

69/2002

Tracing recovery from acidification - a multivariate approach

Norwegian Institute for Water Research

REPORT

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Title Tracing recovery from acidification - a multivariate approach	Serial No. 4564-2002	Date 19 August
	Report No. Sub-No. ICP Waters report 69/2002	Pages Price 39
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	Geographical area	Printed NIVA

Client(s) Norwegian Pollution Control Authority	Client ref.
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Abstract
This report presents a method based on use of multivariate statistics to detect trends in aquatic biota as a results of chemical recovery from acidification. Three datasets from Norway, Sweden and UK are used in the work. The result shows that the method can trace signals of recovery in the benthic community in lakes and rivers that are recovering chemically. This includes localities that are recovering, but still are in a very acidic state. It also includes localities where acidification has been slight, and the recovery has occurred over a pH gradient where the lake or river would be classified as not acidified.

4 keywords, Norwegian 1. 2. 3. 4.	4 keywords, English 1. Biological recovery 2. Acidification 3. Multivariate statistics 4. Invertebrates
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ISBN 82-577-4220-1


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CONVENTION ON LONG-RANGE
TRANSBOUNDARY AIR POLLUTION

INTERNATIONAL COOPERATIVE PROGRAMME ON
ASSESSMENT AND MONITORING OF ACIDIFICATION
OF RIVERS AND LAKES

**Tracing recovery from acidification -
a multivariate approach**

ICP Waters Programme Subcentre
Laboratory of Freshwater Ecology and Inland Fisheries
University of Bergen, July 2002

Preface

The International Cooperative Programme on Assessment and Monitoring of Acidification of Rivers and Lakes (ICP Water) was established under the Executive Body of the Convention on Long-range Transboundary Air Pollution at its third session in Helsinki in July 1985. The Executive Body has also accepted Norway's offer to provide facilities for the Programme Centre, which has been established at the Norwegian Institute for Water Research, NIVA. A programme subcentre is established at the Laboratory of Freshwater Ecology and Inland Fisheries at University of Bergen. The ICP Water programme has been lead by Berit Kvæven, Norwegian Pollution Control Authority.

The Programme objective is to establish an international network of surface water monitoring sites and promote international harmonization of monitoring practices. One of the aims is to detect long-term trends in effects of acidic deposition on surface water chemistry and aquatic biota, and to reveal the dose/response relationship between water chemistry and aquatic biota.

This report presents results on how to detect trends in aquatic biota as a results of chemical recovery from acidification.

ICP Waters Programme Centre,
Oslo, August 15, 2002

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Contents

Summary	5
1. Introduction	6
2. Material and methods.	8
2.1 Norway	8
2.2 United Kingdom	9
2.3 Sweden	10
3. Results and discussion	11
3.1 The Nausta watershed	11
3.2 The Gaular watershed	17
3.3 The Vikedal watershed	21
3.4 United Kingdom	25
3.5 Sweden	25
4. Conclusions	29
5. References	30
Appendix A. Acidity index 2 for all of the localities in the Nausta watershed	32
Appendix B. Reports and publications from the ICP-Waters Programme	35

Summary

Recent research has shown that there has been a significant recovery in water chemistry from acidification in the period 1989 to 1998, following the reduced emissions of sulphur in Northern Europe and North America. This recovery has also been reflected in the macro-zoobenthos in some watersheds in southwestern Norway.

The aim of this work is to see if this recovery can be traced as changes in the abundance of the benthic community during the same period. By use of the multivariate redundancy analysis (RDA) we try to find the proportion of the variation in the abundance data that can be equally well explained by changes in the water chemistry data available as by a linear time variable. If both spring and autumn samples exists for each year, we have also tested how much seasonal changes in water chemistry affects the benthic community by substituting the linear time variable with a seasonal one.

Three watersheds with benthic samples from running water in Norway, six lakes in the United Kingdom, and seven lakes in Sweden, all from the ICP Waters biological database, have been analysed.

The multivariate method found evidence of recovery from acidification in some localities in all of the three Norwegian watersheds, and also in two of the seven lakes analyzed from Sweden. There was no signal of recovery in the benthic community of the ICP Waters lakes in the United Kingdom.

The results shows that

- the multivariate method identifies recovery in the benthic community in lakes and rivers that are recovering chemically.
- the method seems to be quite conservative. It apparently does not overestimate occurrences of recovery.
- the method can trace signals of recovery in the benthic community in lakes that are recovering chemically, but still are in a very acidic state.
- that a recovery can also be traced when the acidification have been slight, and the recovery has occurred over a pH gradient where the lake would be classified as not acidified.

The method only indicate where a recovery in the benthic community has occurred. It does not say anything about how far the site is from its natural unaffected state. However, it may give us an indication of the relative strength of the recovery. It gives a measure of how much of the total change in the abundance data that can be attributed to linear changes in water chemistry. As such, the method may also identify acidification processes equally well as recovery from acidification.

The results also show that the multivariate method can give a relative measure of how much seasonal changes in the water chemistry affects the benthic community, and by this a possible recovery process.

1. Introduction

Recent research has shown that there has been a significant recovery in water chemistry from 1989 to 1998, following the reduced emissions of sulphur in Northern Europe and North America (Stoddard et al, 1999; Skjelkvåle et al., 2000). This recovery has also been reflected in the macro-zoobenthos in some watersheds in southwestern Norway, as documented by the acidification indices developed at the Laboratory for Freshwater Ecology and Inland Fisheries (LFI), University of Bergen (Raddum & Fjellheim, 1984; Raddum, 1999).

In these indices the benthic species / taxa are assigned to four classes based on their tolerance against acid water. These four classes are tolerant, slightly intolerant, moderately intolerant, and intolerant, and they are given values between 0 and 1. A locality with only tolerant species are given the value 0, i.e. the locality are regarded as strongly affected by acidification. A locality with an intolerant species present is given the value 1, that is, unaffected by acidification. The key species in the indices are the mayfly *Baetis rhodani* (Pictet). This is the most common benthic species in riffles in unaffected rivers and streams in southern and western Norway.

Two different indices exist, labeled Index 1 and Index 2. One single specimen of *B. rhodani* or another intolerant species will give a locality an Index 1 value of 1 or unaffected by acidification. Index 2, or the adjusted acidity index, will take the number of specimens of *B. rhodani* into consideration. The locality is given the value 0.5. The number of specimens of *B. rhodani* is then divided by the number of specimens of tolerant stoneflies, and this figure is added to 0.5. If there are many *B. rhodani* present in the sample so that Index 2 exceeds 1, the value is set to 1. This gives an index which is continuous between the categories 0.5 and 1, i.e. between moderately affected and unaffected. In our opinion this index gives a more realistic picture of the effects of acidification, or of a recovery from acidification, than the pure categorical Index 1. In this report we are only referring to the adjusted acidity index or Index 2.

Multivariate methods have been shown to be more sensitive than univariate ones for detecting environmental stress or recovery from stress (e.g. Warwick & Clarke 1991, Yan et al. 1996, Rusak et al. 2001). However, the univariate methods discussed in these papers (e.g. species richness, diversity indices) appears to be different from the acidity indices. They were developed as means for early warning of acidification.

The acidity indices only need a single or a few individuals of an intolerant species in a locality to signal a recovery, and may very quickly indicate biological recovery following improvements in the water chemistry of acidified streams and rivers. On the other hand, this sensitivity may sometimes overestimate a possible recovery.

The aim of our analysis is to see if the recovery indicated by the acidity indices also can be recognized as changes in the total benthic community. The multivariate method we are using does only indicate whether a recovery is or has been occurring. It does not say anything about how far the process of recovery has come, that is, how far from its original predisturbance community the benthic community of a locality is. However, the method may give us an indication of how strong a recovery process is as compared to other factors affecting the abundance of the benthic species.

Preliminary analyses from two watersheds in Western Norway (running water) and six ICP-Waters sites (lakes) in the UK were presented in the 12-year ICP Waters report (Skjelkvåle et al., 2000). This report will go more into detail on these watersheds and sites and also add new ones from Norway and Sweden.

2. Material and methods.

This report uses data from the ICP-Waters database from 1989 to 1998, the same period that was analyzed for water chemistry in Stoddard et al. (1999) and Skjelkvåle et al. (2000). The method and the rationale behind the method used for the multivariate analyses are described in detail in Skjelkvåle et al. (op. cit.). The same procedure is used in this report, with some adjustments described below. In short, we use the program CANOCO 4 (ter Braak & Smilauer, 1998) to run a redundancy analysis (RDA) with the water chemistry variables that are available to see whether the species abundance data gives a significant ordination, i.e. to see if there is a signal in the species data that can be explained with changes in the water chemistry. To examine whether these changes are linear we include a variable called 'Time', which is coded as 0 to 19 for each succeeding sampling date from spring 1989 to autumn 1998 for the Norwegian data sets.

The biological data in the ICP-Waters database have been collected with different techniques in the different countries. Our treatment of the data will be described under a heading for each country included.

2.1 Norway

Three datasets from Western Norway have been analyzed. The Nausta (NO08) and the Gaular watershed (NO07) from the county of Sogn og Fjordane north of Bergen were also included in Skjelkvåle et al. (op. cit.). Both watersheds have been affected by acidification, but to a differing degree, with the Nausta watershed being least acidified. None of the watersheds have been limed, and both have shown a significant recovery in water chemistry during this period (Skjelkvåle et al, op. cit.). In this report we have added the unlimed parts of the Vikedal watershed (NO06) in the county of Rogaland south of Bergen. This watershed has been heavily affected by acidification and had a strongly decimated stock of salmon in the mid 1980ties (Rosseland et al., 1986, Hesthagen, 1986). The anadromous zone of this watershed has been limed with dosers from 1987.

Kick-samples have been used for collecting macrobenthos in all three watersheds, once in May/early June after the snowmelt and once in October/November. All localities were in riffles. Each sample was sorted for one hour, and subsequently identified. Leeches (Hirudinea), molluscs (Mollusca), may-flies (Ephemeroptera), stoneflies (Plecoptera) and trichopterans (Trichoptera) were identified to the lowest possible taxon, i.e. species or genus. In the multivariate analyses we have used the most inclusive taxon with some exceptions. Larsen et al. (1996) showed that the stoneflies *Amphinemura borealis* (Morton) and *A. sulcicollis* (Stephens) had different optima when acidity were concerned, with *A. borealis* being the most sensitive. Small individuals of these species are commonly occurring in the samples, and they cannot be identified to species. Consequently these species have both been ranked as insensitive in the acidification indices. In order to keep the potential signal from the distribution of these species, small individuals identified to *Amphinemura* sp. has been omitted from the multivariate analyses. Also small trichopterans identified to family only were excluded from the analyses. Oligochaeta, Acari, Coleoptera, Chironomidae, Simuliidae,

Ceratopogonidae and remaining Diptera were not identified further, but counted and included in the analysis.

We analysed 20 localities in the Nausta watershed, 17 localities in the Gaular watershed, and in the Vikedal watershed 10 localities were included.

In Skjelkvåle et al. (op. cit.) the species data consisted of the pooled data from all localities in each watershed from each spring and autumn sample. The abundances were expressed as the exact number of individuals of each taxon sorted out during one hour. In this report we present analyses of each locality separately as well as analyses of the pooled data as described above.

