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Spatial and Temporal Variability of Stream Benthic Macroinvertebrates

Implications for Enviromental Assessment

Leonard Sandin

SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

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Abstract

This thesis describes the spatial and temporal variability of benthic macroinvertebrate communities in Swedish streams and its implications for environmental assessment. One of the challenges for environmental assessment is to separate change caused by anthropogenic stress from natural variability, therefore it is essential to have insight in the effects of temporal and spatial variability on the benthic macroinvertebrates. The thesis is based on two stream riffle datasets; one spatial (694 streams) and one temporal (6-11 years sampling of five streams). Classification of macroinvertebrate community composition showed a gradual change in community composition in Sweden. A geographical (ecoregional) classification was tested as a starting point for biomonitoring, but I found that local-scale variables such as stream velocity and substratum type were important descriptors and must be taken into account in assessments of running waters. Despite that temporal variability was small relative to the spatial variability, I argue that the temporal variability is of importance and that the inherent cyclic and seasonal factors affecting benthic macroinvertebrates has to be accounted for in environmental assessment. Surprisingly, the stream macroinvertebrate community composition did not show a large-scale pattern (correlation with the *limes norrlandicus* ecotone, found at ~60° north), whereas a lake dataset showed such a pattern. I speculate that differences between the two ecosystems are caused by differences in detrital retention and stability. Richness indicator measures generally had low variability and high statistical power and needed few samples to detect changes caused by perturbation. In environmental assessment it is important to know what effect size and variability (spatial and temporal) one can expect from an indicator metric (and hence the macroinvertebrate community) so that the money spent in impact studies is used in a cost-effective way.

Key words: benthic macroinvertebrates, streams, running water, environmental assessment, biomonitoring, temporal variability, spatial variability, Sweden

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Key words: benthic macroinvertebrates, streams, running water, environmental assessment, biomonitoring, temporal variability, spatial variability, Sweden

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Contents

Appendix

Papers I-VI

The present thesis is based on the following papers, which will be referred to by their Roman numerals.

I. **Sandin, L.** Community structure, taxon richness, and environmental relations of benthic macroinvertebrates in Swedish streams. Manuscript.

II. **Sandin, L.** & Johnson, R.K. 2000. Ecoregions and benthic macroinvertebrate assemblages of Swedish streams. *J. N. Am. Benthol. Soc. 19(3)*, 462-474.

III. **Sandin, L.** & Johnson, R.K. The importance of local and regional factors for the macroinvertebrate community structure in Swedish streams. In review.

IV. **Sandin, L.** Temporal variability and persistence of benthic macroinvertebrates in five small Swedish streams. Manuscript.

V. Johnson, R.K., **Sandin, L.** & Goedkoop, W. Similarities and differences of hard-bottom macroinvertebrate communities of streams and lakes. Manuscript.

VI. **Sandin, L.** & Johnson, R.K. 2000. The statistical power of selected indicator metrics using macroinvertebrates for assessing acidification and eutrophication of running waters. *Hydrobiologia 422/423*, 233-243.

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Introduction

Background

Running waters are among the most important natural resources, but also among the most threatened on earth. Already in 1970 H.B.N. Hynes wrote: "human activities has profoundly affected rivers and streams in all parts of the world, to such an extent that it is now extremely difficult to find any stream which has not been in some way altered, and probably quite impossible to find any such river". Still aquatic ecosystems have continued to decline worldwide (Karr and Chu, 2000). The most important factors affecting running water ecosystems are: water regulation, abstraction, physical alterations, and different types of pollution.

Sweden has a total of about 300 000 km of streams and rivers and the main threats to these watercourses are hydroelectric power (damming), acidification, and eutrophication (Bernes, 1993). Persson and Eriksson (1996), using data from the National Stream Survey of 1995, estimated that either eutrophication or acidification affected more than 50 % of the Swedish streams. In Sweden 12 000 km of running waters were limed in 1999 and during the year 2000 the government spent 210 million Swedish crowns on liming activities. About 70 % of the large rivers have been greatly affected by the construction of hydroelectric power plants (Friberg and Johnson, 1995).

Since many anthropogenic factors affect running waters, reliable tools are needed to monitor these systems and to distinguish anthropogenic stress from natural variability. The use of biological indicators has the advantage that the organisms directly reflect the overall changes in the environment. The organisms integrate the changes of different types of perturbation over time. In streams and rivers benthic macroinvertebrates, fish, and algae are (and have been) the most common biological indicators used for environmental monitoring and assessment (Hellawell, 1986). In practice, however, benthic macroinvertebrates alone are the most widespread assessment tool for the biological quality of freshwaters (Rosenberg and Resh, 1993).

Benthic macroinvertebrates are "...organisms that inhabit the bottom substrates (sediments, debris, logs, macrophytes, filamentous algae, etc.) of freshwater habitats" (Rosenberg and Resh, 1993). They are usually considered to be organisms that are large enough to bee seen without magnification, i.e., retained in a net with a mesh size of 200 to 500 µm (e.g., Sládecek *et al.,* 1982; De Pauw and Vanhooren, 1983; Rosenberg and Resh, 1993). Although benthic macroinvertebrates have been used for a long time as a biomonitoring tool, the spatial and temporal variability of the communities have often been neglected. Natural variability may confound the results of water quality biomonitoring, possibly resulting in incorrect conclusions of the impact of anthropogenic stress on the benthic fauna. A better understanding of the implications of spatial and temporal variability of benthic macroinvertebrate communities is important for improving our knowledge of the factors influencing the structure and function of aquatic ecosystems, and ultimately the management of aquatic biodiversity.

Environmental assessment using benthic macroinvertebrates

Several authors have summarised the advantages and disadvantages of using benthic macroinvertebrates in biomonitoring (e.g., Hawkes, 1979; Sladecek *et al.*, 1982; Hellawell, 1986; Metcalfe, 1989; Rosenberg and Resh, 1993). In brief, macroinvertebrates are ubiquitous, sedentary and have a relatively long lifespan. Because of these and many other features, they are good representatives of local conditions and integrate changes in environmental conditions over time. There are also several disadvantages of using benthic macroinvertebrates as biomonitoring tools (Hawkes, 1979; Sládecek *et al.*, 1982; Rosenberg and Resh, 1993). For example, it is difficult to sample stream macroinvertebrates quantitatively and their distribution can be affected by other environmental factors than pollution (e.g., water current and sediment content). The identification of some taxa is time-consuming and requires expertise (e.g., some Chironomidae, Trichoptera, and Oligochaeta).

Despite these disadvantages, benthic macroinvertebrates have been used for a long time in environmental assessment of fresh-waters and a large amount of knowledge exists regarding sensitivity and preferences of different macroinvertebrates to pollution. The use of benthic macroinvertebrates in biomonitoring started in the late 19th century in Germany with the idea of saprobity (i.e., assessment of organic pollution and the associated decrease in dissolved oxygen) (Kolkwitz and Marson, 1909). Since the original development of the saprobien system more than 50 other approaches for biomonitoring using macroinvertebrates have evolved (De Pauw and Vanhooren, 1983).

In studies of environmental impact, the objective is to separate the change generated by anthropogenic stress from the natural spatial and temporal variability. If the natural variability is large and the anthropogenically induced change is small it will be difficult to detect a true change in the measured variable(s) caused by the pollutant (Johnson, 1998). A few studies have examined the variability of benthic macroinvertebrate indicator metrics used in water quality assessment, both in running waters (Barbour *et al.*, 1992; Resh, 1994; Hannaford and Resh, 1995; Resh, Rosenberg and Reynoldson, 2000) and in lakes (Johnson, 1995; Johnson, 1998). The variability in the indicator metric(s) can be divided into three parts: i) measurement error, ii) the within-site spatial and temporal variability and iii) among-site variability. The within-site variability can be regarded as noise whereas among site variability often is the parameter of interest in biomonitoring studies. This thesis focuses on the temporal and spatial variability of stream benthic macroinvertebrate community composition and its implications for environmental assessment.

