

# Tracing Recovery from Acidification in the Western Norwegian Nausta Watershed

A novel method, redundancy analysis (RDA), has been used to examine whether chemical recovery from acidification in the western Norwegian Nausta watershed produces detectable recovery within the community structure of the macro-zoobenthos. The RDA results have been compared with measures of recovery based on the changes detected using highly specialized and regionally defined biological acidity indices. We found that the beginning of biological recovery in the Nausta watershed was recognizable during the period 1989–1998. Recovery occurred in the upper reaches and in the tributaries. The multivariate approach proved to complement the acidity indices approach, and much biological information can be gained by their combined use. The RDA method is conservative, i.e. does not overestimate biological recovery, and it is not geographically constrained as are the acidity indices. We also found that seasonal climatic factors strongly influence the benthic community, and may confound the detection of the biological recovery process.

## INTRODUCTION

Chemical recovery of acidified waters due to reductions in emissions has been reported from Europe and North America (1). The western Norwegian river Nausta is an example of a river where chemical recovery was observed during 1989–1998. Continuous monitoring of the zoobenthos using acidification indices indicated that biological recovery has followed chemical recovery (2). The Nausta was, therefore, used in this study to test a multivariate technique of detecting recovery.

The previously established acidification indices are based on data for the acid sensitivity of selected benthic animals from western and southernmost Norway, and were developed as means for early warnings of acidification (3). Briefly, the method is as follows: The different species/taxa are assigned to 4 classes based on their acid tolerance, i.e. very sensitive, moderately sensitive, slightly sensitive, and tolerant taxa, and are then given the acidity scores 1, 0.5, 0.25, and 0, respectively. Localities with one or more specimens of a very sensitive organism are then assigned the acidification score of 1, a locality with moderately sensitive taxa present have the score of 0.5, and so on. Localities with only tolerant species have a score of 0. Using a fixed set of representative localities in a watershed, the mean index for the watershed is calculated on each sampling date. Changes in the index can then be followed over years.

Two slightly different acidity indices based on the same data exist. The one described above is Index 1 (3). Index 2 is a more conservative index. It takes into account the abundance of the mayfly *Baetis rhodani* (Pictet), the dominating sensitive species in the southern and western Norwegian rivers, compared to the abundance of tolerant stoneflies. For example, while Index 1 will give the value of 1 if only one specimen of *B. rhodani* is found in a locality, Index 2 can take any value between 0.5 and 1 based on the abundance of *B. rhodani* as a ratio of the abundance of tolerant stoneflies (4).

There are other general univariate measures that are useful for detecting environmental stress or recovery, but they are not as

sensitive as multivariate methods (5–7). However, the aim of this paper is to examine whether chemical recovery is evident in benthic community structure, not just as changes in acid sensitive taxa as indicated by specialized acidity indices. We use a direct multivariate method, Redundancy Analysis (RDA) to evaluate the 2 different approaches for identifying a recovery process.

It should be stressed that we do not try to identify any recovery target, or how far from such a target we are in the Nausta watershed. The method used only indicates whether a process of recovery is going on. 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 benthos.

## MATERIALS AND METHODS

### Study Area

The Nausta watershed is situated close to the coast in western Norway, about 130 km north of Bergen. Figure 1 shows the lower parts of the watershed and the monitoring sites. The watershed drains 274 km<sup>2</sup>, and the precipitation ranges from 2000 to 3000 mm year<sup>-1</sup>. The geology is dominated by slow weathering bedrock, mainly gneissic granites in the southern and eastern parts, while the northern and western parts have elements of more easily weathering gneisses (8).

The area has not received as much acid precipitation as watersheds further south on the coast of Norway. The Nausta River has never lost its salmon population, but the watershed is exposed to acidic episodes (8). These are mainly acidic pulses associated with snowmelt in the spring, or combinations of strong westerly winds and heavy rainfall with large amounts of sea-salts in the rainwater. Sea-salt episodes are particularly common during the winter and may cause severe temporal acidification effects (9). The watershed was classified as slightly acidified, but vulnerable, in a baseline study from 1985–1986 (8).

