplained variation in macroinvertebrate communities. WISERBUGS, a tool for assessing confidence of ecological status class has been develop within the WISER project. This software assesses the probability of class memberships based on the sampling variation and other sources of unexplained variation.

In this chapter we focus on the variability of metric values using an alternative method to quantify the amount of unexplained variation within the macroinvertebrate community. We conduct a time-for-space analysis in which we use an existing long term data series of 23 years of macroinvertebrate data and abiotic variables of a Dutch shallow lake. Instead of taking multiple replicates at one given time, the samples taken over time serve as replicates. With this technique however, it is important to separate the unexplained variation, which can be caused by 1) replicate sampling variation, 2) sampling processing, 3) natural temporal variation and 4) stochastic events, from the variation that can be explained through changes in abiotic factors.

Separating the two types of variation can be reached through a multiple regression model with metric value as dependent variable and the abiotic factor as independent variables.

2.2 Methods

Study area

Figure 2: Map of Naardermeer Nature reserve (from Boosten, 2006)

Nature reserve “Naardermeer” (700ha) is located between the “Gooische heuvelrug” in the east and the river the “Vecht” in the west (see figure 1). Naardermeer nature reserve is a peat marshland showing a combination of all stages in natural succession from open water to marshland forest. In the 70’s the biodiversity decreased due to eutrophication and low water levels (Boosten, 2006).

Restoration measures started in 1985 with the dephosphorisation of the water inlet, construction of a sewage plant in the area and the hydrological isolation of the Cormorant rookery of Naardermeer.

In addition, the nutrient rich silt was dredged (1992-2000). The restoration measures let to improvement of the water quality, decrease of algae bloom, increase in visibility and increase of macrophytes (Verdonschot and Verdonschot, 2007).

Data collection

We examined one series of macroinvertebrate data which were collected in Groote Meer, one of the larger lakes in Naardermeer, between 1981-2009. We choose this lake over the other sampled lakes in the Naardermeer because it had the longest running data series. During the period of 1981-2003 every year a spring and late summer/ early autumn samples was taken as part of a monitoring program conducted by the Province North-Holland. From 2004 onwards, Alterra took over the sampling and only one sample per year was taken, which was alternating a spring and autumn sample. For the years 1982, 1983, 1997 and 1998 no samples are available.

All samples were taken following the same protocol. Note that interpersonal variation is unavoidable in these kind long data sets, and will be part of the unexplained variation. Within one location 5 m of habitat were sampled with a sweep net. The sampling effort was divided over the different vegetation structures present (*Thypa sp, Stratiotes sp, Carex sp,* and submerged macrophytes) and the collected material was added together into a mixed sample. The material was filtered over a sieve with mesh size 0.5 mm, and one of 250 µm. the residue was sorted and analyzed in the lab. Animals were identified to the lowest possible taxonomic level (van der Hammen, 1992; Verdonschot and Verdonschot, 2007).

Before analyzing the data any issues related to differences in taxonomic level between samples of different years were solved:

- If possible the lowest possible taxonomic level, usually species, was used.
- If the majority of individuals of a certain taxon level (class, family, order or genus) was identified until a lower taxonomic level, the lower taxonomic level was used and the higher taxonomic level was removed.
- If the frequency of individuals identified until the higher level was more than 10% of the frequencies of all the taxa below, the lower taxonomic level was changed into the higher level.
- The rule above is just an indication, in case of doubt the ecological significance of the different taxa levels was taken into account.
- No distinction was made between males, females, pupae, larvae, nymphs and juveniles.

Data analysis

The macroinvertebrate community is described with the commonly used metric % EPT abundance and the multimetrics MM designed by van Riel (in prep.) of the freshwater ecology group of Alterra. The multimetric MM was developed to adequately describe the ecological status of Dutch shallow lakes. This multimetric is the mean of the following standardized metrics:

- Number Coleoptera taxa
- Number of EPT taxa
- Number of exotic species
- Shannon-Wiener diversity index
- % non Insecta taxa
- % EPT abundance
- % amphipoda genera
- % predators
- % sprawlers

- % herbivores
- % coarse detritus
- % trofie III
- % cl 300-1000

The summer means of the following water quality parameters were also available for the period 1981-2004:

pH (dimsls)

Conductivity (mS/m)

Total phosphate (mgP/l)

Ortho-phosphate (mgP/l)

Chlorophyll-a (mg/l)

Ammonium (mgN/l)

Organic Nitrogen (mgN/l)

Chloride (mg/l)

Visibility (m)

Sulfate (mg/l)

Iron (mg/l)

Since we only have the abiotic parameters until 2004, all the statistical analyses were done for the period of 1981-2004. Because we only used the spring samples for our data analysis, we can assume samples are independent from each other (a test for residual autocorrelation confirmed this assumption for all the used models). If we had 2 spring samples per year we took the average of these 2 samples for that year.

Statistics

The aim of the statistical analyses was to detect and quantify unexplained variation within the long data series of macroinvertebrate data. We used a multiple regression model with a metric describing the macroinvertebrate community (i.e. % EPT abundance and the multimetric MM) as dependent variable and the different abiotic factors as independent variables to explain the variation based on known factors. Consequently, the unexplained variation, i.e. the variation not explained by the multiple regression model is expressed by the standard deviation of the residuals (SD_r). The unexplained variation indicates the level of uncertainty, based on the assumption that the abiotic variables explains the majority of the known variation. The variation due to environmental change can be separated from unexplained variation with this technique. The yearly spring samples are used to enable comparison with yearly available abiotic data.

We applied the multiple regression on the data set of 1981-2004 and on a data subset of 1992-2004, i.e. the stable period after the major anthropogenic disturbances in the mid 80s. This was done with MM and %EPT abundance as dependent variables. In total we thus performed 4 analyses; on the data set of 1981-2004 i) Multiple regression model with % EPT as dependent variable, ii) and a multiple regression model with MM as the dependent variable; for the data set of the whole period and on the subset of 1992-2004. For all analyses we selected the model with the highest significant R^2. The statistical analysis was conducted in R 2.11.1

2.3 Results

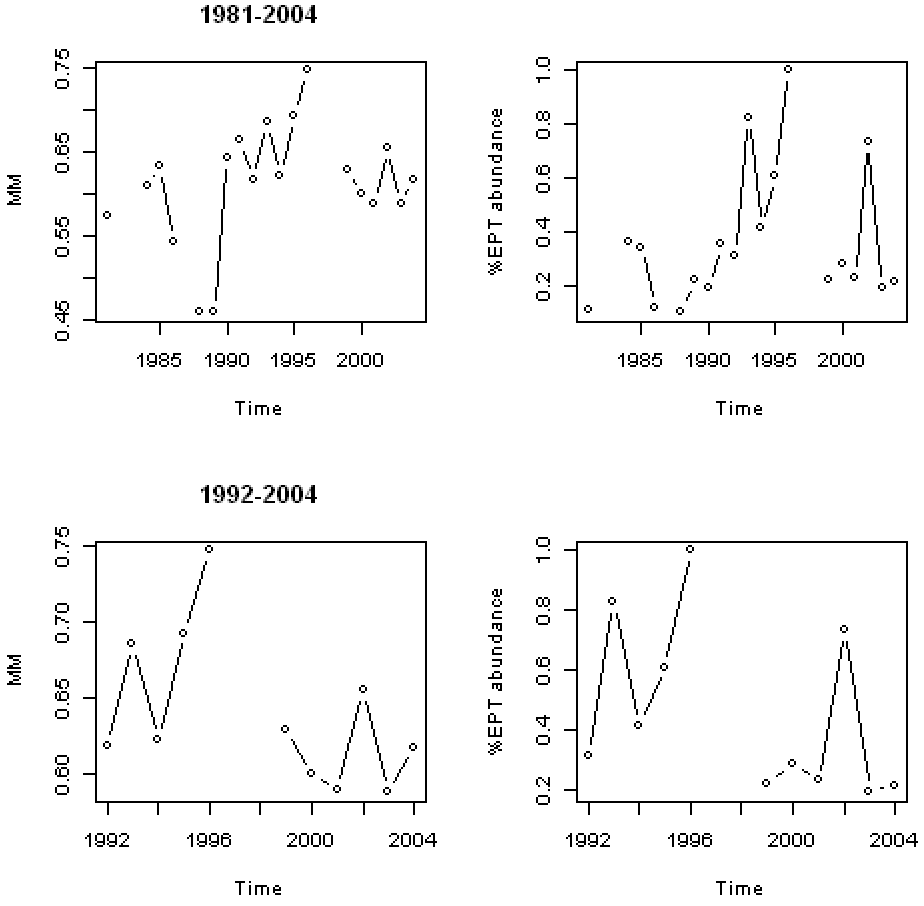

Figure 1 Time series of macroinvertebrate metric scores of Groote meer (Naardermeer). Upper left: Time series of multimetrics MM scores between 1981-2004 (SD_t = 0.07 Mean = 0.61). Upper right: Time series of metric % EPT abundance between 1981-2004. SD_t =0.25, Mean =0.36). Lower left: Time series of multimetrics MM between 1992-2004. SD_t= 0.05, Mean = 0.64). Lower right: Time series of metric % EPT abundance between 1992-2004 (SD_t= 0.28 , Mean = 0.44).

Analysis Multimetric MM dataset 1981 to 2004

Figure 1 shows the change in MM scores in Groote Meer between 1981 and 2004. The total standard deviation (SD_t) of MM over this period is 0.07 and the average MM over this period is 0.61. MM is the lowest between 1985 and 1992, which corresponds with the time the restoration of the lake started. We conducted a multiple regression to explain the known variation in MM. Table 1 shows the results of the multiple regression model with the highest significant R^2 (R^2= 0.70, p= 0.009). The Analysis of Variance confirms that conductivity and visibility explain a significant part of total variance. Standard Deviation of residuals (SD_r) is 0.04, this quantifies the unexplained variation. This unexplained variation can be attributed to different sources, such as sampling variation, taxonomic identification errors, natural temporal variation or unknown sources.

Analysis % EPT Abundance dataset 1981 to 2004

The total standard deviation (SD_t) of %EPT abundance over this period is 0.25 and the average % EPT abundance over this period is 0.36. Table 1 shows the results for the model that explains most of the variation in %EPT (R^2= 0.80, p= 0.003). Analysis of variance shows that Chlorofyll A, ammonium and nitrate explain most of the variation in %EPT. Standard Deviation of residuals (SD_r) as a measure of unexplained variation is 0.11.

Table 1 Regression coefficients of the multiple regression model for the period of 1981-2004.

	Coefficients	Estimate	Std. Error	t value	Pr(>|t|)
MM	Intercept	0.70	0.16	4.36	**<0.01**
	CL	<-0.01	0.00	-0.21	0.84
	COND	<0.01	<0.01	0.12	0.91
	CHLFA	-9.43	2.82	-3.35	**0.01**
	PO4	8.58	8.33	1.03	0.33
	NH4	0.29	0.32	0.91	0.39
	NO3	-0.81	0.59	-1.36	0.21
%EPT abundance					
	Intercept	1.30	0.42	3.09	**0.02**
	CL	<0.01	0.01	0.32	0.76
	COND	<-0.01	<0.01	-0.55	0.60
	CHLFA	-3.92	7.37	-0.53	0.61
	PO4	-14.57	22.07	-0.66	0.53
	NH4	4.93	0.86	5.71	**<0.01**
	NO3	-9.90	1.73	-5.74	**<0.01**

Table 2: Analysis of Variance. Upper: Testing for the effect of the chloride (CL), conductivity (COND), chlorophyll A (CHLFA),ortho-Phosphate (PO4), ammonium (NH4) nitrate (NO3) on the Multimetric MM for the period 1981-2004. Lower: Testing for the effect of the chloride (CL), conductivity (COND), chlorophyll A (CHLFA), ortho-Phosphate(PO4), ammonium (NH4), nitrate (NO3) on the % EPT abundance for the period 1981-2004. Significant p-values are indicated in bold.

	Parameter code	Df	Sum Sq	Mean Sq	F value	Pr(>F)
MM	CL	1	<0.01	<0.01	0.84	0.39
	COND	1	0.01	0.01	3.40	0.10
	CHLFA	1	0.06	0.06	31.07	**<0.01**
	PO4	1	<0.01	<0.01	1.80	0.22
	NH4	1	<0.01	<0.01	0.01	0.76
	NO3	1	<0.01	<0.01	1.85	0.21
	Residuals	8	0.01	<0.01		
%EPT abundance	CL	1	<0.01	<0.01	0.02	0.90
	COND	1	0.03	0.026	2.02	0.20
	CHLFA	1	0.19	0.19	14.67	**<0.01**
	PO4	1	<0.01	<0.01	0.19	0.68
	NH4	1	0.13	0.13	10.21	**0.02**
	NO3	1	0.42	0.42	32.89	**<0.01**
	Residuals	7	0.09	0.01		

Analysis Multimetric MM dataset 1992 to 2004

Here we only analyzed macroinvertebrate data from the stable period in the history of the lake (1992-2004). For this temporal subset of data with MM as dependent variable, the model with the highest significant R^2 (R^2= 0.73, p = 0.001) only include chloride as independent variable. Models which included more abiotic variables were not significant. Standard Deviation of residuals (SD_r) is 0.03.

Analysis %EPT abundance dataset 1992 to 2004

Table 3 shows the regression coefficients of the model with % EPT abundance as dependent variable with the best fit (R^2= 0.92, p= 0.004). Standard Deviation of residuals (SD_r) is 0.07.

Table 3: regression coefficients of the multiple regression model for the period of 1992-2004. chloride (CL), conductivity (COND), chlorophyll A (CHLFA), nitrate (NO3)

		Estimate	Std. Error	t	p
MM	(Intercept)	0.50	0.05	10.97	**<0.01**
	CL	<0.01	<0.01	3.15	**0.01**
%EPT abundance	(Intercept)	1.86	0.34	5.48	**0.01**
	CL	0.033	<0.01	8.27	**<0.01**
	COND	-0.01	<0.01	-7.13	**<0.01**
	CHLFA	87.87	24.57=	3.58	**0.02**
	NO3	-8.25	1.87=	-4.40	**0.01**

Table 4: Analysis of Variance. Upper: Testing for the effect of the chloride (CL), on the Multimetric MM and metric %EPT abundance for the period 1992-2004. Lower: chloride (CL), conductivity (COND), chlorophyll A (CHLFA), nitrate (NO3) on %EPT abundance for the period 1992-2004. Significant p-values are indicated in bold.

		Df	Sum Sq	Mean Sq	F value	Pr(>F)
MM	CL	1	0.01	0.01	9.94	0.01
	Residuals	8	0.01	0.01		
%EPT abundance	CL	1	0.33	0.33	51.37	**<0.01**
	COND	1	0.20	0.20	32.07	**<0.01**
	CHLFA	1	<0.01	<0.01	0.01	0.92
	NO3	1	0.12	0.12	19.42	**0.01**
	Residuals	4	0.03	0.01		

2.4 Discussion and Conclusion

Any measure of ecological quality or status is of little value without some knowledge of its level of uncertainty. To measure the uncertainty on lake assessment, it is important to have an understanding of the variability of the metric value (Clarke et al., 2006). To estimate this variability within metric values and to quantify the different components, especially the variability due to sampling, the WISER project organized a replicate field sampling program. This design allows to accurately estimate the sampling variability of the metric values. Here we conducted a time-for-space analysis in which we use an existing long term data series of 23 years of macroinvertebrate data and abiotic variables of a Dutch shallow lake.

Analysis dataset 1981-2004

When we take the entire dataset into account (1981-2004) a large proportion of the total variation (70%) in the MM values can be attributed to changes in abiotic factors, especially chlorophlyll A. The higher the chlorophyll A concentration the lower the values of MM. The remaining 30 % can't be explained by this model, and is the unexplained variation. This can also be expressed as the standard deviation of the residuals (SD_r) which is 0.04.

For the values of % EPT abundance abiotic factors NH4, NO3 and chlorophyll A explain the majority of the variation (80%).

The unexplained variation in %EPT abundance is related to different sources, such as sampling variation, taxonomic identification errors, natural temporal variation or unknown sources. Due to lack of information about these sources we cannot divide this unexplained variation any further. That's why the value that we found for the SD_r value of %EPT abundance (0.11) is larger than the value that Clarke et al. (2006) found for the average sampling SD for % EPT abundance (0.031) of samples taken with the RIVPACS method in different Austrian river. Afterall, sampling variation is just a part of the total unexplained variation we found.

Similar to Clarke et al. (2006) we find that EPT abundance, one of the individual component metrics of MM has a larger variance than multimetric MM. Because the lower SD_r of MM, the Multimetric MM has a higher precision to estimate the ecological status of a lake.

Analysis dataset 1992-2004

Another method to improve the insights on variability or uncertainty is to use a subset of data of the stable period of the lake. This way the SD_t will be smaller and is a better estimate of unexplained variation. If we look at the values of MM we notice that the total variability in the values of MM is smaller than if we would be in the entire data set. This was expected as the lake went through less changes from 1992 onwards. The regression model indicates that mainly the chloride concentration explains the variability in MM values. An interesting fact is that we see that the SD_r of this model is similar to the SD_r of model run on the entire data set. This result could be an indication that variation caused by unknown sources is irrespective of the total variability in the data set. This is the case for both MM as % EPT abundance. To better investigate this pattern we should test more data series from different lakes.

The variability of the values of the % EPT abundance from the stable period can be largely explained by chloride, conductivity and NO3.

This investigation illustrates the use of a Time-for-Space model to extract the unexplained variation in metric values, which is necessary for the uncertainty estimate of the ecological status of a lake. The information gathered through this Time-for-Space analysis is specific to Groote Meer and can not be used for other sites in contrast to the estimates of variability based on the replicate field sampling. The advantage of this method is that you can apply it on existing long term data series of macroinvertebrate data, instead of conducting a replicate field sampling program.

For management authorities these replicate field sampling programmes are very labor intensive. However, they do often have long term data series of both abiotic variables and macroinvertebrate communities of water bodies under their management. So, this technique can be a good alternative.

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Chapter 3. Comparisons of spatial variability of macrozoobenthos between sublittoral and profundal zones in subAlpine lakes

Francesca Pilotto, Angelo G. Solimini

3.1 Introduction

The structure and composition of macroinvertebrate benthic communities is influenced by several abiotic factors, and communities settled in different lake zones (littoral, sublittoral and profundal) are expected to respond differently to both natural variation and anthropogenic stressors. Therefore it is important to identify how different pressures influence the structure of the communities in the different lake zones in order to define which lake zone is the most informative of the ecological status, and thus the most suitable for the monitoring.

Previous works have been mainly focused on littoral and profundal communities. They showed that littoral communities are mainly influenced by habitat characteristics, especially water level fluctuations, mesohabitat and pH (Johnson et al. 2004, Stendera & Johnson, 2008; Brauns et al., 2008), while profundal communities are influenced by the trophic status, being affected by nutrient concentration and oxygen levels (Rasmussen and Kalff, 1987; Hämäläinen et al., 2003; Stendera and Johnson, 2008). Sublittoral communities have been shown to have lower variation among years than profundal communities in metric values (Johnson, 1998), density and species number (Hämäläinen et al., 2003). These characteristics make sublittoral communities potentially appropriate in detecting anthropogenic impact, such as acidification (Free et al., 2009).

Solimini et al. (2006) reviewed the knowledge about the use of benthic invertebrates as indicators of lake ecological status, focusing on the major anthropogenic pressures affecting lakes: eutrophication, acidification and hydromorphological alterations. The authors hypothesized that: “eutrophication affects the sublittoral zone to a generally less extent that the profundal, and the littoral zone even less (Brauns et al., 2007). In contrast, hydromorphological alterations will affect most strongly the littoral zone, but the sub-littoral to a much lower extent (Brauns et al., 2007). The profundal is probably hardly affected. Similarly, acidification probably mostly affects the upper zones of the lake” (Tab.1).

Tab.1. Hypothesized impact of different stressors on different lake zones (from Solimini et al., 2006)

	Eutrophication	Hydromorphological	Acidification	Combined
Littoral	*	***	***	***?
Sublittoral	**?	*?	**?	**?
Profundal	***	0	?	**?

Despite the awareness about the need of a pressure-specific assessment, a quantitative analysis of the unique effects of different pressures on the communities is still lacking, because communities have high natural spatial and temporal variability and are subjected to different stressors, which may have synergic effects.

When analysing the processes structuring the macroinvertebrate communities, attention must be paid to spatial factors. The spatial component can be responsible for a large part of the variation of the community due to both direct processes such as dispersal, social organization and species interactions and to indirect processes connected to the spatially structured environmental factors (Peres-Neto & Legendre, 2010; Borcard et al., 2004). Therefore the spatial patterns need to be assessed in order to quantify its contribution to the community variance and consequently to be able to distinguish its effects from those due to environmental factors. The spatial pattern of the community can be quantitatively described using Principal Coordinates of Neighbour Matrices (PCNM, Borcard et al., 2004; Leonard et al., 2008, Brind'Amour et al., 2009).

The aim of this chapter is to quantify the effects of eutrophication and morphological pressures on the spatial structure of the macroinvertebrate benthic communities at two depth zones (profundal and sublittoral) in subalpine lakes. We use a set of multivariate procedure to

1) extract the relevant spatial axes
2) select the most relevant environmental variables of each pressure and lake zone
3) partition the biotic variance among spatial and environmental components

The dataset used in this analysis were collected during 2005-2006 within Institutional projects of the Joint Research Centre Ispra (see related publication and reports Free et al., 2008; Free et al., 2009).

3.2 Methods

Study area, and macroinvertebrate sampling

Two datasets from Wiser database were analysed: the first one includes 12 lakes in the Italian subalpine region, sampled in the sublittoral zone (referred as sublittoral 12L), and in the profundal zone (with the exception of lake Montorfano); the other dataset (referred as sublittoral 45L) contained data collected in the sublittoral zone of 45 lakes in the subalpine zone of Italy (15 lakes, different from those of the previous datasets), Germany (15 lakes) and Austria (15 lakes). Macroinvertebrates were sampled on soft substrates (composed by clay, silt or sand fractions). For the 45L dataset the sampling was carried out between April and June 2006 by using an Ekman grab (sampled area: 0.125 m^2) in 3 sites per lake (see Free et al. (2009) for more details), with the exception of lake Hintersteiner See, were 2 sites were sampled. For the 12L dataset the profundal and sublittoral zone were sampled in 3 sites per lake (sampled area: 0.045 m^2) in spring and summer 2005. In each sample 2 replicates were collected. Taxa were identified mostly to species and genus level.

The biotic matrix of each dataset was formed by columns corresponding to taxa and rows corresponding to sampling sites. The cells contain the taxa density, for the 12L dataset this value is the average of the two replicates and the two seasons.

Environmental variables

We started the selection of the environmental variables to be included in the analysis from a set of 27 variables known to be potentially important in structuring macroinvertebrate communities. Those variables were measured at different spatial scales: site, lake and catchment level. Pearson's correlation among variables was calculated and when two o more variables resulted correlated, only one of them was selected for the further analysis. After this selection process, 9 environmental variables were retained and grouped into three sets of variables related to: 1. eutrophication, 2. morphological-pressures, and 3. morphology and geology. The ranges of the variable values for each dataset and their spatial scale are reported in the tab. 3.

Tab.3. Environmental variables: range of the variable values in each dataset and spatial scale of the measurements. The variables are grouped into 3 groups. LOI550=percentage of loss on ignition of sediments at 550 C. ILBS= index of lake basin shape (calculated as maximum depth divided by the square root of lake area).

Variable group	Environmental variable	Spatial scale	12L dataset		45L dataset		
			Profundal	Sublittoral	Italy	Germany	Austria
eutrophication	LOI550 (%)	sample	5.23 - 32.47	1.24 - 63.93	1.52-46.34	1.38-92.18	2.23-41.64
	Mid-lake TP (ug/l)	lake	1.7 - 39.5		9.42-110.13	7.32-31.4	2.99-19.28
morphological pressures	Diversity of macrophyte growth form types	site	-	0 - 5	0-3	0-3	0-3
	Sum of pressures	site	-	0 - 5.5	0-6.5	0-4.5	0-5
	Naturalness of riparian zone	site	-	0.04 – 0.92	0.042-0.96	0.042-0.87	0.042-0.96
	Urban land cover (%)	within 200 m from the lake	0 - 42.7		0-97.84	0-72.94	0-80.44
	Natural land cover (%)	within 200 m from the lake	7.6 - 72.2		0-97.06	0-80.22	0-85
morphology and geology	ILBS	lake	2.39 - 48		5.7-79.42	7.95-41.92	9.47-54.77
	Mid-lake alkalinity (meq/l)	lake	0.89 - 2.72		1.13-4.62	3.07-5.33	1.99-4.27

The eutrophication-related variables were: percentage of loss on ignition at 550 °C of sediments (%LOI550), and mid-lake total phosphorus (mid-lake TP). The morphological-pressure-related variables were 3 indices obtained through a lake habitat survey (LHS; Rowan et al., 2004, 2006) and 2 land use variables (urban and natural land use) within 200 m from the lake, gathered through GIS data. The LHS variables include: diversity of macrophyte growth form types, that records the occurrence of up to ten macrophyte groups; the sum of pressures, that records the presence of 18 potential pressures affecting the riparian zone and the shoreline within a 50 m radius of each site; and the degree of naturalness of the riparian zone, which takes into account riparian vegetation complexity, vegetation longevity and naturalness of land cover (see Free et al. (2009) for more details on the environmental variables). Those LHS indices were based on observation of the riparian/littoral zone and the shoreline close to the sites, therefore they have been calculated only for sublittoral sites since profundal sites could not be matched to any riparian stretch. Morphology and geology are represented by mid-lake alkalinity and by the index of lake basin shape (ILBS), calculated as maximum depth divided by the square root of lake area (Free, 2009).

For each dataset three environmental matrixes have been built, one for each variable group. The environmental matrixes were composed by columns corresponding to the variables and raws corresponding to the sites. The cells contain the variable values.

Data analysis

Prior to analyses, taxa densities were Hellinger-transformed, as suggested by Legendre & Gallagher (2001) and applied in several works (e.g. Brind'Amour et al., 2009; Sweetman et al., 2010; Leonard et al., 2008). The Hellinger transformation preserves the Euclidean distance among rows and therefore allows the use of Euclidean-based ordination methods such as redundancy analysis (RDA). It also offers the advantage of not strongly weighting rare taxa (Legendre & Gallagher 2001). Environmental variables were standardized (Legendre & Legendre, 1998), or the arcsine of the square root was calculated for variables expressed as percentage (Feld and Hering, 2007). This analysis has been performed using the R package Vegan (Osaken et al., 2006; available at http://cran.r-project.org/web/packages/vegan/index.html).Redundancy analysis (RDA) was used in constrained ordination of taxonomic data. This analysis has been performed using the R package Vegan (Osaken et al., 2006).