Scale in running waters

In stream ecology, contention exists as to what factors are considered to be important (i.e., deterministic or forcing) in structuring stream ecosystems. This disagreement might emanate in part from study design, since studies at different spatial and temporal scales are prone to give different answers (Wiley, Kohler and Seelbach, 1997; Fisher, 1994). Recognition of what factor(s) structure stream ecosystems has evolved from single to multiple variables and from structuring forces on the habitat scale to involve factors on the global scale (Minshall, 1988). Running water ecosystems have a high level of spatio-temporal variability and can be divided into four dimensions according to Ward (1989): i) The upstream downstream longitudinal dimension, ii) exchange between the riparian zone, the floodplain and the channel, i.e., the lateral dimension, iii) the vertical dimension, interactions between the running water and the ground water and (iv) time which "...superimposes a temporal hierarchy on the three spatial dimensions" (Ward, 1989).

The ecologically relevant time span in stream ecosystems ranges over 16 orders of magnitude (from seconds to 100 million years), but stream ecologists generally study their systems on scales of days, seasons and years (Minshall, 1988). A number of authors have stressed the need to study temporal variability in aquatic ecological studies (e.g., Strayer, 1986; Resh & Rosenberg, 1989), but the notion that variability in both space and time act in concert in these systems are generally ignored. Studies are commonly carried out either in one stream for a longer time period (during several seasons to years) or are spatially extensive but generally only one sample is taken at each site. These two types of studies each emphasise different mechanisms for explaining the structure of the systems. Spatially extensive studies emphasise abiotic structuring factors, whereas temporally extensive studies emphasise biotic factors such as predation, competition and the effects of pathogens (Wiley, Kohler and Seelbach, 1997).

One caveat in trying to account for the often confounding effect of ecological scale in stream studies is the wide range of spatial scales encountered; spatial dimensions of stream ecosystems range from 1μm (or even smaller) for the smallest particles to 1Mm for the largest rivers or continents (Minshall, 1988). Another problem is that the scale at which a system is observed is important when determining which factor(s) influences the structure of the ecosystem (e.g., Frissell *et al.*, 1986; Minshall, 1988; Carter, Fend and Kennelly, 1996; Wiley, Kohler and Sehlbach, 1997).

Objectives

In this thesis I address questions regarding the spatial and temporal variability of benthic macroinvertebrates in Swedish streams and its implications for environmental assessment of running waters. More specifically, the objectives of this work were the following:

1. To better the understanding of what natural and anthropogenic factors affect the structure and taxon richness of stream benthic macroinvertebrate communities in Sweden. This will be done both by describing the main structure in community composition and by relating the structure and taxon richness to environmental variables and by contrasting these results with data from wind-exposed lake littoral ecosystems.
2. To analyse what level of spatial scale (i.e., local-, catchment-, or regional-scale) is the most important for structuring stream benthic macroinvertebrate communities and thus for environmental assessment using these organisms.
3. To describe the variability and persistence in time of benthic macroinvertebrate communities and analyse what implications this variability has for environmental assessment of running waters.
4. To test some commonly used benthic macroinvertebrate indicator metrics by analysing their spatial, temporal and sample variability, and their efficiency to detect anthropogenic change in running waters.

Materials and Methods

Three different datasets were used in my thesis. One large-scale *spatial dataset* including 694 streams, a *reference dataset* where streams deemed as affected by acidification, eutrophication and liming were removed from the large-scale *spatial dataset*, and a *temporal dataset* with five streams sampled 6-11 years.

Spatial dataset

A Swedish national stream survey of benthic macroinvertebrates was carried out in the autumn of 1995 and the data from this survey form the basis for papers **I** and **VI** of this thesis. Sampling sites were randomly selected from the Swedish Hydrological and Meteorological Institute's watercourse and catchment register. The sites were stratified according to size; 350 within catchments of 15-50 km^2 and 350 within the size 50-250 km^2, 694 of the 700 streams were successfully sampled. Macroinvertebrate samples were taken from a ten-meter sampling area and samples were collected using standardised kick sampling (European Committee for Standardisation 1994) with five samples (1 m x 1 min) taken at each site using a handnet (500-µm mesh), see Wilander *et al.* (1998).

Stream width, depth, stream velocity, substratum and vegetation in the stream were classified. The riparian vegetation, designated as a five-meter wide zone on both sides of the sampling site, was also classified. Water chemistry samples were taken in connection with the biological sampling. Data on land use/cover in the catchment were obtained using GIS and 1:250.000 digital maps. Classification of quaternary deposits and the main geological bedrock type in the catchment were taken from the Swedish National Atlas (Fredén and Wastenson, 1994). Ecoregion delineation of Sweden was obtained from the Nordic Council of Ministers (1984) and climate data from the Swedish Hydrological and Meteorological Institute.

Thirty-nine sites in the county of Västernorrland were removed from all analysis in this thesis, because the sampling protocol was not followed, and in paper **I** an additional 27 sites were removed where one or several environmental variables were not available (Fig. 1).

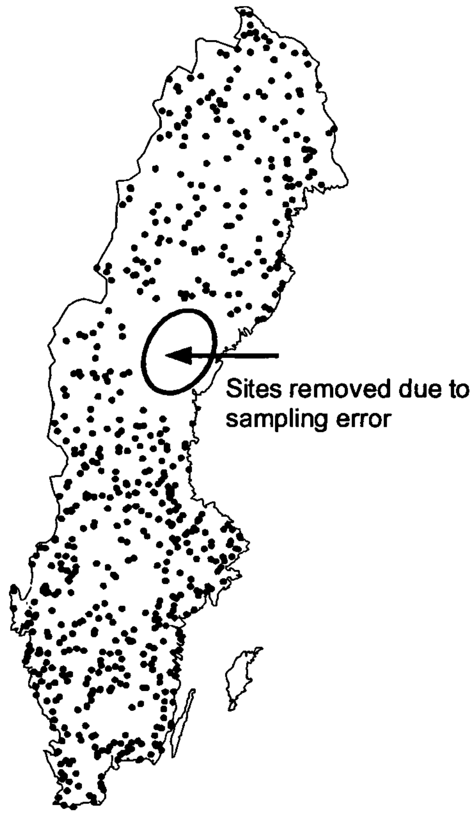

Figure 1. The 628 streams sampled for benthic macroinvertebrates as part of the National Stream Survey and included in paper I.

All benthic macroinvertebrate samples were sorted at the Department of Environmental Assessment according to quality control and assurance protocols (Wilander *et al.,* 1998). When necessary (i.e., if sampling was expected to exceed

2 h) samples were subsampled. Taxa were identified to a predetermined list of 517 operable taxonomic units decided by expert opinion (Wilander *et al.,* 1998). Most of the individuals were identified to species, species-group or genus, but some to a higher taxonomic level (e.g., Oligochaeta and Chironomidae) and all identifications were made by six experienced biologists. An intercalibration of the frequency of misidentified or miscounted taxa was also performed. For a complete list of the taxonomic resolution and the methods used to analyse the chemical variables see Wilander *et al.* (1998).

Reference dataset

Sites judged to be affected by human impacts were removed from all calculations in papers **II**, **III**, **V** and **VI**. Removal of sites directly or indirectly affected by liming, sites classified as eutrophic (containing >20% arable land in the catchment) and sites deemed to be acidified with an exceedance of critical load for S acidity (Henriksen et al., 1992) resulted in a total of 428 streams (Fig. 2a) that were included in the analyses. In paper **V**, the reference stream dataset was compared to a lake spatial dataset of 364 lakes, also sampled as part of the national survey of 1995 (Wilander *et al.*, 1998).

