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Bruk av vassdragets **bunnfauna** i vannkvalitesklassifiseringen

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Abstract: Norwegian watercourses are becoming increasingly acidified. Small animals on the river bottom can be used to classify the biological damage caused by acidification, The report describes the classification systems developed by Raddum and by Engblom/Lingdell. Both systems are based on the presence or absence of indicators that give index values, depending on their tolerance to acidification. Experiments confirm the hypothesis that brief acidification episodes can cause serious reductions in a macroinvertebrate community. Standardised tests showed the macroinvertebrates' tolerance to acid water (pH), acid water containing labile aluminium and acid water containing humus. In general, the mayfly species were most sensitive to acid water, followed by the stoneflies. The caddis flies were most tolerant. Aluminium had a favourable effect on the survival of the mayfly *Baetis rhodani* at low pH. Humus had a favourable effect on the survival of *Baetis rhodani* and the amphipod *Gammarus lacustris* in an acid environment. Based on Raddum's and Engblom/Lingdell's systems, other Nordic publications and our own field and experimental data, NIVA proposes a set of indicators that will be valid for investigations of acidification in most Norwegian watercourses. The water quality is distributed between classes 1-4, where 1 expresses no acidification damage and 4 expresses a locality severely damaged by acidification.

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1. **Bunnfauna**
2. **Forsuring**
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For the Administration

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THE NORWEGIAN INSTITUTE FOR WATER RESEARCH

AND

THE NORWEGIAN STATE POLLUTION CONTROL AUTHORITY

USE OF MACROINVERTEBRATES TO CLASSIFY WATER QUALITY

Report No. 2A.

Acidification

Oslo, November 1990

Torleif Bækken and Karl Jan Aanes

English version,
Oslo, October 1995.

PREFACE

The Norwegian State Pollution Control Authority (Statens Forurensningstilsyn - SFT), in collaboration with the Norwegian Institute for Water Research (Norsk institutt for vannforskning - NIVA) has worked out water quality criteria for freshwater. The handbook "Vannkvalitetskriterier for ferskvann" (Water Quality Criteria for Freshwater) is based on work carried out from 1980 to 1989 (NIVA 1980, 1983, 1986, SFT 1989).

The further development of this work was started in 1987 through the project "Use of macroinvertebrates for monitoring of watercourses". NIVA's task in this survey is to present the information now available and what needs to be done if the macroinvertebrates present in the watercourse are to be used in the further efforts to define criteria for water quality and classify sections of the watercourse. The study should also summarize the current knowledge on the effects of various kinds of pollution on the macroinvertebrates in running water ecosystems. Use of macroinvertebrates as a tool for classifying water quality offers great potential in the further efforts to describe and monitor water quality in lakes and rivers. This is because the macroinvertebrate community, through its composition, is able to integrate the total effect of environmental influences over a long period of time.

In recent years, NIVA has built up a large testing plant, specially designed for carrying out ecotoxicological tests on populations and communities of macroinvertebrates from streams and rivers (Aanes 1989, Aanes and Bækken 1989a, Bækken and Aanes 1990a). This has enabled us, in our work of developing a classification system for acidification damage, to simulate acidification situations and to study, as we do here, the importance of the different hydrochemical parameters for pH-tolerance. These have been important data for determining the tolerance limits of the different organisms to acidification.

In connection with the project "Use of macroinvertebrates for monitoring of watercourses", a report was written in 1989 containing general information on macroinvertebrates, pollution and classification: Use of macroinvertebrates to classify water quality. No. 1. General introduction (Aanes and Bækken 1989b. In Norwegian). In the course of 1990, two reports were published in 1990 on the effects of acidification on macroinvertebrate communities: The present report, No 2a. Acidification (Bækken & Aanes 1990a. In Norwegian), and No. 2b. Effects of acidification on macroinvertebrates in rivers and streams in Sør-Varanger, East Finmark (Bækken & Aanes 1990b. In Norwegian).

We should like to thank Eva Engblom and Per Erik Lingdell, of Limnodata in Sweden, Torgny Wiederholm of the National Environment Protection Agency, Sweden, Arne Fjellheim and Gunnar G. Raddum at the University of Bergen, Per Erik Iversen and Tor Johannessen at SFT, and Dag Berge, Gøsta Kjellberg, Frode Kroglund and Leif Lien at NIVA for useful comments on the report. Arne Henrikesn, NIVA, has willingly provided chemical data from a number of watercourses, collected in connection with the monitoring of acid precipitation in Norway.