### Water Chemistry

The water chemistry data are from the ICP-Waters database at the Norwegian Institute for Water Research (NIVA) in Oslo. Two stations in the Nausta watershed have been sampled throughout the 10-yr period. The main river Nausta has been sampled at locality 11 (Fig. 1), at least once a month, but more frequently during the spring snowmelt. The tributary Trodøla, close to locality 7 (Fig. 1), has been sampled weekly during the whole period. Our dataset is based on 496 samples from Trodøla and 184 samples from Nausta. The spring data are calculated as averages for the months March, April, and May for each variable, whereas the autumn data are averages of measurements from September, October, and November. These data are the same as those analyzed earlier for trends in water chemistry (1, 2). Five variables were used in the analysis: pH, calcium concentration (Ca – mg L<sup>-1</sup>), the concentration of labile aluminum (LAl – µg L<sup>-1</sup>), total organic carbon (TOC – mg L<sup>-1</sup>), and the acid neutralizing capacity (ANC – µekv L<sup>-1</sup>).

### Biological Data

The biological data consist of kick samples from 12 different riffles in the main river and 8 in some of the tributaries. These

localities (Fig. 1) have been sampled with the same methodology every spring and autumn from 1989 to 1998. Each sample was sorted under magnification in the laboratory for 1 hour, and all macroinvertebrates were subsequently identified. Leeches (Hirudinea), molluscs (Mollusca), mayflies (Ephemeroptera), stoneflies (Plecoptera) and trichopterans (Trichoptera) were identified to the lowest possible taxon, i.e. species or genus.

In the multivariate analyses we used the most inclusive taxon with some exceptions. For example, the stoneflies *Amphinemura borealis* (Morton) and *A. sulcicollis* (Stephens) have different pH optima, with *A. borealis* being the most sensitive (10). Small individuals of both species commonly occur in the samples, and they cannot be identified to species. Consequently, small individuals identified as *Amphinemura* sp. have been omitted from the multivariate analyses. Similarly, small trichopterans identified to family only were also excluded from the analyses. Oligochaeta, Acari, Coleoptera, Chironomidae, Simuliidae, Ceratopogonidae and remaining Diptera were not identified further, but counted and included in the analyses. A total of 47 taxa were recognized.

### Numerical Analysis

The relative abundance data, expressed as percentages, were log transformed to reduce the skewness and heteroscedasticity in the data. Downweighting of rare species was not used in order to give weight to the first few appearing individuals of a sensitive organism in the analysis of a potentially recovering system.