Both analyses of exact numbers of individuals and of relative abundance have been carried out. However, we have chosen to present the analyses based on the relative abundances only. This will eliminate some of the noise in the data sets due to the fact that different technicians sorted the samples during the 10-years period. Also, the exploratory detrended correspondence analysis (DCA) run for each dataset showed that the length of the underlying gradient in the species data was on average a little larger when the relative abundance was used. This means that some noise in the dataset has been reduced, and that the potential signal in the species data was stronger.

The water chemistry data used are from the ICP-Waters database. The Nausta watershed has one locality sampled for water chemistry in the main river (NO08) close to the benthic locality 11 (**Figure 1**), and one locality in the tributary Trodøla (NO09) close to the benthic locality 7 (**Figure 2**). The benthic community in the Nausta watershed has been analysed using both water chemistry localities. The Gaular watershed has one sampling station (NO07) close to the benthic locality 5 (**Figure 7**), while the unlimed part of the Vikedal watershed (NO06) has one sampling station above the lime doser some distance downstream of locality 11 (**Figure 11**). The spring data are the average concentrations from the samples collected in the months March, April, May, while the autumn data are the average for September, October and November. The following chemical variables are included in the analyses: pH, acid neutralising capacity (ANC), calcium (Ca), labile aluminium (LAl), and total organic carbon (TOC).

2.2 United Kingdom

The species data from the six lakes in the UK is based on a varying number of kick-samples taken in the littoral of the lakes each spring. No autumn samples were taken.

The following lakes were included in the analyses: Loch Coire nan Arr (UK01), Lochnagar (UK04), Round Loch of Glenhead (UK07), Scoat Tarn (UK10), Llyn Llagi (UK15), and Blue Lough (UK21). For the DCA and RDA, the relative abundances of the different species / taxa from each locality and sampling date expressed as relative abundances were used. In Skjelkvåle et al. (2000) the abundance data were not transformed to relative abundances. The localities and sampling methods are described in Patrick et al. (1991, 1995).

The water chemistry data are from the ICP Waters database, with spring data expressed as averages of measurements from the months of March, April and May. The following variables

were included in the analyses: pH, calcium concentration (Ca), dissolved organic carbon (DOC), and acid neutralising capacity (ANC). The ANC values were lacking for some of the sampling dates in Lochnagar (UK04), so this variable was not included in the analyses of this site.

2.3 Sweden

The biological material consists of qualitative kick-samples from the littoral, and of quantitative samples from the sublittoral and the profundal from 7 lakes. The following lakes have been examined: Tväringen (SE05), Stensjön (SE06), Brunnsjön (SE08), Fiolen (SE09), Storasjön (SE10), Fräcksjön (SE11) and Härsvatten (SE12). The qualitative littoral data analyzed are composed of spring data from 1989-1994, and autumn data from 1995-1998. Sampling strategy changed in 1995, when sampling in the spring was shifted to sampling in the autumn. The quantitative data analyzed are all samples from the autumn.

The water chemistry data are from the ICP Waters database. Spring data are the averages of measurements taken in March, April and May, while autumn data are the averages from September, October and November. The following variables have been included in the analyses: pH, the calcium concentration (Ca), the acid neutralizing capacity (ANC), and total organic carbon (TOC).

3. Results and discussion

3.1 The Nausta watershed

Twelve of the localities and the pooled abundance data from the whole watershed gave significant ordinations with linear time included as the only variable, i.e. a linear trend was apparent in the abundance data from these localities. These were the localities 1, 2, 3, 4, 6, 7, 9, 10, 11, 13, 15, and 16.

The results of the redundancy analyses (RDA) for each of the localities with the water chemistry data from the main river (NO08) are shown in **Figure 1**. Only locality 1 in the upper reaches of the main river gave a significant result in the RDA's when the water chemistry data from the main river was used. The amount of abundance variation explained by linear time was 4.3 %. The nominal variable 'Spring', a coarse measure of the seasonal effects on the macrobenthos, explained 13 % of the abundance variation on locality 1. Locality 5 also gave a significant RDA with the water chemistry data from the main river included as the only variables. However, there was no significant linear trend in the dataset, as stated above. The seasonal variable 'Spring' explained 17.2 % of the variation in the abundance data on locality 5.

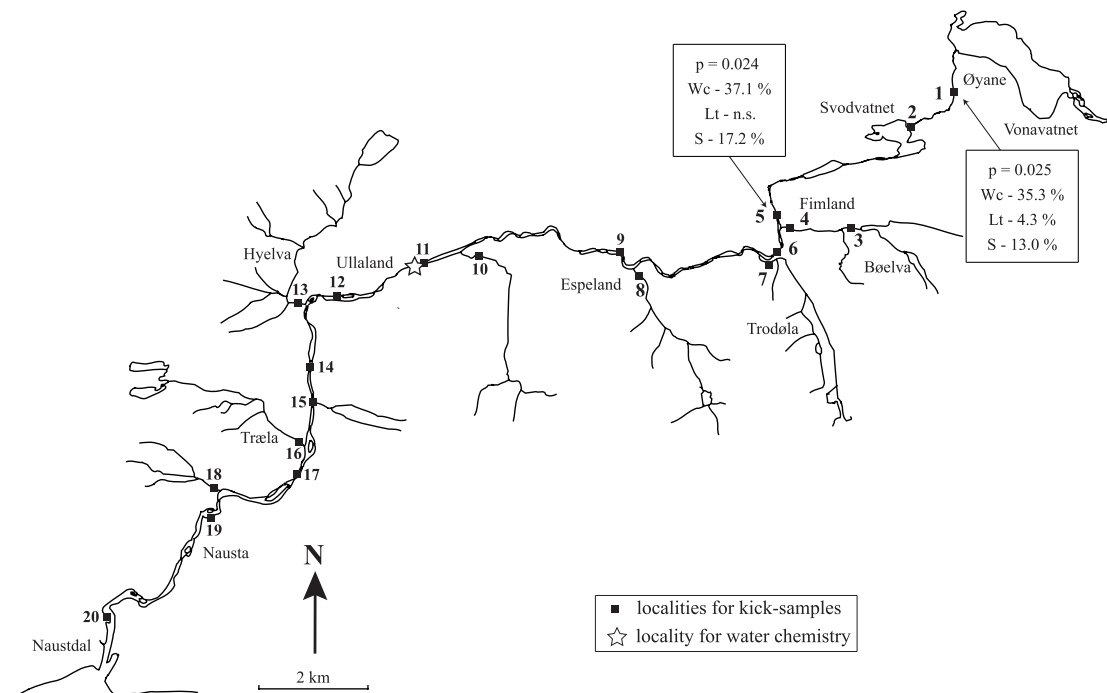


Figure 1. Results from the RDA's of the changes in the benthic communities in the Nausta watershed from 1989 to 1998 with water chemistry data from the main river NO08). Boxes indicate localities with significant results. p = p value from unrestricted permutation test; Lt = amount of abundance variation explained by linear time; S = amount of abundance variation explained by the nominal variable 'Spring'.

Running the analysis with the species data pooled for all localities did not give a significant result, contrary to the result in Skjelkvåle et al. (op.cit) where exact numbers of individuals was used.

A Spearman rank correlation test between the environmental variables used in the RDA's is given in **Table 1**. This shows that there is a significant positive correlation between linear time and pH and ANC, and a significant negative correlation with labile aluminum. The 'Spring' variable on the other hand is correlated with increases in the calcium concentration and with a decrease in total organic carbon.

Table 1. Spearman Rank Correlation matrix between the environmental variables from the main river in the Nausta watershed from 1989 – 1998 used in the RDA's.

	Ca	ANC	TOC	LAI	Time	Spring
pH	0.347	0.749 **	0.624 **	- 0.704 **	0.743 **	- 0.226
Ca		0.344	- 0.015	- 0.212	0.358	0.566 **
ANC			0.690 **	- 0.670 **	0.588 **	- 0.295
TOC				- 0.449 *	0.321	- 0.504 *
LAI					- 0.679 **	0.313
Time						- 0.087

Two-tailed tests. ** significant at the 0.01 level. * significant at the 0.05 level.

Figure 2 shows the results from the similar analyses run with the water chemistry data from the tributary Trodøla (NO09). More localities in the upper reaches of the main river and in the tributaries showed significant results in the RDA's with this dataset. The amount of abundance variation explained by linear time ranged from 4.6 % to 8.5 %, while the seasonal time variable 'Spring' explained from 1.7 % up to 13 %.

When the pooled species data set was analyzed with the water chemistry from the river Trodøla we also found a significant relationship between changes in the water chemistry and relative species abundance. The amount of variation explained by linear time was 8.7 %, while the 'Spring' variable explained 10.9 % of the abundance variation.

Table 2 shows the correlations between the water chemistry variables from Trodøla and the two components of time. Linear time is correlated with an increase in pH and in ANC, and a decrease in labile aluminum. The seasonal variable 'Spring' shows a positive correlation with TOC and a negative correlation with calcium.

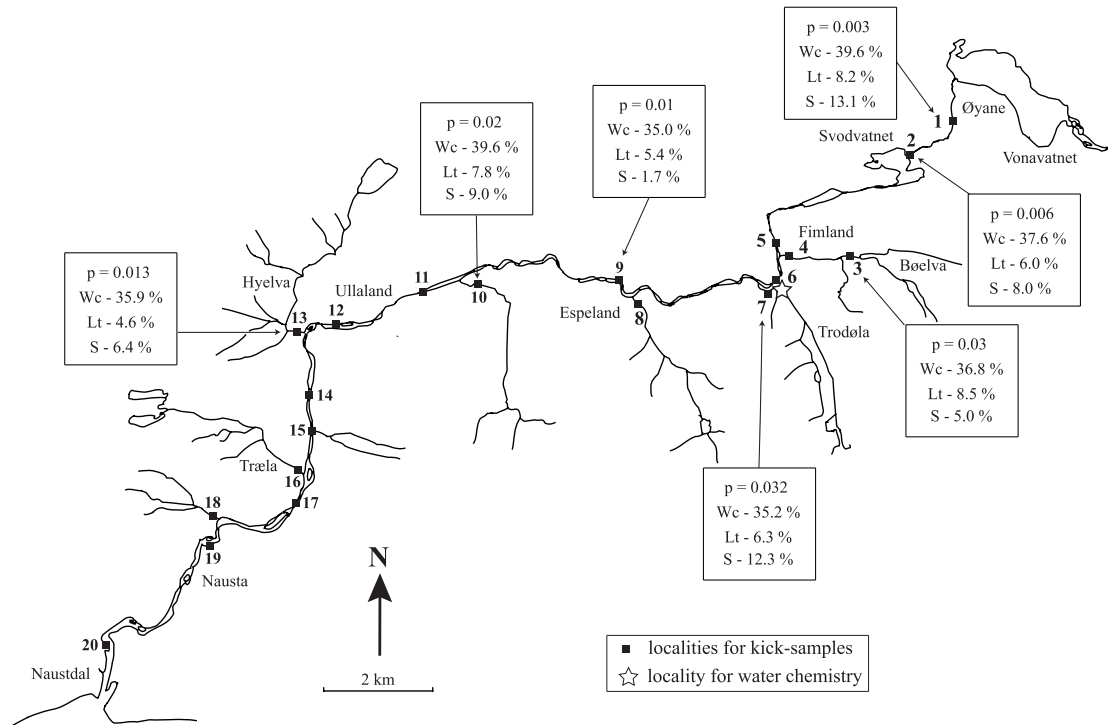


Figure 2. Results from the RDA's of the changes in the benthic communities in the Nausta watershed from 1989 to 1998 with water chemistry data from the tributary Trodøla (NO09). Boxes indicate localities with significant results. p = p value from unrestricted permutation test; Lt = amount of abundance variation explained by linear time; S = amount of abundance variation explained by the nominal variable 'Spring'.