At NIVA, Karl Jan Aanes is head of the project "Use of macroinvertebrates for monitoring of watercourses". Torleif Bækken, NIVA, has collocated the data and prepared the report. The project has been financed partly by SFT and partly from NIVA's own research funds. Our contact at SFT has been Per Erik Iversen.

Oslo, 1 July 1990

Karl Jan Aanes

Many requests for this report, also from countries outside Scandinavia, made SFT/NIVA decide to publish a version in English. Some minor corrections have been made, but in other respects the English version is the same as the Norwegian version published in 1990. Our contact at SFT has been Dag Rosland.

Oslo, 15 October 1995

Karl Jan Aanes
Project leader

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SUMMARY

1. In Norway, one of the most serious pollution problems of our times is acid rain. This has affected Southern Norway in particular, where a large share of the watercourses have become devoid of fish. Different species of macroinvertebrates respond differently to acidification, and can be used to give early warning of acidification, and to classify its biological damage.

2. Acidification indices have been prepared, based on the macroinvertebrates' degree of tolerance to acidification. In Norway, Raddum has worked out an index adapted to the fauna and water quality in Southern and Western Norway. Lingdell has prepared a corresponding index adapted to conditions in Sweden.

3. In the acidification indices, the macroinvertebrates are grouped according to their absence or presence in water with different pH-values. The fauna are placed into 4 tolerance groups designated by pH-interval. Each group is given a numerical value, an "acidification index". The groups characterize the state of acidification in a locality during the most acid episodes and provide information on possible adverse biological effects of the acidification.

4. Data from Raddum and Engblom/Lingdell, from recent publications from Scandinavia and Finland, and from investigations carried out by NIVA in the counties of Buskerud, Akershus and Hedmark, and in East Finnmark, have provided a basis for evaluating the index values given to the different species in relation to their distribution and the water quality. In general, the content of humus seems to influence the tolerance to acid water.

5. Experimental studies have shown that brief episodes of acidification can lead to large reductions in the macroinvertebrate community. It could not be shown that the important indicator species, the mayfly *Baetis rhodani* disappeared completely during these episodes, but the population was considerably reduced. It is not fully known how long an acid period has to last, or how acid the water has to be, to induce demonstrable changes in a specific macroinvertebrate community.

6. Several macroinvertebrate species were tested for sensitivity to acid water (pH), acid water containing labile aluminium and acid water containing humus. The following species were included in one or more of the tests: The mayflies *Baetis rhodani* and *Heptagenia sulphurea*, the stoneflies *Protonemura meyeri*, *Brachyptera risi* and *Leuctra hippopus*, the caddis flies *Polycentropus flavomaculatus* and *Hydropsyche siltalai*, and the crustacean *Gammarus lacustris*.

7. The mayflies were the least tolerant to acid water, followed by the stoneflies. The caddis flies were the most tolerant of the species tested.

8. Aluminium had a favourable effect on the survival of large and small individuals of the mayfly *Baetis rhodani* at pH values of 4.5 and 4.7. No mortality was registered among the other species.

9. Humus had a favourable effect on the survival of large and small individuals of *Baetis rhodani* and on the amphipod, *Gammarus lacustris*, at pH 4.5 and 5.0. The experiments supported the hypothesis that acidified localities with a high humus content are more capable of maintaining macroinvertebrate communities sensitive to acidification than corresponding localities with little humus are. The effect of the humus depends, however, on how long the period of acidification lasts and the degree of acidification.

10. NIVA (Bækken & Aanes 1990a) propose that macroinvertebrates should be placed in acidification classes, defined in terms of pH-intervals and index values, which will be valid to investigate acidification in most Norwegian watercourses. The index values are shown in table 11, on page 43.

11. Through this work for the Norwegian State Pollution Control Authority NIVA has developed a system which, because of its sensitivity and simplicity, can be a valuable tool in monitoring the degree of acidification of our freshwater resources. It can also be applied as a national system for classifying water quality in terms of the effects of acidification on our running water biotopes.

1. INTRODUCTION

In Norway, one of the most serious pollution problems today is acid rain. In Southern Norway, the average pH-value in the rain is less than 4.6. In extreme cases pH-values of 3.6 have been reported. In large areas of the country, the ability of the catchment area to neutralize acid rain is limited, because of the low availability of alkaline cations (calcium, magnesium, sodium and potassium) capable of neutralizing acid depositions (Henriksen et al. 1990). This implies that the watercourses are becoming increasingly acid. The acidification has been most serious in Southern Norway, where a large share of the watercourses have become devoid of fish. But damage from acidification has also been observed in Eastern Norway and East-Finnmark (Bækken & Aanes 1990b). It was recognized early on that acid water could cause death of fish. Later, it was observed that certain species of macroinvertebrates disappeared at an early stage of the acidification, before conditions became critical for the fish in the watercourse. This implied that these species could give early warning of acidification. Moreover, these organisms are important source of food for the fish.