The spatial pattern of the community was quantitatively described using Principal Coordinates of Neighbour Matrices (PCNM, Borcard et al., 2004; Leonard et al., 2008, Brind'Amour et al., 2009). This method produces a set of spatial explanatory variables called PCNM vectors. It consists in the building of a matrix of Euclidan distances from the geographical coordinates of the sampling sites. The matrix of Euclidean distances is truncated at a threshold value, corresponding to the largest among the minimum distances among sites. A principal coordinate analysis on the truncated distance matrix is then computed and only the coordinates corresponding to positive eigenvalues are kept. The resulting principal coordinates are the PCNM vectors. This analysis has been performed using the R package SpacemakeR (Dray, 2008; available at http://r-forge.r-project.org/R/?group_id=195).

The contribution of the spatial factors and the 3 sets of environmental variables in structuring the macroinvertebrate benthic communities has been assessed through the use of variance partitioning with partial RDA. This method allows the decomposition of the variance of the response matrix (taxa density) among sets of explanatory variables in order to identify their pure and shared contributions (Borcard et al., 1992; Legendre & Legendre, 1998). Variance partitioning was performed by applying the varpart function of the R library Vegan, (Osaken et al., 2006) this function computes the RDA-adjusted R^2 values. The adjustment, taking into account the appropriate degrees of freedom, provides a way of comparing models with different numbers of predictors and sample sizes (Peres-Neto et al., 2006).

We included in the variance partitioning analysis only significant spatial (PCNM vectors) and environmental variables, identified by the forward selection procedure implemented in the R package Packfor (Dray, 2005; available at http://r-forge.r-project.org/R/?group_id=195) This procedure, applied to each variable group separately, uses the results of a Monte Carlo permutation test (999 random permutations) to test the significance of the explanatory variables successively entering the model and retains the those variables with P ≤0.05 (Brind'Amour et al., 2009; Leonard et al., 2008).

3.3 Results

A total of 147 macroinvertebrate taxa were identified, 55 in the profundal samples and 83-94 in the sublittoral samples (12 and 45 lakes respectively).

The RDA constrained ordination of taxonomic composition showed that for the profundal zone the proportion of variance explained by environmental variables was 56.77%, while for the sublittoral zone it was 14.8-44.42% for 45L and 12L dataset respectively.

In the profundal zone (Fig.1A) the first RDA axis (explained variance: 38.4%) positively correlated to LOI and mid-lake TP (scores: 0.776 and 0.774) and negatively correlated to ILBS (score: -0.723). The most important components for the second RDA axis (explained variance: 7.41%) were alkalinity and mid-lake TP (scores: -0.703 and -0.502).

In the sublittoral zone 12L dataset (Fig.1B), the first RDA axis (explained variance: 14.85%) resulted positively correlated to ILBS and natural land cover (scores: 0.733 and 0.534) and negatively correlated to mid-lake TP, urban land cover and LOI (scores: -0.598, -0.593 and –0.554). The most important components for the second RDA axis (explained variance: 12.64%) were mid-lake TP in the positive sector (score: 0.602), LOI in the negative sector (score: -0.483).

In the sublittoral zone 45L dataset (Fig.1C), the first RDA axis (explained variance: 6.7%) resulted positively correlated to alkalinity and naturalness of the riparian zone (scores:0.476 and 0.448) and negatively correlated to mid-lake TP and sum of pressures (scores: -0.690 and -0.466). The most important components for the second RDA axis (explained variance: 2.3%) was LOI (score: 0.284), natural land cover and diversity of macrophytes growth form types (score: -0.511 and -0.389).

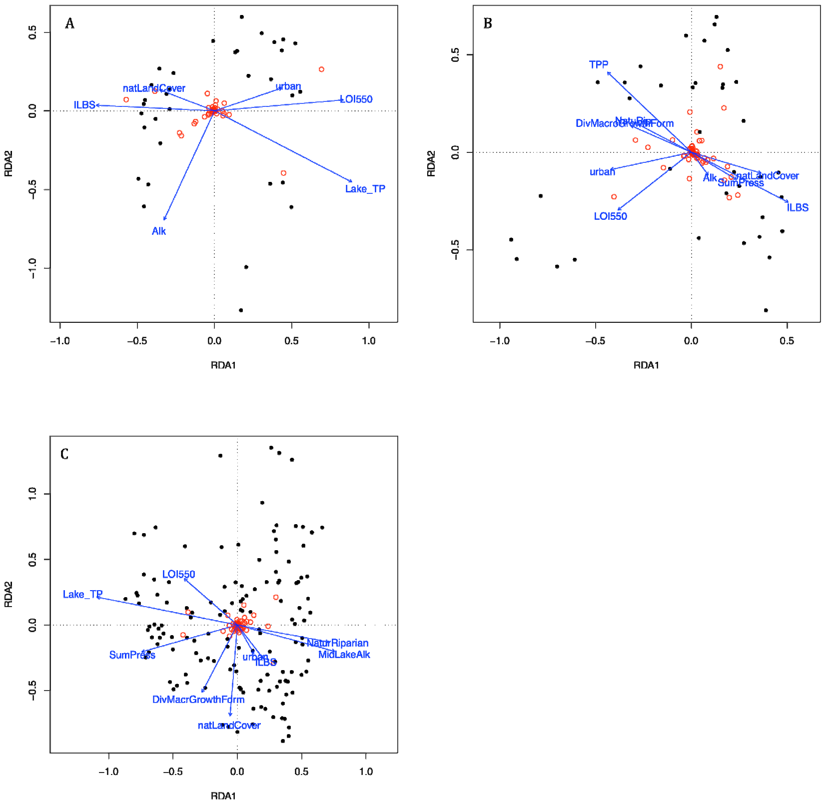

Fig.1. RDA biplot scores for constraining variables. Black dots represent sampling sites, red dots represent species. A. profundal, B. sublittoral 12L and C. sublittoral 45L.

Spatial component:

The PCNM vectors represent a quantification of broad to fine-scale spatial pattern of the study design, the first vectors (broadest/regional scale) depend on the study area surface, while the last vectors (finest/local scale) depend on the truncation distances (Borcard et al., 2004). The truncation distance resulted 146.5 km for the profundal and sublittoral 12L datasets and 125.6 km for the 45L dataset (91.4 km, 42.2 km and 92.3 km for the 15 Austrian, German and Italian lakes respectively). 6 PCNM vectors were produced for the profundal and sublittoral 12L datasets, 37 PCNM vectors for the 45L dataset (11 for 15 the Austrian lakes, 16 for the 15 German lakes and 14 for the 15 Italian lakes).

Variance partitioning:

The explanatory variables selected by forward selection for each variable-group and for each dataset are reported in tab.4.

Tab.4. Results of the forward selection procedure applied to each variable group and each dataset separately. The p values of the significant variables are reported. As regards spatial variables, only significant PCNM vectors are reported.

		Profundal	Sublittoral 12L	Sublittoral 45L
Spatial component	PCNM vectors	1: p=0.001 4: p=0.010 6: p=0.011 5: p=0.022	1: p=0.001 6: p=0.001 5: p=0.024 4: p=0.040	1: p=0.001 3: p=0.001 21: p=0.001 2: p=0.002 8: p=0.009 9: p=0.022 24: p=0.038
Eutrophication	%LOI550	p=0.001	p=0.001	not-selected
	Mid-Lake TP	p=0.001	p=0.001	p=0.001
Morphological pressures	DivMacrGrowthForm	-	p=0.026	p=0.029
	SumPress	-	p=0.019	p=0.002
	NatuRip	-	not-selected	not-selected
	Urban	p=0.05	p=0.003	p=0.044
	NaturalLandCover	not-selected	p=0.027	p=0.022
Lake morphology and geology	ILBS	p=0.001	p=0.001	p=0.001
	Mid-lake Alkalinity	p=0.008	not-selected	p=0.001

The amount of explained variance was and 61.4% for the profundal zone, and ranged between 18 and 32.9% for the sublittoral zone (respectively for the 45L and 12L datasets).

Variance patitioning results are reported in fig.2. Eutrophication resulted an important factor in explaining the taxa variance for the profundal zone, where it accounted for the 9.7%, while in the sublittoral zone it ranged between 0.3-3.2% (45L and 12L respectively). Morphological pressures accounted for the 1% in the profundal zone, and 1.2-3.6% of the taxa variance in the sublittoral zone (45L and 12L respectively). Lake morphology and geology explained the 3.7% in the profundal zone and 1-1.8% in the sublittoral (12L and 45L). The spatial component accounted for the 18.2% in the profundal zone, 8.2-9.1% in the sublittoral (12L and 45L respectively). Interactions among the explanatory variable groups were 28.8% in the profundal, 5.6-16.9% in the sublittoral zone (45L and 12L).

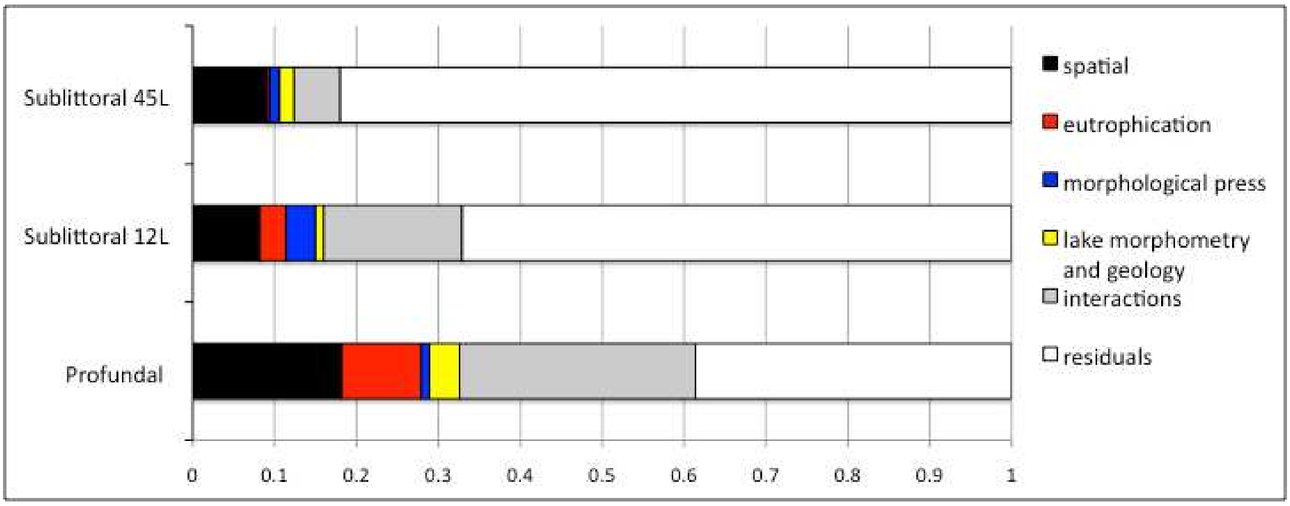

Fig.2. Variance partitioning using pRDA for each dataset. Pure effect of spatial variables (black), eutrophication (red), morphological pressures (blue) and lake morphomentry and geology (yellow). Grey: interanctions among the 4 groups of variables; white: unexplained variance.

The analysis has been performed for the 45-lake dataset separately for each country (Austria, Germany and Italy). The forward selection results are reported in Tab.5.

The total amount of explained variance was 22.1%, 15% and 6.1% for Italian, German and Austrian lakes rispectively. The eutrophication explained 1.5% of the community variance in Italian lakes but was not explicative for German and Austrian lakes. The morphological pressures explained 0.8%, 1.1% and 1.8% of the variance rispectively in Austrian, German and Italian lakes. Morphomentry and geology explained 1.7% and 2.3% of the variance rispectively in Italian and German while none in Austrian lakes. The spatial component 8.5%, 3.5% and 3.9% in Italian, German and Austrian lakes (Fig.3).

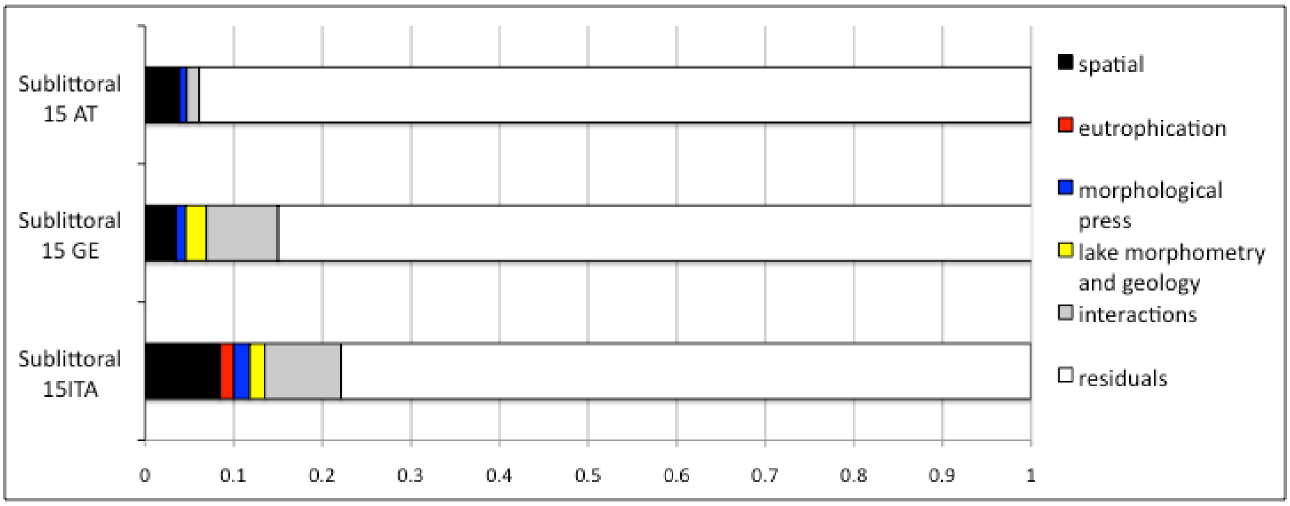

Fig.3. Variance partitioning applied to Austrian, German and Italian lakes separately (from the 45L dataset). The x axis refers to fraction of explained variance.

Tab.5. Results of the forward selection procedure applied to each variable group for Austrian, German and Italian lakes. The p values of the significant variables are reported. As regards spatial variables, only significant PCNM vectors are reported.

Variable group	Variable	Austria	Germany	Italy
Spatial	PCNM vectors	1: p=0.001 4: p=0.047	2: p=0.010 10: p=0.027 15: p=0.030 3: p=0.036 12: p=0.043	3: p=0.009 9: p=0.014 4: p=0.015 10: p=0.015 6: p=0.017 1: p=0.041
Eutrophication	%LOI550	not-selected	not-selected	p=0.004
	Mid-Lake TP	not-selected	p=0.031	not-selected
Morphological pressures	DivMacrGrowthForm	not-selected	p=0.010	p=0.046
	Sum of Pressures	not-selected	not-selected	p=0.042
	NatuRip	not-selected	not-selected	not-selected
	Urban land cover	not-selected	p=0.002	not-selected
	Natural land cover	p=0.012	p=0.002	not-selected
Lake morphology and geology	ILBS	not-selected	not-selected	p=0.006
	Mid-lake Alkalinity	not-selected	p=0.001	p=0.016

3.4 Discussion and Conclusion

Since water bodies are subjected to multiple anthropogenic pressures, it is difficult to assess the unique impact of each pressures on the biota. A fraquent approach to this kind of study is to stratify the sampling design in order to reduce the effects of sources of variaton other than the one of interest. For expample Sandin and Hering (2004), studied the impact of organic pollution on stream macroinvertebrates in a large study across Europe and focused the analysis only on water bodies where organic pollution was the unique dominant stressor. In our study, we applied the variance partitioning approach in order to quantify the combined impact of eutophication and morphological pressures. This method allows to disentangle the problem of interactions among different groups of explaining factors (Peres-Neto and Legendre, 2006). We included in the analysis also variables connected to the spatial pattern (PCNM vectors) and to lake morphology and geology because those factors may be responsible for a large part of the community variance other than anthropogenic derived disturbances and may have synergic effects with the environmental variables related to the two pressure (Borcard et al. 2004). Furthermore, by including those sets of variables in the variance partitioning analysis we could isolate the pure effects of eutrophication and morphological pressures from the effects due to interactions with the other variables.

Spatial and enviornmental variables

In the PCNM analysis of the spatial pattern of the study design, the first vectors are related to the broadest/regional spatial scale while the last vectors to the finest scale (Borcard et al., 2004). Our results highlight the importance of the regional scale in both profundal and sublittoral zones, being the first PCNM vectors highly significant in forward selection in every dataset. However, in both lake zones, the significant PCNM sets represent a mixture of broad, medium and fine scales, indicating the absence of a predominant scale and thus the interdependence between local and regional factors. This result has been reported also for littoral communities by Johnson and Goedkoop (2002), who stated that regional factors, at catchment scale, set upper limits and, within these limits, local factors become important.

In the profundal zone, both environmental variables related to eutrophication pressure (total phosphorous and loss on ignition) resulted highly significant in structuring the invertebrate community, and important variables in defining the gradient of the RDA ordination. The same results were found in the sublittoral 12 lakes dataset, but in the 45 lakes dataset loss on ignition resulted not significant in the forward selection procedure and had only a marginal role in the definition of the RDA gradients.

Among the variables related to morphological pressure, only urban land cover affected the profundal communities. Natural land cover was not significant while the LHS indexes were not taken into account because they are based on observation of the riparian and littoral features, which could not be matched to profundal sites.

Diversity of macrophyte growth form types was statistically significant in both the sublittoral datasets (12L and 45L). This confirms the well known importance of habitat complexity provided by macrophytes in defining the invertebrate community (Weatherhead and James, 2001; McGoff and Irvine, 2009), for example Cheruvelil et al. (2002), demonstrated that macrophyte colonization by invertebrate is influenced by plant architecture. Also land cover on lake surroundings and anthropogenic pressures in the riparian zone and on the shoreline affect the sublittoral community as shown by the forward selection results and by RDA ordinations. The sublittoral community abundance and composition was not affected by the presence and longevity of natural riparian vegetation, synthesized by the index of naturalness of the riparian zone. These features are expected to directly affect littoral communities by providing habitat diversity through the presence of roots and woody debris, known to be important factors for the invertebrate community (Brauns et al., 2007; Brauns et a., 2008).

Lake morphology could be identified highly significant in both profundal and sublittoral zone. The ILBS index synthesizes lake area and slope, being high values typical of small and deep lakes while low values typical of large and shallow lakes. Lake area and slope have been demonstrated to be indicators of anoxia (Nürnberg, 1995) and to indirectly affect invertebrate communities by influencing fine sediment distribution and macrophyte growth (Rasmussen and Kalff, 1987). In fact, lake area is strictly related to wind fetch, which determines wave height and thus fine sediment distribution (Smith and Sinclair, 1972). Slope influences the ability to retain fine sediments and has been found negatively related to the biomass of rooted submerged macrophytes (Duarte and Kalff, 1986). Alkalinity was identified to significantly explain the variance in community structure in the profundal zone and in the 45 lakes dataset but not in the sublittoral 12 lakes.

Variance partitioning:

The most striking results of our analysis are eutrophication-profundal and hydromorphological-sublittoral pressure- biota relationships. Although this differential effect of the two pressures on the different zones of lakes has been hypothesized (Solimini et al., 2006), to date no direct quantification was available. Our results showed that the profundal communities are mainly affected by eutrophication, with explained variance 10 times higher than that of morphological pressure. The relationship between lake trophic state and profundal communities is well known, as reported in Solimini et al. (2006). The input of nutrients enhances littoral and pelagic productivity and leads to an increase of organic matter in the sediments. The degradation of the organic matter causes a decrease of oxygen in the hypolimnion which has direct effects on the profundal macroinvertebrate community (Rasmussen and Kalff, 1987; Dinsmore et al., 1999). This process affects the structure of the community, through a decrease of diversity and sensitive-taxa abundances and increase of tolerant-taxa abundances (Bazzanti et al., 1994). Our results showed that in addition to euthrophication, also the morphological signal could be tracked in the profundal, although with much less extent.

In the sublittoral zone the pure effect of eutrophication resulted lower than in the profundal zone, accounting for the 0.3-3.2% of the explained variance. This is consistent with previous studies which demonstrated that the profundal zone is more suitable for detecting early signs of eutrophication than the sublittoral zone (Bazzanti et al., 1994; Hämäläinen et al., 2003).

The pure effect of morphological pressures is up to 3.6 times higher in the sublittoral than in the profundal zone. However, variance partitioning is different among regions. The 12 lakes dataset and the 15 Italian lakes showed a similar pattern, with a similar importance of eutrophication and morphological pressure. On the contrary, the sublittoral communities of the 15 German and 15 Austrian lakes were not affected by eutrophication. This may be due to the lower trophic gradient of these lakes then that of Italian lakes.

The spatial component was the dominant factor in both the lake zones, representing the 18.2% of the explained variance in the profundal zone and the 8.2-9.1% in the sublittoral. This fraction accounts for three possible causal factors: spatially-structured environmental or biotic factors not included in the analysis, spatially-structured historical events and spatial autocorrelation in the response matrix (Legendre and Legendre, 1998).

This study provides a quantification of the pure effects of eutrophication and morphological pressure on profundal and sublittoral macroinvertebrate communities. It will be interesting to integrate it for the littoral communities by including in the analysis the data gathered during the WISER project.

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Chapter 4. Analysis of invertebrate diversity metrics sensitive to eutrophication in different depth zones of natural Mediterranean lakes

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4.1 Introduction

In the past two decades, multimetric indexes of biological integrity have been widely adopted as tools for the monitoring of ecological status and they are increasingly being incorporated into regulations of Member States as numeric biological criteria (See Wiser Deliverable 2.2-2: Guidelines for indicator development). Multimetric indexes are the combination of several single metrics, each taping distinct aspects of the biological response to a given pressure. At the community level, the metrics most often used include those indicative of the abundance of organisms, their diversity, their sensitivity/tolerance to a given pressure, and their ecological traits (Karr and Chu, 1999).

Analysis of presence/absence data alone can allow the calculation of several qualitative metrics, some of which are widely applied for the benthic fauna. For example, macroinvertebrate species richness is a fundamental component of several multimetric indices of ecological integrity assessment of rivers (Maxted et al. 2000, Klemm et al. 2002), lakes (White & Irvine, 2003, O'Toole et al. 2008, Donohue et al. 2009a) and ponds (Solimini et al. 2008, Trigal et al. 2009). Some of these multimetric indices, include in their calculation several (single) "richness" metrics based on the taxa richness of the total macroinvertebrate assemblage (Lenat 1988), or only of a portion of it. For example, several insect groups can be considered together in a metric, such as Ephemeroptera, Plecoptera and Trichoptera (EPT, Morse et al. 1993) or Ephemeroptera, Plecoptera, Trichoptera and Coleoptera (EPTC, Compin & Céréghino 2003). Other metrics comprise single taxonomic groups such as chironomids (Trigal et al. 2009), ephemeropteran Heptagenidae (Clements et al. 2000), diving beetles (Nilsson & Södenberg 1996). The qualitative approach might be useful when abundance data are not available or too sparse to be comparable among lakes.

Benthic studies traditionally focus separately on the different lake zones, that are approached as different subsystems. The rationale of this relies on the fact that the structure of the invertebrate assemblage in eulittoral, infralittoral and sublittoral/profundal zones (*sensu* Hutchinson 1967 and later adopted by O'Sullivan & Reynolds 2004) are driven by different abiotic factors and can respond differently to different pressures. However, this lake feature complicates the task of quantifying the relative sensitivity of macroinvertebrates species to pressures and what metric is really indicative of a given pressure. Especially in the Mediterranean region, cohemprensive enumerations of macroinvertebrate species present in natural lakes are lacking, hindering the finalisation of biological classification systems. For example, no classification system based on invertebrates has been intercalibrated.

In this chapter we: 1) compare the response of the macroinvertebrate assemblage based on presence/absence data to euthrophication pressure in natural Mediterranean lakes, 2) test the effectiveness of several qualitative metrics (sensitivity/tolerance and richness metrics) in responding to eutrophication pressures, and 3) identify species typically associated with trophic levels. Our analysis compares not only different metrics and eutrophication levels but also different lake zones. A large dataset was assembled based on a collection of 22 papers (published from 1981 to 2008) and 5 unpublished studies on benthic assemblages of sandy eulittoral, vegetated infralittoral and sublittoral/profundal zones of natural lakes located in central Italy. This region includes a large number of volcanic lakes (8) with different degrees of anthropogenic disturbance and measures of environmental protection.

The approach developed in this report aims to be a useful contribution to the implementation of richness and sensitivity metrics into multimetric indexes of ecological status in natural Mediterranean lakes. Moreover, as biodiversity is one of the main criteria used to establish protection priorities and to propose management actions regarding the conservation of continental water (Ramsar Convention Bureau 2005), this work can offer basic data on potentially target species for those actions.

4.2 Methods

Study area and environmental features of lakes

The macroinvertebrate dataset used in this report refers to 5 different lakes and sampling campaigns carried out in the last 30 years. The lakes (Bracciano, Martignano, Vico, Albano, Nemi) are located close to Rome (Fig. 1) and their main morphometric features are showed in Table 1. They are all monomictic lakes with different levels of euthrophication pressure (Table 2). The following studies focus on the sandy eulittoral and vegetated infralittoral: Mastrantuono 1986a and 1986b, 1990, 1991, 1995a, 1995b, Mastrantuono & La Rocca 1988, Mastrantuono 2000, Mastrantuono et al. 2001, Mastrantuono & Mancinelli 2003, 2005, Mastrantuono & Sforza 2008, Mastrantuono et al. 2008, while the following studies refer to the sublittoral/profundal: Bazzanti & Loret 1982, Bazzanti & Seminara 1987a and 1987b, 1995, Bazzanti et al. 1993, 1994a and 1994b, 2001.

Regarding eutrophication, Lake Bracciano showed the best conditions (lowest Total P concentration, higher water transparency and well oxygenated hypolimnetic water throughout the year), whereas, at the opposite end, Lake Nemi during 1976-77 and 1982-83 presented a very poor ecological condition (higher Total P values, lower water transparency, and long lasting hypolimnetic anoxia during summer-early autumn). The trophy levels of Lakes Martignano, Vico, Albano and Nemi (the latter during 2001-02) were placed among these extremes, gradually shifting from the oligo-mesotrophic to the meso-eutrophic lakes. Most of the study lakes showed a marked or a total deoxygenation in the hypolimnion during the summer stratification period (Tab. 2 and see also the original papers for details). In addition, Lake Albano suffered from a meromictic state (Lami et al. 1994) localized in the layer from 120 to the maximum depth (175 m), lacking in this zone of any forms of macroinvertebrates.

A part from eutrophication, some hydrological changes affect lake Albano, Lake Bracciano (which showed a water level lowering of about 1 m depth in 2003; Mastrantuono et al. 2008), and Lake Nemi (water level lowered of about 1,5 m in 2000-2001; Mastrantuono & Sforza 2008).