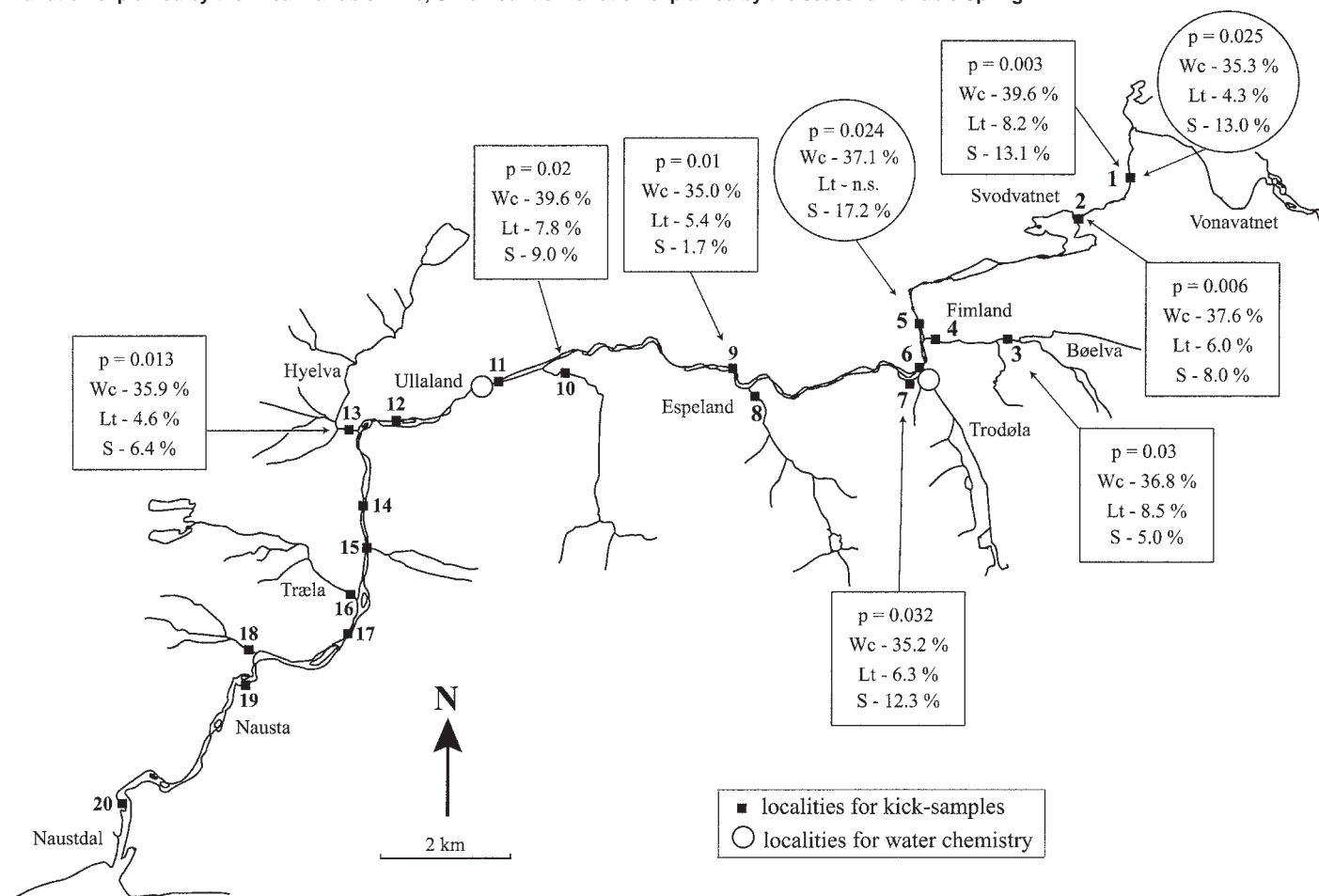
A detrended correspondence analysis (DCA) using CANOCO 4 (11) identified the length of the first axis to be less than 2 standard deviations. Consequently, a redundancy analysis (RDA), and a partial RDA was selected as most appropriate (12–14).

All water chemistry variables except pH were log-transformed. In addition, a time component was included as a variable in some analyses. A linear variable called Time was coded successively as 0 to 19 from the spring of 1989 to the autumn of 1998, and a dummy variable called Spring was coded as 0 for the spring samples and as 1 for the autumn samples. The two time-related variables were included to compare a possible long term trend with seasonality.

Our first objective was to test whether changes in water chemistry were reflected in changes in the benthic community. For this assessment, we completed a RDA with the 5 water chemistry variables included. Due to constant row sums in the abundance data, a log-ratio analysis (15) was computed by centering and standardization on the sample scores in the RDA. Furthermore, the RDA was focused on interspecies correlations (14). A Monte-Carlo permutation test (999 permutations) was performed on both the first axis and the canonical axes combined, to evaluate the significance of the RDA analysis (13). Given significant results, the sum of canonical eigenvalues reflects the variation in the benthic community explained by the water chemistry variables.

Subsequently, we examined the correlation of time and benthic species composition by a RDA with the variable Time included.