Table 2. Spearman Rank Correlation matrix between the environmental variables from the tributary Trodøla in the Nausta watershed from 1989 – 1998 used in the RDA's.

	ANC	ANC	TOC	LAI	Time	Spring
pH	0.253	0.890 **	0.523 *	-0.860 **	0.666 **	0.295
Ca		0.391	-0.168	-0.042	0.311	-0.503 *
ANC			0.624 **	-0.684 **	0.546 *	0.295
TOC				-0.430	0.277	0.520 *
LAI					-0.669 **	-0.434
Time						0.087

Two-tailed tests. ** significant at the 0.01 level. * significant at the 0.05 level.

Nausta is the watershed in the Norwegian monitoring program where the benthic fauna has been least affected by acidification (SFT, 2000). The water chemistry has, however, shown a significant recovery during the decade from 1989 to 1998 (Skjelkvåle et al. 2000). The changes in pH in the main river are shown in **Figure 3a** and for the tributary Trodøla in **Figure 3b**.

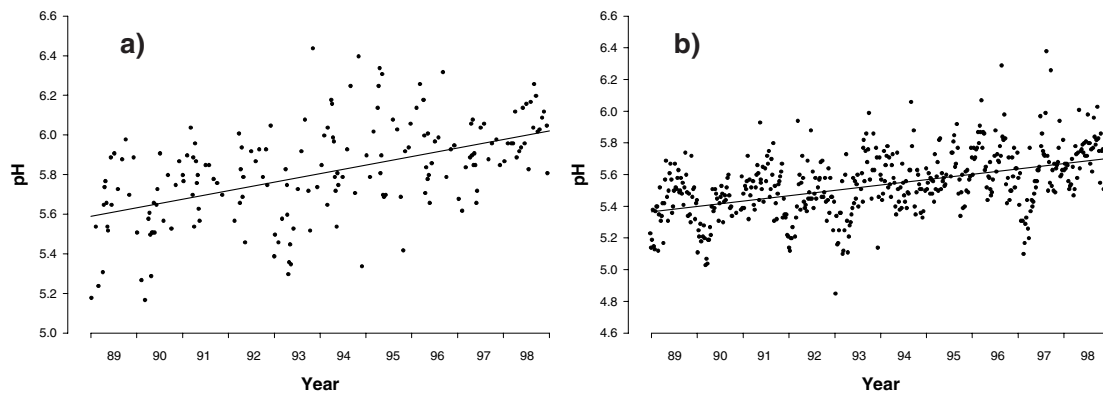


Figure 3. Simple regressions of pH upon time in the Nausta watershed. a) the main river Nausta (NO08) (locality 11). b) the tributary Trodøla (NO09) (locality 7).

As the figure shows the pH has increased from about 5.6 to 6.0 in Nausta, while in Trodøla the increase has been from 5.4 to 5.6. The increasing trend in pH at both stations, measured as a decrease in hydrogen ion content, has been shown to be significant at both stations (Skjelkvåle et al., 2000).

The remaining water chemistry variables examined are shown in **Figure 4**. The figure shows simple regressions of the means of the measurements from the spring and the fall data used in the analysis. In Nausta the ANC showed a significant increase. The TOC also increased, but not significantly. In Trodøla the TOC increased significantly. The ANC also increased, but not significantly. The LAI decreased in both localities, but the trends have not been tested for significance (Skjelkvåle et. al, op.cit).

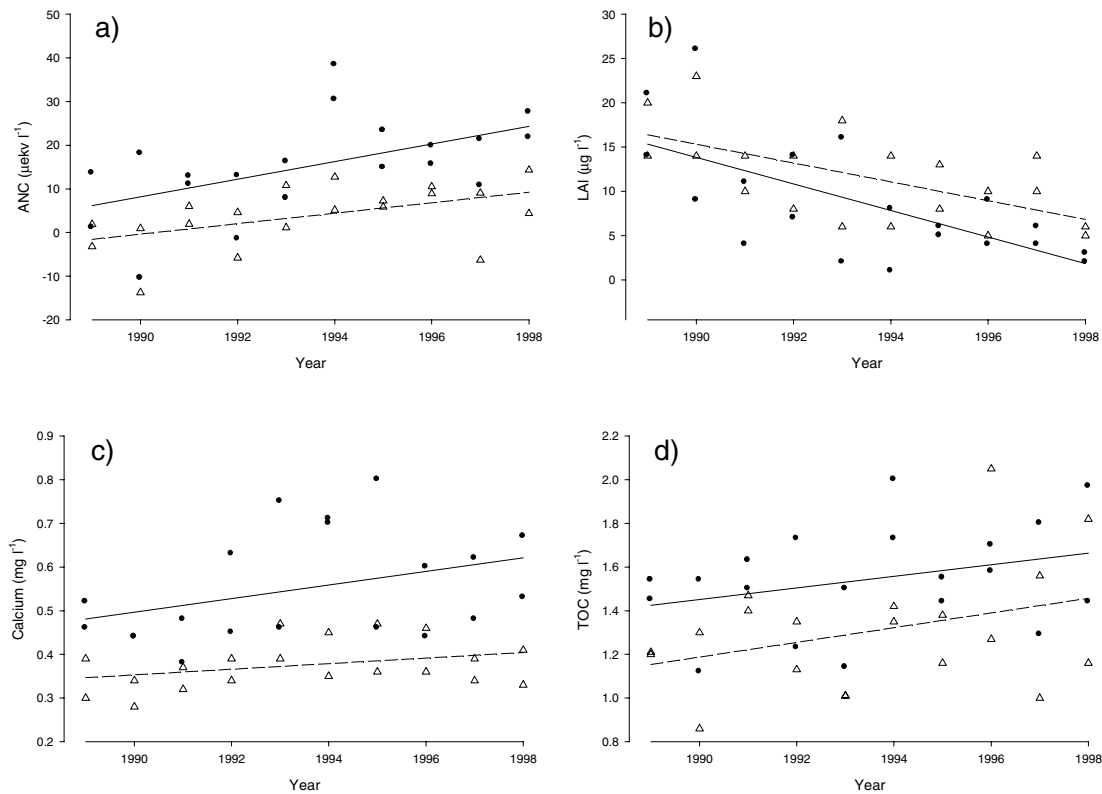


Figure 4. Simple regressions of the averages of the water chemistry variables measured in the spring (March, April, May) and the fall (September, October, November) upon time. **a)** = acid neutralizing capacity (ANC), **b)** = labile aluminum (LAI), **c)** = calcium, **d)** = total organic carbon (TOC). Filled circles and solid lines represents the data from the main river Nausta (locality 11), while open triangles and dashed lines represents data from the tributary Trodøla (locality 7).

The recovery in water chemistry is reflected in the acidity indices. **Figure 5** shows the averages of index 2 from all of the localities during the period from 1983 to 1998.

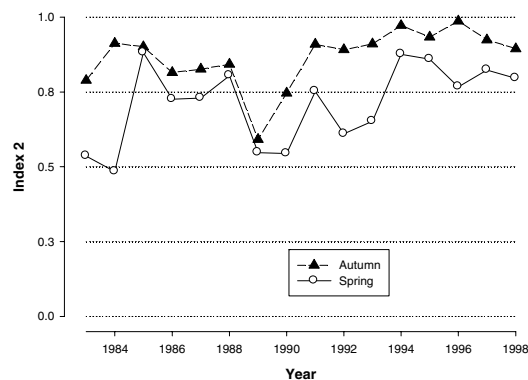


Figure 5. Mean values of acidity index 2 for the 20 localities in the Nausta watershed from 1983 to 1998.

The index fluctuates before 1989, but from 1989 to 1998 there is an increase in the values. The slight decrease from 1996 to 1998 may signal that the watershed is still vulnerable. The dominating sensitive species in the calculation of the acidity indices, the mayfly *Baetis rhodani* (Pictet), has not yet established large populations in all of the localities, and some of the localities are still affected by seasonal snowmelt and acidifying episodes like winter storms. This is also evident from the years from 1998 to 2001 (unpublished data).

Only two of the localities in the Nausta watershed can be directly linked to the water chemistry measurements, so we will consider these first. The analysis from locality 11 in the main river showed no signal of change in the benthic community that can be attributed to a possible recovery from acidification. The changes in the water chemistry during the 10-years period, however, showed a significant effect of reduced emissions of sulphur (Skjelkvåle et al., op. cit). The pH interval where the increase has happened, i.e. from about 5.6 - 6.0 (**fig. 3a**), is regarded as close to, but above the tolerance limit for the most sensitive organisms (Lien et al., 1996). This locality has probably never been seriously affected by acidification, and other variables are responsible for the variations in the community structure. This is also consistent with the acidification indices from the locality, calculated from 1983 to present. They indicate minor acidification episodes during spring in 1989, 1991 and 1996, but otherwise stays at or close to the 'no acidification' level (**fig. 6a**).

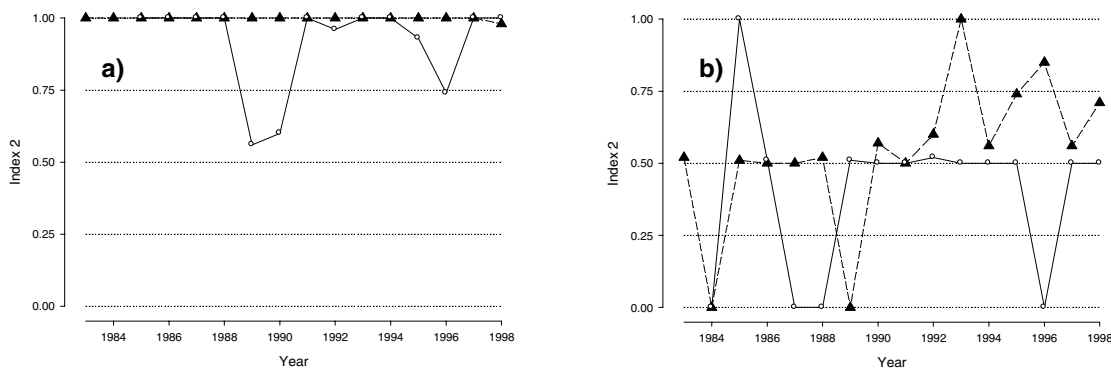


Figure 6. Acidity index 2 in Nausta and Trodøla from 1983 to 1998. **a)** Locality 11 in the main river. **b)** Locality 7 in the tributary Trodøla. Open circles and solid lines show the spring values, filled triangles and dashed lines shows the autumn values.

The tributary Trodøla at locality 7 gave a signal that can be interpreted as a recovery. The overlap between the linear variable 'Time' and the water chemistry (**Fig. 1**) amounts to 6.3 % of the variation explained in the abundance data, i.e. this may be interpreted as a response to a linear change in the water chemistry variables. This is also consistent with the acidity indices (**Fig. 6b**). This tributary has been more strongly affected by episodic acidification. Before 1989 the indices oscillated strongly. After 1989 the very sensitive mayfly *Baetis rhodani* have been present in the river, but in low numbers and almost exclusively in the fall samples. It is important to realise that there has been an increase in ANC (**Fig. 4a**), but that the values are still below the estimated tolerance limit of the most sensitive species for at least long periods of the year (Lien et al., 1996). **Table 2** shows that significant correlations exists between the

linear variable 'Time' and increases in pH and ANC, and a decrease in LAI, characteristics that are associated with recovery from acidification.