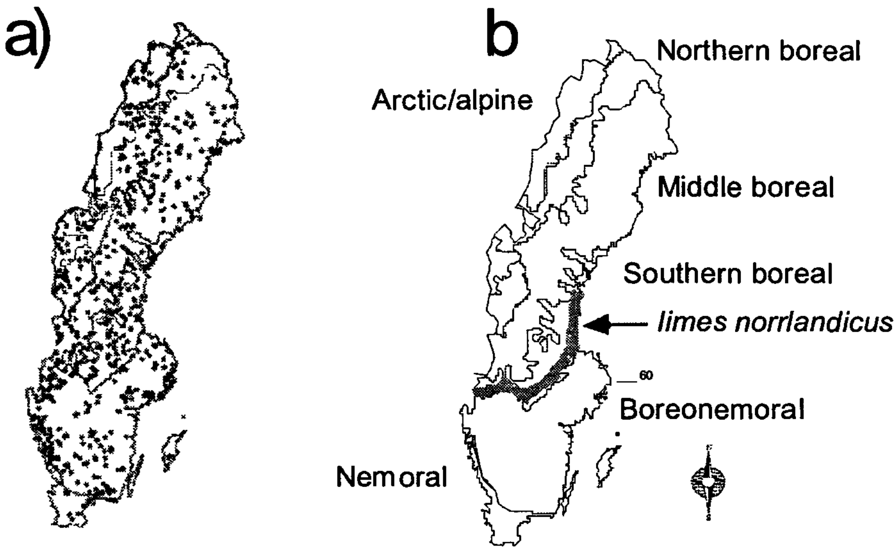

Figure 2. a) The reference streams used in papers II, III, IV, and V, b) the 6 main ecoregions in Sweden and the limes norrlandicus ecotone.

Temporal dataset

A temporal dataset from five small streams was used in papers **IV** and **VI**. They are all part of the long-term monitoring program; International Co-operative Programme on Integrated Monitoring on Air Pollution Effects (Pylvänäinen, 1993), and cover a large climatic and depositional gradient in Sweden (Fig. 3). The streams are situated from 65° 47′ North and 19° 05′ East (Laxtjärnsbäcken) to 50° 04′ North and 12° 48′ East (Pipbäcken). All streams had small (0.93 - 10.9 km^2) forested catchments. The sites are situated in catchments that have been "protected" from forestry activity for several decades (they are unsuitable for forestry since they have unproductive soils or are found in steep and rocky terrain). Climate and deposition are thus the only factors governing the runoff chemistry of the streams.

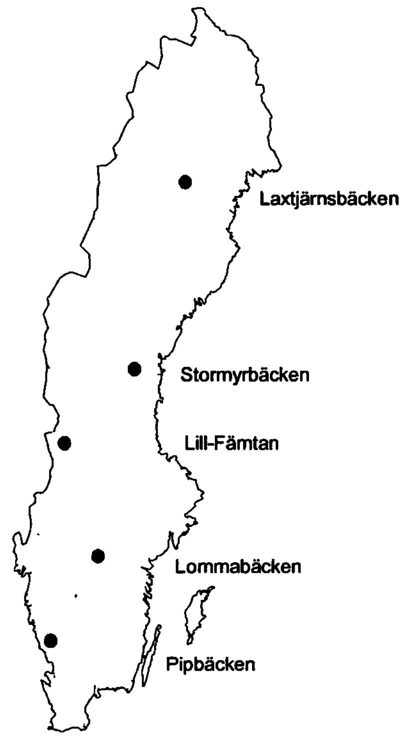

Figure 3. The five (temporal) sampled streams along a climatic and depositional gradient from south to north. Benthic macroinvertebrate samples were taken once a year in spring or summer (between April and July). Macroinvertebrate sampling began in 1986 (Lill-Fämtan, Lommabäcken), 1987 (Pipbäcken, Stormyrbäcken), and 1990 (Laxtjärnsbäcken).

Macroinvertebrate samples were taken once a year in spring or summer (between April and July). Macroinvertebrate sampling began in 1986 to 1990 in the different streams, and since sampling was terminated in 1996, the number of available samples (years) varied between six and eleven.

At Laxtjärnsbäcken, only samples from six and not seven years were available since the 1994 sample was taken in the autumn. Samples were collected from riffle habitats using standardised kick sampling (European Committee for Standardisation, 1994), with five samples (1 m x 1 min) taken at each site using a handnet (500-µm mesh). Benthic macroinvertebrate samples were sorted and the organisms identified at the Department of Environmental Assessment according to quality control and assurance protocols. All taxa were identified to a predetermined taxonomic level, mainly to species or genus, but some to higher taxonomic levels. Since the taxonomic resolution of identified individuals had increased somewhat during the sampling period; some taxa identified during the latter stage of the project were merged to a higher taxonomic level.

Streamwater chemical samples were collected once (at low flow) or twice (at high flow) each month resulting in 15-20 samples per year. All chemical samples were analysed by certified laboratories according to European (EN) or International (ISO) standards where applicable (see Wilander *et al.,* 1998). Runoff for each site was calculated using a dynamic hydrological model that was calibrated against the continuously measured water level at a calibrated gauge. Driving variables were daily precipitation and temperature taken from a nearby meteorological station within the national Swedish meteorological network (Hans Kvarnäs pers. comm.).

Ecoregions and *limes norrlandicus*

In paper **II** the use of ecoregion classification as a basis for environmental assessment of benthic macroinvertebrates is tested. Sweden can be divided into 8 main ecoregions according to the Nordic Council of Ministers (1984). Here I used 6 ecoregions (Fig. 2b), the arctic region was combined with the alpine region, and the northern boreal region was combined with the northern-southern boreal region. The nemoral region consists mainly of deciduous forests and the boreo-nemoral region consists of mixed forests. North of these mixed forests there is a rather abrupt transition zone known as *limes norrlandicus* (Fig. 2b). To the north of this ecotonal zone, the vegetation consists of predominantly pine and spruce forests, whereas in the arctic-alpine region the vegetation is characterised by heaths and very sparse vegetation cover in the high mountain areas.

Multivariate methods

Classification, ordination, and discriminant analysis are some of the most widely used multivariate techniques in water quality assessment using macroinvertebrates (Norris and Georges, 1993). Classification comprises a group of methods where the investigated objects are arranged into small homogenous groups or clusters (Everitt and Dunn, 1991). Two Way INdicator SPecies ANalysis (TWINSPAN) is a polythetic, divisive, hierarchical classification method (Hill, 1979), that has often been used in macroinvertebrate studies. Unweighted Pair Groups Using ArithMetic Average (UPGMA) (Sneath and

Sokal, 1973) is another classification technique that is agglomerative and hierarchical. Belbin, Faith and Milligan (1992) have further developed this method and introduced flexible UPGMA, a clustering technique which has been shown to work well in the classification of benthic macrofauna (e.g. Marchant, Barmuta and Chessman, 1994; Marchant *et al.*, 1997; Pardo and Armitage, 1997; Parsons and Norris, 1996). FLEXCLUS (van Tongeren, 1986) is a non-hierarchical clustering technique where the sites are relocated until stability between the clusters is reached.

"Ordination is a procedure for adapting a multidimensional swarm of data points in such a way that when it is projected onto a two-space (such as a sheet of paper) any intrinsic pattern the swarm may possess becomes apparent" (Pielou, 1984). Indirect and direct gradient analysis are two types of ordination techniques. In indirect gradient analysis the axes are constructed from the variation among the sampled communities and thereafter interpreted in terms of environmental gradients (ter Braak and Prentice, 1988). These techniques include methods such as Principal Component Analysis (PCA) and Correspondence Analysis (CA) (Hill, 1974). CA has been developed further into Detrended Correspondence Analysis (DCA) where the ordination axes are detrended in order to counteract the so called arch effect, a defect of CA. Further Semi-Strong Hybrid Multidimensional Scaling is a unconstrained ordination method based on a similarity matrix.