**Figure 1.** The lower part of the Nausta watershed. Filled squares (■) indicate benthic localities, open circles (○) indicate water chemistry stations. Significant ordinations using RDA with water chemistry from the main river (locality 11) is presented in circles, whereas the results with water chemistry from the tributary Trodøla (locality 7) are presented in squares. p = significance value from the RDA with water chemistry, Wc = amount of variation in the benthic community explained by the water chemistry variables, Lt = amount of variation explained by the linear variable Time, S = amount of variation explained by the seasonal variable Spring.



The results were tested for significance, using the same options as in the analysis above.

A partial RDA using Time as a covariable and the Water chemistry as environmental variables extracts the variation explained by Water chemistry that correlate with a linear trend in time, i.e. due to a long-term recovery over the 10-yr study period. Sum of constrained eigenvalues in this analysis gives the amount of variation explained by water chemistry after Time has been accounted for.

Because RDA does not have a direct method for estimating the proportional influence of different sets of environmental variables that are correlated, we used an indirect approach (16–18). The procedure is illustrated in Figure 2. The axis represents the total variation in species data expressed in percent. Amount 'a' is the variation in the species data that can be explained by the water chemistry variables. This quantity is the sum of the constrained eigenvalues when the water chemistry variables are the only ones included. Likewise, the quantity 'b' is the amount of variation correlated with Time, and 'c' is the variation in species composition extracted when both the Water chemistry variables and Time are included in the RDA. The overlap between the amount explained by Water chemistry and Time is of interest, as it represents the amount of variation in the benthic community associated with temporal changes in water chemistry. The overlap then equals  $((a + b) - c)$ , a proportion attributable to the long-term linear recovery in water chemistry.

The same procedure was used to examine possible seasonal

effects of sea-salt episodes, and the effects of snowmelt in the spring when large amounts of acid waters enters the river. In these analyses the linear variable Time was replaced by the dummy variable Spring.

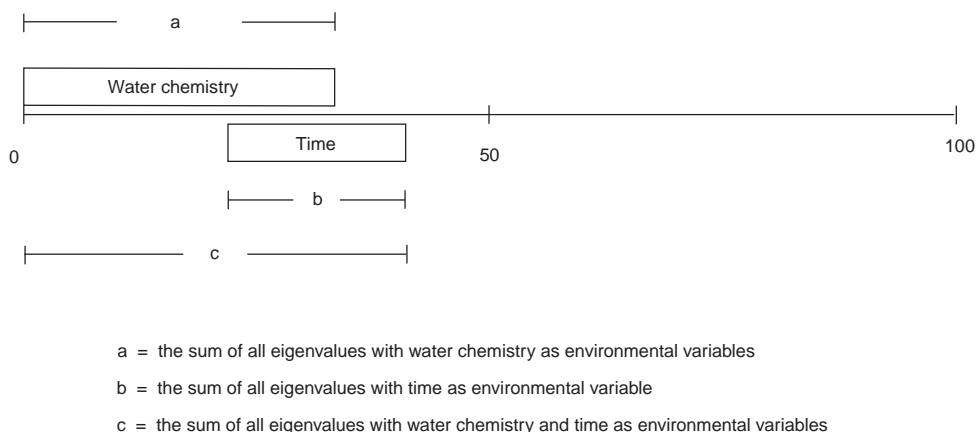
A two-tailed Spearman rank correlation was used to evaluate correlations between the individual water chemistry variables and the 2 time variables.

Two strategies were applied with respect to the abundance data. Because we do not have water chemistry data from each benthic locality, the 20 localities were first treated as subsamples of the total watershed. The pooled abundance data for all the benthic localities in the watershed were included in the analyses together with the water chemistry data from both water chemistry sampling stations. Secondly, each benthic locality was analyzed separately with both of the 2 water chemistry datasets, the 2 datasets regarded as representative for the changes of the water chemistry in the watershed.