The seasonal signal in Trodøla was stronger than the linear component of time in our analyses. The nominal variable 'Spring' explained 12.3 % of the variation in the abundance data. This is also reflected in the acidity index (**Fig. 6b**), where the index values from the spring samples is almost always lower than in the autumn samples.

The analyses of the remaining localities are based on the assumption that the two water sampling stations we have are representative for the variation in the water chemistry on these other localities. Since we do not have data from each site sampled for macrobenthos, the results must be interpreted with care. However, some general trends may be found. Recovery from acidification was indicated at more localities when the water chemistry from Trodøla was used in the analyses, than when the data from the main river were included. Although the increases in pH and ANC and the decrease in LAI were slightly larger in the Nausta than in the Trodøla dataset, the latter apparently gives a better signal. The analyses indicate that a recovery from acidification have taken place in the upper reaches of the main river and in some of the tributaries. This is also indicated in the acidification indices of some of the localities (**Appendix A**).

The magnitude of the recovery appears, however, to be quite small. The linear trends in water chemistry explain only from 4.3 % to 8.5 % of the total variation in abundance on the different localities (**Figs. 1 & 2**). This means that other processes have been more important in ordering the benthic community, than the linear change in water chemistry interpreted as a recovery.

On average the spring - fall variability is stronger than the linear trend, ranging from 1.7 % to 17.2 %. This support the notion expressed in Lien et al. (1988) that the Nausta watershed is vulnerable to episodic events of acidification. It also means that a possible biological response to the recovering water chemistry in the watershed may still be inhibited by such episodes.

3.2 The Gaular watershed

The pooled data from the watershed gave a significant ordination (RDA) with linear time included as the single environmental variable, as did also the data from the localities 4, 6, 7, 8, 11, 12, 15, and 16.

The results from the RDA's with the water chemistry data included are shown in **Figure 7**. Only one locality, the tributary Neselva, gave a significant ordination. The water chemistry data explained 36.7 % of the variation in the benthic abundances in this locality. The linear component in the water chemistry explained 3.7 % of the variation, while the seasonal component explained 10.7 %.

The water chemistry data is from locality 5 in the southern branch of the watershed. This branch of the river showed the largest acidification damages in a baseline study from 1984 (Raddum & Fjellheim, 1986). No localities in this branch showed any signal of recovery in our analyses and neither did the pooled biological data from all of the localities in the watershed.

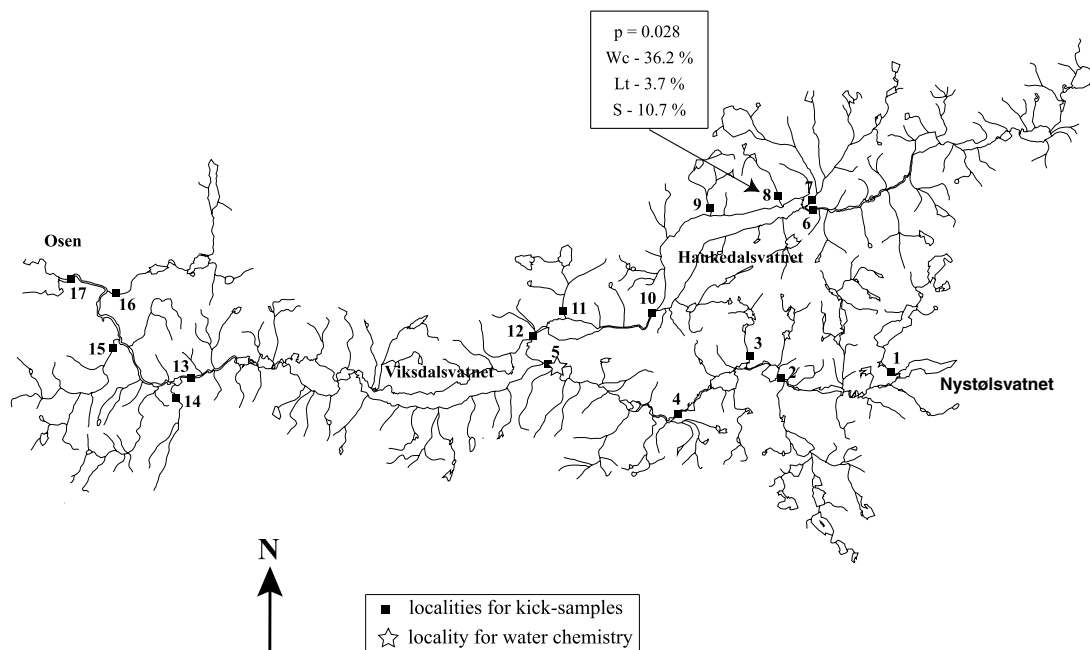


Figure 7. Results from the RDA's of the changes in the benthic communities in the Gaular watershed from 1989 to 1998 with water chemistry data from the southern side branch at locality 5 (NO07). Boxes indicate localities with significant results.

p = p value from unrestricted permutation test; Wc = amount of variation explained by the water chemistry variables; Lt = amount of abundance variation explained by linear time; S = amount of abundance variation explained by the nominal variable 'Spring'.

Table 3 shows the correlations between the water chemistry variables from Gaular at locality 5 and the two components of time. Linear time is correlated with an increase in pH and in ANC, and a decrease in labile aluminum, while the seasonal variable 'Spring' shows a positive correlation with calcium.

Table 3. Spearman Rank Correlation matrix between the environmental variables from the Gaular watershed from 1989 – 1998 used in the RDA's.

	Ca	ANC	TOC	LAI	Time	Spring
pH	-0.029	0.735**	0.328	-0.773**	0.655**	-0.278
Ca		0.150	0.239	0.202	0.141	0.774**
ANC			0.557*	-0.611**	0.532*	-0.208
TOC				-0.256	0.391	-0.113
LAI					-0.635**	0.400
Time						-0.087

Two-tailed tests. ** significant at the 0.01 level. * significant at the 0.05 level.

The pH data from the Gaular watershed at locality 5 is shown in **Figure 8**, and the remaining water chemical variables are given in **Figure 9**.

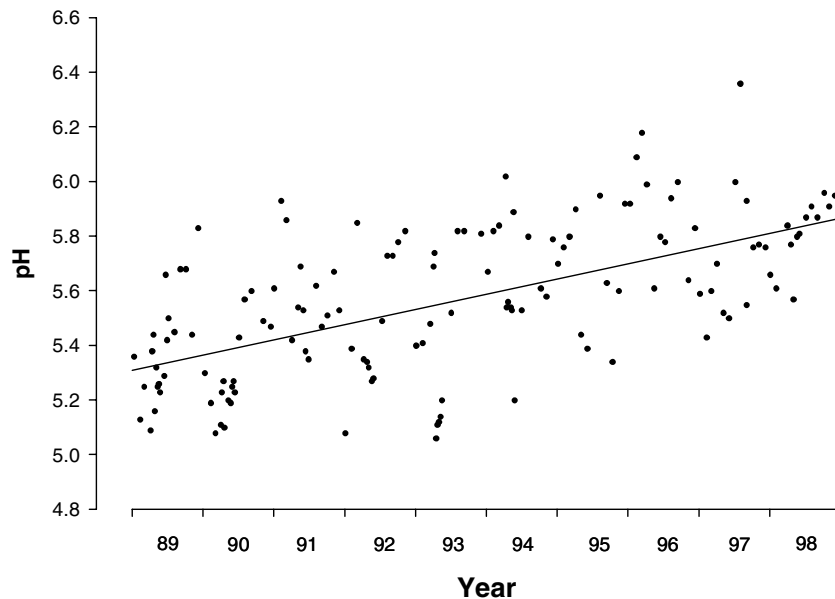


Figure 8. Simple regression of pH upon time in the Gaular watershed (NO07) at locality 5.

The pH has increased from about 5.3 to about 5.8 in this ten years period. This increasing trend has been shown to be significant measured as a decrease in H^+ ions. Also the ANC showed a significant increasing trend in this period (Skjelkvåle et al., 2000).

In the baseline study from 1984, the southern branch of the river and some of the tributaries in the northern branch were described as strongly affected by acidification (Raddum & Fjellheim, 1986). This is illustrated in **Figure 10a** which gives the average of index 2 for the southern branch (locs. 1-5) from 1984 to 1998. The index indicates a slight recovery in the benthic fauna in this part of the river, but the branch is still strongly affected by acidification. **Figure 10b** shows the index for locality 8. The index oscillates strongly between years, and the spring values are also much lower than the autumn values, indicating that the locality is vulnerable to acid episodes. However, our results indicate that there has been a slight recovery.

The northern branch and the main river after the confluence of the two branches were described as being slightly affected and not affected by acidification respectively in the baseline report (Raddum & Fjellheim, 1986).

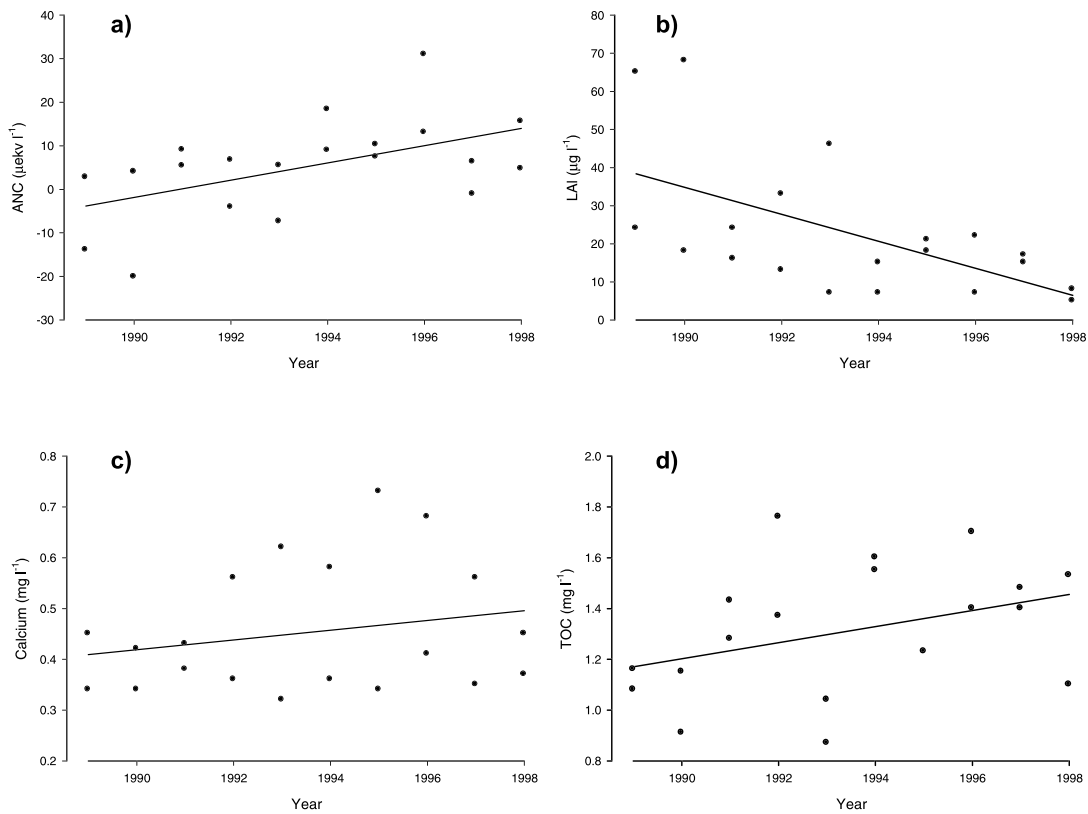


Figure 9. Simple regressions of the water chemistry variables from spring and autumn upon time from the Gaular watershed at locality 5. The spring variables are the averages measured in March, April, and May, and the autumn variables are the averages from the measurements in September, October, and November. **a)** = acid neutralizing capacity (ANC), **b)** = labile aluminum (LAI), **c)** = calcium, **d)** = total organic carbon (TOC).