In direct gradient analysis, species abundance or probability of occurrence is described directly as a function of the measured environmental variables (either with a linear or unimodal response). It includes methods such as Redundancy Analysis (RDA) (Rao, 1964; van den Wollenberg, 1977) and Canonical Correspondence Analysis (CCA) (ter Braak, 1986; 1987). Direct gradient analysis has been widely used in ecology and benthic macroinvertebrate studies (see Birks, Peglar and Austin, 1994 for a review). Partial constrained ordination (pRDA or pCCA) is a procedure where known or unwanted variables are removed from the computations by means of multiple linear regression (ter Braak, 1988). This method has been used to partition the variation of species abundance data into different environmental and spatial variable groups (e.g. Borcard, Legendre and Drapeau, 1992; Økland and Eilertsen, 1994; Liu and Bråkenhielm, 1995). Discriminant analysis is an ordination technique used to test whether a set of variables (e.g., environmental factors) can discriminate among a number of predefined groups (e.g., a TWINSPAN classification).

Results

The large-scale picture of community composition, taxon richness and environmental relations of benthic macroinvertebrates in Swedish streams is described (paper I) and an ecoregional delineation as a basis for environmental assessment was tested (paper II). The spatial variability in benthic macroinvertebrate community composition was decomposed into local, catchment, and regional parts (paper III), and the temporal variability and persistence of the macroinvertebrate community was examined (paper IV). Structure and function of stream (riffles) and lake (wind-exposed) littoral communities are compared in relation to environmental variables (paper V), and a number of benthic macroinvertebrate indicator metrics commonly used in environmental assessment of running waters are tested for their statistical power (using information on their temporal and spatial variability) and number of false positives (type I error) and false negatives (type II error) errors (paper VI).

Community composition and taxon richness (paper I)

Rarefaction was used to compare taxon richness of sampling sites with a varying number of individuals. Rarefaction was calculated for a common abundance level of 300 individuals. The richness of the sample and the 'rarefied' sample richness were strongly correlated ($r^2 = 81\%$) and factors such as [K], alpine vegetation in catchment and total phosphorous [TP] were negatively correlated, whereas pebble substratum, pH, stream velocity, April air temperature, and catchment area were positively correlated with taxon richness. Taxon richness was lowest in the arctic/alpine ecoregion in the northwestern parts of the country and in the boreo-nemoral and nemoral ecoregions in the south-central parts of the country (Fig. 4). The southeastern parts of the country also had surprisingly low taxon richness, despite the fact that at least the northern parts of this area are well buffered against acidification. One reason that richness was lower in this area might be the influence of urbanisation and agriculture.

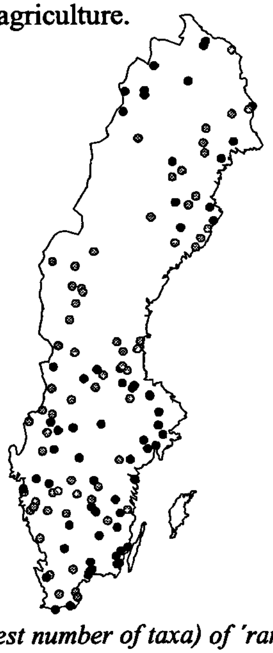

Figure 4. The 10 percentile (lowest number of taxa) of 'rarefied' taxon richness of benthic macroinvertebrates (black circles) and the 90 percentile (highest number of taxa) (grey circles) in the national stream survey of 1995.

In order to describe the stream macroinvertebrate community composition, the biological data were classified using TWINSPAN and related to environmental variables. The TWINSPAN classification resulted in 14 terminal groups. The ability to predict the biologically derived classification groups using a few environmental variables differed considerably between the groups (Fig. 5). In the north, the groups were well defined and had a high correct prediction rate (>70%), whereas some of the groups in the south had a very low correct prediction rate (<20%). In total only 37% of the sites in the final classification were correctly predicted into one of the 14 groups. Classification thus showed that there are no truly distinct macroinvertebrate community groups or types, but rather a gradual change in taxon composition and related environmental variables along the gradients found in the study.

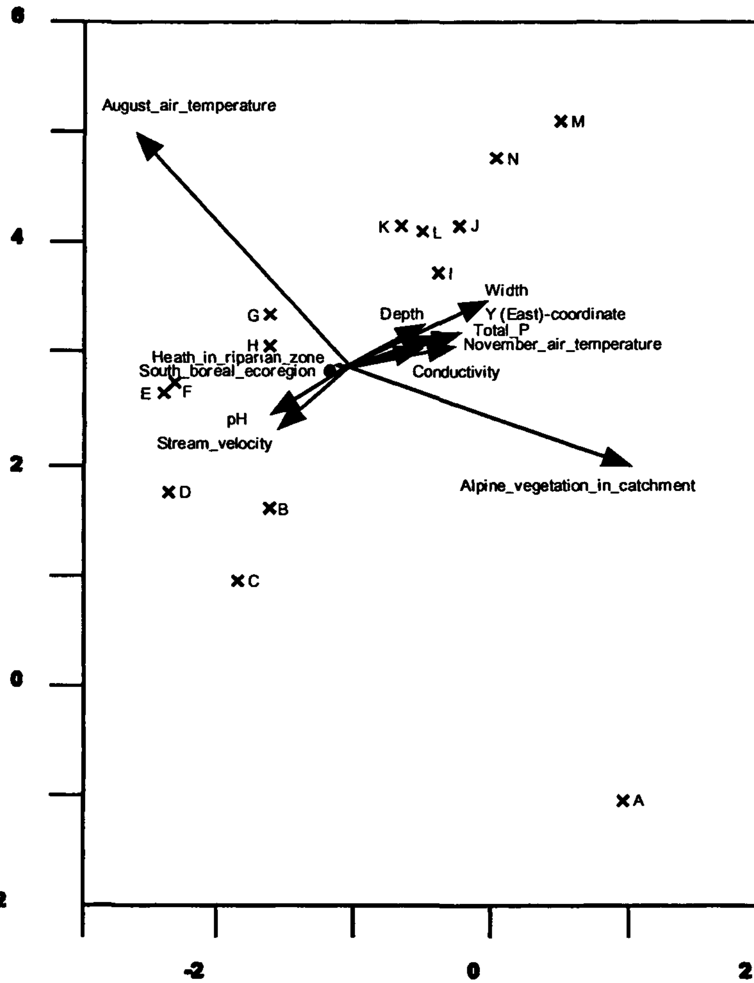

Figure 5. Discriminant analysis of 14 TWINSPAN groups (centroid A - N) and environmental variables that discriminated among the groups.

Ecoregional classification (paper II)

Geographical classifications can be a useful tool in partitioning the natural spatial variability of the response variable (e.g., macroinvertebrates), thereby optimising environmental assessment and conservation programs. In paper **II**, 428 unimpacted streams were used to test the concordance between an ecoregional delineation of the six main ecoregions of Sweden and metrics of benthic macroinvertebrates, i.e., taxon richness, abundance and diversity.

Taxon richness, abundance and diversity all differed significantly among ecoregions. The main difference was found between the arctic-alpine ecoregion that had lower taxon richness, abundance and diversity than the other five ecoregions. A permutation test was used to compare the agreement between six UPGMA groups and a classification based on the six main ecoregions of Sweden (Fig. 6).

a)

b)

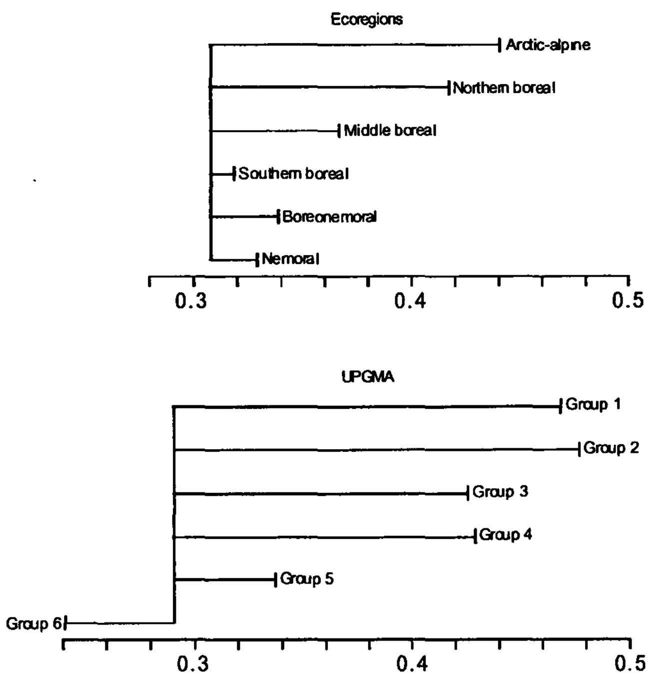

Figure 6. Mean similarity dendrograms based on the Sørensen coefficient for benthic macroinvertebrate communities in a) the six main ecoregions of Sweden and b) the 6 UPGMA (unweighted pair-group method using arithmetic averages) cluster groups. The vertical lines represent the mean between-class similarity and the horizontal lines terminate at the mean within-class similarity for each ecoregion or UPGMA group.