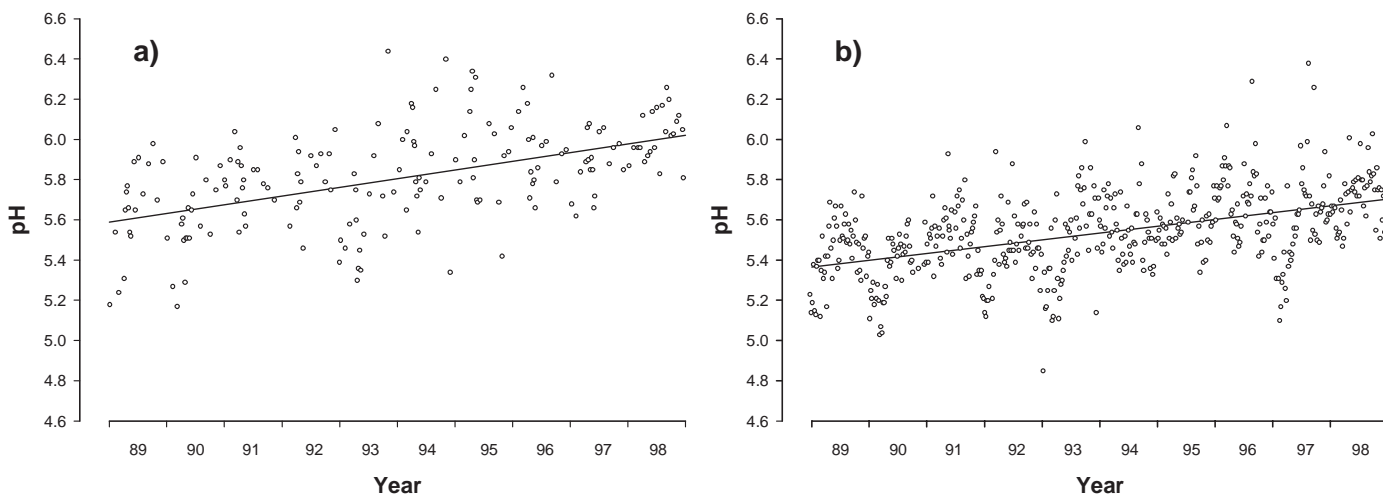
## RESULTS

Changes in pH at the 2 water chemistry stations from 1989 to 1998 are illustrated by the regressions in Figure 3. The pH in Trodøla has increased from about 5.4 to about 5.7 (Fig. 3b), whereas in Nausta the increase was slightly larger from about 5.6 to just above 6 (Fig. 3a). The increasing trend in pH was significant at both stations based on a seasonal Kendall tau test

**Figure 2. Boxplot to illustrate the partitioning of the variation in the abundance data. The axis represents the total variation in the species data in percent. 'a' is the amount of variation in the species data that can be explained by the water chemistry variables. 'b' is the amount of variation explained by linear time, and 'c' is the variation explained by both water chemistry variables and linear time. The overlap between variation explained by water chemistry and linear time,  $((a + b) - c)$ , represents the possible amount of response by the benthic community to a linear recovery in water chemistry.**



**Figure 3. Regressions of all of the pH measurements on time from 1989 to 1998 in the Nausta watershed. a) the main river Nausta at locality 11. b) the tributary Trodøla at locality 7.**