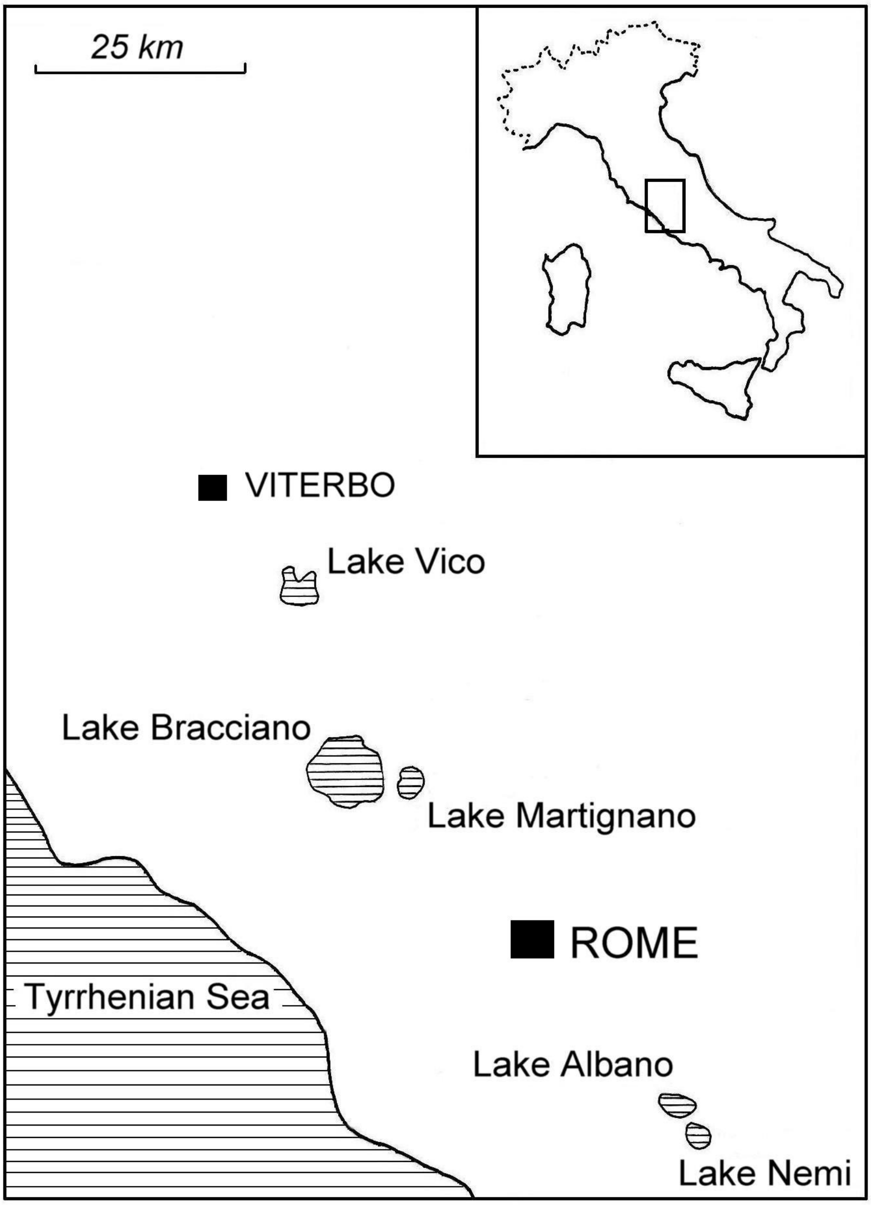


***Figure 1**. Location of the 5 lakes included in this study.*

Characteristics of the macroinvertebrate dataset

The sampling campaigns on benthic invertebrates are described in details in the studies included in the reference list at the end of the chapter and refer to three lake depth zones: sandy eulittoral (0.5 m), vegetated infralittoral (from 3 to 8 m) and sublittoral/profundal (from 10 or 20 m to the maximum depth). For all lakes and sampling campaigns we assembled presence/absence data of macroinvertebrate samples collected during 4-6 different sampling dates over a year, including the summer/early autumn stratification and the winter/early spring water mixing resulting in more than 1000 samples. After aggregation at lake, site and season levels, a total of 371 samples are included in the database. The final presence/absence list include more than 200 taxa (mostly to species or genera) of which 99 were insects (47 chironomids), 56 oligochaetes and 18 molluscs. For detailed information on study sites and materials and methods, see the original articles cited in references of this chapter.

Table 1. Morphometric characteristics of the study lakes (from Gaggino et al. 1985; Ciccacci et al.1987); tw = theoretical water renewal time.

Lake	altitude m a.s.l.	area km2	volume m3	mean depth m	max. depth m	tw years
Albano	293	6.0	464	77	175	47.6
Bracciano	164	57.0	5050	88.6	165	137
Martignano	207	2.4	72.3	29.64	60	29.6
Nemi	316	1.6	26.5	16.5	31	15
Vico	510	12.1	260.6	21.6	48.5	17

Table 2. Annual mean values of Total P, NH4, conductivity (as range of annual means in the water column), transparency, and minimum hypolimnetic dissolved oxygen (D.O.) in the studied lakes. Trophic evaluation was calculated according to Total P and transparency values (OECD, 1982).

Lake	Bracciano	Martignano	Vico	Nemi	Albano	Nemi
study year	1998-99	1996-97	1985-86	2001-02	1985-86	1982-83
Total P (µg l-1) range	5-15 (mean=8.7)	13-25 (mean=18)	14-35 (mean=21)	13-105 (mean=36)	69-263 (mean=164)	97-279 (mean= 193)
NH4 (µg l-1)	9-20	20-80	3-62	13-795	110-884	488-1587
Conductivity (µm cm-1)	476-502	336-397	373-381	290-314	446-491	535-641
Transparency (m)	9.6	7.9	6.6	5.4	5.8	1.8
D.O. (mg l-1)	3.1	0.7	0.2	0.1	0	0
Trophic evaluation	oligotrophy	oligo-mesotrophy	oligo-mesotrophy	meso-eutrophy	meso-eutrophy	eutro-hypereutrophy

Data analysis and selection of metrics

To visualise the community structure based on presence/absence data in 2 dimensions, we generated an association matrix between samples using Bray–Curtis similarity and we ran a non-metric multidimensional scaling (N-MDS) to produce two-dimensional ordinal plots (Clarke & Warwick 2001). Non-metric multidimensional scaling (N-MDS) was chosen as ordination analysis to assess differences in species composition between zones and lakes. N-MDS ordination was selected because of its non-restrictive assumptions (not assuming random sampling, multivariate normality and non-clustering of observations). Taxa present only one time in a zone of a lake were excluded from the analysis.

To test the hypotheses that the invertebrate community structure (based on presence/absence data) differed between lakes/year with different nutrient levels grouped following OECD

(1982) classification, we computed a nonparametric one-way analysis of similarity (ANOSIM, ANalysis Of SIMilarities; Clarke & Warwick 2001) for all samples and for each lake zone in turn. Analysis of similarities has been widely used for testing hypotheses about spatial and temporal differences in assemblages and for detecting environmental impacts (Chapman & Underwood 1999). Pairwise permutation tests followed each ANOSIM.

To select the macroinvertebrate species typically associated with lake/year trophic groups, we used Similarity Percentage analysis (SIMPER; Clarke & Warwick 2001). The SIMPER algorithm determines the relative contribution of each species to the average similarity within a group and the average dissimilarity between groups. According to Clarke & Warwick (2001), if a species consistently contributes to between group dissimilarity between pairs of samples (lakes of different trophic status), then its percentage contribution to similarity is high and it can be considered a good discriminating species and, therefore, a useful indicator of the lake trophic status. All statistical analyses were done with PRIMER Version 6.1 (PRIMER-E Ltd., Plymouth**,** UK) and Vegan package for R (Oksanen 2007).

To verify the response of potential metrics to eutrophication we selected metrics indicative of diversity and sensitivity/tolerance of taxa from published literature. The results of the metrics were plotted by box-plots to detect potential differences between lakes of different euthrophication status and zones. Statistical significance was tested by Kruskal-Wallis rank sum test and by post hoc multiple comparison test with significance at $p < 0.05$. While a description of the metrics tested here can be found in Cuffney (2003), particularly relevant richness metrics are described below.

Taxonomic richness metrics. The total number of taxa (RICH, Trigal et al. 2006), the number of taxa belonging to Ephemeroptera + Trichoptera + Odonata + Coleoptera (EOTC), a variant of EPTC (Compin & Céréghino 2003) in which Odonata was added instead of Plecoptera uncommon in lentic waters, the number of molluscan + large crustacean taxa (MOLCRUR, Burton et al. 1999, Kashian & Burton 2000), the number of chironomid taxa (CHIR, Trigal et al. 2006) and of oligochaete species (OLIGOR, Slepukhina 1984, Lafont et al. 2010). The assumption inherent in their use is that lakes having high environmental quality should be also have high values of richness of total and of some taxonomic groups (i.e., Reynoldson et al. 1997, Kashian & Burton 2000).

Sensitive/Tolerant taxa metrics. Sensitive and tolerant taxa were selected with the SIMPER procedure and from the well known data reported in the literature, and then summed up in sensitive and tolerant taxa metrics. This calculation was made for the three zone separately, because of the different species composition per zone and the fact that same species can be both sensitive or tolerant depending by the zone (littoral or profundal) in which can be found (see for example O'Toole et al. 2008, Donohue et al. 2009b). Additionally we calculated the Biological Monitoring Working Party (BMWP) and the Average Score Per Taxon (ASPT) scores. Both metrics were firstly developed in UK for the classification of water quality in rivers (e.g., Armitage et al. 1983) and later modified for Mediterranean ecosystems (Alba-Tercedor & Sanchez-Ortega 1988). Their use for lakes is reported in Johnson (1998) and O'Toole et al. (2008).

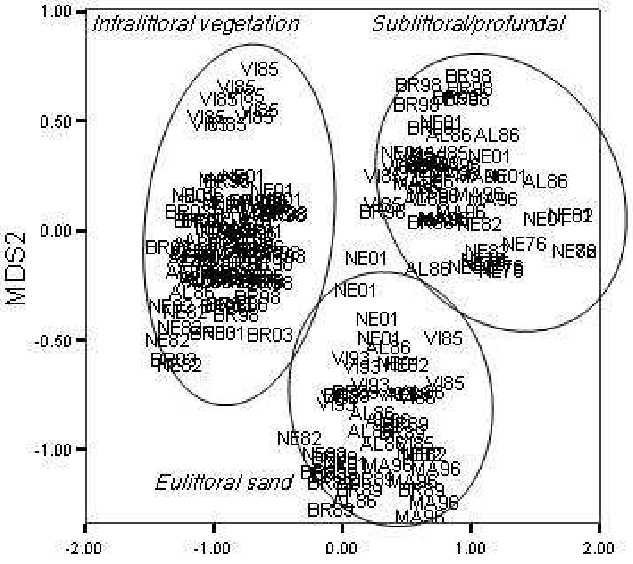
4.3 Results

Relationships among macroinvertebrate taxa, depth zones and trophic levels of lakes

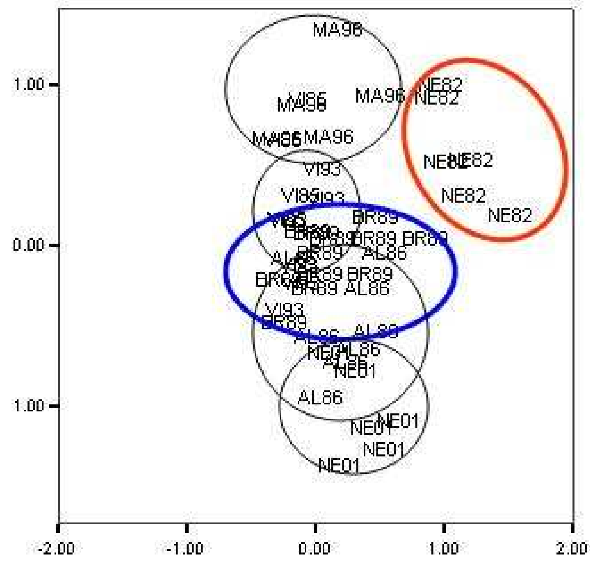
The NMDS plot (Figure 2a) shows a separation of the three lake zones (ANOSIM: R-statistic =0.98 p <0.01), suggesting that the depth zone assumes more importance than the different lakes in structuring the macroinvertebrate assemblage. Separate NMDS for each lake zone (Figure 2b, c, d) locates lakes/years along an eutrophication gradient, from the nutrient-poor and transparent lake/year conditions (Bracciano) to nutrient-rich and turbid lake/year conditions (Nemi in 1982 and 1976). Lakes were also arranged along gradients of deoxygenation for sublittoral/profundal zones and of water transparency for the infralittoral vegetation zones. The ANOSIM analysis shows significant differences among lake/years for all the three benthic zone (eulittoral: R=0.57, p<0.01; infralittoral: R=0.76, p<0.01; sublittoral/profundal: R=0.55, p<0.01). SIMPER results indicate for each depth zones those taxa contributing at higher level to dissimilarity between pairs of lake/years grouped following OECD (a cut-off of a cumulative percentage of dissimilarity of 60% was applied, Table 4). In the eulittoral, 23 taxa were selected of which 11 were oligochaete species and 9 chironomid taxa. In the infralittoral zone, 31 taxa appeared to be indicators of eutrophication level, representing diverse taxonomic groups (oligochate naidids, large crustaceans, acariens and several order of insects). Finally, in the profundal zone 15 taxa are potential indicators of lake trophic level, mostly belonging to oligochaetes, crustaceans and chironomids. The species traditionally referred as "eutrophic" species (such as those belonging to *Potamothrix* and *Limnodrilus*, other species or group of species belonging to *Chironomus* and *Procladius*) showed no clear trophic preference in all the three zones. Exception were *Potamothrix heuscheri* in the eulittoral and *Chaoborus flavicans* and *Chironomus plumosus* group in the sublittoral/profundal zone that were classified in the meso-eutrophic group.

Figure 2*. Plot of the first two axes of NMDS on taxa presence/absence of all 3 lake zones together (a) and separately (b-d). Ellipses depict samples belonging to the same lake/year (in blue: oligotrophic state, in red: eutrophic-hypereutrophic state).*

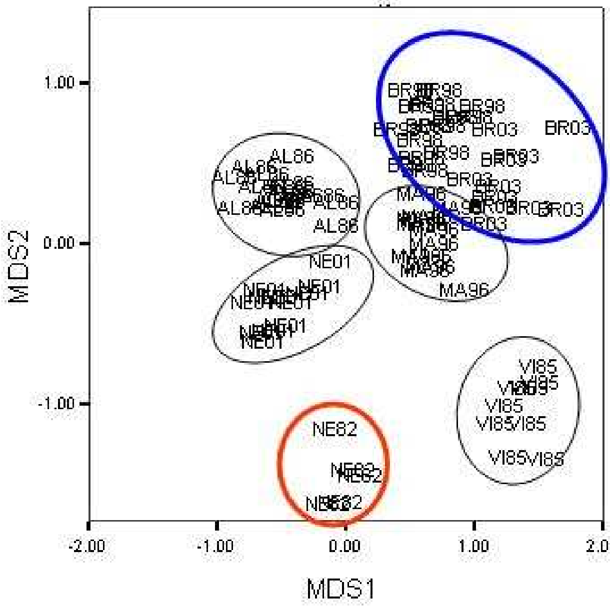
3a all lakeyear



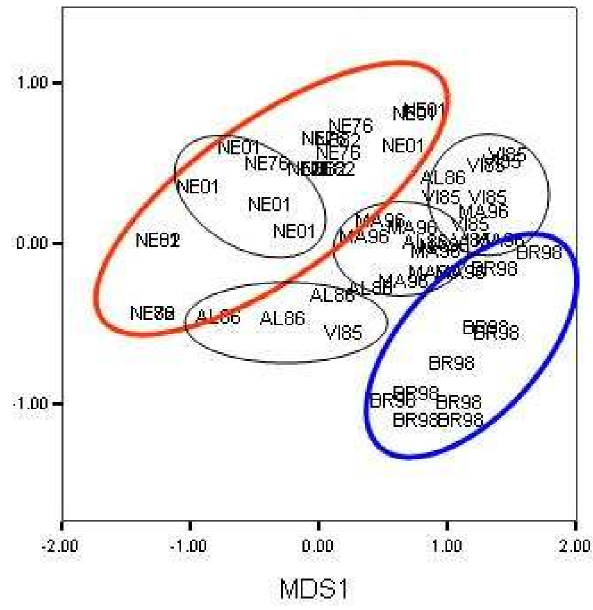
3b Eulittoral sand



3c infralittoral vegetation



3d sublittoral/profundal



Testing of selected metrics based on macroinvertebrates

We adopted the following criteria to test if a given qualitative metric could be a useful indicator of lake trophic level: the metric either coherently increases or decreases with lake trophic group (defined by OECD scheme) and the metric shows a statistical significant difference at least between oligotrophic and euthrophic conditions (no overlap between, at least, the values for the two extremes of the trophic gradient). All metrics tested here are showed in Table 4 and only metrics meeting those requirements are showed in Figure 5. The richness of both sensitive and tolerant taxa and molluscan+large crustacean taxa, showed the above mentioned patterns with increasing of eutrophication in all the three lake zones, with the exception of the number of tolerant taxa in the sublittoral/profundal zone which showed differences only between oligotrophic and oligo-mesotrophic lakes. For sublittoral/profundal zone also total taxa richness, BMWP and ASPT score discriminated lakes of different trophic condition. It is interesting to note that in the sublittoral/profundal zone the number of molluscan+large crustacean taxa and the ASPT score seemed to be more sensitive to eutrophication than other metrics because they discriminated more trophic levels.

Table 3. *List of sensitive (oligotrophic and oligo-mesotrophic taxa) and tolerant (meso-eutrophic and eutro-hypereutrophic taxa) macroinvertebrates based on the dissimilarity (SIMPER analysis) between pairs of different lake typologies (a cut-off of a cumulative percentage of dissimilarity of 60% was applied) and on the literature data. Taxa reported only in the literature are indicated by a number referring to references as following: 1= Saether (1979), 2 = Wiederholm (1980), 3 = Mouthon (1993), 4 = Lang (1990), 5 = Milbrink (1993), 6 = Lafont et al. (2010).*

SENSITIVE TAXA

Eulittoral sand

Aelosoma hemprich, Chaetogaster diaphanous, Amphichaeta leydigii, Nais christinae, Pristina longiseta, Paratendipes, Stictochironomus, Paracladopelma,Tanytarsus,Cladotanytarsus, Micropsectra [1, 2]*,Microtendipes* [1, 2]*,Pisidium* spp. [2]

Infralittoral vegetation

Nais christinae, Dero sp., *Echinogammarus veneris,Palaemonetes antennarius,Centroptilum* sp., *Cloeon simile* gr., *Procloeon* sp., *Psectrocladius* sp., *Larsia* sp., *Paratendipes* sp., *Dicrotendipes, Tanytarsus* sp., *Ecnomus tenellus, Leptocerus* sp., *Limnesia* sp**.,** *Acercus* sp., Halacaridae indet., *Theodoxus fluviatilis,* Hydrobiodea indet.,*Micropsectra* [1, 2]

Sublittoral/Profundal

Psammoryctides barbatus ,Aulodrilus pluriseta, Spirosperma velutinus, Lumbriculidae indet., *Echinogammarus veneris ,Niphargus* sp., *Proasellus coxalis* gr., *Micropsectra* sp. *,Tanytarsus* sp., *Microtendipes pedellus* gr., *Paratendipes albimanus* gr., *Pisidium* sp., *Theodoxus fluviatilis* [3] *,Bithynia tentaculata* [3] *,Potamopyrgus antipodarum* [3] *,Valvata piscinalis* [3] *,Physella acuta* [2] *,Planorbella* sp. [3,] *Acroloxus lacustris* [3] *,Belgrandia* latina [3]

TOLERANT TAXA

Eulittoral sand

Hydra sp., *Nais barbata, Nais pardalis, Branchiura sowerbyi, Potamothrix heuscheri, Psammoryctides barbatus, Caenis luctuosa, Micronecta, Psectrocladius sordidellus, Procladius, Polypedilum nubeculosum* gr., *Glyptotendipes,* Ceratopogonidae, Culicoidinae, *Chironomus* sp. [1, 2] *,Dero digitata* [6] *Tubifex tubifex* [4, 5, 6]

Potamothrix hammoniensis [4, 5, 6] *Limnodrilus hoffmeisteri* [4, 5, 6] ,*Limnodrilus claparedeianus* [4, 5, 6] ,*Limnodrilus udekemianus* [4, 5, 6] *Limnodrilus* immatures [4, 5, 6]

Infralittoral vegetation

Nais barbata, Nais pseudobtusa, Caenis luctuosa, Pyrrhosoma nimphula, Erythromma viridulum, Ischnura elegans, Psectrocladius sordidellus, Cricotropus sylvestris , Ceratopogonidae ,Culicoidinae, Coleoptera indet., *Lymnaea auricularia (Radix* cf. *auricularia), Planorbella* sp.,

Sublittoral/Profundal

Chironomus plumosus gr., *Chaoborus flavicans, Chironomus* sp.[1, 2], *Dero digitata* [6], *Tubifex tubifex* [4, 5, 6], *Potamothrix heuscheri* [4, 5, 6], *Potamothrix hammoniensis* [4, 5, 6], *Limnodrilus hoffmeisteri* [4, 5, 6], *Limnodrilus claparedeianus* [4, 5, 6], *Limnodrilus udekemianus* [4, 5, 6], *Limnodrilus* immatures [4, 5, 6]

4.4 Discussion and Conclusion

Our results showed that the driving factors affecting macroinvertebrate diversity were first the lake zone and second the eutrophication status. This probably reflect the fact that macroinvertebrate taxa of the three zones are taxonomically and functionally (as habit and food preferences, cf. Cummins & Wilzbach 1985, Merritt & Cummins 1996) different. In the sandy eulittoral, most of taxa (oligochaetes, bivalves and some chironomids) were borrowing and collector/shedder elements, whereas the macrophyte-associated taxa (such as acariens, large crustaceans, several order of insects, gastropods) containing high numbers of mobile forms (climbers, sprawlers and swimmers) and scraper/shredder/piercer forms. In the profundal zone, macroinvertebrates showed again a predominance of burrowers and collectors/shedders. Predators were present in all three zones. Despite these general observations, no *qualitative* metric based on functional feeding group was correlated coherently with the pressure gradient, suggesting that those metrics might be valuable only coupled with organism abundance estimates.

The multivariate analysis also suggest that communities belonging the three zones should be treated separately to get an unbiased information on macroinvertebrate responses to disturbances. It is known that, besides the nutrient enrichment, the profundal community can be affected by hypolimnetic oxygen depletion and/or sediment toxic contamination (Bazzanti & Seminara 1987b, Lafont et al. 2010). The macroinvertebrate associated with the infralittoral vegetation are mainly affected by water transparency related to trophic status (Cyr & Downing 1988, Pieczyńska et al. 1999**)**, whereas the eulittoral community can be also affected by hydromorphological alterations **(**Brodensen et al. 1998, Brauns et al. 2007).

Metrics based on lake zone specific sensitive and tolerant taxa and on molluscan+large crustacean taxa (MOLCRUR and related) are potentially suitable to assess ecological status because their values decreased with disturbance and provided separation of the lakes situated at the extremes of the trophic scale. Because the presence of species along the lakes with different trophic levels depends by the availability of suitable habitats, the list of trophic sensitive taxa varies according to the lake zone. Additionally, as already stated, the assemblages of the three lake zones are not responding in the same way to eutrophication. As previously stated, in addition to eutrophication pressure, the eulittoral can be influenced by hydromorphological modifications of lake shores and the simultaneous effects of these two pressures are probably the cause of the weaker response of selected metrics in this zone with respect to the other two zones. These results and those reported in the literature (Brodensen et al. 1998, Brauns et al. 2007) highlight that further studies on different eulittoral substrates are necessary to correctly assess the ecological status of the eulittoral of lakes.

The other taxa richness metrics tested in this study seemed less efficient in depicting correctly the trophic gradient in the three lake zones. In particular, chironomid taxa richness showed not significant results along lake eutrophication gradient in none of the three benthic zones. This is a surprising finding because chironomids represent one of the major numerical constituent, both as species number and abundances, of freshwater macroinvertebrates, and have been widely used for biological assessment of lentic waters (cf. Saether, 1979; Wiederholm 1980; Rosenberg & Resh 1993). In this paper, especially for both vegetated and sublittoral/profundal zones we individuated some good indicators belonging to this dipteran family, such as *Micropsectra* sp., *Patatendipes albimanus* group, *Microtendipes pedellus* group, which well discriminated oligo-mesotrophic conditions from eutrophic ones. Although the adoption of chironomid species as metric might pose some difficulties of taxonomic identification, we advocate the need to include this group for lake bioassessment studies considering also the information coming from their abundances. Also the metric EOTC showed no clear relation to eutrophication, because all these four orders of insects contain both sensitive and tolerant taxa. It is the case, for example, of the ephemeropteran *Caenis luctuosa* which is indicator of eutrophic waters in our study both for eulittoral sand and infralittoral vegetation zones, whereas *Cloeon simile* resulted more sensitive to eutrophication. Some metrics like richness of Odonata species (ODONOR) showed a barely significant difference among trophic levels only in the infralittoral vegetation. The total number of taxa, the BMWP score, and especially the ASPT score, gave good results only for sublittoral/profundal zone of the studied lakes. This is a surprisingly result because most of pollution-sensitive families of insects usually colonize the littoral of lakes and a good response of these indices should be expected there. Indeed, the BMWP and ASPT methods are widely adopted for running waters, but their application to lake macroinvertebrates are still infrequent (i. e. Johnson 1998, O'Toole et al. 2008), so further studies need to test their applicability to lake littoral. In the sublittoral/profundal, especially deoxygenation plays a major role as a structuring agent of the communities and, consequently, as determinant of these qualitative indices values. BMWP and ASPT score, therefore, could be very promising tools for the evaluation of ecological condition of this benthic zone, where the biological diagnosis usually requires generic or specific identification of the most abundant and diversified groups, such as oligochaetes and chironomids (Saether 1979, Wiederholm 1980, Lang 1990, Lafont et al. 2010) with great processing effort and taxonomic expertise.

In conclusion, we have used a statistical approach to individuate potential metrics and macroinvertebrate taxa indicative of eutrophication in the three zones of Mediterranean lakes. The list proposed in Tables 3 and 4 can be used as a starting point in developing indicator species and indices to be assembled into along with other abundance based metrics to formulate a multimetric index for lake eutrophication proposals. At this stage, these indices can represent valid diagnostic tools to be used for surveillance of the same lakes in the future and of other lakes with different typologies, as their benthic components are commonly distributed in most European lakes (and also elsewhere).