The stream macroinvertebrate assemblages were more homogeneous within than between the ecoregions (Fig. 6). Macroinvertebrate assemblages were most homogeneous in the arctic-alpine ecoregion, whereas streams situated in the southern boreal region were the least homogeneous. Pairwise comparisons revealed that the arctic-alpine and the northern boreal regions differed from the 4 remaining ecoregions ($p < 0.001$). The nemoral region differed from the middle boreal region ($p < 0.05$), but not from the southern boreal and boreo-nemoral regions. Correspondence between the 6 UPGMA groups and the 6 ecoregions was relatively poor. A gradual change in the percentage of sites belonging to the different ecoregions was noted for each UPGMA group, but no group was composed of sites from < 4 ecoregions, and 5 of the groups contained sites from 5 of the 6 ecoregions.

These results indicate that the benefit of an ecoregional classification for biomonitoring or assessment of streams using benthic macroinvertebrates is not convincing, because there is a gradual change in community composition from north to south. Benthic macroinvertebrate stream communities are structured both by large-scale factors (i.e., on a geographical scale), and by small-scale factors (i.e., on a local scale). Ecoregion classifications alone, therefore, may not sufficiently partition the variance in community composition (i.e., large differences are found within different habitat types within an ecoregion). A nested approach, including factors such as altitude, stream size, and catchment characteristics, is probably needed to improve ecoregion classifications and environmental assessment that use stream benthic macroinvertebrates.

Spatial scale (paper III)

The question of whether benthic macroinvertebrate communities are structured by local-, catchment- or large-scale factors is currently being debated. To study the importance of different spatial scales, the variance in macroinvertebrate community composition was decomposed into parts explained by these different spatial scale levels using partial constrained ordination (Fig. 7).

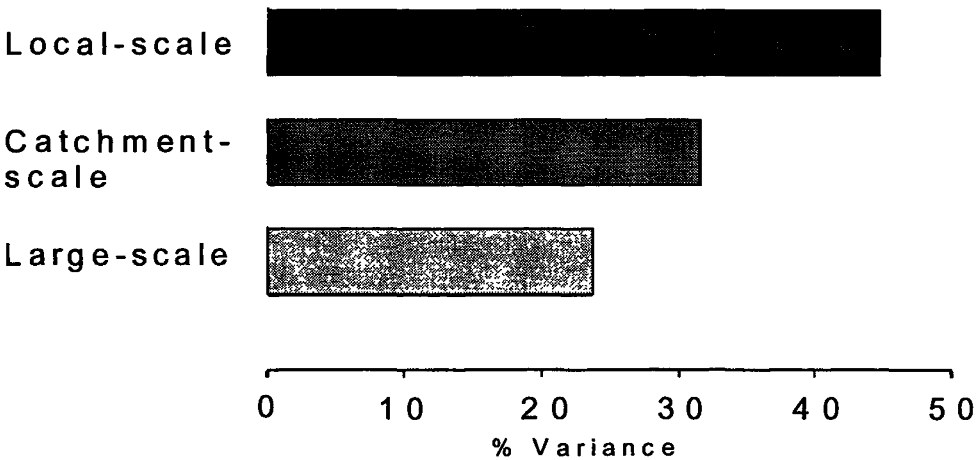

Figure 7. Proportion of variance explained by environmental variables on three spatial scales.

The 80 environmental variables that were tested for inclusion in the partial constrained ordination model were divided into seven environmental variable groups: local physical, local chemical, catchment land use/cover, catchment bedrock geology and quaternary geology in catchment, large-scale (regional) factors (e.g., ecoregions) and spatial position. The pure effects of the seven variable groups accounted for 69% of the total explained variance and combinations of variable groups (i.e., interaction terms) accounted for the remaining 31% of the total explained variance. Local scale variables such as substratum, vegetation in and near (riparian) the stream and some chemical variables were most important for explaining the among-site variance. Local physical (24%) and local chemical (20%) variables were the two factors explaining the largest part of the among-site variability in community composition.

Although local-scale factors explained much variance, regional scale (physical) processes can impose substantial control, confounding the interpretation of the individual (or unique) importance of scale-related variables. The scale at which observations are made is another factor that may confound interpretation of scale-related processes, since the scale at which a process or object is studied can also affect what factors will be recognised as important. My study clearly showed the importance of local-scale variables such as substratum, in-stream and riparian vegetation and some chemical variables in explaining the among-site variance of stream benthic macroinvertebrate communities. Large-scale variables were also significant (although of less importance). Hence a combined understanding of both local- and large-scale processes is needed to assess the factors structuring communities of running waters.

Temporal variability and persistence (paper IV)

In total, 109 taxa were recorded at the five sites during the eleven years of the survey. The mean number of taxa differed significantly between the streams, with the highest number of taxa in northern and lowest in southern Sweden. The northernmost stream had the highest mean similarity value during the sampling period, on average 50% of the community structure was the same between the sampled years. The other streams had similarity values between 26% and 43%. Both cluster analysis, unconstrained and constrained ordination showed that the streams were well separated from each other in benthic macroinvertebrate composition both in time and space. The constrained ordination revealed that many of the monthly measured chemical variables were (not surprisingly) strongly correlated. pH was the environmental variable that could explain most of the variation in macroinvertebrate data. Partial Canonical Correspondence Analysis was used to partition the total explained variance in the species data into seven parts; space, time, environment and interactions between these three groups. The interaction of space and environment (chemical and hydrological variables) explained 48% and the pure environmental variables accounted for 42% of the total explained variance. The north-south climatic and pH gradients are thus so strong that the between stream (spatial) variability is much larger in comparison to the within stream (temporal) variability (Fig. 8).

Although taxon composition within each stream differed between years, samples from the same stream taken in different years generally clustered together suggesting that a number of 'indicator' taxa tend to characterise each stream. This was also seen by the fact that pH alone explained the main part of the variation along the first ordination axis in CCA. Trying to understand what factor(s) determines the within-stream among-year variability in macroinvertebrate community composition was more difficult. Different nutrient variables (in all five streams) and metal(s) (in four streams) explained significant parts of the within-stream among-year variability. Whether these variables structure community composition or are correlated with other structuring variables could not be ruled out .