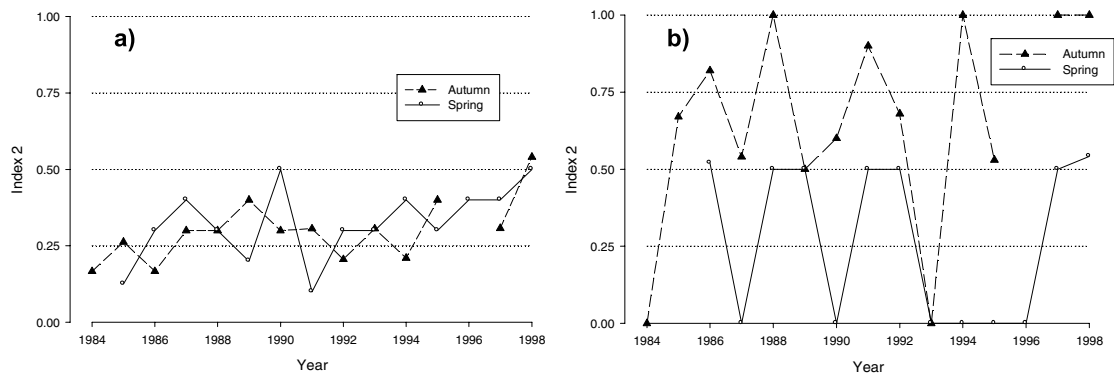


Figure 10. The acidity index 2 in parts of the Gaular watershed from 1984 to 1998. **a)** average values from the localities 1 - 5 in the southern branch of river. **b)** locality 8 (Neselva)

3.3 The Vikedal watershed

The pooled dataset from all the unlimed localities and the localities 3 and 11 gave significant ordinations (RDA) with linear time included as the single variable.

The results from the RDA's with water chemistry included are shown in **Figure 11**.

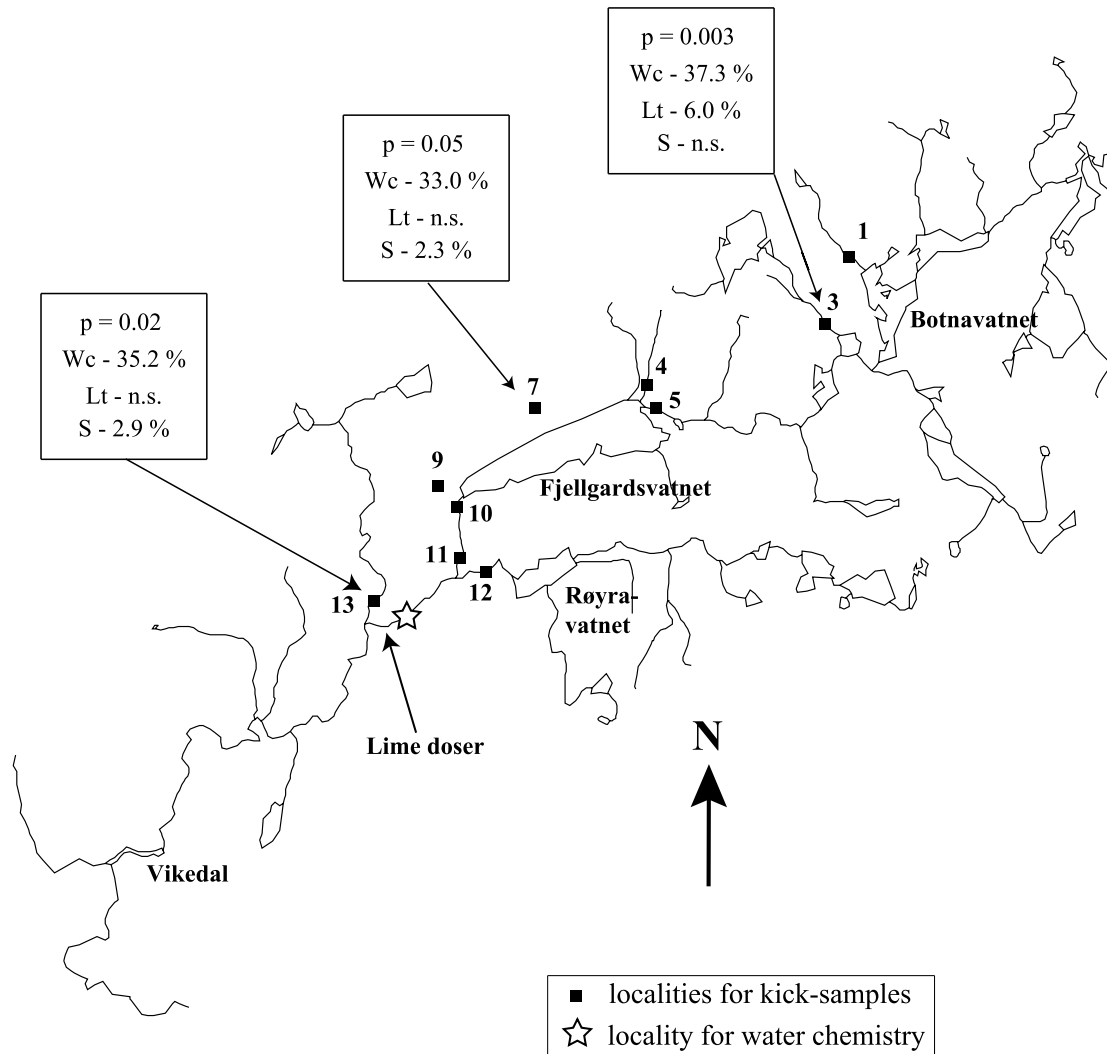


Figure 11. Results from the RDA's of the changes in the benthic communities in the Vikedal watershed from 1989 to 1998 with water chemistry data from the unlimed part above the lime-doser (NO06). Boxes indicate localities with significant results. p = p value from unrestricted permutation test; Wc = amount of variation explained by the water chemistry variables; Lt = amount of abundance variation explained by linear time; S = amount of abundance variation explained by the nominal variable 'Spring'.

The RDA gave significant ordinations for three localities where the water chemistry explained from 33.0 % to 37.3 % of the variation in the abundance data. The pooled data did not give significant results. Only locality 3 had a significant linear trend in water chemistry. This linear component explained 6 % of the abundance variation at the locality. In contrast to the analyses from the Nausta and Gaular watersheds explained the seasonal changes in the water chemistry little of the abundance variation. The 'Spring' variable explained only 2 - 3 % of the variation at the localities 7 and 13, where no linear trend or recovery was found..

The correlations between the time variables and the water chemistry variables are shown in **Table 4**.

Table 4. Spearman Rank Correlation matrix between the environmental variables from the Vikedal watershed from 1989 – 1998 used in the RDA's.

	Ca	ANC	TOC	LAI	Time	Spring
pH	0.681**	0.716**	0.435	-0.931**	0.853**	-0.156
Ca		0.708**	0.078	-0.624**	0.672**	-0.191
ANC			0.298	-0.707**	0.790**	-0.017
TOC				-0.390	0.354	-0.156
LAI					-0.836**	0.087
Time						0.087

Two-tailed tests. ** significant at the 0.01 level. * significant at the 0.05 level.

The linear variable 'Time' show strong correlations with increases in pH, calcium and ANC, and a decrease in labile aluminum. The seasonal variable 'Spring' did not show significant correlations with any of the water chemistry variables. Skjelkvåle et al. (2000) showed that the increasing trends in pH, the sum of base cations, and ANC were significant. The development of the pH in the unlimed parts of the watershed is shown in **Figure 12**.

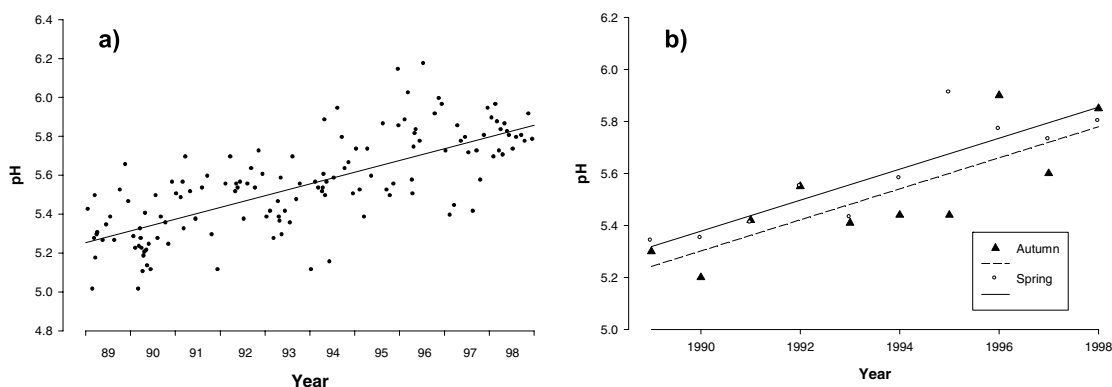


Figure 12. pH in the unlimed part of the Vikedal watershed (NO06) **a)** simple regression of pH upon time based on all measurements **b)** simple regression of pH upon time based on the spring and autumn means from the same period.

The minor role of the seasonal variable in this watershed as compared to the two other Norwegian watersheds may be that this river has its headwaters at lower altitudes than the others do. It is also situated in an area of Norway with less snow during the winter than in the two more northern watersheds. **Figure 12 b** shows that the mean values of pH are higher or equal during the spring months than in the autumn for most of the years. **Figure 13** gives the other water chemistry variables from the watershed.