**Table 1. Spearman rank correlation matrix between the environmental variables from Nausta (upper right triangle) and Trodøla (lower left triangle) from 1989–1998 used in the RDA's. Two-tailed tests. (\*\* significant at the 0.01 level. \* significant at the 0.05 level).**

	pH	Ca	ANC	TOC	LAI	Time	Spring
pH		0.347	0.749 **	0.624 **	-0.704 **	0.743 **	0.226
Ca	0.253		0.344	-0.015	-0.212	0.358	-0.566 **
ANC	0.890 **	0.391		0.690 **	-0.670 **	0.588 **	0.295
TOC	0.523 *	-0.168	0.624 **		-0.449 *	0.321	0.504 *
LAI	-0.860 **	-0.042	-0.684 **	-0.430		-0.679 **	-0.313
Time	0.666 **	0.311	0.546 *	0.277	-0.669 **		0.087
Spring	0.295	-0.503 *	0.295	0.520 *	-0.434	0.087	

at the 0.005 significance level (2). The trends in the other water chemistry variables included in this analysis were correlated with the change in pH (Table 1). The ANC, Ca, and TOC increased in both localities, while the LAI decreased (2).

The pooled dataset using the average of all sites from the Nausta watershed showed a significant ( $p = 0.004$ ) Time effect in the RDA, when Time was the only included variable. Individual benthic localities 1, 2, 3, 4, 6, 7, 9, 10, 11, 13, 15 and 16 also provided significant results with Time ( $p < 0.025$ ), indicating changes in the benthic community over the 10-yr period.

The RDA with the pooled dataset and the water chemistry from the main river (locality 11) did not give a significant result. Using the water chemistry data from the tributary Trodøla (locality 7), gave a significant ordination ( $p < 0.001$ ). Changes in water chemistry (a in Fig. 2) explained 35.3% of the total variation in the benthic community. The amount explained by Time (b in Fig. 2) was 16.1%, whereas the amount of change accounted for by Water chemistry and Time together (c in Fig. 2) was 47.1%. The amount of change that can be interpreted as a response to long-term changes in water chemistry ((a + b) - c) is then 8.7%. The seasonal variation explained by the dummy variable Spring was 10.9%.

The results from the RDA with Time and Spring for the separate localities are presented in Figure 1. Only those localities where water chemistry gave significant ordinations are shown. Locality 1 and 5 in the upper reaches of the main river gave significant ordinations using water chemistry data from locality 11 (results presented in circles), and only locality 1 shows a linear trend. The amount of change accounted for by Time was 4.3% of the total variation in the benthic community.

Seven localities showed significant ordinations when the water chemistry data from Trodøla were used (locality 7, results presented in squares). Two of these localities are found in the upper parts of the main river (localities 1 and 2), 4 are in tributaries (localities 3, 7, 10 and 13), while 1 is in the intermediate stretch of the main river (locality 9). The amount of change in the benthic community attributable to a long-term trend of recovery in water chemistry lies between 4.6% and 8.5% of the total variation, whereas the seasonal variable explains from 1.7% to 13.1% of the total variation. The seasonal variable explains, on the whole, more of the change in the benthic community than the linear one.

The upper right panel of Table 1 shows Spearman rank correlations between the water chemistry data from the main river sampling station and the 2 different time variables, while the lower left panel shows the correlations for the water-chemistry data from the tributary Trodøla. Time is correlated with an increase in pH and ANC, and with a decrease in LAI in both datasets, while the dummy variable Spring is correlated with a decrease in Ca and an increase in TOC.

## DISCUSSION

The 2 localities from the Nausta watershed where water chemistry was measured will be considered first. Results from local-

ity 11 in the main river gave no indication of change in the benthic community that could infer recovery from acidification using our analysis, although there was a significant trend when Time was included as the only variable. The species scores from this analysis also showed that increases in the abundances of sensitive taxa played an important role in the ordination. The observed pH interval change from about 5.6 - 6.0, is close to, but above the tolerance limit for the most sensitive organisms (19). This locality has probably never been seriously affected by acidification, and other variables are likely responsible for the observed variation in the benthic community structure. This result is also consistent with the acidification indices from that locality. They were calculated from 1983 to 1998, and Index 2 indicates 2 minor acidification episodes during the spring of 1989 and of 1990, and 1 episode in the spring of 1996. Apart from these episodes the locality is regarded as not acidified (20).