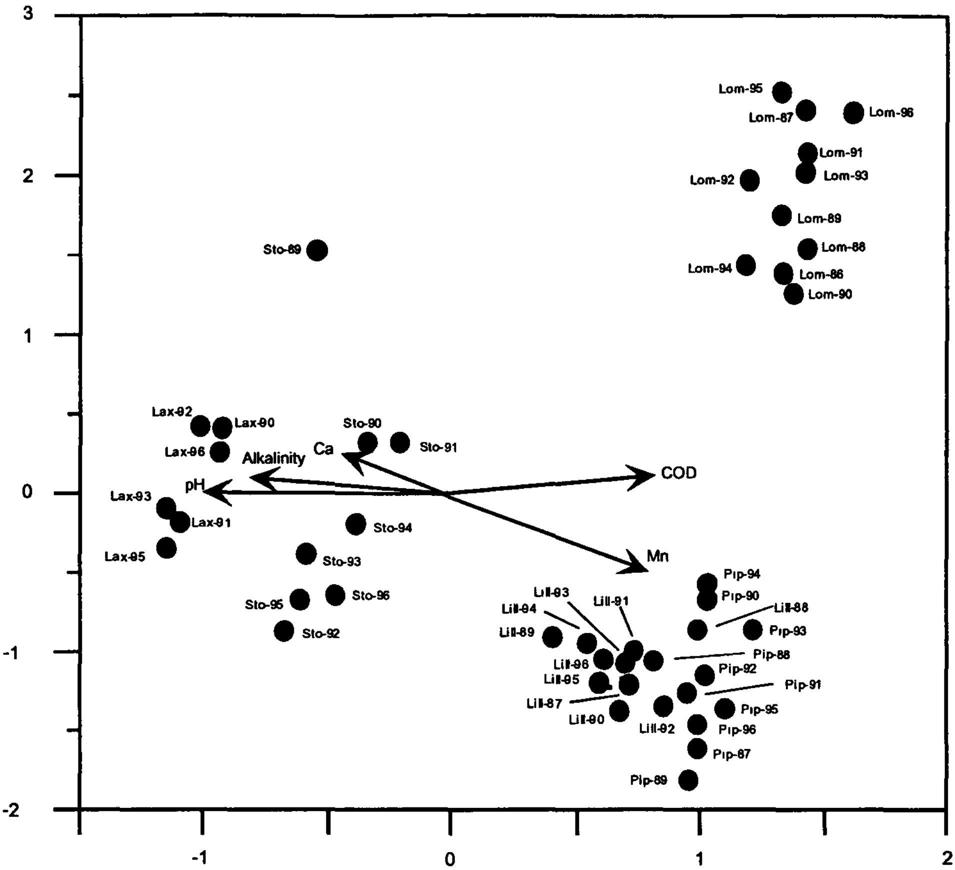

Figure 8. Canonical correspondence analysis with forward selection of environmental variables. Only significant environmental variables are shown in the ordination. The environmental variables were pH measured 5 months prior to benthic macroinvertebrate sampling, minimum [Mn] measured in the stream, alkalinity measured 11 months prior to the biological sampling, [Ca] measured at the biological sampling occasion and chemical oxygen demand (COD) of the stream measured 4 months prior to the biological sampling. The eigenvalues for the first two CCA axes were 0.612 and 0.416, respectively. Lax = Laxtjärnsbäcken, Sto = Stormyrbäcken, Lill = Lill-Fämtan, Lom = Lommabäcken, Pip = Pipbäcken.

Comparison of streams and lakes (paper V)

In general, stream (riffle) communities were more diverse and species-rich than lake (wind-exposed littoral sites) and had a higher proportion of grazers, shredders and passive filter-feeders. Conversely, lake communities had a higher proportion of predators and detritivores. Surprisingly, lakes and streams only shared three of the ten most common taxa; namely, the mayfly genus *Leptophlebia*, clams (i.e., Sphaeriidae) and the isopod *Asellus aquaticus* (Linnaeus). In both lakes and streams, habitat-level descriptors, i.e., substratum and vegetation explained a large amount of unique among-site variance (i.e., 11%

for lakes and 14% for streams) (Fig. 9). However, the large-scale pattern differed between the two ecosystems.

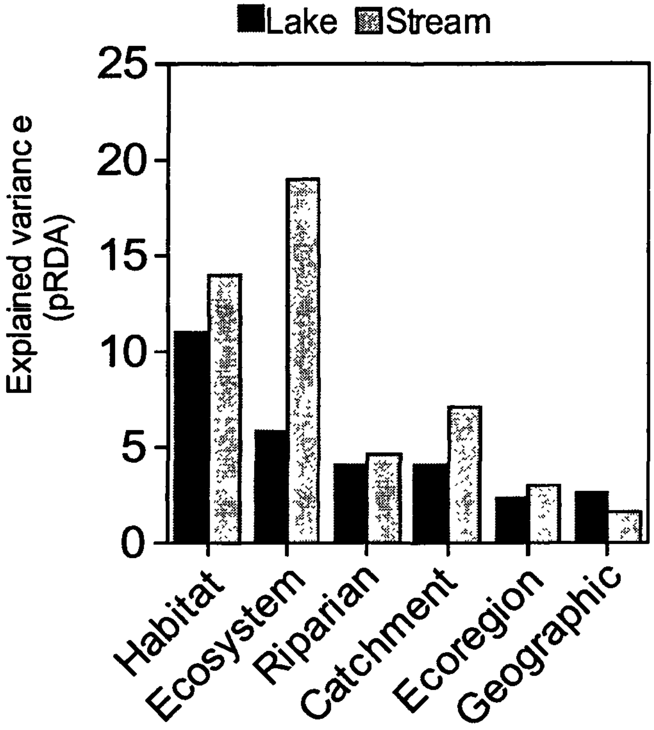

Figure 9. The unique variance in benthic macroinvertebrate community composition (lakes and streams) explained by six spatial scales.

Lake communities were strongly correlated with the *limes norrlandicus* ecotone (found at ~60° north), whereas rather surprisingly streams did not show such a pattern. These findings indicate that detrital inputs are similar between lakes and streams, but that retention and presumably processing of CPOM differ. In paper **V** we propose that differences in detrital trapping and retention between stream riffles and lake litoral habitats should result in stronger ecological linkages between lakes and their surrounding landscapes than between streams and their surrounding landscapes.

Indicator metrics (paper VI)

Tools for environmental assessment need to be robust against natural spatial and temporal variability, but at the same time sensitive to changes caused by anthropogenic perturbation. In the last paper of this thesis ten benthic macroinvertebrate indicator metrics commonly used in the assessment of running water were evaluated for their ability to detect change caused by anthropogenic stress. Taxon richness, total density, number of EPT taxa, Shannon's index, Simpson's index, Average Score Per Taxon (ASPT), Danish Fauna Index (DFI), and three acidity indices (Raddum's, NIVA's and the LD index, see Sandin and

Johnson, 2000) were evaluated for effect size, standardised effect size, statistical power and the number of samples needed to detect an impact of either eutrophication or acidification.

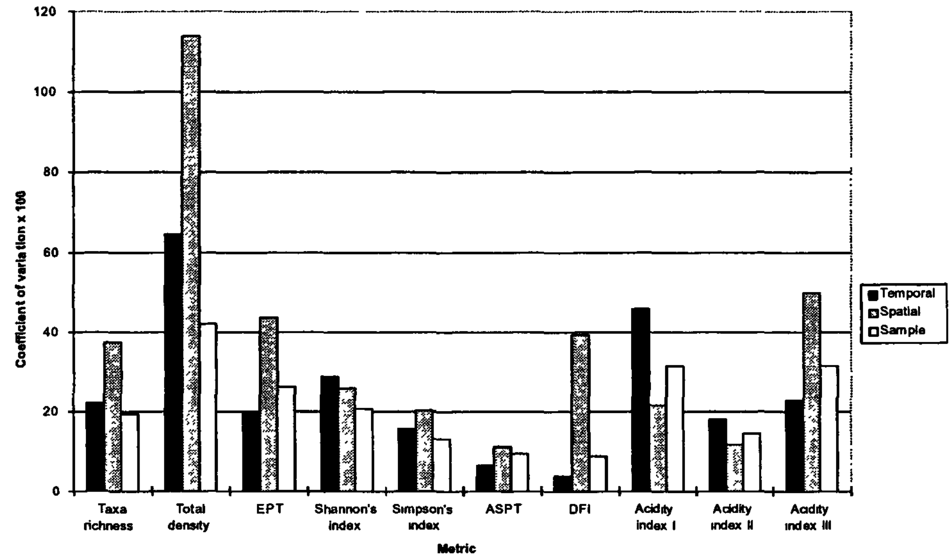

Figure 10. Spatial, temporal and sample variability (Coefficient of Variation, CV) of ten biological indicator metrics commonly used in the assessment of running waters. Spatial variability calculated from 246 sites classified as non-affected by acidification or eutrophication. Temporal variability and sample variability calculated from five time-series reference streams sampled between six and eleven years. Temporal variability was calculated using the mean of five kick samples at each sampling location, whereas the sample variability was calculated using all the individual samples.