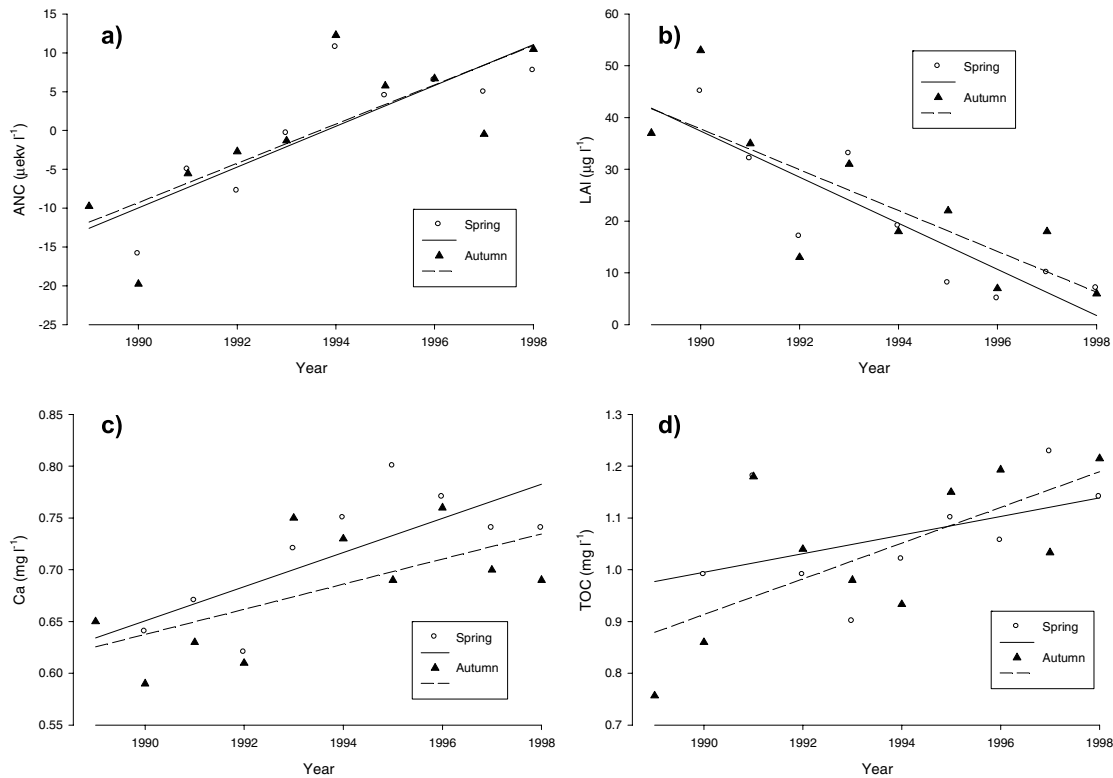


Figure 13. Simple regressions of the water chemistry variables from spring and autumn upon time from the Vikedal watershed. The spring variables are the averages measured in March, April, and May, and the autumn variables are the averages from the measurements in September, October, and November. **a)** = acid neutralizing capacity (ANC), **b)** = labile aluminum (LAl), **c)** = calcium, **d)** = total organic carbon (TOC).

The watershed has, however, been more affected by acid rain than the two more northern ones. The river nearly lost its population of salmon during the 1980ies, and as a consequence the anadromous stretch of the river was limed from the mid 80ties.

The acidification is also shown in the acidification index. **Figure 14** shows index 2 for the unlimed part of the watershed from 1982 to 1998. The index is still low, but shows an increase in the 1990ties.

The three localities where changes in water chemistry give significant results in our analyses are quite different. Locality 13 (**fig. 15 c**) has been strongly affected, and a lime-doser was installed below the benthic locality in the late 1990ties. The acidity index shows no sign of recovery for this locality, and neither do the multivariate analysis. Locality 3 has also been strongly affected. The acidity index shows no clear sign of recovery (**fig. 15 a**), however, the

multivariate analysis indicates that a recovery has been going on from 1989 to 1998. Locality 7 is different from the two others. This locality is a small, springfed stream that has been a refuge for the most acid sensitive fauna in the watershed. **Figure 15 b** shows that this locality also has been affected by acidification. The autumn values of the index drop in several of the years, a pattern that must be connected with heavy rainfall during the autumn.

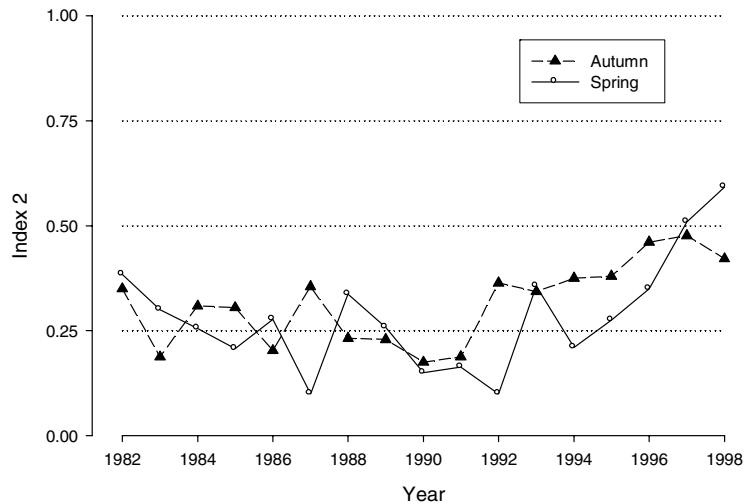


Figure 14. The mean of the acidity index 2 in the unlimed parts of the Vikedal watershed from 1982 to 1998.

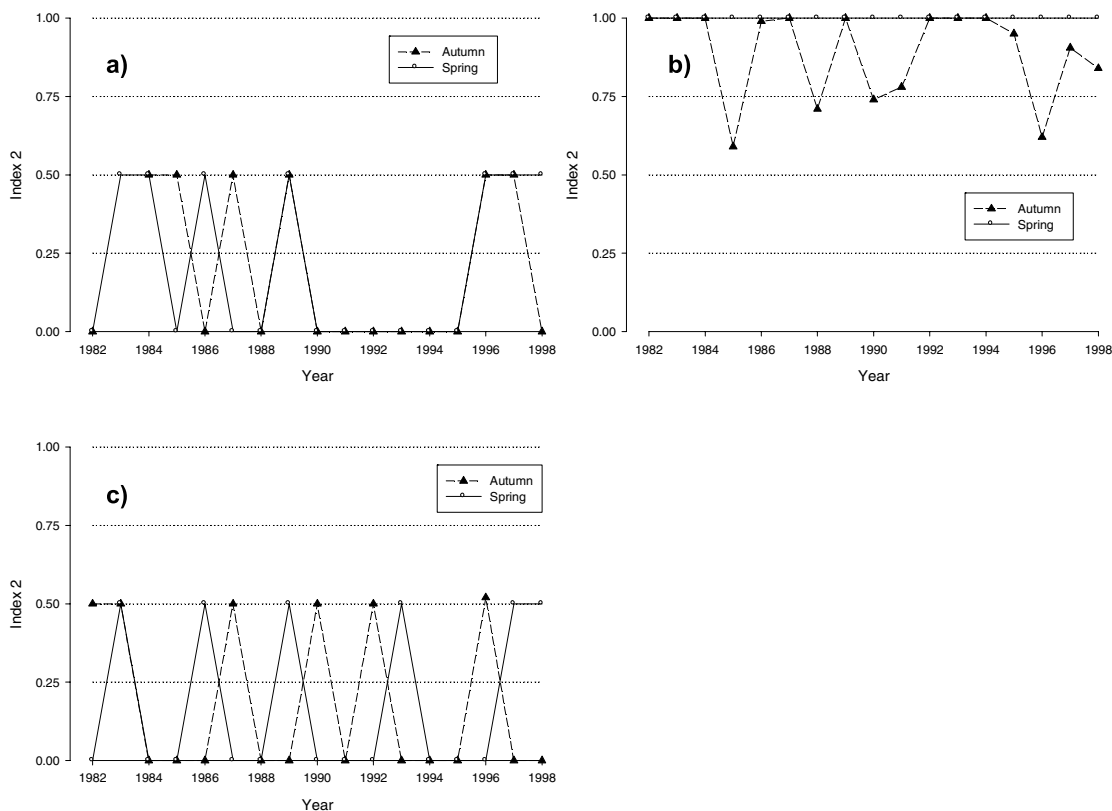


Figure 15. The acidity index 2 from 1982 to 1998 in the three localities in the Vikedal watershed that showed signals of recovery. *a)* locality 3, *b)* locality 7, *c)* locality 13.

3.4 United Kingdom

Skjelkvåle et al. (2000) reported no signal of recovery in the UK localities when analysed with the abundance data expressed as individuals per sample. None of the localities showed any significant signal of recovery when the data were reanalysed with the abundance data expressed as relative abundances either. The RDA from Lochnagar (UK04) was, however, very close to the significance level ($p = 0.054$). What is happening in this locality, though, is a continued acidification process rather than a recovery from acidification according to the 10 year report from the UK monitoring (Evans et al., 2000).

3.5 Sweden

The initial RDA's of the littoral samples from the localities Härsvatten (SE12) and Stensjön (SE06) gave significant results. However, in 1995 the sampling design was changed from taking the littoral samples during spring to autumn sampling. When the analyses were corrected for this (i.e. running the RDA's with the sampling period as covariable), we got no significant results.

The quantitative samples from the sublittoral and the profundal of the lakes analysed were all samples from the autumn. Five lakes (Fiolen, Härsvatten, Fräcksjön, Stensjön and Brunnsjön) had sublittoral samples. Of these gave the RDA's from 3 lakes significant results when linear time was the only included environmental variable. Only 2 lakes showed significant results when the water chemistry was included. The results of the RDA's are shown in **Table 5**.

Table 5. Results from the redundancy analyses of the Swedish ICP Waters lakes. Only those lakes that showed significant results are listed.

	Variation in abundance explained by	
	Water chemistry (%)	Linear time (%)
Fiolen (SE09)		
Littoral	ns	
Sublittoral	54.1 %	17.5 %
Profundal	ns	
Härsvatten (SE12)		
Littoral	ns	
Sublittoral	73.8 %	34.4 %
Profundal	ns	

Härsvatten is the most acid of the lakes in the Swedish dataset. The sublittoral of this lake did not have abundance data from 1989 so the period analysed are from 1990 to 1998. Also the water chemistry data from the autumn of 1990 were missing, so the data included in the analysis are the means of the autumn data from 1989 and 1991. We also did an alternative analysis with the spring data from 1990. These results are not presented here, but they were very close to the results with the calculated values.

The pH of the lake has increased from about 4.3 to 4.7 in the ten year period. The analyses in Skjelkvåle et al. (2000) showed that there has been a significant decreasing trends in hydrogen ion concentration, in sulphate and the sum of base cations. In addition, nitrate has also decreased significantly. These trends in water chemistry are strongly mirrored in the analysis of the abundance data. Changes in the water chemistry explained 73.8 % of the variation and 34.4 % of this variation could equally well be explained by the linear variable 'Time'. This shows that the sublittoral community has reacted on the linear changes in water chemistry over the 9 years. The most likely candidate of this is the increase in pH, which is strongly correlated with linear time, or variables not measured, but correlated to this increase. This means that a recovery may have taken place in the sublittoral community, although the lake is still very acid. **Figure 16** shows the development in the water chemistry in Härsvatten.