***Figure 3**. Box-plots of six selected metrics of the study lakes cumulated according to their trophic states (O=oligotrophic, OM=oligo-mesotrophic, ME=meso-eutrophic and EH=eutro-hypereotrophic).*

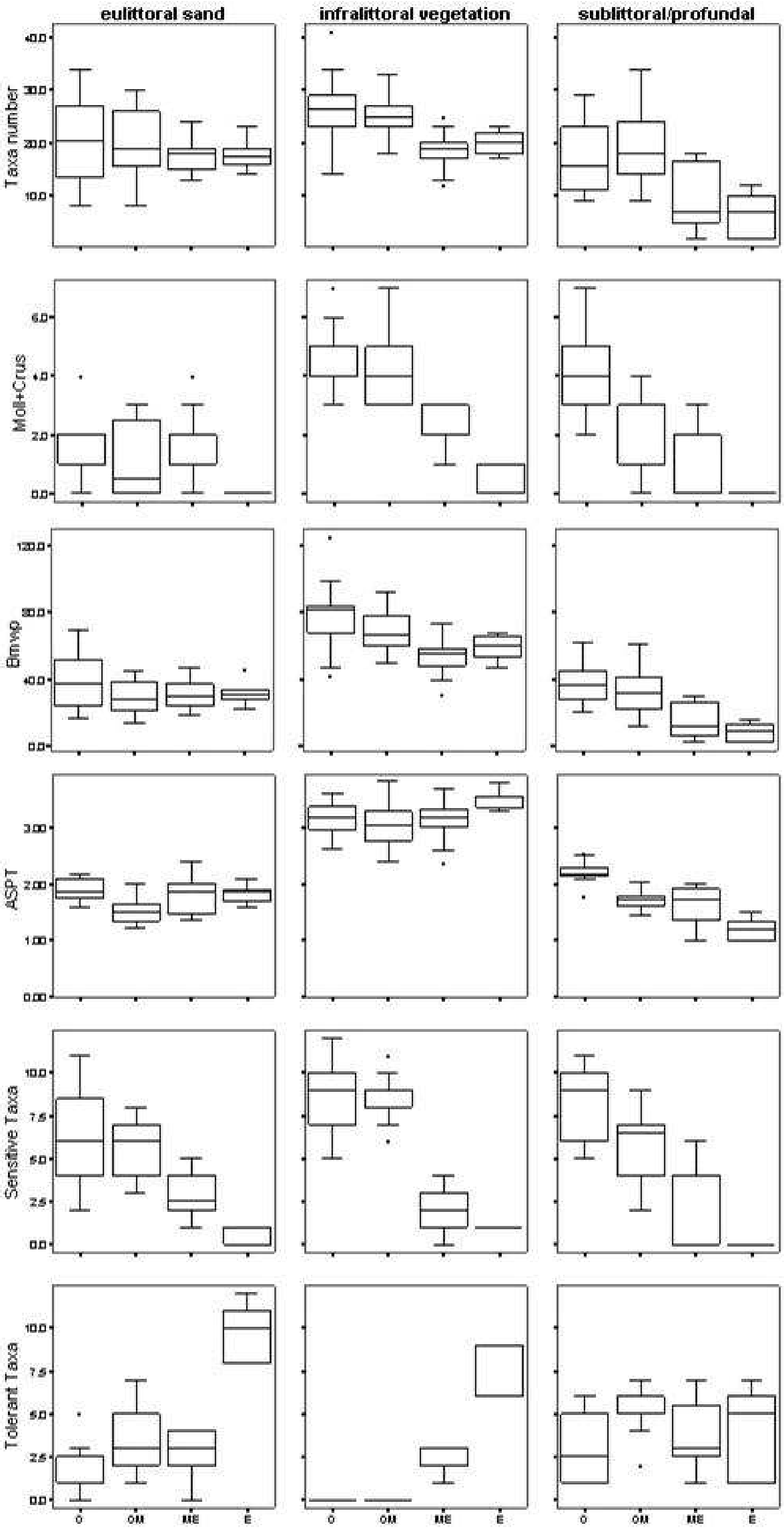


Table 4. List of metric tested in this study. We report here as yes or no the coherence of relationship with eutrophication and the statistically significant differences between the oligotrophic lake/years and eu-hypereuthrophic lake/years.

Metric	Relationship coherent with ecological theory	Eulitoral sand	Infralitoral Vegetation	Sublittoral / Profundal
CHR Richness composed of midges	yes	no	no	no
COLEOPR Richness composed of Coleoptera	yes	no	no	no
DIPR Richness composed of Diptera	yes	no	no	no
EPEMR Richness composed of mayflies	yes	no	no	no
EPTCBO Richness composed of mayflies, stoneflies, caddisflies, Coleoptera, Bivalvia and Odonata	yes	no	no	yes
EPTR Richness composed of mayflies, stoneflies, and caddisflies	yes	no	no	no
ETO Richness composed of mayflies, caddisflies and Odonata	yes	no	no	no
ETO_CHR Ratio of ETO richness to midge richness	yes	no	no	no
ETOC Richness composed of mayflies, caddisflies,Odonata and Coleoptera	yes	no	no	no
GASTROR Richness composed of Gastropoda	yes	no	no	no
MOLCRUR Richness composed of molluscs and crustaceans	yes	yes	yes	yes
NCHDIPR Richness composed of non-midge Diptera	yes	no	no	yes
NONINSR Richness composed of non-insects	yes	no	no	no
ODONOR Richness composed of odonates	yes	no	yes	no
OLIGOR Richness composed of Oligochaeta	yes	no	no	no
ORTHO_CHR Ratio of orthoclad richness to midge richness	no			
ORTHOR Richness composed of Orthocladinae midges	yes	no	no	no
RICH Total richness (number of non-ambiguous taxa)	yes	no	no	yes
TANY_CHR Ratio of Tanytarsanii richness to midge richness	yes	no	no	no
TANYR Richness composed of Tanytarsanii midges	yes	no	no	no
PR_Rich Richness composed of predators	No			
OM_Rich Richness composed of omnivores	No			
GC_Rich Richness composed of collector-gatherers	No			
FC_Rich Richness composed of filtering-collectors	No			
SC_Rich Richness composed of scrapers	No			

Table 4. Continue

Metric	Relationship coherent with ecological theory	Eulittoral sand	Infralittoral Vegetation	Sublittoral / Profundal
BMWP Biological Monitoring Working Party metric	yes	no	no	yes
ASPT Average Score per Taxon	yes	no	no	yes
EUL_SAND_TOL Richness of tolerant taxa in eulittoral according to Table 3	yes	yes	no	no
VEG_TOL Richness of tolerant taxa in infralittoral according to Table 3	yes	yes	yes	no
PROF_TOL Richness of tolerant taxa in profundal according to Table 3	yes	yes	yes	no
EUL_SAND_SEN Richness of sensitive taxa in eulittoral according to Table 3	yes	yes	yes	yes
VEG_SEN Richness of sensitive taxa in eulittoral according to Table 3	yes	yes	yes	no
PROF_SEN Richness of sensitive taxa in eulittoral according to Table 3	yes	no	yes	yes
EUL_SAND_sen_tol Richness sensitive / richness tolerant taxa in eulittoral	yes	yes	no	no
VEG_sen_tol Richness sensitive / richness tolerant taxa in infralittoral	yes	no	no	no
PRO_sen_tol Richness sensitive / richness tolerant taxa in profundal	yes	no	no	yes

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Chapter 5. Effect of catchment land use on littoral macroinvertebrate response to local habitat structure and trophic state

Eleine McGoff, Leonard Sandin

5.1 Introduction

Human pressures can result in clear changes to macroinvertebrate communities in lakes. Anthropogenic eutrophication continues to be a major threat for many lakes, substantially altering the ecological communities (Brauns et al., 2007a). Agriculture, for example, can greatly affect macroinvertebrate communities both directly through cultural eutrophication (Brodersen et al., 1998; White and Irvine, 2003) and indirectly through habitat alteration of lake shores (Brauns et al., 2007b) or through reduction in structural complexity in the littoral zone of lakes (Donohue et al., 2009a; Egertson et al., 2004; Scheffer et al., 1993). However, clear patterns in the response of littoral macroinvertebrates to eutrophication are lacking, possibly due to the structural complexity and patchiness of the littoral environment (Tolonen et al., 2001). Previous work in Danish lakes found only a few species related to trophic status, whereas lake morphometry influenced the majority of the species (Brodersen et al., 1998), and Johnson and Goedkoop (2002) found that trophic state was not the main predictor for Swedish lakes, with other environmental factors explaining most of the variation from stony wave washed shores.

Previous studies have highlighted the importance of both the littoral and the riparian zone for macroinvertebrates. Littoral zones of lakes are made up of a multitude of habitats, comprising various sediment size, plant species, and communities of submerged and emergent vegetation. Work done by White and Irvine (2003) on different habitats within one lake found distinct community compositions for specific littoral mesohabitats. A few studies have attempted to link macroinvertebrate community structure with changes in trophic status of lakes and substrate characteristics, with conflicting views emerging. Tolonen et al. (2001) examined effects of various substrate types and trophic status within lakes on the macroinvertebrate community. They found that substrate had a more important role to play in invertebrate assemblages. This conflicts with the findings of White and Irvine (2003) who found that physical, chemical and environmental variables had a greater impact on invertebrate assemblages than substrate type. This may be owing to the smaller size of the lakes in the White and Irvine study (Tolonen and Hämäläinen, 2010) or the limited nutrient range in the Finnish lakes (White and Irvine, 2003).

Riparian zones also comprise a mosaic of landforms and environments undergoing a variety of natural disturbances. The characteristics of the riparian zone have been found to affect abundance, composition, richness and distribution of macroinvertebrates owing to habitat availability and the nutritional resources in the area (Giudicelli and Bournaud, 1997). This gives rise to an environmental mosaic with few parallels in other systems, causing these aquatic-terrestrial ecotones to display highly differentiated environments (Naiman and Décamps, 1997). However, there is a scarcity of available information on lake ecotones compared with either the terrestrial or open water environments alone (Pieczyńska, 1990). Habitat features and complexity, both in the riparian and littoral zones can greatly influence the composition and abundance of macroinvertebrates. Macroinvertebrate assemblages integrate changes in the physical, chemical and ecological environment of their habitat over time and space (Pinel-Alloul et al., 1996). As such they are likely to change in response to differing aspects of lake hydromorphology among sites and lakes, and the characteristics of the area can affect abundance, composition, richness and distribution of macroinvertebrates owing to habitat availability and the nutritional resources in the area (Giudicelli and Bournaud, 1997).

Lake habitat alteration is a major stressor for lakes worldwide, and Paulsen (1997) stated that physical habitat alteration is likely the biggest threat to aquatic ecosystems, exceeding all other anthropogenic pressures. The text of the European Water Framework Directive (European Commission, 2000) requires the use of freshwater biota for determining the quality and status of fresh and marine waters, and recognises hydromorphological alteration as a potential impact on the composition and abundance of those communities. When using such organism groups it is essential to determine how much effect both natural variation and anthropogenic stress has on the communities (Trigal et al., 2007), and knowledge of the ecological linkages is necessary to understand how changes in land use will affect aquatic communities (Johnson and Goedkoop, 2002).

This study sought to elucidate which group of variables was the most important for describing the Swedish littoral biotic data: trophic status, substrate variables or riparian variables, and what influence each of these groups of variables have on each other. Different features in both the riparian zone and the littoral zone have been found to be highly influential for various macroinvertebrate groups (Gerrish and Bistrow, 1979; Harrison and Hildrew, 1998; Harrison and Harris, 2002; McGoff and Irvine, 2009; Taniguchi and Tokeshi, 2004; Tolonen et al., 2003; Tully et al., 1991; Winterbourn and Crowe, 2001), but to the authors knowledge there has been no previous attempt in the published literature to partition the variance in littoral macroinvertebrates into that independently explained by riparian habitat variables, littoral substrate variables and trophic status of a lake. The impact of large scale land use patterns will also be investigated, to determine if macroinvertebrates respond to different variables in different land use classes. To investigate this our lakes are divided into two groups, those termed agricultural lakes, which have greater than 10% agriculture in their catchment and are judged to be antropogenically impaired, and those termed forestry lakes, which have more than 70% forestry in their catchment, which are judged to be natural lakes. Our hypothesis is that the signal would be clearer in the natural lakes, with more variation being explained in the macroinvertebrate community and less noise from anthropogenic influence. We also hypothesise that the agricultural lakes will be influenced more by the nutrient variables than the forestry lakes, with the habitat variables explaining more of the variation in the forestry lakes as nutrient levels should be lower and thereby exerting less influence. Community heterogeneity is also expected to be lower in the agricultural lakes owing to a homogenisation effect of the nutrients. We will investigate whether macroinvertebrate communities in impaired and natural lakes differ in their response to local habitat and nutrient descriptors, and how the different environmental descriptors affect the variation in the macroinvertebrate data set among different land use types.

5.2 Methods

Data collection and taxonomic resolution

The data set used was a subset of the Swedish national lake survey on 678 lakes (Johnson, 2000; Wilander et al., 1998), carried out in 2000. Measures were taken to ensure the reliability of this data set: macroinvertebrates were sampled from stony bottom littoral regions in the autumn of 2000 to reduce both spatial and temporal variability. Samples were collected using a standard 0.5 mm mesh, 25x25 cm kick net. Five kick samples were taken in each site, comprising a total area of 1.25 m2, and each kick sample comprised a kick along a 1 m stretch for 20 seconds. All five samples were then pooled to one sample for analysis. Samples were subsampled if it was judged that sorting would exceed 2 hours. Taxonomic identification was carried using a predetermined list of 517 operable taxonomic units decided by expert opinion (Wilander et al., 1998). Taxa were identified to the lowest taxonomic unit possible, generally species, except for oligochaets and chironomids. All samples were sorted and identified according to quality control and assurance protocols. For more detailed description of the sample processing see Wilander et al. (1998). The data set was taxonomically harmonised prior to analysis to ensure coherence throughout the data.

Sample sites

For this study we wanted to test if there was a difference in benthic communities between disturbed and natural catchment land use lakes, and decided to compare those lakes affected by agriculture with the more natural forestry lakes. As there is large differences between the ecoregions in Sweden (Johnson and Goedkoop, 2002), it was decided to only compare lakes within the same region, which in this case was the mixed forest region of Sweden, Illes ecoregion 14, comprising sites in the nemoral, boreonemoral and sourthern boreal, done in accordance with Johnson (2003). Any lake catchment with more than 10% agriculture was deemed an ‘agricultural’ lake, and lake catchments with more than 70% forestry were classified as ‘forestry’ lakes. Corine information was used to determine the percentage of land use falling into both of the chosen land use types using Swedish Land Cover Data or

Svenska Marktäckedata (SMD) which is a product of the CORINE land cover data from 2000. 80 lakes were randomly chosen in each land use class, giving a total of 160 lakes.

Habitat descriptors and chemical analysis

The sites where macroinvertebrates were sampled were classified according to substrate type and vegetation cover. Six substrate classes, ranging from silt/clay to block, two classes of detritus cover, fine and coarse, and eight classes of vegetation, e.g. emergent vegetation, floating leaves etc were classified using four categories: <5%, 5-25%, 25-75% and >75%. Using this same categorical classification scheme the riparian zone was also surveyed in shoreline stretches 50 m long and 30m, adjacent to the sampling site. Eleven categories were used for riparian land use and vegetation cover, e.g. mixed forest, arable, clear-cut etc. Furthermore, shoreline characteristics were also assessed in a band 5m and 50 m long alongside the sampling site, 5 categories were used, e.g. presence of riparian trees and canopy cover. These substrate and vegetation classes are further outlined in Table I, Appendix. The habitat descriptors were divided into two groups for further analysis 1) substrate variables, which described the in lake substrate and vegetation present, and 2) riparian variables, incorporating the shore and riparian habitat variables. In addition, water samples were taken for each site for analysis, and were analysed according to international (ISO) or European (EN) standards, when available. Nutrient variables were extracted from the water chemistry data for analysis of the 160 lakes, these were total phosphorus, total nitrogen, NO2, NH4 and total organic carbon.

Statistics

Community composition was analysed using PERMAVOVA in the PRIMER statistical package (Anderson et al., 2008). PERMANOVA tests for the simultaneous response of one or more variables to one or more factors in an ANOVA design, on the basis of any resemblance measure, using permutation methods. PERMANOVA takes a geometrical approach to MANOVA, by calculating the distance among points within a group, and then calculating the distance among groups, and using these as the sum of squares, similar to the ANOVA measures. A pseudo F statistic is calculated, which is analogous to the F statistic in ANOVA, but it does not have a known distribution under the true null hypothesis. The p value is calculated by permutation (or randomization) technique. For our data, the community composition was first 4th root transformed to down-weight the importance of abundant species, and allow rarer species to exert some influence, and a Bray Curtis similarity matrix was generated. Non Multi Dimensional Scaling (MDS), based on the transformed Bray-Curtis resemblance matrix, was used to visualise the community composition (Clarke and Warwick, 2001). PERMDISP (Anderson, 2006) was used as a measure of compositional heterogeneity of macroinvertebrates. PERMDISP is a distance based test of homogeneity of multivariate dispersions among groups of a single factor, and is essentially a multivariate extension of Levene’s test (Levene, 1960)

RELATE was also used in the PRIMER package to measure how closely related two sets of multivariate data are (Clarke and Warwick, 2001). The underlying resemblance matrices of two sets of multivariate data are compared using a Spearman rank correlation coefficient, similar to a Mantel test but with rank correlations instead of Pearson correlations. The matching coefficient, rho, falls in the range of -1 to 1, with values around zero indicating no match between the two matrices. The significance of this result is tested with a non parametric form of the Mantel test, where the sample labels are randomly permuted and rho is recalculated to build up a frequency histogram with which the true values of rho can be compared, and a p statistic generated (Clarke and Gorley, 2006).

Detrended correspondence analysis (DCA) (ter Braak, 1988; ter Braak, 1990) with downweighting of rare taxa, detrended by segments was used to determine the biological turnover, indicating that a unimodal model would best suit the data (gradient length >2), and hence CCA was used. The downweighting option was chosen to reduce the influence of rare taxa. Forward selection with Monte Carlo permutation tests ($p < 0.05$) was used to select the least number of important variables. This analysis was performed using CANOCO version 4.5 (ter Braak and Smilauer, 2002).

Partial CCA (pCCA) analysis was used to decompose the variance explained by each of the variable groups for the macroinvertebrate community composition, as well as residual unexplained variation. All significant variables as chosen by the permutation tests were included in the analysis. Total variation was partitioned into 1) that explained by substrate variables after removing the effect of riparian and nutrient variables 2) that explained by riparian variables removing co-variation from the other groups 3) that explained by nutrient variables removing co-variation from other groups and 4) the variance explained by the crossover of the variable groups. This was determined by running the variable group of interest as a predictor variable, and the other variable groups as the co-variables. pCCA analysis was done with the Vegan package (Oksanen et al., 2008) using the R program (version 2.12.1).

5.3 Results

Land use effects on the environmental variables

PERMANOVA analysis indicated significant differences between forestry and agriculture in all variable groups (substrate, riparian, nutrients) (Table 1)

Table 1: PERMANOVA table of results for the differences between agriculture and forestry lakes in terms of substrate, riparian and nutrient variables.

Source	df	SS	MS	Pseudo-F	P(perm)

Substrate					
Landuse	1	69.5	69.5	3.9	0.001
Residual	158	2792.5	17.7		
Total	159	2862			
Riparian					
Landuse	1	77.2	77.2	4.94	0.001
Residual	158	2466.8	15.3		
Total	159	2544			
Nutrients					
Landuse	1	99.4	99.4	22.6	0.001
Residual	158	695.6	4.4		
Total	159	795			

As expected, the TP values from agricultural lakes were much greater than the forestry lakes (Agriculture TP range: 3-118 µg/l, TP mean: 23.7 +/- 2.6 µg/l; Forestry TP range 4-33 µg/l, TP mean: 12.2 +/- 2.3 µg/l). However, primer RELATE indicated no relationship between either substrate variables and riparian variables, or substrate and nutrient variables, or riparian and nutrient variables, with values close to zero for all of these matches (substrate vs. riparian: rho=0.11, p=0.6%; nutrient vs. substrate: rho=-0.027, p=75.8%; nutrient vs. riparian: rho=0.047, p=13.7%).

Macroinvertebrate community composition was also significantly different between agriculture and forestry (PERMANOVA p<0.05) (Table 2) (Figure 1), indicating that the catchment land use had a significant impact on community composition. Although the data is not well represented in the 2 dimensional MDS, as indicated by the high stress value, the difference in dispersion of sites between agriculture and forestry can be deciphered (Figure 1). The 3D representation more clearly depicts the significant difference between agriculture and forestry community composition (Figure 1). Mean Euclidian distance of samples to the group centroid was greater for agriculture compared with forestry lakes, indicating a higher dispersion, or compositional heterogeneity, in the agricultural macroinvertebrate community composition than that in the forestry lakes (PERMDISP p<0.05) (Table 2).

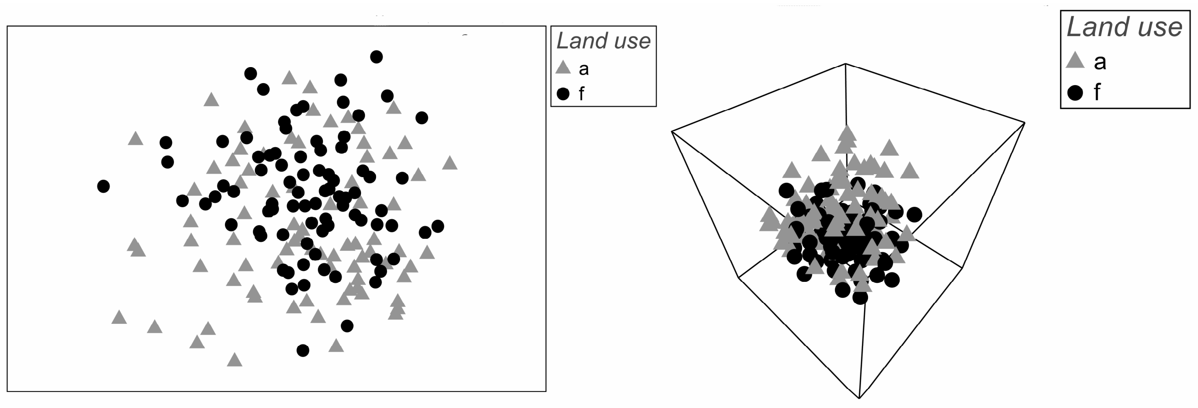

Figure 1: MDS 4th root transformed, Bray Curtis similarity of agriculture and forestry community composition (2D stress=0.28, 3D stress=0.2)

Table 2: PERMANOVA and PERMDISP table of results for the macroinvertebrate community composition between agriculture and forestry

PERMANOVA	df	SS	MS	Pseudo-F	P(perm)
Landuse	1	10027	10027	4.3	0.001
Residual	158	366750	2321.2		
Total	159	366750			
PERMDISP	Mean Euclidean distance	S.E.	P (perm)		
Agriculture	49.3	0.74	0.001		
Forestry	46.3	0.72			

Variance partitioning

Fourteen variables were retained in the pCCA model after forward selection and Monte Carlo permutation tests for all lakes; nine variables were retained in the CCA model for agricultural lakes, and ten were retained for forestry lakes (Table 3).

When all lakes were analysed together, 13% of the variation in the macroinvertebrate community composition could be explained by our three categories; substrate had a significant effect, accounting for 52% of the explained variation. The same pattern was found in both agricultural and forestry lakes, with 10 % and 13% of variance explained respectively by all three categories, and with substrate as the primary driver of this variance. Substrate variables explained over 57% of the variance in agricultural lakes, and 71% of the variance in forestry lakes (Figure 2). Riparian variables explained the most variance after substrate, with nutrients explaining the least fraction of the three, less than 15% of variance in all cases (Figure 2).

Table 3: Variables retained in the direct gradient analysis by forward selection for 1) all lakes, 2) agricultural and 3) forestry lakes with % variance explained as given by forward selection in CANOCO

Variable category	**Variable**	**p**	**λ**	**% explained**
1) All Lakes				
Substrate	pebble	0.001	0.10	9.40
Substrate	sand	0.001	0.05	5.04
Riparian	mire	0.001	0.04	4.25
Riparian	arable	0.001	0.04	4.06
Riparian	upland	0.008	0.03	3.36
Substrate	rosette	0.001	0.03	3.26
Substrate	FOM	0.005	0.03	2.87
Substrate	coarse dead	0.005	0.03	2.77
Nutrient	TN	0.015	0.03	2.57
Nutrient	TOC	0.01	0.03	2.57
Substrate	Emergent veg	0.015	0.03	2.47
Nutrient	NH4	0.025	0.03	2.47
Nutrient	NO2	0.048	0.02	2.37
Substrate	Gravel	0.031	0.02	2.37

2) Agricultural lakes	**Variable**	**p**	**λ**	**% explained**
Substrate	Pebble	0.001	0.13	7.08
Substrate	Moss	0.01	0.06	3.48
Substrate	Sand	0.011	0.06	3.15
Substrate	Rosette	0.032	0.05	2.87
Nutrient	NO2	0.001	0.09	4.83
Nutrient	TP	0.001	0.08	4.33
Riparian	Arable	0.001	0.07	4.04
Riparian	Mire	0.006	0.06	3.54
Riparian	Artificial	0.031	0.05	2.87

3) Forestry lakes	**Variable**	**p**	**λ**	**% explained**
Substrate	Gravel	0.001	0.11	7.61
Riparian	Mire	0.001	0.08	5.67
Riparian	Upland	0.004	0.06	3.88
Substrate	Cobble	0.001	0.05	3.60

Substrate	Coarse dead	0.003	0.05	3.53
Substrate	Sand	0.008	0.05	3.25
Substrate	Emergent	0.004	0.05	3.25
Substrate	FOM	0.009	0.05	3.18
Substrate	Fine sediment	0.022	0.04	2.98
Nutrients	NO2	0.041	0.04	2.84

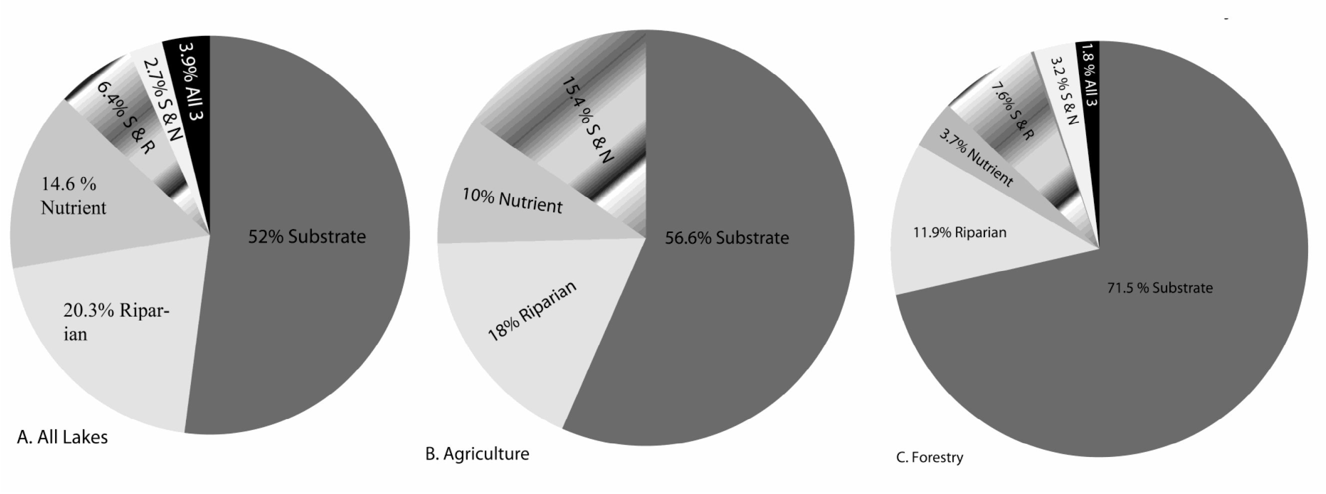

Figure 2: Partitioning of the explained variance of the macroinvertebrate community composition in all lakes, agriculture lakes and forestry lakes according to three categories, substrate, riparian and nutrient independently, with interactions among all three sets of variables (S=Substrate, R=Riparian and N=Nutrient).