In connection with the work of monitoring and characterizing the water quality in Norwegian rivers and lakes, it has become increasingly necessary to coordinate the chemical and biological data into a system for classification of water quality.

In this cooperative project, SFT and NIVA's aim has been to find out how macroinvertebrates can be used to classify water quality (Aanes & Bækken 1989, SFT 1989a). Such a system is based on the fact that the macroinvertebrates show differing degrees of tolerance to pollution, for example, acid water. The most sensitive animals die out at an early stage of the acidification, while tolerant species can sometimes survive in very acid water. Therefore, investigations of the benthic faunas show to what degree acid episodes may have harmed the fauna normally found in the watercourse, and the remaining fauna can indicate how acid the water has possibly been. Therefore, just one sample of the macroinvertebrate fauna can often indicate the pH-range during the most acid period.

The objectives of the report are as follows:

1. To describe the existing systems that use macroinvertebrates to classify acidification.

2. To assess the macroinvertebrates' pH-tolerance as defined in the classification systems in relation to other field and experimental data.

3. To present information on how large the episodic reductions in pH must be to cause demonstrable changes in the fauna.

4. By means of controlled tests, to provide accurate information on pH-tolerance and on how this is affected by other hydrochemical parameters.

5. Taking into account the above points, to propose how macroinvertebrates can be used to classify acidification in our watercourses.

2. MACROINVERTEBRATES AND ACIDIFICATION

2.1 Background.

Most of the information on the tolerance of macroinvertebrates to acid water has been obtained from field studies in watercourses in Norway, Sweden, Finland and some other European countries (Anttila 1985, Bækken & Aanes 1990b, NIVA 1988, Degerman et al. 1987, Engblom & Lingdell 1983, 1987, Huttunen et al. 1987, Lien et al. 1989, Näslund 1987, Nyman et al. 1986, Otto & Svensson 1983, Raddum 1979, Raddum & Fjellheim 1984, 1986, Økland, J. 1969, 1980, 1983, Økland, J. & Økland, K.A. 1986, Økland, K. A. 1980). Because the hydrochemistry and the composition of the fauna varies geographically, data from reports published in other countries cannot be used without reservation to study the connection between acidification and macroinvertebrate species in Norway. Therefore, in this report, the most important basic data have been taken from publications from Scandinavia and Finland.

Ever since scientists became aware of the problems associated with acidification of watercourses, many registrations of hydrochemical parameters have been undertaken, and also of certain hydrobiological parameters. As far as Norway is concerned, most of the studies have been carried out in Southern and Western Norway, because these are the areas most exposed to acidification. It was gradually discovered that the various small animals living on the river bottom respond differently to acidification; some species could tolerate only a small degree of acidification, while others were much more tolerant to this form of pollution. Scientists therefore started to use macroinvertebrates to characterize the biological status of the watercourses.

Traditionally, damage caused to the fauna by acidification, and the fauna's tolerance limits, have been studied in relation to the degree of acidity of the water (pH). However, the pH-tolerance can be influenced by other hydrochemical parameters such as calcium, aluminium, bicarbonate and the content of organic material. By using the capacity of the water to neutralize acid, these different parameters are combined to give a single parameter; ANC (Acid Neutralization Capacity) (Lien et al. 1989). This gives a more correct picture of the macroinvertebrates' tolerance to acidification than when pH is used alone. If the macroinvertebrate fauna are classified in relation to ANC, it may be necessary to change some of the pH/macroinvertebrate classes, but the changes will probably be only small ones.

Most of the studies on macroinvertebrates and acidification use pH to indicate the tolerance limits (see references above). Therefore, in this report, we shall also describe the groups of macroinvertebrates and tolerance values seen in relation to pH. In addition, we want to extract some specific factors from the ANC-concept, to test how they affect pH-tolerance.

A non-acidified (and otherwise unpolluted) river will always contain a wide range of species and groups of organisms that are either tolerant to acidification or sensitive to acidification. When a community is exposed to acidification, the sensitive species will disappear first, and the remaining macroinvertebrate community will contain fewer species, and usually also fewer individuals, and will consist of tolerant species. It is always the species that are sensitive to acidification in a locality that decide which acidification class the locality should be placed in. For example, if the mayfly *Baetis rhodani* (figure 1) is found in a river in Western Norway, it is almost certain that the locality has not been damaged by acidification, even if many species that are tolerant to acidification are found there as well.