Richness measures (i.e., taxon richness and number of EPT taxa), and the two eutrophication indices (i.e., ASPT and the Danish Fauna Index) had the highest standardised effect sizes as well as a high statistical power. Consequently, these indices needed fewer samples to detect changes caused by perturbation. Total density was the least informative metric, with the lowest standardised effect size and the highest spatial, temporal and sample variability (Fig. 10). These findings are consistent with those of other studies where enumeration and functional measures have been found to be more variable than richness measures (e.g., Barbour *et al.*, 1992; Resh, 1994).

The spatial and temporal variability of indicator metrics often confound interpretation and data analysis of environmental impact assessment due to the inverse relationship between natural variability and detection of impact. My findings support those of earlier studies in that it is not only important how large an effect size a metric has, but it is also very important how the metric is affected by natural variability. If the human impact is small compared to the natural variability, it will be difficult to detect change with a high degree of confidence.

The acidity indices tested here may have a higher power to detect improvements of a site (since only colonisation of a site by one sensitive taxon will improve the score of that site. On the other hand, they may have a low power to detect impoverishment of a site, since all individuals of all sensitive taxa used in the index have to disappear before the site is deemed as affected. In environmental assessment studies it is important to have some knowledge of the effect size and variability one can expect from a metric so that the money spent in an impact study is used in a cost-effective way.

Discussion

Implications for environmental assessment

A better understanding of what factors are important, both natural and anthropogenic, for determining the structure of running water ecosystems should result in improved environmental assessments. In this thesis, two datasets of stream benthic macroinvertebrates were analysed to examine which factors were the most important for explaining the variability in stream benthic macroinvertebrate composition between (spatial) and within (temporal) sites. The analysis of macroinvertebrate composition and taxon richness in paper **I** showed that a few strong environmental gradients could explain most of the variance. Stream velocity, substratum composition, and catchment area were correlated with the first gradient, whereas the second gradient was related to pollution (i.e., indicated by pH and total phosphorous concentration) of the stream. This could also be seen as an east-west gradient (in the north, from the Scandinavian mountain chain in the west towards the Baltic Sea in the east) and also for the whole country as a north-south gradient, with streams more affected by urbanisation and agriculture in the south. Classification showed no truly distinct macroinvertebrate community groups or types, but rather a gradual change in taxonomic composition and related environmental variables along these longitudinal and latitudinal gradients.

Many biotic indices or score systems are based on taxon richness and taxonomic composition of the macroinvertebrate community. Stratification in space (e.g., using an ecoregional classification as a basis, as in paper **II**, or only taking samples in one habitat) and time (e.g., by only take samples in season) should decrease the effects of natural variability, thereby increasing the probability that if change occurs it will be detected (i.e., high statistical power). In both papers **I** and **III**, local scale factors such as stream velocity and substratum composition were found to be of principal importance for structuring community composition and taxon richness. This was to some degree unexpected since sampling was both stratified according to catchment size and substratum type. As can be seen from paper **I**, however, a stream velocity gradient is evident from north to south.

Stream velocity and factors related to the velocity (e.g., width, depth, catchment area, discharge, and substratum composition) were among the most important structuring factors in papers **I**, **II** and **III**. Certain species are confined to certain substratum types (e.g., the mayfly *Ephemera danica*, a acid-sensitive species, is found mainly at particle sizes 0.05-3 mm) (Percival and Whitehead, 1926) and it is well known that community composition changes with in substratum type (e.g. Berg *et al.,* 1948). Stream velocity not only represents a direct physical force to the organisms, but it also affects other factors such as substratum composition, food availability, and the response of the organisms to different stressors (e.g., metals and pesticides). These (possible) effects should be regarded when designing monitoring programs.

Few studies have formally addressed the ability or sensitivity of different sampling protocols to detect human-induced change. Kerans and Karr (1992) found distinct differences among the sampling protocols that they analysed. Likewise, Gurtz and Wallace (1984) showed that substratum type was an important factor in determining the direction and magnitude of the response of many taxa to disturbance by clear-cutting. For example, they found that 10 of the 30 taxa examined had a positive (density) response in one type of substratum, but a negative response in another type of substratum. The positive responses were generally found with coarser substrate types, whereas negative responses were found in the fine fractions. Hence, the stability and heterogeneity of the substratum are two important factors determining diversity, biomass and benthic fauna density in streams. Stability increases with mean particle size, and high amounts of organic material found on the inorganic substrate also increases abundance and diversity (Giller and Malmqvist, 1998). This was also seen in paper **I**, where pebble substratum and stream velocity were positively correlated with taxon richness and in papers **II** and **III** were substratum-type explained the among site variance in macroinvertebrate community composition.

A comparison of macroinvertebrate communities of hard-bottom stream (riffle) and lake (wind-exposed littoral) habitats also supports the conjecture that habitat stability and heterogeneity are two important factors for species richness and diversity (paper **V**). Whereas streams are seen as systems more directly reflecting the catchment and the changes within it, lakes have been thought of as more stable and less influenced by their surroundings. In paper **V**, habitat-level descriptors such substratum and vegetation explained a large amount of unique among-site variance (i.e., 11% for lakes and 14% for streams). However, large-scale, landscape level patterns differed between the two ecosystems. Lake communities were strongly correlated with the *limes norrlandicus* ecotone (found at ~60° north). Surprisingly, streams did not show such a pattern. This finding might indicate that stronger ecological linkages exist between lakes and their adjacent landscape. In brief, detrital retention may differ between ecosystems; namely, in lakes leaf detritus is retained for a longer time than in streams and thus has a greater influence on the macroinvertebrate community. Stream macroinvertebrate community composition, on the other hand, seems to be more determined by the local-scale variability of environmental variables such as stream velocity and substratum composition.

Environmental assessment of running waters has much to gain by bettering our understanding of what factors affect macroinvertebrate community composition and taxon richness. The effect of several (simultaneous) stressors (both natural and anthropogenic) must also be taken into account as well as how stress-related response is associated with habitat characteristics. For example, since streams are often affected by different types of natural disturbance (mainly connected to hydrology), stream organisms can be regarded as more tolerant and thus also more resistant to other types of disturbances (e.g., anthropogenic) than lake organisms. On the other hand, stream organisms might be more susceptible to other types of disturbances when they are already stressed by one factor (e.g., Courtney and Clements, 2000). Improving our understanding of factors that are important for determining natural variability should result in better management practices of running waters that are more cost-effective and scientifically sound.

Spatial variability

Geographical classifications (e.g., by ecoregions) can be a useful tool in partitioning natural spatial variability of the response variable (e.g., macroinvertebrates) thereby optimising monitoring, assessment, and conservation programs. If the relationship between large- (regional) and small-scale (habitat) variability is known, this knowledge can be used to underpin generalisations thus enabling more confident predictions. How well ecoregion classification is able to partition the biological variance of stream macroinvertebrate communities was tested in paper **II**. In this study we found that an ecoregional classification can be used as a good starting point for environmental assessments of streams using benthic macroinvertebrates, but also that local-scale variables such as stream velocity and substratum must also be taken into account (see above and papers **I**, **III** and **V**).