Substrate

Substrate, as the most important category for explaining macroinvertebrate variation, was further examined. Four substrate classes were chosen which were determined to be indicative of either hard and soft substrates (Table 4). The 25 lakes sites which scored highest, i.e. had the largest amount of this habitat, in the soft categories in the original habitat survey were classified as soft, and the same applied for 25 lakes in hard categories.

Table 4: Substrate classes used to classify sites into hard and soft

Soft substrate categories	Hard substrate categories
Fine sediment	Gravel
Fine organic matter	Cobble
Coarse organic material	Pebble
Emergent vegetation	Coarse block

Significant differences in community composition were found between hard and soft substrate communities in both the agricultural and forestry sites (PERMANOVA, $P<0.05$) (Table 5, Figure 3). These differences are visible in the MDS, despite the high stress level for both agriculture and forestry lakes (Figure 3). This indicates that although sites were already stratified to be stony wave washed shores, there was still a substrate gradient leading to differences in the community composition.

Table 5: PERMANOVA table of results for macroinvertebrate communities from agriculture and forestry lakes

Agriculture					
Source	df	SS	MS	Pseudo-F	P(perm)
Su	1	10386	10386	4.5678	0.0001
Res	48	109140	2273.7		
Total	49	119520			
Forestry					
Source	df	SS	MS	Pseudo-F	P(perm)
Su	1	6193.4	6193.4	2.9973	0.0001
Res	48	99185	2066.4		
Total	49	105380			

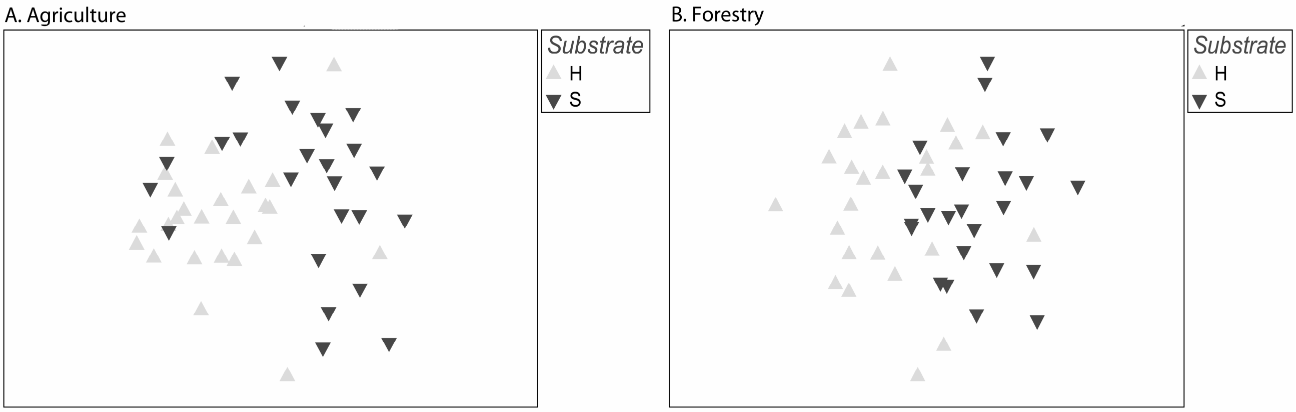

Figure 3: MDS of 4th root transformed community composition with Bray Curtis similarity of agriculture and forestry hard and soft sites. (agriculture stress= 0.22, forestry stress=0.27)

When the 25 hard sites for each agriculture and forestry were assessed using forward selection, nutrients, in the form of nitrogen, were important for stratifying the macroinvertebrates in the agricultural sites, but not in the forestry sites (Table 6). Riparian variables explained more variation in both forestry and agricultural lakes. Mire was the individual variable explaining the most variation in both lake types, with canopy cover and presence of trees also explaining significant amounts of the variation in agricultural lakes.

Table 6: Variables retained in the direct gradient analysis by forward selection for riparian and nutrient variables in 25 hard sites from agriculture and forestry

		p	λ	% explained
Agriculture	Mire	0.01	0.193	10.7
	NO2	0.01	0.175	9.7
	NH4	0.01	0.134	7.4
	Canopy cover	0.01	0.134	7.4
	Trees	0.05	0.119	6.6
	TN	0.05	0.105	5.8
Forestry	Mire	0.01	0.266	14.5

5.4 Discussion

Catchment land use differences

Macroinvertebrate community composition differed significantly from agriculture to forestry lakes. This is in keeping with findings by Johnson and Goedkoop (2002), which also highlighted the importance of ecoregion and catchment characteristics for predicting macroinvertebrate communities in Swedish lakes. They conjectured that catchment characteristics may set the upper limits and that nested within these limits the local factors become important. However, not all researchers agree, and although we found a significant difference in this study, this is not always the case. This is highlighted in the review by Hawkins et al (2000) who summarised that although ecoregion and catchment characteristics may account for more than a chance amount of biotic variability, the classification strength of these for macroinvertebrates community composition was still weak, and the relationship between benthic invertebrates and catchment characteristics was poorly understood.

The substrate and riparian variables also differed significantly between land use types. This is not unexpected, as landscape scale variables can influence the smaller scale habitat level characteristics, and hence the community composition (Johnson and Goedkoop, 2002). However, interestingly, when tested, there was no clear relationship between the riparian and substrate variable groups themselves. Previous work by Brauns et al (2007b) has shown the relationship between shoreline habitat alteration and a consequent reduction in the habitat heterogeneity in the littoral zone of the lake, with knock on effects on the littoral macroinvertebrates. However, in this case, possibly owing to the absence of a clear alteration pressure within the riparian zone, the relationship between riparian and substrate variables was not found.

No relationship was found between the nutrients and the substrate or riparian variables, but the nutrients did differ significantly with land use. Several studies have demonstrated the impact of land use on water chemistry in lakes (Dodson et al., 2005; Harper and Stewart, 1987; Kizuka et al., 2008), indicating that lake nutrients are often related to land use over a much larger area, such as the catchment. As expected, the nutrient levels were higher in agricultural lakes compared with forestry lakes. However, interestingly, the compositional heterogeneity of macroinvertebrate community composition was greater in agricultural sites than in forestry sites. This is surprising given findings by Donohue et al (2009a) who found that nutrient rich lakes has significantly more homogenous benthic assemblages than nutrient poor lakes. However, the nutrient rich lakes in Donohue et al (2009a) were those with a TP value above 35 µg/l, and the average TP for our agricultural lakes was 23 µg/l. Tolonen (2005) found a unimodal relationship between taxa richness and trophic gradient, possibly indicating that intermediate disturbance enhances species richness (Cornell and Lawton, 1992), and in our case possibly leading to increased compositional heterogeneity.

Substrate and nutrient effects on macroinvertebrates

This study highlighted the importance of substrate above either riparian or nutrient variables for macroinvertebrates in Swedish lakes, with substrate explaining the largest proportion of the variation in community composition in all lakes. Several other studies have attempted to link macroinvertebrate community structure with changes in trophic status of lakes and substrate characteristics, with conflicting views emerging. Tolonen *et al.* (2001) examined effects of various substrate types and trophic status within lakes on the macroinvertebrate community. They found, similar to our findings, that substrate had a more important role to play in invertebrate assemblages than nutrients. This conflicts with the findings of White and Irvine (2003) who found that trophic state had a greater impact on invertebrate assemblages than substrate type. White and Irvine (2003) highlight that the trophic range of the lakes within the Tolonen *et al*. (2001) study were much narrower than those examined by them, and may lead to misrepresentative data based on too narrow a range. Tolonen & Hämäläinen (2010) concluded that the differing results may be owing to the size of the lakes studied, with a more heterogeneous network of substrate patches in the larger Finnish lakes. However, in this study we also highlighted the importance of substrate, and the Swedish lakes were comparable in size to White and Irvine (2003) and had a much greater TP range than the Finnish lakes studied by Tolonen (2001). Our TP range of 4-118 µg/l, the White and Irvine (2003) study was 1-344 µg/l, and the Tolonen et al. (2003) study was 3-26 µg/l. This would indicate that while lake size was not such an important factor, trophic gradient might still be, as although our trophic range was greater than Tolonen et al’s (2001) study, the TP gradient was still significantly shorter than in the White and Irvine (2003) study. Whatever the mechanism, in this study habitat variables are more important for describing the bentic community than nutrient variables, in keeping with Johnson and Goedkoop (2002) who also found that environmental factors other than nutrient effects were the most important for the biotic communities of wave washed stony shores of Swedish lakes.

Substrate stratification

When the lakes were further stratified according to substrate hardness, significant differences in macroinvertebrate community composition were found between the hard and the soft sites in both the agriculture and forestry sites. Similar differences in macroinvertebrate community composition between soft vegetation-rich habitats and harder habitats have been found in other studies (McGoff and Irvine, 2009; Tolonen et al., 2001; White and Irvine, 2003). Hard habitat macroinvertebrates samples have been previously found to be the most indicative of trophic pressure (Donohue et al., 2009b), and when examined in this study the macroinvertebrates in the hard sites from agriculture and forestry were responding to different riparian and nutrient variables. Individual nutrient variables were responsible for explaining much of the variation in the agriculture data set, but none of the variation in the forestry data set. The lack of response to nutrient variables in the forestry lakes may be owing to the shortness of the nutrient gradient, similar to what White and Irvine (2003) concluded was happening in the Tolonen et al (2001) study. The range in forestry lakes was just 1-33 µg/l TP, compared with 1-118 µg/L TP in agricultural lakes. Therefore, as predicted, the macroinvertebrates in agricultural lakes were more influenced by antropogenic pressure than those in the forestry lakes, but this only becomes obvious when sites are strongly stratified for substrate, as without this stratification substrate was the explanatory factor.

In forestry lakes the only measured variable of the riparian and nutrient variables examined which contributed to explaining the variation was the presence of mire/wetland habitat in the riparian band. Interestingly, riparian mire habitat also explained the largest amount of variation in the agricultural lakes. This may be owing to organic matter input, as wetlands can be a significant source of organic matter for lakes and streams, and even small riparian wetland areas can have a dominant effect on the organic budget of a waterway (Dosskey and Bertsch, 1994; Gergel et al., 1999). Riparian variables had more influence than nutrient variables, even in these stratified sites, collectively accounting for 24% of the variation in the agricultural data set, and 14.5% of the variation in the forestry data set. The important variables were the presence of mire and/or the presence of trees and canopy cover. Riparian vegetation, and overhanging canopy have been previously shown to be important for macroinvertebrate community composition as it alters the oviposition behaviour of the adult phase of many macroinvertebrates (Harrison and Hildrew, 1998; Harrison and Harris, 2002). Brauns et al (2007a) also highlighted the importance of the riparian zone in their study, with grassland next to the lake being significantly related to community composition, over and above trophic effects. The authors hypothesise that the mechanism was that the reduction in the amount of trees and scrub in the riparian zone led to a concomitant reduction in the amount of coarse woody debris entering the lake. Similar results were found for North American lakes: with an increase in anthropogenic pressure on the lakes shore, in the form of lake cabins, the amount of riparian vegetation and hence coarse woody debris entering the lake also decreased (Christensen et al., 1996). This mirrors the findings of our study, which also highlighted the importance of riparian trees and canopy cover for describing macroinvertebrate community composition.

Management implications

These results have some interesting implications for standard monitoring and assessment. These sites were initially stratified to be taken from stony, wind exposed littoral areas, however, as evidenced they fell along more of a gradient, leading to changes in the macroinvertebrate community which were related to substrate, not nutrients, thereby masking the nutrient signal. While it has been previously recommended to stratify samples according to hard substrate (Donohue et al., 2009b; White and Irvine, 2003), these results indicate that, at least in the Swedish setting, greater care needs to be taken in classifying the substrate in order to see this pressure response relationship. White and Irvine (2003) acknowledge that variation among mesohabitats would increase the inherent noise in a dataset, which is clear from our dataset. Likewise, variation in the riparian zone also needs to be considered, particularly in relation to mire and the presence of riparian trees, as these add additional noise to the data set. Our results suggest that in order to reduce variability among sites, particular care needs to be taken to stratify according to either the presence or absence of riparian trees.

Although the Water Framework Directive requires the use of benthic macroinvertebrates to classify the ecological status of lakes (European Commission, 2000), there has been ongoing debate in the literature as to whether littoral macroinvertebrates are too hetergenous to use in standard monitoring (Harrison and Hildrew, 1998; Moss et al., 2003; Rasmussen, 1988). This study would suggest that in the absence of strict stratification by substrate, the macroinvertebrates of Swedish lakes would not provide a robust classification of the trophic status of a lake. This is similar to findings for lowland lakes in Germany (Brauns et al., 2007a), which found that different mesohabitats within the lake were responding to different pressures, often unrelated to trophic status. However, separating the effects of nutrient enrichment and hydromorphological alteration is difficult as they are often interrelated (Solimni et al., 2006), and if macroinvertebrates are to provide a useful assessment of lake quality, then there is a need for a considerable increase in the knowledge of how they respond to nutrient and hydromorphological pressures, and how these factors interact with each other.

5.5 Conclusion

In conclusion, our results suggested that trophic effects are nested within substrate habitat effects, and were masked by local habitat heterogeneity. Once substrate was stratified more strictly the nutrient signal could be deciphered. However, the macroinvertebrates were also responding to riparian vegetation, and the importance of riparian vegetation has been shown by many studies (Brauns et al., 2007a; Christensen et al., 1996; Harrison and Hildrew, 1998; Harrison and Harris, 2002). The absence of riparian trees and canopy cover can be used as a proxy for anthropogenic alteration of lake shores. Therefore, as outlined in Brauns et al (2007a), although macroinvertebrates may not be strong indicators of trophic pressure in all cases or habitats, they may be useful indicators for other anthropogenic pressure on lake shores.

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Appendix

Table I: Riparian and substrate variables measured as part of the Swedish national survey. 0/1 denotes presence/absence, 0-3 indicates a range of surface cover of a particular variable where 0=none present, 1= < 5%, 2=5-50% and 3=>50%. Zone indicates if it was measured in the 50 m or the 5 m riparian band

Zone/ m	Riparian variables	Measure	Littoral variables	Measure
50	Decidious	0- 3	Fine sediment	0-3
50	Arable	0- 3	Sand	0-3
50	Artificial	0- 3	Gravel	0-3
50	Coniferous	0-3	Pebble	0-3
50	Rough grassland	0- 3	Cobble	0-3
50	Other	0-3	Fine block	0-3
50	Mixed forest	0-3	Coarse block	0-3
50	Upland grassland	0-3	Bedrock	0-3
50	Clearcut	0-3	Emergent veg	0-3
50	Mire/wetland	0-3	Floating leaved veg	0-3
50	Boulder field	0-3	Fontinalis veg	0-3
5	Riparian trees	0 or 1	Rosette veg	0-3
5	Bushes	0 or 1	Mosses	0-3
5	Open land	0 or 1	Epiphytes	0-3
5	Amount of canopy cover	0-3	Fine organic material	0-3
5	Amount of shading	0-3	Coarse organic material	0-3
			Fine dead wood	0-3
			Coarse dead wood	0-3
			Fine sediment	0-3

Chapter 6. Implications of shoreline development on lake macroinvertebrates and consequences for assessment of lake ecological status

Gwendolin Porst, Steffen Bader, Elise Münch

6.1 Introduction

Lakeshore zones are characterized by a complexity of habitats (Hall et al. 1992, Pickett and White 1985, Strayer and Findlay 2010, Wetzel 2001) owing to a variety of abiotic and biotic factors. This natural variability is threatened, however, by a multitude of human activities including shoreline developments associated with human settlements or industrial purposes (Brauns et al. 2007b, Strayer and Findlay 2010). One of the consequences of anthropogenically caused shoreline alterations is the loss of habitats and here particularly the loss of macrophyte beds, root or woody debris habitats (Christensen et al. 1996, Elias and Meyer 2003, Radomski and Goeman 2001). While ecological consequences of shoreline development such as loss of habitat complexity have mainly been studied for fish assemblages (Jennings et al. 1999, Scheuerell and Schindler 2004), implications this has on the less mobile macroinvertebrate assemblages have rarely been quantified to date. Alteration of lake shorelines such as erosion control structures or recreational beaches are, however, expected to have an important influence on littoral macroinvertebrates (Bänziger 1995, Solimini et al. 2006, Brauns et al. 2007b). Macroinvertebrates strongly depend on littoral habitats as habitat diversity provides a variety of ecological niches (O'Connor 1991, Schneider and Winemiller 2008, Taniguchi et al. 2003), lowers predation risk by foraging predators (Schneider and Winemiller 2008) and provides refuge from physical disturbances such as wind- or ship-induced waves (Gabel et al. 2008).

Littoral macroinvertebrates are one of the major components of lake ecosystems. The European Water Framework Directive (WFD), therefore, requires member states to assess lake ecological status by monitoring these biological quality elements alongside other biological groups (EC 2000). Thus, understanding major environmental influences such as implications of hydromorphological shoreline alterations on macroinvertebrate diversity, abundance and community composition have to be assessed prior to the development of lake monitoring protocols and for the development of suitable metrics for the assessment of lake ecological status. While the understanding of ecological impacts of shoreline development forms a prerequisite for a scientifically based ecological monitoring system for lakes, cost and time are often an additional important factor when choosing appropriate sampling methods for the monitoring of lakes using benthic macroinvertebrates. Several studies have identified habitat stratification as a requirement to overcome the problem of inherent habitat heterogeneity in the lake littoral (Brauns et al. 2007a, Tolonen et al. 2001, Weatherhead and James 2001). While habitat stratification can reduce variability within macroinvertebrate samples and, thus, possibly improve signal precision, the collection of pooled ‘composite’ macroinvertebrate samples could, however, present a feasible cost and time effective alternative to the time-intensive habitat-specific sampling approach.

The objectives of this study were 1.) to quantify the impact of hydromorphological shoreline alterations on the community structure and diversity of lake macroinvertebrates by comparing unmodified with soft (recreational beaches) and hard (retaining walls, ripraps) altered shorelines 2.) to test whether a composite macroinvertebrate sample could represent a sampling site adequately when compared with stratified habitat specific macroinvertebrate samples and can, thus, serve as a cost and time effective alternative methodology for the monitoring of lakes using the example of lake Werbellin.

6.2 Methods

Macroinvertebrate sampling

This study was conducted at lake Werbellin (52°55.577' N, 13°42.525' E), a large oligo- to mesotrophic lake with a surface area of 7.95 km^2, situated in north-eastern Germany. Samples were collected in April 2010 from three morphologically differing shoreline types following the WISER WP 3.3 lake macroinvertebrate sampling protocol. Morphological alterations were classified as ‘soft alteration’ (recreational beaches) and ‘hard alteration’ (e.g. retaining walls, ripraps). Within the lake three unmodified shoreline sites, three sites with soft alterations and three sites with hard alterations were sampled for macroinvertebrates. Each sampling site comprised a shoreline section of minimum 25 m length representing either soft alteration, hard alteration or unmodified sites. At each sampling site 3 habitat specific samples, ideally from sand, stones and macrophytes plus one composite sample were collected. In case one of these habitats was not present at a certain sampling site, a second sample of the dominant habitat at this site was collected. If only one habitat was present at a site i.e. only sand habitats at recreational beaches, three sand habitat samples were collected. Whenever present, the habitats roots and woody debris were sampled for macroinvertebrates additionally to samples collected according to the WISER WP 3.3 sampling protocol. Habitat-specific samples comprised the collection of 1 m² samples per habitat. Composite samples involved a standardised 1 min sampling for macroinvertebrates by sampling all habitats present proportional to their availability within each sampling site. Sampling of different habitats followed the methods described in Brauns et al. (2007a). Habitat and composite samples were preserved in ethanol and processes in the laboratory. Macroinvertebrates were identified to species level, whenever possible, except Chironomidae (sub-family), other Diptera (family) and Oligochaeta (order).

Statistical Analysis

The non-parametric Kruskal-Wallis test (PASW 17, SPSS-Inc. 2009) was used to test for differences in macroinvertebrate taxon richness among alteration types and among sand habitat samples collected from different shoreline alteration types. Non-metric multidimensional scaling (MDS) tested for similarities in macroinvertebrate community structures among alteration types and sand habitats within alteration types as well as among habitat specific and composite macroinvertebrate samples (PRIMER$^{®}$ 6, PRIMER-E Ltd, Ivybridge). MDS constructs a ‘map’ of all macroinvertebrate samples and represents these as points in a low dimensional space, so that the relative distance of points corresponds to the same rank order of dissimilarities measured by the underlying similarity matrix (Clarke and Warwick 2001). While the comparisons of macroinvertebrate community structures from different alteration types and sand habitats within alteration types were based on log(x+1) total abundance macroinvertebrate data and a Bray-Curtis similarity matrix, for the comparison of sampling methodologies the ordination method was based on log(x+1) transformed proportional abundance data using a Bray-Curtis similarity matrix to account for different sampling methodologies. A one-way analysis of similarities (ANOSIM, PRIMER$^{®}$ version 6, PRIMER-E Ltd, Ivybridge) tested for significant differences in macroinvertebrate community structures among alteration types, sand habitats within alteration types, and habitats and composite samples using 9999 permutations.

In order to identify taxa contributing most to dissimilarities in macroinvertebrate community structures among alteration types, sand habitats within alteration types and among single-habitat and composite samples, the similarity percentage routine SIMPER (PRIMER® 6, PRIMER-E Ltd, Ivybridge) was used. SIMPER computes the percentage contributions of individual species to respective sample differences (Clarke and Warwick 2001). Indicator species analysis (INdVal) identified macroinvertebrate taxa which are characteristic for a habitat or alteration type by means of the programme R (R Development Core Team 2009) and the statistical package labdsv (Roberts 2010).

To test whether variability of macroinvertebrate community structures within composite samples was significantly different from variability within habitat specific samples, we tested the homogeneity of dispersion of each individual habitat sampled using permutational analysis of multidimensional dispersion (PERMDISP, PRIMER® 6 with PERMANOVA+, PRIMER-E Ltd, Ivybridge). PERMDISP compares the average dissimilarity of replicate samples to their group centroid based on an F statistic while calculating significance levels using permutation of least-squares residuals (9999 permutations) (Anderson et al. 2008). Analysis was based again on log(x+1) proportional abundance data using a Bray-Curtis similarity matrix. PERMDISP was furthermore used to test the adequacy of composite samples for monitoring of lake ecological status by comparing the composite samples collected in the field with artificially computed composite samples. Artificial composite samples were generated exemplarily for unmodified sampling sites by accumulating single habitat samples according to their proportional availability at respective sampling sites.

6.3 Results

Spatial variability

Number of habitats available at sampling sites varied among different sites and alteration type (Table 1). Highest average taxon richness over all habitats sampled was found at unmodified sampling sites (30 ind./m^2) and lowest at recreational beaches (21 ind./m2). Macroinveretbrate taxon richness at hard alteration sites reached an average of 24 ind./m^2. However, while a tendency of decreasing macroinvertebrate taxon richness with increasing alteration of shorlines was noted, this could not be identified to differ significantly among alteration types or in sand habitat samples among alteration types (Kruskal-Wallis test, Chi-Square = 5.52, $P > 0.5$ and Chi-Square = 1.74, $P > 0.05$, respectively).

Macroinvertebrate samples from different habitat types showed higher similarities within alteration type than within habitat type (Figure 1). ANOSIM identified significant differences among macroinvertebrate community structures of different alteration types including all habitat samples (ANOSIM, R-statistic = 0.364, $P < 0.01$), with macroinvertebrate assemblages from unmodified sampling sites differing stronger from soft alteration sites (ANOSIM, R-statistic = 0.482, $P < 0.01$) than from hard alteration sites (ANOSIM, R-statistic = 0.306, $P < 0.01$). Assemblages from hard and soft alteration sites also differed significantly from each other (ANOSIM, R-statistic = 0.337, $P < 0.01$).

Table 1: Number of habitat types sampled for macroinvertebrates at different shoreline alteration types.

	Unmodified sampling sites	Soft alteration sampling sites	Hard alteration sampling site
Sand	4	9	8
Macrophytes	4	-	-
Stones	1	-	1
Roots	2	1	-
Woody debris	3	-	-

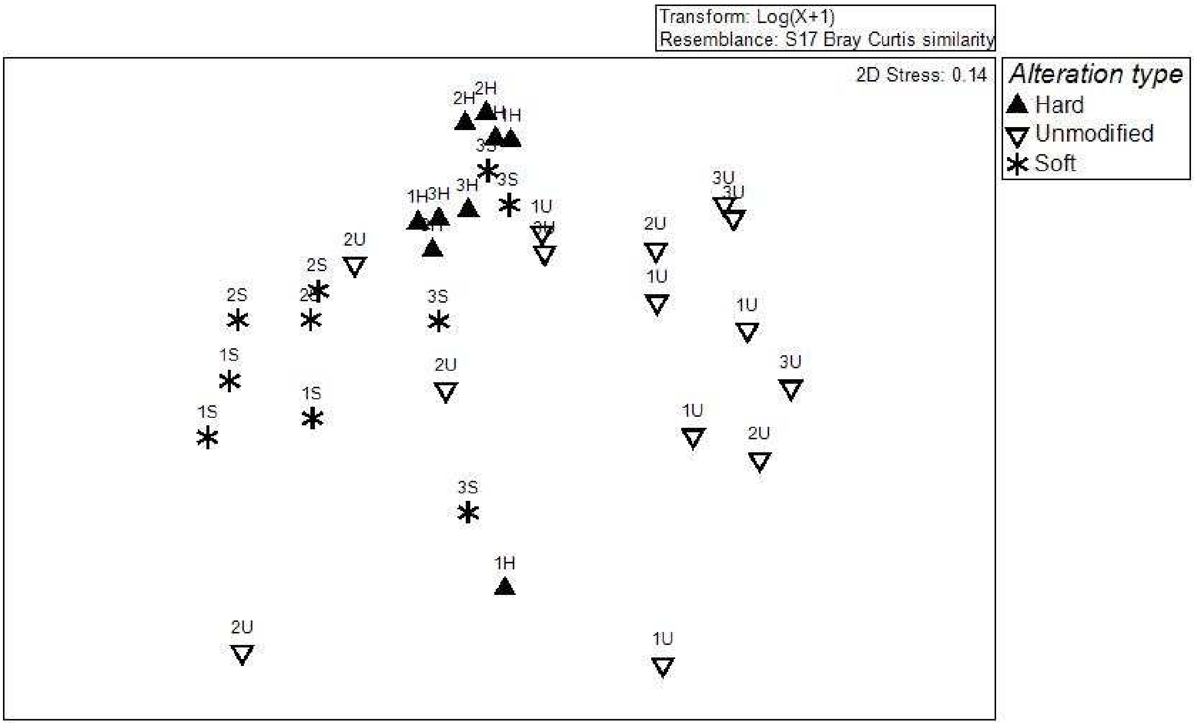

Figure 1: MDS-plot of macroinvertebrate species log(x+1) total abundance data from sand, root, macrophyte, stone and woody debris habitats collected at unmodified, soft and hard alteration sampling sites at lake Werbellin.