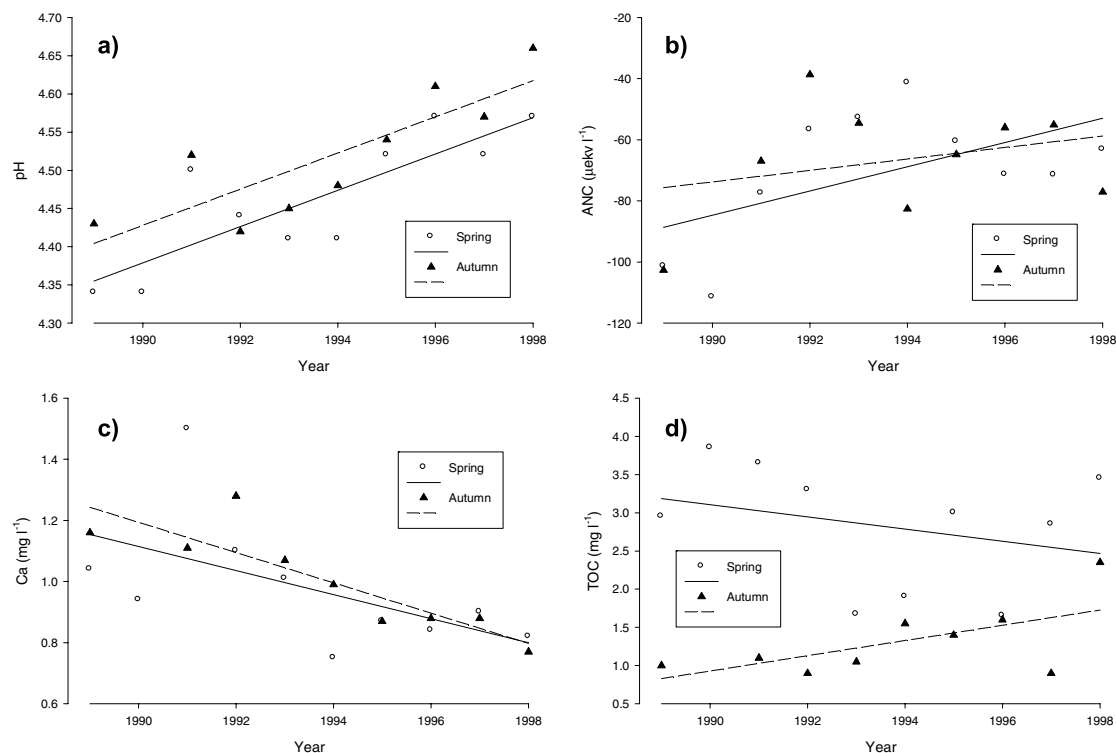


Figure 16. Simple regressions of the water chemistry variables from spring and autumn upon time from the lake Härsvatten (SE12). **a)** = pH, **b)** = acid neutralizing capacity (ANC), **c)** = calcium, **d)** = total organic carbon (TOC).

Table 6 gives the correlations between the water chemistry variables included in the analysis and linear time component. The decreases in Ca and TOC is also correlated with linear time, so these changes may also be responsible for parts the linear change in the abundance data.

Table 6. Spearman Rank Correlation matrix between the environmental variables from Härsvatten (SE12) from 1990 – 1998 used in the RDA's.

	Ca	ANC	TOC	Time
pH	-0.417	-0.150	-0.350	0.833**
Ca		-0.367	0.533	-0.733*
ANC			-0.467	0.267
TOC				-0.667*

Two-tailed tests. ** significant at the 0.01 level. * significant at the 0.05 level.

The sublittoral community in the lake Fiolen also gave a significant RDA (**Table 5**). Changes in the water chemistry variables explained 54.1 % of the abundance variation, and the linear time component of this trend explains 17.5 %, i.e. there is a signal of a recovery in the sublittoral data.

The pH ranges from about 6.1 to about 6.6 during the 10 year period. However, Skjelkvåle et al. (2000) found significant decreasing trends in the hydrogen ion concentration and in calcium content. Also the nitrate concentration showed a significant decreasing trend. The ANC and TOC did not show any significant trends. The water chemistry variables used in the RDA are shown in **Figure 17**.

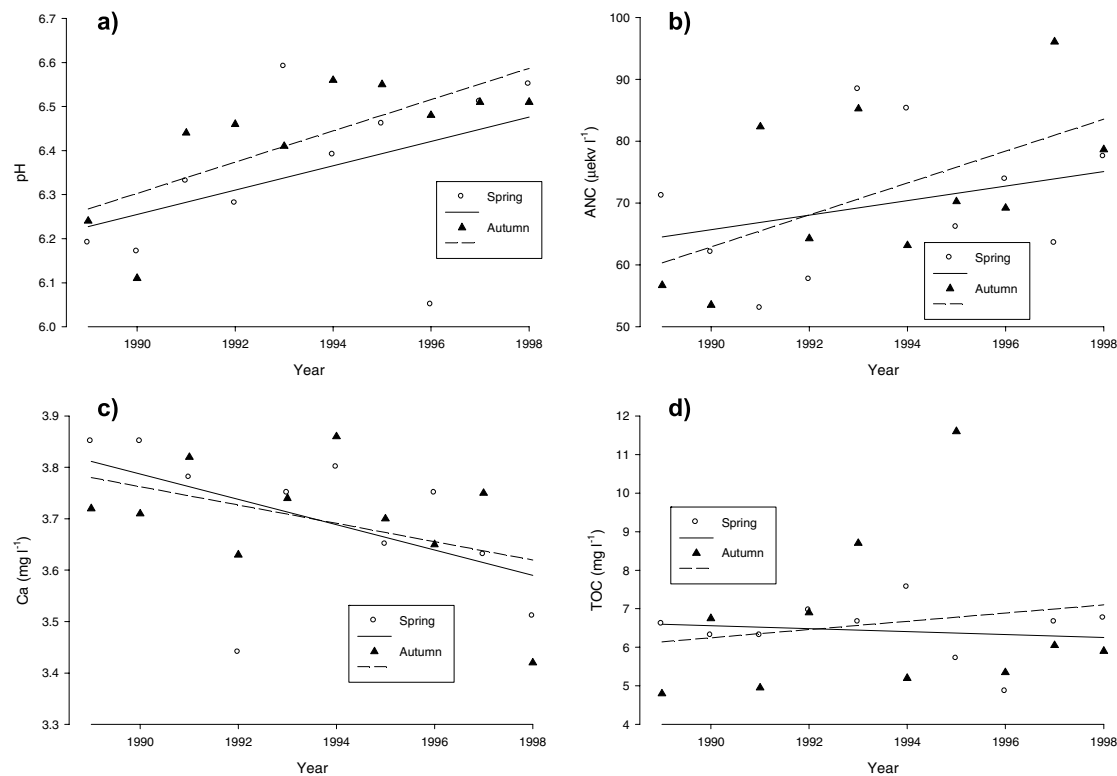


Figure 17. Simple regressions of the water chemistry variables from spring and autumn upon time from the lake Fiolen (SE09). **a**) = pH, **b**) = acid neutralizing capacity (ANC), **c**) = calcium, **d**) = total organic carbon (TOC).

The correlations between the water chemistry variables and the linear time variable from Fiolen are given in **Table 7**. The linear variable of time is significantly correlated with an increase in pH and a decrease in the calcium concentration. Although the acidification seems to have been weak, the sublittoral community apparently reacts to the increasing pH.

Table 7. Spearman Rank Correlation matrix between the environmental variables from Fiolen (SE09) from 1989 – 1998 used in the RDA's.

	Ca	ANC	TOC	Time
pH	-0.490	0.596	0.006	0.717*
Ca		0.006	0.167	-0.693*
ANC			-0.012	0.491
TOC				-0.366

Two-tailed tests. ** significant at the 0.01 level. * significant at the 0.05 level.

All seven lakes had profundal samples, but only the data from the lake Fiolen showed significant results in the RDA with linear time included as the single environmental variable. However, no significant result was found when the water chemistry variables were included.

4. Conclusions

The multivariate method found evidence of recovery from acidification in some localities in all of the three Norwegian watersheds, and also in two of the seven lakes analyzed from Sweden. There was no signal of recovery in the benthic community of the ICP Waters lakes in the United Kingdom.

The results from Norway is in good accordance with the results based on the acidity indexes. The northernmost Nausta watershed shows more signs of recovery than the other two watersheds analysed here. This watershed is the one of the Norwegian ICP Waters sites that has been least affected by acidification. It appears that the biological recovery in the acidified watersheds in western Norway, following improvements in water quality, is in its beginning.

The result from the Swedish sites shows that the multivariate method can trace signals of recovery in the benthic community in lakes that are recovering chemically, but still are in a very acidic state. Also, the results from Fiolen shows that a recovery can be traced when the acidification has been slight, and the recovery has occurred over a pH gradient where the lake would be classified as not acidified. In cases like this, methods based on indices would probably have indicated no change at all.

The method do only indicate where a recovery in the benthic community has occurred. It does not say anything about how far the site is from its natural unaffected state. However, it may give us an indication of the relative strength of the recovery. It gives a measure of how much of the total change in the abundance data that can be attributed to linear changes in water chemistry. As such, the method may also identify acidification processes equally well as recovery from acidification.

The Norwegian dataset shows that the method can give a relative measure of how much seasonal changes in the water chemistry affects the benthic community, and by this a possible recovery process. The results from the Nausta and the Gaular watershed shows that seasonal changes in water chemistry connected to snowmelt in spring and / or sea-salt episodes during the winter may confound a recovery process. In the Vikedal watershed, however, the seasonal changes appears to be of less importance. Here, rainfall during the autumn seems to be a more important factor in affecting a biological recovery than snowmelt or sea-salt episodes.

The multivariate method appears to be conservative in the context of indicating recovery. The number of localities which shows linear trends when the linear time variable is included in the analyses as the only variable, is higher than when the water chemistry variables are included as well. This means that the method can be used in cases like the Norwegian monitoring sites, where we do not have water chemistry samples from each benthic locality, but have to rely on one or a few sampling sites as representatives from each watershed. The method does apparently not overestimate signals of recovery.

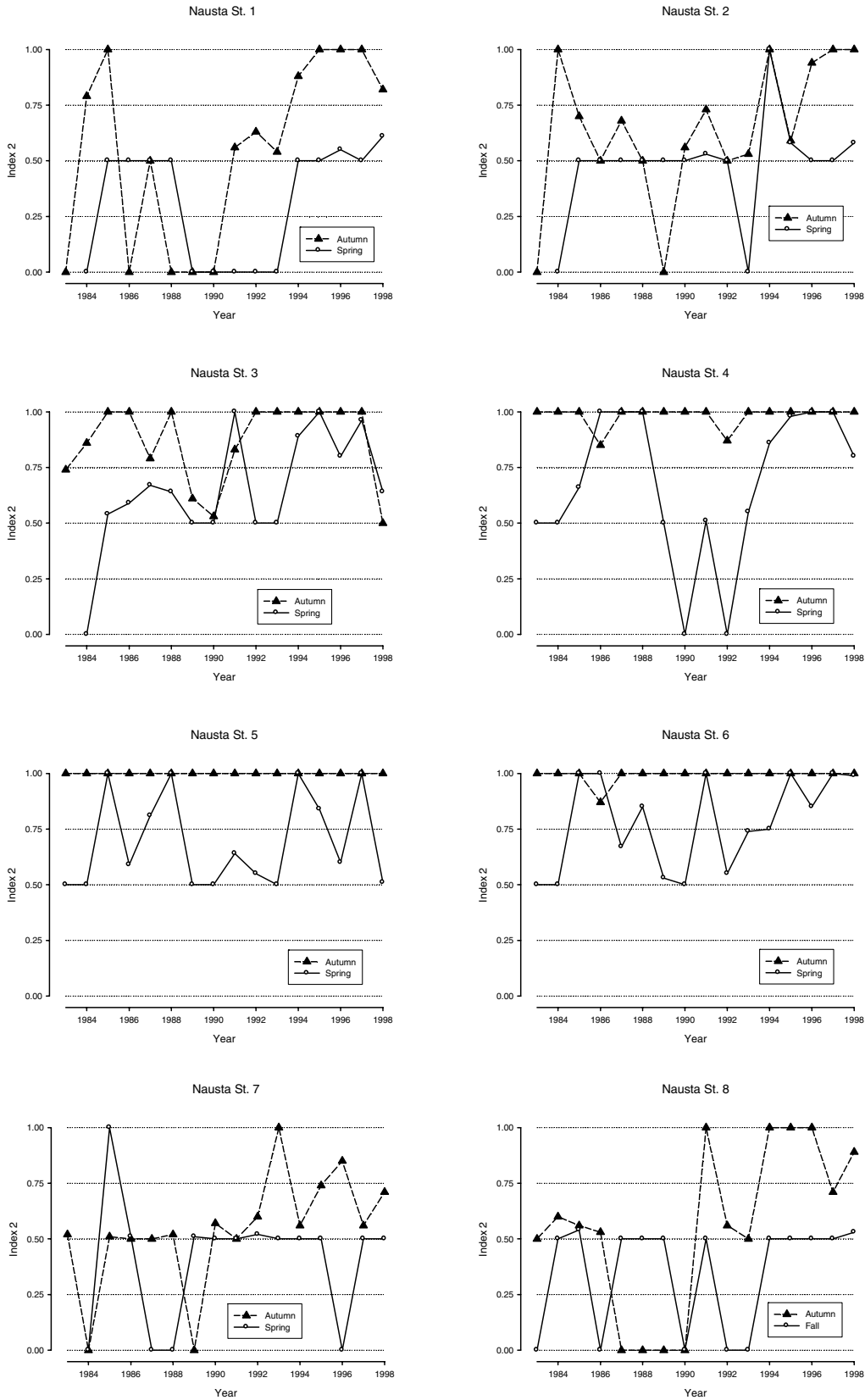
We regard the multivariate method to be complementary to methods based on acidity indices. It may corroborate a signal of recovery indicated by the indices, and most important, it may also discover signals of recovery at water chemistry levels where the indices have no resolution.