Integrating pattern and scale is a central theme in ecology, yet surprisingly few studies have focused on the correspondence between community structure and function at different levels of spatial scale (e.g., from habitat to landscape). In paper **III**, we studied whether benthic macroinvertebrate communities are mainly structured by local-, catchment- or large-scale factors using partial constrained ordination. Most of the variance in the benthic macroinvertebrate dataset was explained by local-scale variables, less by catchment-scale variables, and the smallest amount was accounted for by the large- (regional) scale characteristics. However, one caveat in using this approach is that variable classifications are not independent. For example, environmental variables such as nutrients, sediment, and hydrology (in our study defined as local-scale variables), are more influenced by regional scale characteristics, whereas other variables, for example the vegetation cover at a site is more locally controlled (Allan, Erickson and Fay, 1997). This was recently exemplified by Allan and Johnson (1997) who wrote: "the entire catchment influences the rivers and lakes within its boundaries and on a larger scale controls chemistry as well as hydrology and sediment delivery". This implies that environmental variables can be seen as spatially nested within a hierarchical model (Allen and Starr, 1982), and hence each variable can not be simply classified as controlled locally or regionally.

Temporal variability

Although a number of authors have stressed the need to study temporal variability in aquatic ecological studies (e.g., Strayer, 1986; Resh & Rosenberg, 1989), the concurrent effect of spatial and temporal variability are generally ignored. Indeed, studies are commonly focused either on temporal or spatial variability, but seldom on both. Simply whether a study is spatially or temporal extensive will emphasise different aspects of ecosystem structure and function. Spatially extensive studies will emphasise abiotic structuring factors, whereas temporally extensive studies will emphasise biotic factors such as predation, competition and the effects of pathogens (Wiley, Kohler and Seelbach, 1997).

In paper **IV**, the explained variance in species data was partitioned using constrained ordination and environmental variables indicative of spatial and temporal variability. I found that environmental variables as well as the interaction of space and environmental variables explained the main part of the variance. Space and environmental variables interacted to a large degree. Northern streams had a higher pH, lower precipitation and mean annual temperature compared to the central and southern streams. The pure time factor and the interaction of time with space and environment explained only a small part of the total explained variance. This can be explained by the fact that the space-environment (north-south, high pH-low pH) gradient between the streams was strong and that the within-stream between-year variability was low in comparison.

However, these findings do not imply that temporal aspects can be ignored in environmental assessment of running waters. For example, work in the UK has shown that using data from three sampling seasons (spring, summer, and autumn) gave better assessment results (RIVPACS model predictions) than sampling only one or two seasons. Including multi-seasonal samples resulted in an increase in the number of taxa found at each site, a decrease in the inter-site variation, and a better measure of inherent cyclic and seasonal factors affecting benthic macroinvertebrates (Furse *et al.,* 1984). The relatively large temporal variability (among seasons) found in running waters can be attributed to the fact that there are large fluctuations of environmental features, such as discharge in these systems (Resh and Rosenberg, 1989). If samples are taken once a year at a stream site, differences between years can be caused by changes in the anthropogenic stress on the system (e.g., improved removal of nutrients from waste-water) or alternately by the fact that the sampled years differed in climate (e.g., one being relatively 'dry' and the other 'wet').

Indicator variability

In paper **VI**, a number of indicator metrics commonly used in environmental assessment of impact were analysed for their robustness to detect anthropogenic change. We found that richness measures had the highest standardised effect sizes as well as a high statistical power, and therefore needed fewer samples to detect change. Conversely, total density was the least informative metric of those analysed, with the lowest standardised effect size and the highest spatial, temporal, and sample variability. Similar results have been found in earlier studies evaluating benthic macroinvertebrate metrics. However, though taxon richness may seem as a "simple" measurement, it is affected by (among other things) spatial and temporal variability. This natural variability may confound the use of metrics in the assessment of running waters. For example, Brönmark *et al.* (1984) found a positive relationship between the catchment area and taxon richness, as was also seen in paper **I** of this thesis. A number of other 'natural' environmental variables such as substratum size, predation, annual temperature range, and biome type also affect taxon richness of benthic macroinvertebrates in streams (Vinson and Hawkins, 1998). In my studies, taxon richness was found to vary regionally. Low taxon richness was found in the north-western parts of the country and also in the boreo-nemoral and nemoral ecoregions in the south-central parts of the country (see paper **I**). In paper **V**, higher taxon richness was found in the northernmost neutral streams (in the boreal region), compared to the southernmost streams (in the boreo-nemoral region). Spatial stratification (by biogeographic or ecological regions) can be used to improve detection of differences in taxon richness caused by natural factors so that detection of impact increases. However, as mentioned local scale factors such as substratum types need also to be considered (see papers **I**, **II**, and **III**).

Conclusions and future perspectives

Since both spatial and temporal variability of stream benthic macroinvertebrates may confound the environmental assessment of streams, these factors should be scrutinised when planning and implementing monitoring of running waters. One such factor not accounted for in the present thesis is the importance of biotic factors (e.g., predation). Whether predation affects the results of environmental assessment of streams using benthic macroinvertebrates have to my knowledge not been studied. Vinson and Hawkins (1998) suggest that predation causes a reduction in the abundance of benthic macroinvertebrates, but not the disappearance of taxa altogether. The statistical power for detecting anthropogenic impacts could thus be reduced by biotic factors such as predation.

The indicator metrics used in environmental assessment of streams today may be far too simple and have inherently high false positive (type I) or false negative (type II) associated errors. For example, many of the metrics that are commonly used are probably better at revealing improvement than degradation in stream condition. For example, the occurrence of one organism of a sensitive taxa is often all that is needed to classify a site as improved, whereas a number of taxa or organisms may disappear before the site is deemed as being more degraded. By using 'black box' methods such as Baeysian methods or Neural Networks, it might be possible to better predict the occurrence of species at a site (e.g., Mastrorillo *et al.,* 1997) or improve the performance of metrics commonly used today (Walley and Hawkes, 1996; 1997). This however, however, also includes a trade-off between the user-friendliness of an analysis method and the complicated reality of the stream ecosystem.

In the 1995 national stream survey only one riffle (reach) was sampled in each stream. How representative this one composite sample is of the stream is difficult to assess. Friberg *et al.* (1977) found that two sites sampled in the same stream were not more similar to each other than two different sites in two different streams. The representativity of a single riffle sample could be evaluated by a nested approach where several reaches within several streams could be sampled and the spatial variability and similarity within and between the spatial levels evaluated using a geostatistical or nested approach.

Ecosystems are often experiencing the effects of multiple stressors (e.g., organic enrichment and metal loadings). Designing and implementing monitoring and assessment programs to meet present-day and future environmental problems is a challenge confronting applied ecologists. Two areas of interest that deserves a greater attention are the use of complementary indicator groups (or metrics) and improved diagnostic tools. Although more study is needed in determining stressor-response relationships, it is known that indicator groups react differently to different stressors. For example, periphyton in a stream may be a reliable indicator of nutrient enrichment, but a poor indicator of changes in temperature. Conversely, fish may be considered as an early-warning indicator of temperature, but a poor indicator of nutrient enrichment. Incorporating this knowledge into field assessment protocols should result in a lower frequency of false negative errors. Another area that deserves more attention is the development of better diagnostics tools. For example, simple univariate or multivariate statistical approaches may indicate deviation from an expected (reference) condition, but trying to ascertain cause-and-effect relationships are often difficult. Better diagnostic tools (e.g., using Baeysian methods, Neural Networks or Expert Systems) may improve our understanding of correlative relationships between potential stressor and response variables.

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Since both spatial and temporal variability of stream benthic macroinvertebrates may confound the environmental assessment of streams, these factors should be scrutinised when planning and implementing monitoring of running waters. One such factor not accounted for in the present thesis is the importance of biotic factors (e.g., predation). Whether predation affects the results of environmental assessment of streams using benthic macroinvertebrates have to my knowledge not been studied. Vinson and Hawkins (1998) suggest that predation causes a reduction in the abundance of benthic macroinvertebrates, but not the disappearance of taxa altogether. The statistical power for detecting anthropogenic impacts could thus be reduced by biotic factors such as predation.

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