MDS identified a clear clustering of macroinvertebrate assemblages from sand habitats (the most dominant habitat present across all sampling sites; $n \geq 3$ for all alteration types) according to alteration types (Figure 2). This was confirmed by the results from ANOSIM, which identified community structures of sand habitats to strongly differ among alteration types (ANOSIM, R-statistic = 0.48, P < 0.01). Macroinvertebrate communities from hard alteration sites differed strongest from unmodified sites (ANOSIM, R-statistic = 0.772, P < 0.01), while differences between unmodified and soft (ANOSIM, R-statistic = 0.437, P < 0.01) and soft and hard alteration sampling sites (ANOSIM, R-statistic = 0.461, P < 0.01) still showed clear differences among groups. Taxa contributing most to dissimilarities among alteration types and among sand habitat samples from different alteration types are summarized in Table 2 and 3. IndVal analysis identified a total of eighteen characteristic species for different habitat types (Table 4) with highest numbers of characteristic species found for macrophytes (8) and woody debris (6). A total of twenty-five characteristic taxa were identified for different alteration types with the majority of characteristic taxa found in unmodified sampling sites (Table 5).

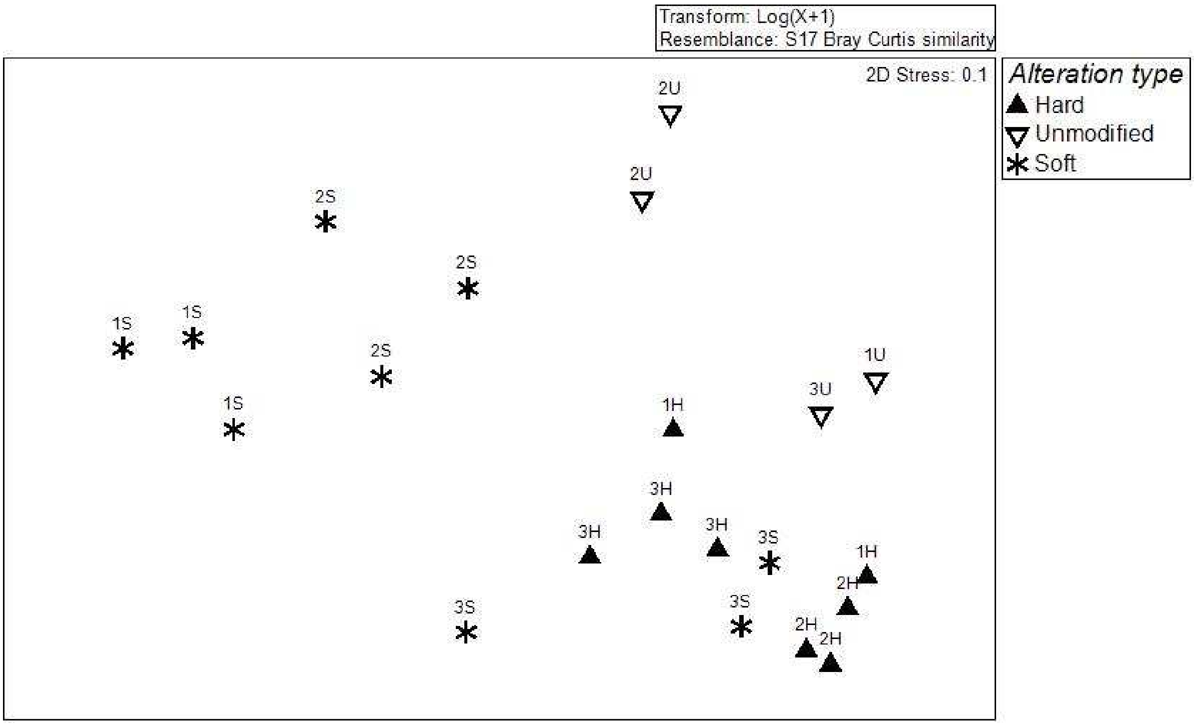

Figure 2: MDS-plot of macroinvertebrate species log(x+1) total abundance data from sand habitat samples collected at unmodified, soft and hard alteration sampling sites at lake Werbellin.

Table 2: Summary results from SIMPER analysis showing cumulative contributions (Cum.%) of first 20 taxa contributing to dissimilarities (in %) among alteration types (unmod=unmodified sampling sites; soft=soft alteration sampling sites; hard=hard alteration sampling sites).

Hard alteration & unmodified sampling sites			Hard & soft alteration sampling sites			Unmodified & soft alteration sampling sites		
Average dissimilarity = 52.63			Average dissimilarity = 46.26			Average dissimilarity = 56.75		
Species	Higher abundance	Cum.%	Species	Higher abundance	Cum.%	Species	Higher abundance	Cum.%
Dreissena polymorpha	unmod	3.95	*Potamopyrgus antipodarum*	hard	7.42	*Micronecta* sp*.*	soft	5.71
Potamopyrgus antipodarum	hard	7.86	*Pisidium* sp.	hard	14.13	*Dreissena polymorpha*	unmod	11.04
Tanypodinae sp.	unmod	11.77	*Caenis luctuosa*	hard	19.44	*Potamopyrgus antipodarum*	unmod	15.63
Orthocladiinae sp.	unmod	15.46	*Micronecta* sp.	soft	24.56	*Tanypodinae* sp.	unmod	19.88
Caenis luctuosa	hard	19.09	*Dreissena polymorpha*	hard	29.65	*Orthocladiinae* sp.	unmod	23.81
Pisidium sp.	hard	22.63	*Ceratopogonidae* sp.	hard	33.7	*Pisidium* sp.	unmod	27.58
Chironominae sp.	hard	26.05	*Oligochaeta* sp.	hard	37.59	*Sphaerium* sp.	only unmod	31.05
Mystacides azurea	hard	29.38	*Caenis horaria*	hard	41.46	*Gammaroidea* sp.	unmod	34.15
Diptera sp. *(pupae)*	hard	32.68	*Chironominae* sp.	hard	44.93	*Pontogammarus robustoides*	soft	36.84
Sphaerium sp*.*	only unmod	35.76	*Mystacides azurea*	hard	48.39	*Dikerogammarus villosus*	unmod	39.5
Ceratopogonidae sp.	hard	38.77	*Athripsodes cinereus*	hard	51.8	*Oligochaeta* sp.	soft	41.96
Oligochaeta sp.	hard	41.78	*Diptera* sp. *(pupae)*	hard	54.95	*Caenis horaria*	soft	44.43
Caenis horaria	hard	44.52	*Pontogammarus robustoides*	soft	57.91	*Tinodes waeneri*	only unmod	46.87
Micronecta sp.	hard	47	*Gammaroidea* sp.	hard	60.73	*Chironominae* sp.	soft	49.31
Gammaroidea sp.	unmod	49.39	*Haliplus* sp. *(larvae)*	hard	63.5	*Mystacides azurea*	soft	51.72
Tinodes waeneri	unmod	51.68	*Molanna angustata*	hard	65.91	*Dikerogammarus haemobaphes*	unmod	53.96
Dikerogammarus villosus	unmod	53.9	*Acentria ephemerella*	hard	68.17	*Limnephilus lunatus*	unmod	56.12
Limnephilus lunatus	unmod	55.96	*Orthocladiinae* sp*.*	soft	70.25	*Athripsodes cinereus*	unmod	58.25
Dikerogammarus haemobaphes	unmod	58.02	*Gyraulus crista*	hard	72.29	*Caenis luctuosa*	soft	60.33
Athripsodes cinereus	hard	60.06	*Tanypodinae* sp.	hard	74.22	*Gyraulus crista*	unmod	62.37

Table 3: Summary results from SIMPER analysis showing cumulative contributions (Cum.%) of first 20 taxa contributing to dissimilarities (in %) among sand habitat samples from different alteration types (unmod=unmodified sampling sites; soft=soft alteration sampling sites; hard=hard alteration sampling sites).

Hard alteration & unmodified sampling sites			Hard & soft alteration sampling sites			Unmodified & soft alteration sampling sites		
Average dissimilarity = 40.61			Average dissimilarity = 44.40			Average dissimilarity = 46.24		
Species	Higher abundance	Cum.%	Species	Higher abundance	Cum.%	Species	Higher abundance	Cum.%
Diptera sp. *(pupae)*	hard	5.63	*Potamopyrgus antipodarum*	hard	7.58	*Potamopyrgus antipodarum*	unmod	8.56
Caenis luctuosa	hard	11.04	*Pisidium* sp.	hard	14.3	*Micronecta* sp.	soft	16.73
Mystacides azurea	hard	16.25	*Caenis luctuosa*	hard	20.45	*Pisidium* sp.	unmod	22.72
Tanypodinae sp.	unmod	21.37	*Dreissena polymorpha*	hard	25.93	*Sphaerium* sp.	only unmod	28.47
Sphaerium sp.	only unmod	26.48	*Micronecta* sp.	soft	30.89	*Tanypodinae* sp.	unmod	34.2
Ceratopogonidae sp.	hard	31.13	*Ceratopogonidae* sp.	hard	35.13	*Dreissena polymorpha*	unmod	38.13
Dreissena polymorpha	hard	35.69	*Caenis horaria*	hard	39.32	*Mystacides azurea*	soft	41.62
Caenis horaria	hard	39.92	*Oligochaeta* sp.	hard	43.14	*Gammaroidea* sp.	soft	45.03
Micronecta sp.	hard	43.71	*Mystacides azurea*	hard	46.81	*Ceratopogonidae* sp.	soft	48.17
Chironominae sp.	hard	47.24	*Chironominae* sp.	hard	50.43	*Pontogammarus robustoides*	soft	51.29
Haliplus sp. (larvae)	hard	50.4	*Athripsodes cinereus*	hard	53.86	*Diptera* sp. *(pupae)*	soft	54.3
Pisidium sp.	hard	53.27	*Haliplus* sp. (larvae)	hard	57.01	*Caenis luctuosa*	unmod	57.25
Oligochaeta sp.	hard	56.1	*Diptera* sp. *(pupae)*	hard	60.1	*Mystacides longicornis/nigra*	unmod	60.19
Acentria ephemerella	hard	58.85	*Pontogammarus robustoides*	soft	63	*Oligochaeta sp.*	unmod	63.1
Gammaroidea sp.	hard	61.44	*Acentria ephemerella*	hard	65.68	*Caenis horaria*	soft	65.93
Potamopyrgus antipodarum	hard	63.95	*Molanna angustata*	hard	68.16	*Molanna angustata*	unmod	68.76
Mystacides longicornis/nigra	unmod	66.27	*Gammaroidea* sp.	hard	70.62	*Athripsodes cinereus*	unmod	71.51
Pontogammarus robustoides	hard	68.53	*Gyraulus crista*	hard	72.83	*Chironominae* sp.	unmod	73.77
Gyraulus crista	hard	70.74	*Tanypodinae* sp.	hard	74.96	*Dikerogammarus villosus*	unmod	75.79
Cloeon dipterum	only hard	72.9	*Mystacides longicornis/nigra*	soft	76.85	*Gyraulus crista*	soft	77.51

Table 4: Taxa identified characteristic for different habitat types using IndVal analysis (IV=indicator value, 1=sand, 2=macrophytes, 3=stones, 4=roots, 5=woody debris).

Taxon	*Habitat*	*IV*	*P*
Micronecta sp	1	0,80	0,024
Oligochaeta sp	1	0,58	0,042
Pisidium sp	1	0,58	0,028
Sphaerium sp	2	0,91	0,004
Gyraulus crista	2	0,74	0,013
Mystacides longicornis nigra	2	0,70	0,001
Coenagrion puella pulchellum	2	0,69	0,020
Gyraulus albus	2	0,69	0,024
Valvata piscinalis	2	0,69	0,030
Molanna angustata	2	0,60	0,007
Goera pilosa	2	0,60	0,037
Planorbis carinatus	4	0,48	0,034
Lype sp	5	0,98	0,003
Dikerogammarus haemobaphes	5	0,87	0,022
Orectochilus villosus (larvae)	5	0,75	0,018
Dikerogammarus villosus	5	0,74	0,039
Haliplus flavicollis (adult)	5	0,67	0,024
Oulimnius sp *(adult)*	5	0,64	0,025

Table 5: Taxa identified characteristic for different alteration types using IndVal analysis (IV=indicator value, 1=unmodified sampling sites, 2=soft alteration sampling sites, 3=hard alteration sampling sites).

Taxon	*Alteration type*	*IV*	*P*
Sphaerium sp	1	0,71	0,001
Dreissena polymorpha	1	0,71	0,019
Limnephilus lunatus	1	0,70	0,002
Tanypodinae sp	1	0,69	0,009
Dikerogammarus villosus	1	0,69	0,003
Halesus radiatus	1	0,65	0,004
Orthocladiinae sp	1	0,64	0,002
Orectochilus villosus (larvae)	1	0,57	0,007
Lype sp	1	0,57	0,006
Tinodes waeneri	1	0,54	0,006
Dugesia lugubris polychroa	1	0,53	0,007
Coenagrion puella pulchellum	1	0,50	0,006
Goera pilosa	1	0,48	0,008
Acroloxus lacustris	1	0,43	0,027
Planorbis planorbis	1	0,39	0,022
Ancylus fluviatilis	1	0,36	0,034
Limnephilus rhombicus	1	0,33	0,049
Limnephilus stigma	1	0,29	0,039
Radix balthica	1	0,29	0,041
Micronecta sp	2	0,82	0,001
Ischnura elegans	2	0,39	0,039
Diptera sp *(pupae)*	3	0,81	0,001
Chironominae sp	3	0,77	0,006
Caenis luctuosa	3	0,76	0,004
Potamopyrgus antipodarum	3	0,70	0,001
Haliplus sp *(larvae)*	3	0,69	0,002
Acentria ephemerella	3	0,64	0,002
Athripsodes cinereus	3	0,61	0,014
Mystacides azurea	3	0,55	0,011
Cloeon dipterum	3	0,47	0,026

Methodological comparison

The proportion of individual habitats at each unmodified sampling site varied among sites (Table 6). While sand and macrophyte habitats were the most dominant habitats found at all unmodified sampling sites, root, stone and woody debris habitats accounted only for up to 10% when present.

Table 6: Proportional availability of each habitat at each unmodified sampling site (WE= Lake Werbellin; 1U=unmodified site 1; 2U=unmodified site 2; 3U= unmodified site 3).

Sampling site	*Habitat*	*Proportional abundance of habitat at sampling site (%)*
WE 1U	Sand	50
WE 1U	Stone	5
WE 1U	Macrophytes	25
WE 1U	Roots	10
WE 1U	Woody debris	10
WE 2U	Sand (1)	50
WE 2U	Sand (2)	50
WE 2U	Macrophytes	30
WE 2U	Roots	10
WE 2U	Woody debris	10
WE 3U	Sand	40
WE 3U	Macrophytes (1)	50
WE 3U	Macrophytes (2)	50
WE 3U	Woody debris	10

For the processing of habitat specific samples collected from unmodified sampling sites an average of 10.2 h for sorting per macroinvertebrate sample was needed. Processing of composite samples collected from unmodified sites accounted for 10 h on average (Table 7).

Table 7: Sorting effort for samples collected at unmodified sampling sites (WE=Lake Werbellin; 1U=unmodified site 1; 2U=unmodified site 2; 3U= unmodified site 3; CO=composite sample; SA=sand habitat; ST=stone habitat; MP=macrophyte habitat; EW=root habitat; TH=woody debris habitat).

Sample	*Sorting time (h)*		*Sum*
WE 1U CO	**12**		
WE 2U CO	**10**		
WE 3U CO	**8.5**		
WE 1U SA (1)	14	Sum U1	**49.25**
WE 1U ST (1)	8		
WE 1U MP (1)	12		
WE 1U EW (1)	4.25		
WE 1U TH (1)	11		
WE 2U SA (1)	6.5	Sum U2	**44.25**
WE 2U SA (2)	7		
WE 2U MP (1)	12		
WE 2U EW (1)	7.5		
WE 2U TH (1)	11.25		
WE 3U SA (1)	18	Sum U3	**46.5**
WE 3U MP (1)	9		
WE 3U MP (2)	10		
WE 3U TH (1)	9.5		

MDS identified variability within habitat and composite replicate samples from unmodified sampling sites to be smaller than variability among samples from individual unmodified sites (Figure 3). With the exception of root habitat samples (n=2), samples from the same habitat generally grouped closely together, illustrating high similarities among habitat types. Composite samples showed highest similarities to macrophyte and sand habitat replicate samples, the habitats which made up the largest proportion of habitats sampled at all unmodified sampling sites and, thus, also the largest proportion of the composite samples. Root, woody debris and stone habitats, which made up only a minor proportion of each sampling site showed stronger dissimilarities to composite samples at unmodified sampling sites.

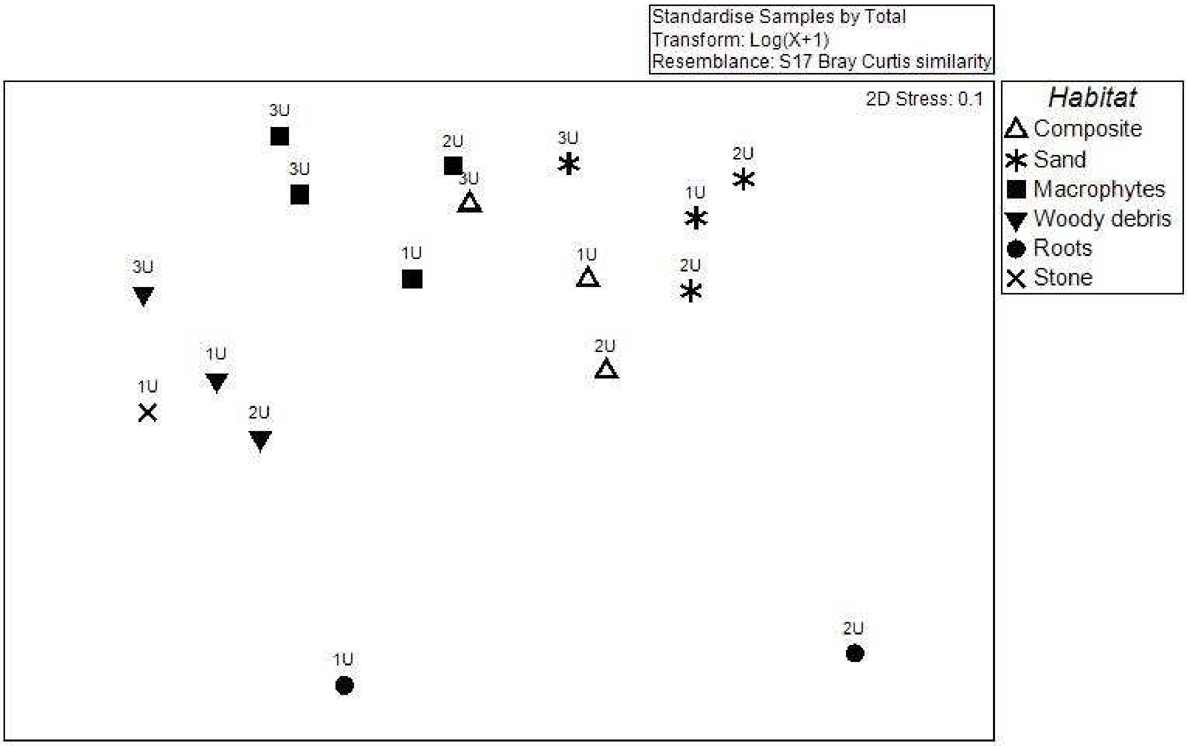

Figure 3: MDS-plot of macroinvertebrate species log(x+1) proportional abundance data from sand, root, macrophyte, stone and woody debris habitats and composite samples from unmodified sampling sites at lake Werbellin (1U=unmodified site 1; 2U=unmodified site 2; 3U=unmodified site 3).

ANOSIM identified no significant differences among habitat-specific and composite sample community structures from unmodified sites, while macroinvertebrate community structures from individual habitats differed significantly from each other (Tab. 8). Owing to the low number of replicate samples (n < 3) the habitats roots and stones could not be included in the ANOSIM and PERMDISP analyses. No significant differences were found neither among habitat-specific and composite samples collected from soft (ANOSIM, R-statistic = 0.137, p > 0.05) nor from hard alteration sites (ANOSIM, R-statistic = 0.27, p > 0.05).

*Tab 8: ANOSIM R-statistic of different habitat-specific versus composite samples. Significant results are marked with * (P < 0.05).*

	Composite	*Macrophytes*	*Sand*
Macrophytes	0,352		
Sand	0,074	0,854*	
Woody debris	1	0,963*	1*

PERMDISP identified no significant differences in homogeneity of variances among habitat specific and composite sample macroinvertebrate community structures (PERMDISP, F = 1.1034, P(perm) = 0.5031, N = 14). However, variability within composite samples was higher (19.81 ± 2.03) than variability within habitat-specific samples (Figure 4). Among habitat-specific samples variability was highest in macrophyte habitats (19.02 ± 0.12), followed by woody-debris (18.86 ± 2.12) and sand habitat with the lowest variability (16.43 ± 1.29).

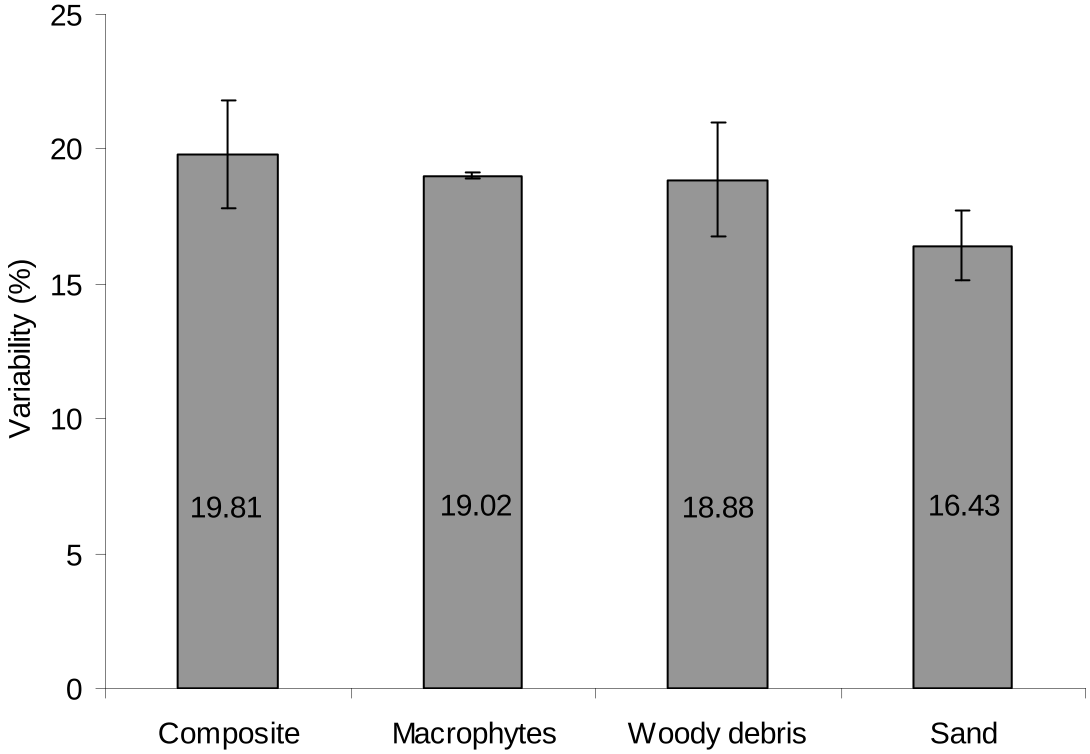

Figure 4: Variability in macroinvertebrate community composition of individual habitats (macrophytes: n=4; sand: n=4; woody debris: n=3 and composite samples: n=3) expressed as Bray-Curtis similarity of habitat/composite replicate samples to its group centroid.

ANOSIM identified no significant differences among macroinvertebrate community structures from artificially calculated and collected composite samples (ANOSIM, R-statistic = -0.333, p > 0.05). Furthermore, no significant differences could be detected among homogeneity of variances of macroinvertebrate communities from artificially calculated and collected composite samples (PERMDISP, F = 1.7428, P(perm) = 0.1976, N = 6). Artificial composite samples, however, showed lower variability in macroinvertebrate community structures compared with collected composite samples (Figure 5).

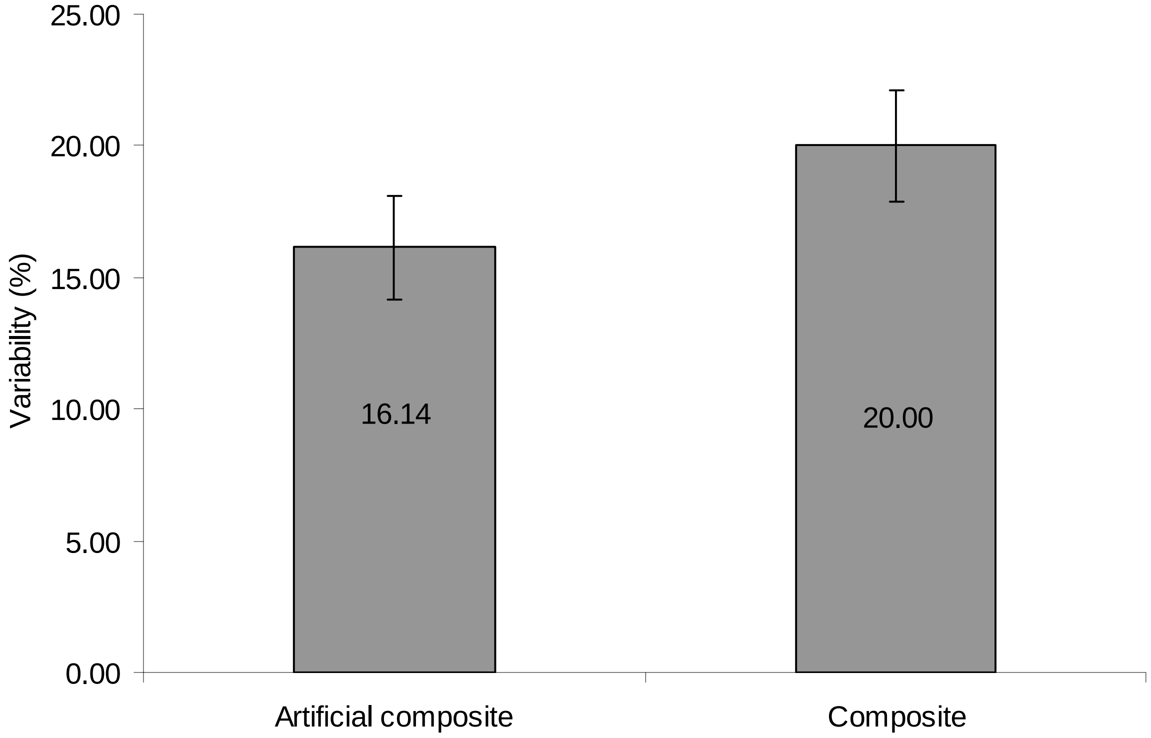

Figure 5: Variability in macroinvertebrate community composition of artificial and collected composite samples (n=3, respectively) expressed as Bray-Curtis similarity of habitat/composite replicate samples to its group centroid.