The tributary Trodøla at locality 7 gave a signal that is consistent with a recovery interpretation. The overlap between Time and 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 temporal change in water chemistry. This finding is also consistent with the acidity indexes. Before 1989 the indexes oscillated strongly, indicating strongly episodic acidification events. After 1989 the very sensitive mayfly *Baetis rhodani* (Pictet) have been sporadically present in the river, but in low numbers and almost exclusively limited to the autumn samples (20). Although there has been an increase in ANC, the values are still below the estimated tolerance limit of the most sensitive species for long periods of the year (19). Table 1 shows that significant correlations exists between Time and increases in pH and ANC, and a decrease in LAI, characteristics that are associated with recovery from acidification.

The RDA using the pooled abundance data representing the whole watershed did not give any significant result with the water chemistry data from the main river. However, the pattern in the water chemistry from the tributary Trodøla gave a significant result, showing that 8.7% of the changes in the abundance data can be explained as an effect of temporal linear changes in water chemistry. Although the increase 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 correlation with the biological signal.

Recovery from acidification was indicated at more localities when the water chemistry from Trodøla was used in the analyses. The analyses with chemistry data from the main river added locality 5 only, a locality that did not give a significant result when the chemistry dataset from Trodøla was used. However, the data from this locality did not yield a significant ordination with linear time. The variation in the abundance data at this locality is explained by seasonal changes, the seasonal variable Spring explaining 17.2% of the variation.

The analyses indicate that biological changes over time consistent with recovery are occurring in the upper reaches of the main river and in some of the tributaries. This result was also shown in the acidification indices (20). There is, however, no



indication with this approach that the benthos at a given location has recovered. That is, there is no endpoint for recovery in this analysis.

The watershed was described as being vulnerable to acid episodes (8). The amount of abundance variation explained by the dummy variable Spring ranges from 1.7% to 17.2%. This percentage is a crude estimate of the impact of seasonal factors like snowmelt in the spring and of possible sea-salt episodes due to storms in the winter. The fact that this variable explains more variation in most of the localities, is a strong indication that the acidification in this watershed has been episodic and that biological recovery may be confounded by episodic events linked to climatic factors.

We regard the method used here as complementary to the traditional methods of acidity indices based on the presence/absence of key organisms. The multivariate method (RDA) shows the changes in species composition as a function of water chemistry through time, whereas the acidity indices provides information about the earliest time the localities host sensitive species. The acidity indices will recognize a single occurrence of a sensitive species in a locality, which may overestimate recovery. Compared to this, the multivariate method as used here seems to be conservative. In the Nausta watershed, 12 of the localities showed a significant trend to the linear variable Time. However, this number was reduced to 7 localities where the species composition correlated with both Time and Water chemistry from the tributary Trodøla, and only one when the water chemistry

from the main river was used. This indicates that the multivariate method does not overestimate biological recovery when data from one or a few localities have to be used to model the water chemistry in the watershed. On the other hand, the Redundancy Analysis has the possibility to discover recovery where the acidity indices are insensitive, as has been shown for Swedish lakes (20). Furthermore, the acidity indices are regionally defined as they relate to the species pool of a specific geographical area. The multivariate method is taxonomically and geographically universal. The RDA restructures the observations based on species composition along axes defined as a linear combination of the predictor variables. The acidity indices and the multivariate method (RDA) give different biological information, and it is important to realize that much can be gained by their combined use in a study of a recovery process.

Biological recovery from acidification in the Nausta watershed following reduced emissions of sulfur is still just beginning. At this point, seasonal factors play an important role in the observed structure of the benthic community in the watershed. These factors may obscure signals of biological recovery, or even confound the process itself. A similar multivariate analysis of 2 other watersheds in western Norway, the neighboring Gaular watershed and the more southern and stronger affected Vikedal watershed, indicated ongoing biological recovery in only 1 locality in each watershed. The acidity indices in these 2 watersheds also indicates recovery, but to a lesser degree than in the Nausta watershed (20).

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