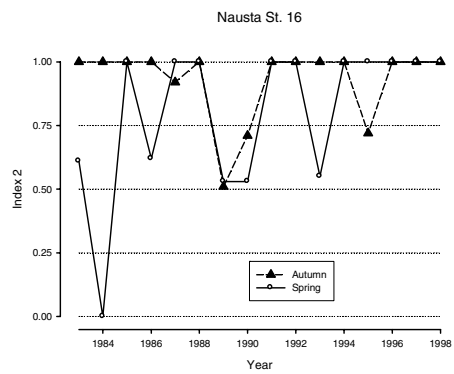
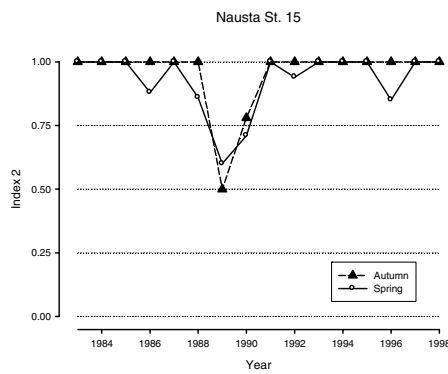
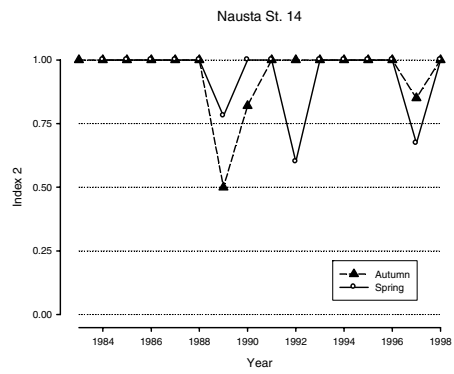
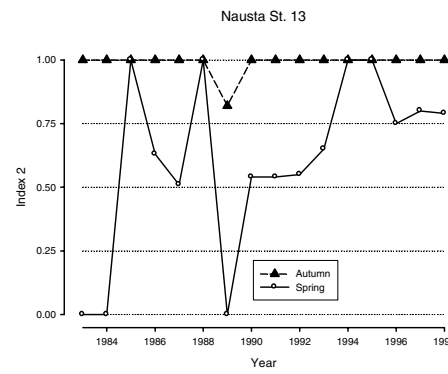
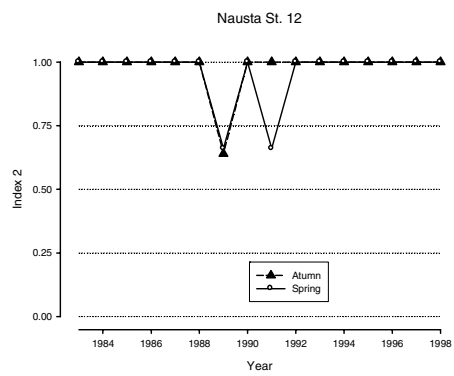
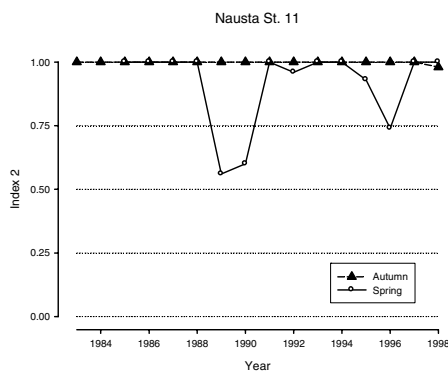
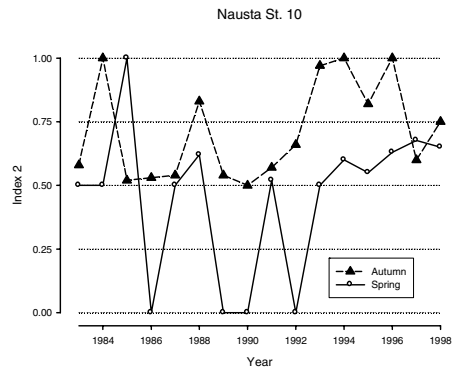
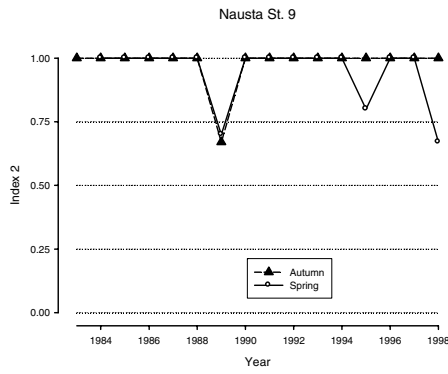
5. References

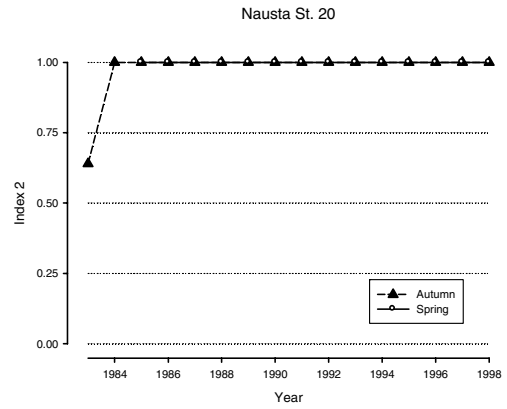
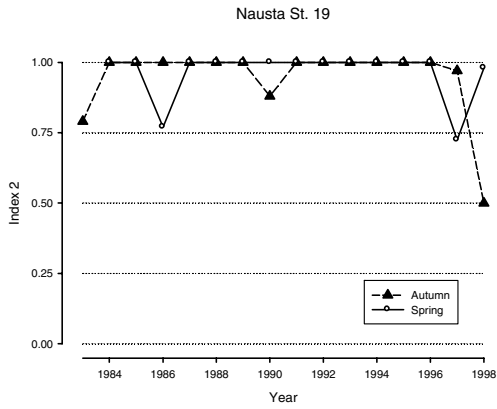
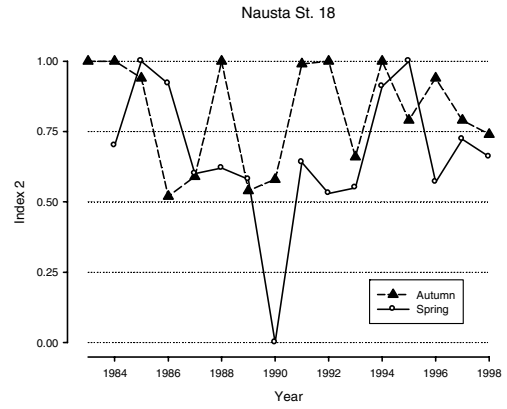
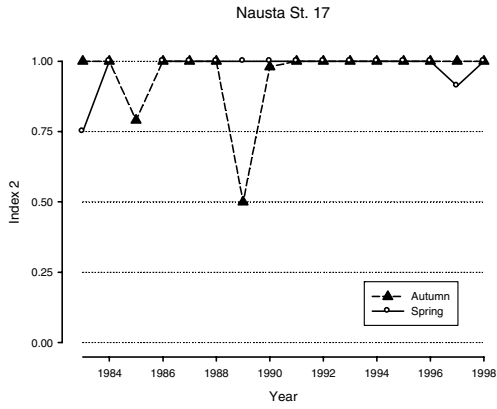
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Appendix A. Acidity index 2 for all of the localities in the Nausta watershed







Appendix B. Reports and publications from the ICP-Waters Programme

1. Manual for Chemical and Biological Monitoring. Programme Manual. Prepared by the Programme Centre, Norwegian Institute for Water Research. NIVA, Oslo 1987.
2. Norwegian Institute for Water Research, 1987. Intercalibration 8701. pH, Ks, SO₄, Ca. Programme Centre, NIVA, Oslo.
3. Norwegian Institute for Water Research, 1988. Data Report 1987 and available Data from Previous Years. Programme Centre, NIVA, Oslo.
4. Norwegian Institute for Water Research, 1988. Intercalibration 8802. pH, K₂₅, HCO₃, NO₃, SO, Cl, Ca, Mg, Na, K. Programme Centre, NIVA, Oslo.
5. Proceedings of the Workshop on Assessment and Monitoring of Acidification in Rivers and Lakes, Espoo, Finland, 3rd to 5th October 1988. Prepared by the Finnish Acidification Research Project, HAPRO, Ministry of Environment, October 1988.
6. Norwegian Institute for Water Research, 1989. Intercalibration 8903: Dissolved organic carbon and aluminium fractions. Programme Centre, NIVA, Oslo. NIVA-Report SNO 2238-89. ISBN 82-577-1534-4.
7. Note: Some reflections about the determination of pH and alkalinity. Prepared by the Programme Centre, Norwegian Institute for Water Research. Håvard Hovind, NIVA, Oslo October 1989.
8. Hovind, H. 1990. Intercalibration 9004: pH and alkalinity. Programme Centre, NIVA, Oslo. NIVA-Report SNO 2465-90. ISBN 82-577-1776-2.
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9. Johannessen, M. 1990. Intercalibration in the framework of an international monitoring programme. Proceedings of the third annual Ecological Quality Assurance Workshop, Canada Centre for Inland Waters, Burlington Ontario. Programme Centre, NIVA, Oslo.
10. Norwegian Institute for Water Research, 1990. Data Report 1988. Programme Centre, NIVA, Oslo.
11. Norwegian Institute for Water Research, 1990. Data Report 1989. Programme Centre, NIVA, Oslo.
12. Proceedings for the 5th Meeting of the Programme Task Force Freiburg, Germany, October 17 -19, 1989. Prepared by the Umweltbundesamt, Berlin July 1990.
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