SIMPER also identified highest similarities of macroinvertebrate community structures among composite and sand and composite and macrophyte samples of unmodified sampling sites, respectively (Table 9). Taxa contributing most to differences among habitat specific and composite samples from unmodified sampling sites are summarized in Table 9.

Table 9: Summary results from SIMPER analysis showing cumulative contributions (Cum.%) of first 20 taxa contributing to habitat/composite (CO= Composite; SA=Sand; MP=Macrophytes; WD=Woody debris; ST=Stone; RO=Roots) dissimilarities (in %).

Composite & Sand			Composite & Macrophytes			Composite & Woody debris		
Average dissimilarity = 31,70			Average dissimilarity = 39,71			Average dissimilarity = 47,44		
Species	Higher abundance	Cum.%	Species	Higher abundance	Cum.%	Species	Higher abundance	Cum.%
Dreissena polymorpha	CO	11,11	*Dreissena polymorpha*	MP	11,47	*Potamopyrgus antipodarum*	CO	13,19
Tanypodinae sp.	CO	19,73	*Potamopyrgus antipodarum*	CO	19,44	*Dreissena polymorpha*	WD	25,01
Chironominae sp.	SA	27,44	*Chironominae* sp.	CO	25,79	*Pisidium* sp.	CO	33,82
Gammaroidea sp.	CO	34,43	*Orthocladiinae* sp.	MP	31,93	*Lype* sp.	WD	38,74
Pisidium sp.	CO	41,19	*Sphaerium* sp.	MP	38,01	*Gammaroidea* sp.	WD	43,66
Potamopyrgus antipodarum	SA	46,19	*Pisidium* sp.	CO	43,16	*Tanypodinae* sp.	WD	48,41
Orthocladiinae sp.	CO	51,02	*Gammaroidea* sp.	CO	47,53	*Orthocladiinae* sp.	WD	53,13
Sphaerium sp.	SA	54,58	*Tanypodinae* sp.	CO	51,68	*Dikerogammarus haemobaphes*	WD	57,57
Dikerogammarus villosus	CO	58,10	*Oligochaeta* sp.	CO	55,60	*Chironominae* sp.	CO	61,54
Oligochaeta sp.	SA	61,26	*Tinodes waeneri*	MP	58,51	*Dikerogammarus villosus*	WD	65,18
Mystacides longicornis/nigra	CO	64,31	*Gyraulus crista*	MP	61,18	*Oligochaeta* sp.	CO	68,68
Dikerogammarus haemobaphes	CO	67,17	*Mystacides longicornis/nigra*	MP	63,59	*Tinodes waeneri*	WD	71,06
Mystacides azurea	CO	69,84	*Caenis horaria*	MP	65,94	*Molanna angustata*	WD	72,86
Molanna angustata	CO	72,22	*Dikerogammarus villosus*	CO	68,26	*Mystacides longicornis/nigra*	CO	74,62
Ceratopogonidae sp.	SA	74,19	*Athripsodes cinereus*	CO	70,26	*Sphaerium* sp.	only CO	76,24
Gyraulus crista	CO	75,99	*Limnephilus lunatus*	MP	72,10	*Mystacides azurea*	CO	77,80
Limnephilus lunatus	CO	77,66	*Goera pilosa*	MP	73,85	*Athripsodes cinereus*	CO	79,30
Athripsodes cinereus	CO	79,32	*Mystacides azurea*	CO	75,60	*Orectochilus villosus (larvae)*	WD	80,75
Goera pilosa	CO	80,92	*Dikerogammarus haemobaphes*	CO	77,32	*Limnephilus lunatus*	WD	82,14
Micronecta sp.	CO	82,10	*Molanna angustata*	CO	78,99	*Dendrocoelum lacteum*	only WD	83,32

Table 9 (contd.): Summary results from SIMPER analysis showing cumulative contributions (Cum.%) of first 20 taxa contributing to habitat/composite (CO= Composite; SA=Sand; MP=Macrophytes; WD=Woody debris; ST=Stone; RO=Roots) dissimilarities (in %).

Composite & Stone			Composite & Roots		
Average dissimilarity = 50,63			Average dissimilarity = 56,21		
Species	Higher abundance	Cum.%	Species	Higher abundance	Cum.%
Potamopyrgus antipodarum	CO	17,59	*Potamopyrgus antipodarum*	CO	8,47
Dreissena polymorpha	ST	28,31	*Pontogammarus robustoides*	RO	16,79
Gammaroidea sp.	ST	35,96	*Halesus radiatus*	RO	24,04
Tinodes waeneri	ST	43,60	*Gammaroidea* sp.	RO	31,19
Ancylus fluviatilis	only ST	50,66	*Dreissena polymorpha*	CO	37,68
Pisidium sp.	CO	57,27	*Chironominae* sp.	CO	42,72
Orthocladiinae sp.	ST	62,96	*Pisidium* sp.	RO	46,96
Tanypodinae sp.	CO	66,96	*Echinogammarus ischnus*	RO	51,11
Chironominae sp.	CO	69,88	*Tanypodinae* sp.	CO	55,10
Athripsodes cinereus	CO	72,36	*Dikerogammarus villosus*	RO	59,08
Oligochaeta sp.	CO	74,72	*Sphaerium sp.*	RO	62,38
Dikerogammarus villosus	ST	76,88	*Valvata cristata*	RO	65,41
Mystacides longicornis/nigra	only CO	78,69	*Orthocladiinae* sp.	CO	67,96
Molanna angustata	only CO	80,46	*Haliplus* sp. (larvae)	only RO	70,20
Mystacides azurea	only CO	81,97	*Oligochaeta* sp.	CO	72,17
Sphaerium sp.	CO	83,34	*Diptera* sp. *(pupae)*	RO	73,89
Dikerogammarus haemobaphes	ST	84,54	*Athripsodes cinereus*	CO	75,44
Caenis luctuosa	ST	85,62	*Limnephilus lunatus*	RO	76,96
Acentria ephemerella	only ST	86,52	*Mystacides longicornis/nigra*	only CO	78,45
Gyraulus crista	CO	87,39	*Dikerogammarus haemobaphes*	only CO	79,93

6.4 Discussion and Conclusion

Spatial variation

Our study demonstrates that hydromorphological shoreline alterations such as erosion control structures or recreational beaches have a significant influence on littoral macroinvertebrate community structures. While no significant difference could be detected among macroinvertebrate taxon richness within alteration types or within sand habitats collected at different alteration types, macroinvertebrate community structures, however, did change considerably among different shoreline alteration types. The difference in community structures among alteration types, and among sand habitats across alteration types, which was identified with MDS and ANOSIM, is attributable to differences in abundances and occurrences of certain macroinvertebrate taxa. While some taxa such as *Sphaerium* sp. or *Orectochillus villosus* larvae were encountered only at unmodified lakeshores, others such as *Potamopyrgus antipodarum* were found at all sites but in varying abundances. The lack of certain taxa at structurally modified shore zones can primarily be attributed to the reduction of habitat complexity, as structurally complex macrophyte or woody debris habitats at unmodified shorelines were replaced by sand habitats with a comparatively low structural complexity at soft and hard alteration sites. As habitat complexity is one of the major factors influencing macroinvertebrate assemblages by providing *inter alia* important ecological niches and shelter from predation (O'Connor 1991, Thompson 1985), and has been shown to foster diverse community structures (White and Irvine 2003), it is not surprising that unmodified shorelines harboured quite a number of characteristic species many of which were also found to be characteristic for the structurally complex macrophyte or woody debris habitat. Such structurally complex habitats additionally offer a high variety of food sources such as periphyton or decaying organic matter which supports the abundance of functional feeding groups such as piercers, shredders, grazers or xylophagous species (Moog 1995). The high diversity in food resources together with physical attributes of macrophyte and woody debris habitats supports comparatively high inveretbrate diversity and also highest numbers of characteristic taxa recorded in both of these habitats.

We found a decrease in characteristic macroinvertebrate species from unmodified (14) over hard (9) to soft alteration sites (2), reflecting the loss of almost all but the sand habitat at both alteration types, and thus, a loss of habitat complexity. The identification of characteristic taxa for hydromorphologically altered shorezones together with a non-significant difference in taxon richness among shore types suggests that taxa characteristic for unmodified sites are not only lost but are furthermore, at least partly, substituted by other invertebrate species at hydromorphologically altered shore zones. Not only the loss of structurally complex habitats such as macrophytes, woody debris or roots causes the observed changes in macroinevertebrate community structures at structurally altered sites. The additional anthropogenic disturbance associated with recreational beaches and hard altered shore zones such as increased recreational pressures (especially trampling) and an increase in wave action through wave reflection (Solimni et al. 2006) also have a major influence on the occurrence and abundances of certain macroinvertebrate taxa. The strongest difference in community structure between unmodified shoreline sites and recreational beaches observed in this study supports the assumption by Brauns et al. (2007b) that alterations of this kind most profoundly affect lake macroinvertebrate communities in the littoral zone.

Abundances of Chironominae and Diptera pupae (both identified characteristic for hard alteration sampling sites) increased with increasing structural shoreline alteration from unmodified, over soft to hard shoreline alteration sites. This chironomid subfamily is characterised by its tolerance to low oxygen conditions (Armitage et al. 1995). This tolerance favours its occurrence at sand-dominated hard alteration sites, which can frequently be covered by an anoxic-layer of organic matter. Brauns et al. (2007b) also identified a significant increase in abundances of Chironomidae in the littoral on recreational beaches, but could not identify a significant difference in chironomid numbers on hard alteration sites such as retaining walls or riprap when compared to natural shore zones. Abundances of Chrironomidae subfamily-groups Tanypodinae and Orthocladinae, however, showed highest abundances in unmodified sites, with a decrease from hard to soft alteration shore zones. These groups are not able to tolerate anoxic conditions and are predominantly characterised as gathering collectors, shredders or scrapers but also predators (Moog, 1995). Armitage et al. (1995) furthermore identified a preference of Orthocladiinae for macrophyte habitats, which was also the habitat in which this sub-familiy was predominantly found in this study. This supports the identification of both sub-families as characteristic for unmodified shore zones, which offer a greater habitat diversity and, thus, food resources and possibly better oxygen conditions when compared with altered shore zones.

High densities of *Potamopyrgus antipodarum* at hard altered sites are in accordance with its generalist feeding behaviour, which allows its occupation of degraded sites (Moog 1995, Schreiber et al. 2003, Keransi et al 2005). The comparatively low abundance of other gastropod taxa at recreational beaches and hard altered sites again reflects the loss of complex habitats through shoreline modification. Gastropod taxa such as *Gyraulus albus* and *Gyraulus crista* encountered in unmodified shore zones of lake Werbellin and identified as characteristic macrophyte species are predominantly grazers (Moog 1995) which can not find adequate food sources in the dominant sand habitats of recreational beaches and structurally alteratered shorelines. Exposed sand habitats furthermore do not offer enough shelter for the fragile gastropod shells (Bänziger 1995) or from detachment owing to increased wave action (Gabel et al. 2008), which is reflected in the absence or very low densities of gastropod species (except *P. antipodarum*) in all sand habitats in this study. Thus, it is mainly the habitat characteristics but also the increased anthropogenic pressure at altered sites creating unsuitable conditions for this taxa group.

Taxa identified characteristic for unmodified lakeshore zones such as *Sphaerium* sp., *Orectochillus villosus* (larvae), *Limnephilus stigma*, *Coenagrion puella pulchellum*, *Ancylus fluviatis*, *Radix balthica* were not recorded at hydromorphologically altered sampling sites. This loss of certain taxa at altered shore zones once again highlights the loss of structurally complex habitats such as macrophytes as an important impact of anthropogenically caused alteration of lake shores for the littoral macroinvertebrate community and especially on habitat specialists. A high number of Trichoptera taxa were identified as characteristic for unmodified shorezones and habitats associated with those sites. The loss of more than half of these taxa at hydromorphologically altered sites, again, reflects the high exposition to increased wave action and anthropocenic disturbaces associated with exposed sand-habitats, leading to detachment of organisms or deterioration of fragile caddis-fly cases.

Micronecta sp. (larvae) showed highest abundances at structurally altered shore zones, decreasing from soft over hard to unmodified sites. This taxon which was identified as characteristic for recreational beaches as well as the here dominantly occurring sand-habitat has been described to feed mainly on organic material associates with sand (gatherer-collector) (Moog 1995). The classification of *Ischnura elegans* as characteristic for recreational beaches is in contrast with findings by Brauns et al. (2007b) who identified this taxon as characteristic for natural, unmodified shorelines but with a strong preference for root habitats. Its classification as characteristic for recreational beaches in our study is based on its finding in high abundances in the only root habitat found in this alteration type and adjacent sand habitats. Thus, *I. elegans* seems more dependant on the occurrence of root habitats, which are usually associated with natural shorelines, rather than natural shorelines as such.

Brauns et al. (2007b) furthermore identified erosion control structures to have no effect on infralittoral macroinvertebrate community structures, which is somehow in contrast with the results of this study. While in the earlier study the extend of retaining wall or ripraps where limited, hard alteration structures assessed in our study, also comprising retaining walls and ripraps, were covering considerable stretches along shorelines sampled. This suggests that the extent of alteration structures along lake shorelines is an important factor shaping the influence of these modifications on macroinvertebrate community structures as demonstrated in this study.

Methodological comparison

We were able to demonstrate that a macroinvertebrate composite sample collected by sampling all present habitats proportional to their availability within a sampling site can represent each individual sampling location adequately. Habitat specific and composite sample macroinvertebrate community structures did not differ significantly, while individual habitat samples harboured distinct macroinvertebrate communities. The importance of the proportional sampling regime used was demonstrated by the relatively strong similarity of macroinvertebrate communities from sand and macrophyte habitats to the composite samples. These habitats were the ones with highest proportional distribution at unmodified sampling sites, thus, making up the largest part of each composite sample. Community structures in woody debris habitats differed stronger from composite sample macroinvertebrate assemblages, but the difference was still not significant. While these results encourage the use of composite samples for the assessment of lake ecological status, it should be noted that important sensitive taxa which are known to inhabit habitats such as woody debris or roots, normally making up only a fraction of the area of each sampling site, could be overlooked and, thus, their loss owing to anthropogenic disturbances be missed.

The adequacy of composite samples for routine monitoring programmes is supported by the results from the PERMDISP analysis which identified no significant differences in homogeneity of variances among habitat specific macroinvertebrate community structures and composite samples. Yet Schreiber and Brauns (2010) found the variability within macroinvertebrate samples from individual habitats to differ significantly from pooled samples. The authors, however, did not take into account the proportional abundance of habitats at each sampling site. This once more underlines the importance of this approach for the collection of representative macroinvertebrate composite samples. The comparison of artificial and collected composite macroinvertebrate samples concurs with the previously stated results. While artificially generated composite samples showed slightly lower variability within community structures, this difference was still not significant and supports the adequacy of using composite samples for monitoring purposes.

Despite the fact that the processing time for composite samples was on average not so much different from the time needed to process an individual habitat sample, the time needed to process individual habitats needed in order to represent a site was approximately fivefold higher than for composite samples. If we assume the same to be true for collection of samples in the field and macroinvertebrate identification, the much higher working effort and, thus, higher cost associated with habitat specific sampling becomes even more evident.

Conclusions

One of the consequences of morphological shoreline alterations is the loss of habitats, especially structurally complex ones, which foster characteristic and sensitive macroinvertebrate taxa. Increases in recreational pressures and other anthropogenically caused disturbances associated with hydromorphologically altered shorezones furthermore intensify the severe effects of habitat loss on faunal communities. The strength of these influences on the littoral community structure of a whole lake depends, however, on the extent to which the shoreline is altered and, thus, associated losses of habitat complexity and diversity in the lake littoral as a whole. Our results show that littoral macroinvertebrates are responsive to the influence of hydromorphological alterations, and could, thus, be used for the assessment of lake ecological status relating to hydromorphological pressures. It should be further elaborated whether identified characteristic species can serve as indicator taxa for hydromorphological shoreline alterations as part of a lake assessment regime. An elaborated sampling regime could consequently serve as a basis for restoration measures to protect the integrity of a lake ecosystem with the aim of actively protecting habitat complexity and diversity in the lake littoral. While shoreline alterations proved to have an important influence on macroinvertebrate community structures, it has yet to be tested whether cost and time-effective macroinvertebrate composite samples are sensitive enough to discriminate among alteration types and would, thus, be suitable for routine monitoring of lake ecological status.

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Chapter 7. Description of Wiser data analysis strategies to account for spatial variability

Mike Dunbar, Ralph Clarke

Note: this chapter should be considered alongside WISER Deliverable D6.1-1 “Report on a workshop to bring together experts experienced with tool development and uncertainty estimation”, and presentations from Mike Dunbar and Ralph Clarke at the Wiser mid-term meeting, available on the WISER Intranet.

7.1 Introduction

Fieldwork has been undertaken in WISER WP3.3 partly to gain understanding of the importance of spatial, within-lake variation in macroinvertebrate community composition, on the estimation of lake-level values for particular metrics, and ultimately, on lake status assessments using these metrics. Because there are data collected across and within lakes, the collected data are hierarchically-structured. Using the information on this structure, one can fit statistical models which partition the total variance of any available metric into within-lake variation and among-lake variation. It is important to note that, to the extent allowed by the data, these hierarchical variance estimates correctly partition the total variance in the dataset. Simply subtracting (from total variance) an estimated between-lake variance estimated by averaging all values within a lake, will not give correct values at either level in the hierarchy and can be misleading.

There is a wide-ranging terminology used to describe such statistical models. Hierarchical analyses of variance, using nested terms, have been used in statistics for over 60 years. Traditionally, such ANOVA models were fitted using least squares, with adjustments to account for the fact that grouping variables (such as lake identity or sites within a lake), are more appropriately modelled as random effects (samples drawn from a wider population) rather than as fixed effects for which parameters are estimated directly for each group and no generalisation is possible.

More recently, a newer class of models, known alternatively as mixed-effects or multilevel models, fitted using generalised least squares and maximum likelihood have superseded nested least squares ANOVAs, although the latter are still widely applied. Henceforth we shall term these models mixed-effects, there are subtle (and unimportant here) differences with multilevel models, and other terms such as random coefficient and REML models. Mixed-effects models, which also represent hierarchical structure directly in the model, have considerable advantages over the aforementioned ANOVA models. Firstly, they are far less sensitive to any imbalance in the analysed dataset (such as variable numbers of samples per site and sites per lake). Secondly, they are able to fit explanatory (e.g. environmental) variables (termed fixed effects) in the same framework. Thirdly, they extend to generalised linear models (glms), where error terms are not normally distributed. When fitted using no fixed effects, such models are often termed variance components analyses, the term mixed refers to the mixing of both fixed and random effect in the same analysis.

It may not initially be obvious why fitting explanatory variables might be necessary in a variance partitioning exercise such as this. A simple example relates to the initial selection of lakes to survey in WISER. These were not selected at random, rather they were selected to be along a trophic gradient. With approx 3 lakes per country, simply considering lake identity as a random effect will over estimate between-lake variance compared to within-lake variance. However it is simple to account for this by including a measure of trophic status (e.g. TP concentration) in the model. TP status will explain part of the between-lake variance, leaving a lower residual between-lake variance. Fixed-effects may also be used at the site or sample level to describe habitat type, degree of bank profile alteration etc.

At the WISER mid-term meeting in Sept 2010, Mike Dunbar and Ralph Clarke presented some ideas as to how to undertake analysis of among and within-lake variance components. Mike presented his examples using the R software package. There are many packages capable of undertaking these analyses, including SAS, Statistica, Genstat, Minitab and Stata. Often people will be best placed to use the package they are already comfortable with, but there are some advantages to using R which are worth noting:

- R is free and open source so if you learn how to use it, no-one can take it away from you
- R runs on Windows and Unix (including Mac OS X)
- You can extract, manipulate, plot and analyse your data all in one package
- The R language is very powerful and *elegant*, it doesn’t take much typing to do complex things
- If you learn the basics you can then teach yourself
- R can do every basic statistical analysis, and also has an incredible breadth due to the thousands of add-on packages (including several very useful for ecological data)
- New methods are constantly becoming available
- R analyses are written as scripts, this means you have an entire record of your analysis, you can send it to someone else
- Some analyses (e.g. resampling species) effectively require programming, or very specialist packages

However it is equally important to point out disadvantages:

- R's programming language paradigm means that learning R can take some time
- The syntax can be confusing and frustrating, especially if you haven’t done any computer programming before
- It can be especially difficult if you are used to doing analyses by “point and click”
- Error messages may not be helpful for beginners
- There’s often many ways of doing the same thing
- Packages can be of variable quality

- People new to R should seriously consider undertaking some formal training in order to get the best start

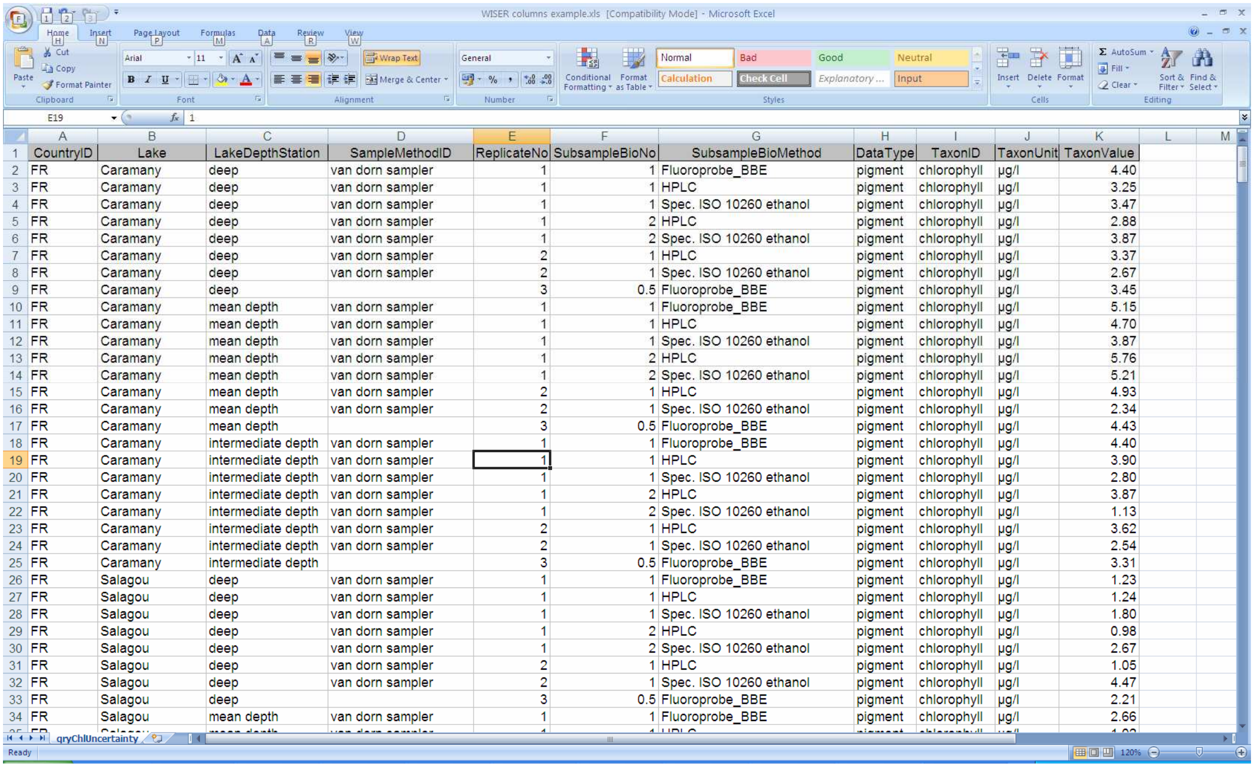
Hence If you only dip into statistics and just want to do a quick one-off analysis you are probably better off using a point and click package such as Minitab or Genstat.

7.2 Data formatting

The WISER database structure lends itself well to undertaking analyses of this sort, however queries need to be written to organise the data to be analysed into a single flat-file structure. Figure x gives an example of this structure for WP3.1 data. All of the required information for each macroinvertebrate sample needs to be a separate single row in the table. A separate column is needed for the values for each response variable (macroinvertebrate metric) and separate columns are needed for the sample descriptive data. At a minimum, such descriptive data only needs to be a column for lake identity of the sample and a columns distinguishing individual sampling stations within the lake. However, it is highly beneficial to include explanatory variables as mentioned above. Where these correspond to higher-level groupings (e.g. lake), they will be repeated: for example:

Lake	Replicate (station)	Metric	Total phosphorus for lake (ug/ml)
A	1	4.1	100
A	2	6.7	100
A	3	8.5	100
B	1	2.1	200
B	2	3.6	200
B	3	3.7	200

When reading in data, any coding system that uses numbers for higher-level grouping variables must be converted into factors. In the above example, replicate is the lowest level of the model, hence it forms the residual and is not specified explicitly in the model.



1	CountryID	Lake	LakeDepthStation	SampleMethodID	ReplicateNo	SubsampleBioNo	SubsampleBioMethod	DataType	TaxonID	TaxonUnit	TaxonValue
2	FR	Caramany	deep	van dorn sampler	1	1	Fluoroprobe_BBE	pigment	chlorophyll	µg/l	4.40
3	FR	Caramany	deep	van dorn sampler	1	1	HPLC	pigment	chlorophyll	µg/l	3.25
4	FR	Caramany	deep	van dorn sampler	1	1	Spec. ISO 10260 ethanol	pigment	chlorophyll	µg/l	3.47
5	FR	Caramany	deep	van dorn sampler	1	2	HPLC	pigment	chlorophyll	µg/l	2.88
6	FR	Caramany	deep	van dorn sampler	1	2	Spec. ISO 10260 ethanol	pigment	chlorophyll	µg/l	3.87
7	FR	Caramany	deep	van dorn sampler	2	1	HPLC	pigment	chlorophyll	µg/l	3.37
8	FR	Caramany	deep	van dorn sampler	2	1	Spec. ISO 10260 ethanol	pigment	chlorophyll	µg/l	2.67
9	FR	Caramany	deep		3	0.5	Fluoroprobe_BBE	pigment	chlorophyll	µg/l	3.45
10	FR	Caramany	mean depth	van dorn sampler	1	1	Fluoroprobe_BBE	pigment	chlorophyll	µg/l	5.15
11	FR	Caramany	mean depth	van dorn sampler	1	1	HPLC	pigment	chlorophyll	µg/l	4.70
12	FR	Caramany	mean depth	van dorn sampler	1	1	Spec. ISO 10260 ethanol	pigment	chlorophyll	µg/l	3.87
13	FR	Caramany	mean depth	van dorn sampler	1	2	HPLC	pigment	chlorophyll	µg/l	5.76
14	FR	Caramany	mean depth	van dorn sampler	1	2	Spec. ISO 10260 ethanol	pigment	chlorophyll	µg/l	5.21
15	FR	Caramany	mean depth	van dorn sampler	2	1	HPLC	pigment	chlorophyll	µg/l	4.93
16	FR	Caramany	mean depth	van dorn sampler	2	1	Spec. ISO 10260 ethanol	pigment	chlorophyll	µg/l	2.34
17	FR	Caramany	mean depth		3	0.5	Fluoroprobe_BBE	pigment	chlorophyll	µg/l	4.43
18	FR	Caramany	intermediate depth	van dorn sampler	1	1	Fluoroprobe_BBE	pigment	chlorophyll	µg/l	4.40
19	FR	Caramany	intermediate depth	van dorn sampler	1	1	HPLC	pigment	chlorophyll	µg/l	3.90
20	FR	Caramany	intermediate depth	van dorn sampler	1	1	Spec. ISO 10260 ethanol	pigment	chlorophyll	µg/l	2.80
21	FR	Caramany	intermediate depth	van dorn sampler	1	2	HPLC	pigment	chlorophyll	µg/l	3.87
22	FR	Caramany	intermediate depth	van dorn sampler	1	2	Spec. ISO 10260 ethanol	pigment	chlorophyll	µg/l	1.13
23	FR	Caramany	intermediate depth	van dorn sampler	2	1	HPLC	pigment	chlorophyll	µg/l	3.62
24	FR	Caramany	intermediate depth	van dorn sampler	2	1	Spec. ISO 10260 ethanol	pigment	chlorophyll	µg/l	2.54
25	FR	Caramany	intermediate depth		3	0.5	Fluoroprobe_BBE	pigment	chlorophyll	µg/l	3.31
26	FR	Salagou	deep	van dorn sampler	1	1	Fluoroprobe_BBE	pigment	chlorophyll	µg/l	1.23
27	FR	Salagou	deep	van dorn sampler	1	1	HPLC	pigment	chlorophyll	µg/l	1.24
28	FR	Salagou	deep	van dorn sampler	1	1	Spec. ISO 10260 ethanol	pigment	chlorophyll	µg/l	1.80
29	FR	Salagou	deep	van dorn sampler	1	2	HPLC	pigment	chlorophyll	µg/l	0.98
30	FR	Salagou	deep	van dorn sampler	1	2	Spec. ISO 10260 ethanol	pigment	chlorophyll	µg/l	2.67
31	FR	Salagou	deep	van dorn sampler	2	1	HPLC	pigment	chlorophyll	µg/l	1.05
32	FR	Salagou	deep	van dorn sampler	2	1	Spec. ISO 10260 ethanol	pigment	chlorophyll	µg/l	4.47
33	FR	Salagou	deep		3	0.5	Fluoroprobe_BBE	pigment	chlorophyll	µg/l	2.21
34	FR	Salagou	mean depth	van dorn sampler	1	1	Fluoroprobe_BBE	pigment	chlorophyll	µg/l	2.66

Figure 7.1. Example of flat file data structure suitable for importing to R.

7.3 Examples of simple analyses

Here examples will be presented using R code, using the lme package in R.

A simple variance components model would take the form:

```
my.model.1 <- lme(MetricValue ~ 1, random=~1|CountryID/Lake,
data=mydata)
```

```
summary(my.model.1)
```

MetricValue is the response variable. ~1 refers to the fact that an overall intercept (i.e. an overall mean) is the only fixed effect that is fitted. The random= part of the model specifies the structure of the data. In this case there are two grouping variables, “country” and “lake within country”. Replicate within lake is the lowest level of grouping and this forms the residual, it does not need to be specified explicitly. This model estimates independent variance components for country, lake and within-lake location. The latter variance is what is needed for further incorporation of uncertainty, for example in the new WISERBUGS software (see WISER web-site) for assessing uncertainty of status class . Where simulating the effects of different sampling strategies such as number of stations per lake (n), the central limit applies, the appropriate variance being σ^2/n, where σ^2 is the variance between stations within a lake.

A more complex model would be

```
my.model.2 <- lme(MetricValue ~ LakeTP + StationBankMod,
random=~1|CountryID/Lake, data=mydata)
```

```
summary(my.model.2)
```

In this case there are two fixed effects, LakeTP is measured at the lake level (one Total Phosporus (average) value per lake) and explains (hopefully) part of the between lake variance in the metric. It possibly also partly explains variance between countries, but cannot explain variance within lakes. StationBankMod is a bank modification metric with is measured for each station at each lake, hence it may explain within-lake variance and potential some variance at higher levels as well.

Chapter 8. Design of the WISER sampling campaign

Martin Pusch, Gwendolin Porst

8.1 Introduction

Lakes may be impacted by a variety of anthropogenic influences, e.g. by the alteration of water levels, by acidification and other deterioration of water quality, by the development of lake shores, or by the introduction of alien species. Among those, nutrient enrichment is the most widespread pressure affecting European lakes (Solimini et al. 2006), leading to their eutrophication. Therefore, currently most approaches assessing the ecological status of lakes traditionally focus on its primary producers, i.e. phytoplankton and macrophytes. In contrast, the ecological effects produced by hydrological and morphological alterations to lakes have been studied to a much less extent (Solimini et al. 2006).

However, recent research has demonstrated that the functioning of lake ecosystems is fundamentally influenced by terrestrial inputs of organic carbon, which often forms a quantitatively dominating component of lakes that is also significantly used by food webs and thus largely shapes biotic assemblages (Vadeboncoeur et al. 2002, Pace et al. 2004, Carpenter et al. 2005, Jansson et al. 2007). The dominance of pelagic production has obviously developed in many lakes only after anthropogenic eutrophication (Vadeboncoeur et al. 2003).

Urban development of lake shores accompanied by alterations of lake shores tends to interrupt functional linkages between the pelagic zone of the lake and adjacent aquatic and terrestrial shoreline habitats (Christensen et al. 1996, Radomski & Goeman 2001, Larson et al. 2011, Brauns et al. accepted). Moreover, the use of lakes for boating and navigation creates artificially increased hydraulic stress in littoral zones through the ship-induced waves that affect wind-sheltered shorelines, too (Gabel et al. 2008).

The composition of the assemblages of aquatic invertebrates in lakes basically respond to all major natural and anthropogenic factors relevant for the lake ecosystem. Their sensitivity to these factors gradually changes along a depth gradient. The assemblages of profundal invertebrates are mostly shaped by the availability of dissolved oxygen in the hypolimnic zone, which in turn may be significantly reduced by eutrophication (Bazzanti & Seminara 1995, Rossaro et al. 2006). In the sublittoral, and even more in the littoral zone, the effects of natural or anthropogenic hydrological and morphological (summarized as hydromorphological) conditions dominate (O'Connor 1991, Brauns et al. 2007b, Brauns et al. 2008, Gabel et al. 2008, Free et al. 2009), while eutrophication may affect even littoral invertebrates, especially in specific meso-habitats that are structurally affected by eutrophication (Mastrantuono & La Rocca 1988, Pieczynska et al. 1999, Brauns et al. 2007a, Donohue 2009a, b).

Hence, ecological assessment tools based on lake invertebrates may potentially indicate the effects of several modes of human pressure. As a working assessment system for eutrophication already exists, it was decided to target efforts within the WISER workpackage 3.3 to develop an assessment approach for morphological alterations of lake shores. These seem to be quite widespread within the more densely populated regions of Europe. Other hydromorphological alterations, as the manipulation of water levels of lakes, or the creation of ship-induced waves, seem to be regionally more restricted, or mainly occur in lakes, which are defined as artificial water bodies by the EU Water Framework Directive.

The decision to use littoral invertebrates for bioindication purposes was encouraged by studies that recommended their use, as the inherent spatio-temporal dynamics of their community composition (Picket & White 1985) can be met by an appropriate design of sampling and analytical efforts (Brauns et al. 2007a,b, Donohue 2009 a,b, Free et al. 2009, Johnson 1998, 2003, White & Irvine 2003, Johnson & Goedkop 2002, Solimini et al. 2008).

The work approach of workpackage 3.3. ‘Lake invertebrates’ thus implicitely assumes that

i) hydromorphological alterations constitute important impacts to the ecological integrity of European lakes,

ii) that pressure-specific assessment tools should be preferred to unspecific ‘general’ approaches, as the latter do not support the identification of promising restoration strategies, and

iii) that lake invertebrates include sufficient and abundant taxa or functional guilds (Feld & Hering 2007) that respond sensitively to hydromorphological alterations, and thus can be taken as indicator groups.

The WISER ‘Lake invertebrate’ work package thus undertakes an innovative effort, which will provide the first available assessment principle that enables to assess the ecological effects of morphological alterations on lake shores.

8.2 Design of sampling schedule

In contrast to other biological quality elements, there were few databases existing in European member states that contained results from surveys on littoral invertebrates, with related data on potential morphological degradation of the sampling sites. Hence, WP 3.3 was scheduled to analyze on one hand existing (mostly heterogeneous) data from mostly national monitoring activities, but on the other hand to conduct a comprehensive field sampling campaign within WISER.

According to the WISER Description of Work, ‘the ultimate aim of the field exercise will be to quantify the confidence in classification of BQE metric results. Variability in metric scores associated with spatial, temporal and analytical variability will be examined. Spatial variability may include within-type (different lakes), within-lake (different locations) and within-location (sample and sub-sample) variability. Temporal variability (seasonal, inter-annual) will be examined, where possible, through analysis of existing long-term datasets.' WISER WP 3.3 has a focus on hydromorphology assessment, and it will be a major task to disentangle effects of eutrophication and hydromorphological degradation. Hence, the field sampling campaign follows a strategy to produce a dataset that will hold observations within the full range of these variables (independent variation), allowing sound statistical analyses. The resulting homogeneous dataset enables unbiased analyses on the pressure sensitivity of metrics to hydromorphological alterations, and also the assessment of uncertainty associated with sampling procedures used. The effect of uncertainty produced from various sources of spatial, temporal and analytical variability can also be studied.

Using estimates of time (cost) per sample and the uncertainty associated with each technique, it will also be possible to quantify the cost associated with varying levels of precision – the cost-effective precision of sampling.

Thus, the database resulting from the WP3.3 field sampling campaign should

- be based on a homogeneous sampling methodology, i.e. a common sampling protocol,
- focus on sampling European lakes belonging to a similar type across climatic gradients.
- contain independent parallel records on the structural integrity of sampled lake shores, which serve as external standards, and may enable to develop methodological elements of low-cost assessment by supplementing biological with rapid abiotic assessment surveys,
- allow the analysis of various sources of uncertainty during the various methodological steps to be conducted until an assessment score is established,
- enable estimation of time (cost) needed to take and process samples, which together with the information of uncertainty will allow estimating the relationship between cost and assessment precision,

In addition, it was agreed at the WISER kick-off meeting not only to apply the standard (habitat-specific 1 m^2) sampling protocol, but to take a number of additional samples with a low-cost method, i.e. composite habitat samples (1 min time-limited sampling) at each invertebrate sampling site. In order to produce a nested, hierarchically structured dataset that facilitates analyses of uncertainty, it was agreed that sampling should be performed once at 9 sites per lake, with 3 sites representing low hydromorphological pressure (reference/high status), intermediate hydromorphological pressure (good/moderate status), and high hydromorphological pressure conditions (poor/bad status).

This sampling scheme was meant to be applied to 9 lakes per country, i.e. 3 at reference eutrophication level, 3 at intermediate eutrophication level, and 3 at high eutrophication level. As there are 4 major partners in the WP capable to perform field campaigns, 36 lakes were planned to be sampled, with a maximum of 5 habitat-specific samples and one additional composite sample collected at each sampling site, summing up to a maximum of 486 samples to be collected in each country.

From the 36 lakes to be sampled for the purposes of WP 3.3 15 lakes were initially planned to be sampled for the cross-BQE exercise, in order to enable harmonisation of assessment results.

However, it turned out during the WISER kick-off meeting that there were hardly any lakes available (with existing monitoring data for several Biological Qality Eements already existing) that met

- the requirements for the WP 3.3 field campaign on one side (focusing on hydromorphological pressure)
- and at the same time for WPs 3.1. 3.2 and 3.4 (focusing on eutrophication pressure).

Hence, it was not possible to identfy 15 cross-BQE lakes as part of the WP 3.3 specific lake list. Additional sampling was performed voluntarily by IGB in Denmark, SYKE in Finland and by CEH in the UK. Lakes were selected from three common lake types representing three European regions (GIG regions):

- Northern – Low alkalinity, deep lake (L-N2b)
- Central / Atlantic: High alkalinity, shallow lake (L-CB1 / L-A1/A2)
- Mediterranean: High alkalinity, deep reservoir (L-M8)

8.3 Implementation of the sampling campaign

A common WP 3.3 lake macroinvertebrate sampling protocol was agreed among all WP 3.3 partners during a WP 3.3 workshop in Berlin in April 2009. Morphological alterations were classified as “soft alteration” (e.g. riparian clear-cutting, recreational beaches) and “hard alteration” (e.g. retaining walls, rip-rap). Macroinvertebrate samples should be collected from 3 soft alteration, 3 hard alteration and 3 unmodified sites within each lake. Each sampling site should represent a shoreline section of minimum 25 m length representing either soft alteration, hard alteration or unmodified sites. If either of the two alteration types was not present at a lake, the number of sampling sites was still kept constant (9 sites per lake). Sampling was carried out in the season commonly used for aquatic invertebrate surveys in each ecoregion.

At each sampling site a number of habitat samples (minimum number of habitats = 3; number of habitat samples kept constant among all sampling sites and lakes in each country, even at sites which only showed one or two habitats) plus one composite sample had to be collected. Composite samples comprised a standardised 1 min macroinvertebrate sample including sampling of all available habitats proportional to their availability within each sampling site. Habitat-specific samples comprised the collection of 1 m² samples per habitat, which is an area that will comprise most of the species present (Schreiber & Brauns 2010).

This agreed sampling schedule also reflects the outcome of extensive discussions on a balanced sampling scheme held with WP 6.1 (Uncertainty) at the WISER kick-off meeting in Mallorca. Originally it was planned that nine lakes should be selected in Sweden, Ireland, Germany and Italy which should cover a range of trophic pressures (oligotrophic, mesotrophic, eutrophic states represented ideally by 3 replicates each) and ideally show two different shoreline morphological alteration types in each of the selected lakes.

The actually planned number of 36 lakes to be sampled in WP 3.3 increased to 51 lakes, as 15 additional lakes selected for the WISER uncertainty field exercise, which partly did not possess all necessary morphological alteration types, had to be sampled for macroinvertebrates additionally. In Italy, 2 additional lakes were sampled, in order to adequately cover both Italian lake areas in Northern and Central Italy. This sums up to a total of 39 lakes, which were sampled according to the agreed WISER WP 3.3 sampling protocol. Further 12 lakes were sampled for macroinvertebrates in order to meet the requirements of the WISER uncertainty field exercise, including cross-BQE comparisons. Only those cross-BQE lakes which fitted the WISER WP 3.3 sampling protocol were sampled accordingly. From the additional lakes only composite samples were collected.

During the sampling protocol workshop in Berlin an introduction to the Lake Habitat Survey (LHS) methodology (Rowan et al. 2004, 2006) was given, and it was agreed to conduct a complete LHS for each lake as well as hab-plot/site specific LHS at each macroinvertebrate sampling site.

Sampling for lake benthic macroinvertebrates using the agreed WP 3.3 common sampling protocol has been accomplished in all countries (Finland: 4 lakes, September/October 2009; Germany/Denmark: 11 lakes, April/May 2010; Ireland: 9 lakes, April/May2009; Italy: 15 lakes, August-November 2009; Sweden: 9 lakes, November 2009; UK: 3 lakes, October 2009). In some lakes, the general sampling schedule had to be modified, as not all pressure levels were encountered and sometimes only composite samples were taken in order to keep total number of samples within feasible limits. Whole lake and hab-plot/site-specific LHS has been carried out in all lakes in all countries (Finland: September/October 2009; Germany: August 2010; Ireland: September 2009, Italy: October 2009; UK: October 2009). For details on the number of samples collected in each lake and country and records from LHS please see Table 1.

Table 1: Overview on the 51 WISER WP 3.3 lakes sampled for macroinvertebrates according to WP 3.3 sampling protocol, including number of stations and habitats sampled per lake. ‘Cross-BQE’ = Lake sampled for WISER cross-BQE intercalibration exercise. No. per country = Total no. of samples per country.

Country Code	Cross-BQE	Lake Name	Latitude (WGS 84)	Longitude (WGS 84)	Shoreline types sampled	Number of habitats sampled	No. per Country
DE	**X**	Glindower See	52°21.413' N	12°55.760' E	3 Soft, 3 Hard, 3 Unmodified	Up to 3 per site plus 1 composite	
DE	**X**	Grienericksee	53°06.406' N	12°53.289' E	3 Soft, 3 Hard, 3 Unmodified	Up to 3 per site plus 1 composite	
DE		Müggelsee	52°26.274' N	13°36.750' E	3 Soft, 3 Hard, 3 Unmodified	Up to 3 per site plus 1 composite	
DE		Röblinsee	53°10.966' N	13°07.320' E	3 Soft, 3 Hard, 3 Unmodified	Up to 3 per site plus 1 composite	
DE	**X**	Roofensee	53°06.697' N	13°02.168' E	3 Soft, 3 Unmodified	Only composite	
DE		Stienitzsee	52°30.219' N	13°49.399' E	3 Soft, 3 Hard, 3 Unmodified	Up to 3 per site plus 1 composite	
DE		Werbellinsee	52°55.446' N	13°42.837' E	3 Soft, 3 Hard, 3 Unmodified	Up to 3 per site plus 1 composite	
DE		Schwedtsee	53 11.335' N	13 9.538' E	3 Soft, 3 Hard, 3 Unmodified	Up to 3 per site plus 1 composite	
DE		Unteruckersee	53°16.699' N	13°52.276' E	3 Soft, 3 Hard, 3	Up to 3 per site plus	**294**

					Unmodified	1 composite	
DK	**X**	Fussingsø	56°28.264' N	9°52.300' E	3 Soft, 3 Unmodified	Only composite	
DK	**X**	Nordborgsø	55°03.479' N	9°45.645' E	3 Soft, 3 Hard, 3 Unmodified	Up to 3 per site plus 1 composite	**42**
FI	**X**	Sääksjärvi	62°10.458' N	25°44.010' E	3 Soft, 3 Hard, 3 Unmodified	Up to 3 per site plus 1 composite	
FI	**X**	Vuojärvi	62°24.814' N	25°56.289' E	7 Soft, 2 Unmodified	Up to 3 per site plus 1 composite	
FI		Jyväsjärvi	62°14.477' N	24°12.432' E	3 Soft, 3 Hard, 3 Unmodified	Up to 3 per site plus 1 composite	
FI	**X**	Iso-Jurvo	62.609154' N	25.938997' E	9 Unmodified	Up to 3 per site plus 1 composite	**144**
UK	**X**	Rostherne Mere	53°21.240' N	2°23.100' W	3 Soft, 3 Unmodified	Only composite	
UK	**X**	Loweswater	54°34.980' N	3°21.360' W	3 Hard, 3 Soft, 3 Unmodified	Only composite	
UK	**X**	Grasmere	54°27.000' N	3°1.320' W	3 Hard, 3 Soft, 3 Unmodified	Only composite	**24**
IE		Muckno	54°07.252 N	6°43.749 W	3 Hard, 3 Soft, 3 Unmodified	Up to 3 per site plus 1 composite	
IE		Oughter	54°00.702 N	7°27.351 W	3 Hard, 3 Soft, 3 Unmodified	Up to 3 per site plus 1 composite	
IE		Brackley	54°08.337 N	7°43.100 W	3 Hard, 3 Soft, 3 Unmodified	Up to 3 per site plus 1 composite	
IE		Garadice	54°03.029 N	7°41.755 W	3 Hard, 3 Soft, 3 Unmodified	Up to 3 per site plus 1 composite	
IE		Scur	54°01.992 N	7°57.756 W	3 Hard, 3 Soft, 3 Unmodified	Up to 3 per site plus 1 composite	
IE		Rinn	53°53.955 N	7°51.347 W	3 Hard, 3 Soft, 3 Unmodified	Up to 3 per site plus 1 composite	
IE		Rea	53°11.854 N	8°34.489 W	3 Hard, 3 Soft, 3 Unmodified	Up to 3 per site plus 1 composite	
IE		Cullin	53°58.961 N	9°11.852 W	3 Hard, 3 Soft, 3 Unmodified	Up to 3 per site plus 1 composite	
IE		Carra	53°42.610 N	9°13.484 W	3 Hard, 3 Soft, 3 Unmodified	Up to 3 per site plus 1 composite	**324**
IT	**X**	Segrino	45°49.875' N	9°16.146' E	3 Soft, 3 Hard, 3 Unmodified	Up to 3 per site plus 1 composite	
IT		Bolsena	42°35.908' N	11°56.321' E	3 Soft, 3 Hard, 3 Unmodified	Up to 3 per site plus 1 composite	
IT	**X**	Montorfano	45°46.943' N	9°8.311' E	3 Soft, 3 Hard, 3 Unmodified	Up to 3 per site plus 1 composite	
IT	**X**	Alserio	45°47.159' N	9°12.905' E	3 Soft, 3 Hard, 3 Unmodified	Up to 3 per site plus 1 composite	
IT		Candia	45°19.471' N	7°54.694' E	3 Soft, 3 Hard, 3 Unmodified	Up to 3 per site plus 1 composite	
IT		Piediluco	42°32.006' N	12°45.313' E	3 Soft, 3 Hard, 3 Unmodified	Up to 3 per site plus 1 composite	
IT		Nemi	41°42.712' N	12°42.106' E	4 Soft, 1 Hard, 4 Unmodified	Up to 3 per site plus 1 composite	
IT	**X**	Monate	45°47.707' N	8°39.809' E	3 Soft, 3 Hard, 3 Unmodified	Up to 3 per site plus 1 composite	
IT		Martignano	42°6.742' N	12°18.927' E	4 Soft, 5 Unmodified	Up to 3 per site plus 1 composite	
IT		Pusiano	45°48.026' N	9°16.416' E	3 Soft, 3 Hard, 3 Unmodified	Up to 3 per site plus 1 composite	
IT		Albano	41°44.928'N	12°40.119' E	4 Soft, 5 Unmodified	Up to 3 per site plus 1 composite	

IT	Vico	42°19.117' N	12°10.616' E	3 Soft, 3 Hard, 3 Unmodified	Up to 3 per site plus 1 composite	
IT	Varese	45°48.684' N	8°44.350' E	3 Soft, 3 Hard, 3 Unmodified	Up to 3 per site plus 1 composite	
IT	Bracciano	42°7.255' N	12°13.588' E	3 Soft, 3 Hard, 3 Unmodified	Up to 3 per site plus 1 composite	
IT	Iseo	45°43 N	10°05 E	3 Soft, 3 Hard, 3 Unmodified	Up to 3 per site plus 1 composite	**540**
SE	Vällen	60° 3’33.710 "N	18°19’0.530 "E	3 Hard, 3 Soft, 3 Unmodified	Up to 3 per site plus 1 composite	
SE	Öjaren	60° 41’53.264 "N	16°49’3.608 "E	3 Hard, 3 Soft, 3 Unmodified	Up to 3 per site plus 1 composite	
SE	Hedesundafjärden	60° 22’10.870 "N	17°1’31.102 "E	3 Hard, 3 Soft, 3 Unmodified	Up to 3 per site plus 1 composite	
SE	Storfjärden	60° 28’51.244 "N	17° 22’43.165 "E	3 Hard, 3 Soft, 3 Unmodified	Up to 3 per site plus 1 composite	
SE	Storsjön	60° 31’49.498 "N	16° 44’17.682 "E	3 Hard, 3 Soft, 3 Unmodified	Up to 3 per site plus 1 composite	
SE	Färnebofjärden	60° 14’21.034 "N	16° 47’15.569 "E	3 Hard, 3 Soft, 3 Unmodified	Up to 3 per site plus 1 composite	
SE	Magelungen	59° 13’51.488 "N	18°6’17.395 "E	3 Hard, 3 Soft, 3 Unmodified	Up to 3 per site plus 1 composite	
SE	Runn	60° 35’10.111 "N	15°45’3.402 "E	3 Hard, 3 Soft, 3 Unmodified	Up to 3 per site plus 1 composite	
SE	Svärdsjön	60° 46’33.841 "N	15° 53’25.541 "E	3 Hard, 3 Soft, 3 Unmodified	Up to 3 per site plus 1 composite	**324**
Total						**1692**

Subsequently, the macroinvertebrate samples were processed, which took 1 – 1.5 years at the various partners. During the WISER kick-off meeting in Mallorca it was agreed to collect additional pooled “composite” macroinvertebrate samples (pooling all habitats) and to test the usefulness of composite sampling as alternative cost-efficient assessment method. With the purpose to assess the costs saved and the possible loss in assessment precision by collecting composite instead of habitat specific macroinvertebrate samples, at each sampling site one composite sample was collected additionally to the three habitat specific samples during the field campaign of the WP 3.3 lake macroinvertebrate team.

The collection of composite samples and habitat samples does involve generally the same amount of time with the average time to collect each composite or habitat sample accounting for 0.4 hours. For the composite sampling method the collection of only 1 sample per site would be necessary, the habitat specific sampling method, however, involves the collection of at least 3 different habitat samples (ideally sand, stone and macrophytes) and is, thus, more time and cost intensive (composite sampling per site = 0.4 hours; habitat specific sampling per site = 1.2 hours; average over all countries). The time and cost-effectiveness of the composite sampling method is, moreover, supported by time estimates for sorting of macroinvertebrate samples per site (example from lake Werbellin, Germany: average time to sort a composite sample per site = 10.2 hours; average time to sort 3 habitat specific sample per site = 30 hours). The usefulness of the apparently more cost-efficient composite sampling method for monitoring of lakes, however, stills needs an in depth analysis of the complete WISER WP 3.3 lake macroinvertebrate data set.

It has to be assessed whether the results generated using the composite sampling method are equally precise when compared with the more time intensive habitat specific sampling method.

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