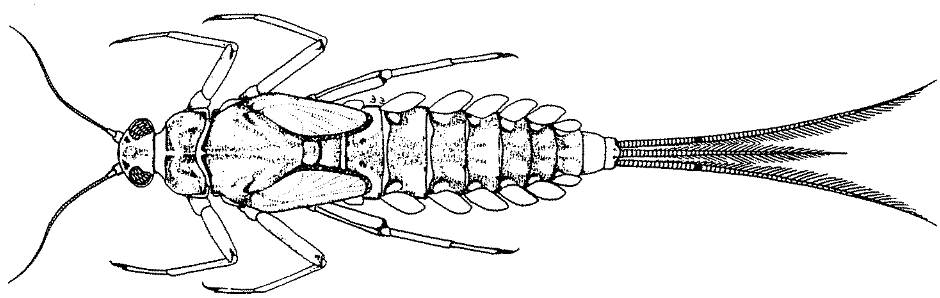


Figure 1. The nymph stage of the mayfly *Baetis rhodani*. This is one of the most important indicators of acidification in the macroinvertebrate community. (Drawn by Eva Engblom).

2.2 pH-tolerance and acidification index

It has been found useful to group the macroinvertebrates by their pH-tolerance. The animals are associated with a specific pH-interval, so that the composition of the macroinvertebrate community can, in addition to classifying the damage caused by acidification, also provide information on the degree of acidity of the water courses during the most acid periods. For the sake of simplicity, the animals within each pH-group have been given a numerical value, an "acidification index", instead of operating with pH-values.

In Norway, Raddum developed this kind of index system for the fauna in Southern and Western Norway (Fjellheim & Raddum 1984, Raddum et al. 1988). In Sweden, a corresponding system was developed by Engblom and Lingdell (1987). In principle, Raddum's and Engblom/Lingdell's systems are organized in the same way. They are both based on the presence/absence of specific species or groups with a known tolerance to acid water.

The fauna are grouped into four tolerance categories with specified pH-intervals. These categories describe the degree of acidification and any damage caused by acidification in the localities:

I. Animals that are sensitive to acid water.
Engblom/Lingdell define the tolerance interval for this group as pH > 5.4, while Raddum uses pH > 5.5. In R's system this group is given an acidification index 1, but E/L give it an index of 4.

II. Animals that are moderately sensitive to acid water.
Both Engblom/Lingdell and Raddum define the tolerance interval for this group as pH $\geq$ 5.0.
E/L give an acidification index 3, and R a value of 0.5, which cover respectively the intervals 5.0 $\leq$ pH $\leq$ 5.4 and 5.0 $\leq$ pH $\leq$ 5.5.

III. Animals that are relatively tolerant to acid water.
Engblom/Lingdell define the tolerance interval for this group as pH > 4.5, while Raddum uses pH > 4.7.
E/L gives this group an acidification index 2, while R gives it the index 0.25, which cover respectively the pH ranges 4.5 $\leq$ pH < 5.0 and 4.7 $\leq$ pH < 5.0.

IV. Animals that are very tolerant to acid water.
Engblom/Lingdell define the tolerance range for this group as pH < 4.5, while Raddum uses pH < 4.7.
E/L gives this group an acidification index 1, while R. uses an index value 0.

In their tables of species and index values, both Engblom/Lingdell and Raddum use 0 to indicate lack of knowledge on a species' tolerance to acid. In Raddum's system, this means that persons without the necessary knowledge of the subject may confuse very tolerant species with species for which knowledge is lacking. However, this does not have any practical consequences for use of the system.

A prerequisite for using this kind of system is that the tolerance limits used are correct. In the case of certain species, the tolerance values stated by different authors and from different countries may sometimes vary considerably (table 9, page 39). The most probable reason for this variation is that the tolerance to acid water also depends on other water quality parameters. The water quality may vary from country to country, but also between different ecological regions in the same country. This applies especially to Norway, where most of the watercourses in Southern and Western Norway are relatively deficient in ions and humus, while the watercourses in Eastern Norway and parts of Finnmark have a larger content of both ions and humus. Populations that live far distant from each other may exhibit varying tolerance to acid water for purely genetic reasons, but the different stages in the life cycle may also respond differently to acid water (figure 2). Moreover, species that live in the centre of their area of distribution react in a different way to this type of environmental stress than species living on the edge of the area. The presence or absence of a particular species can be influenced by various biological conditions, such as pressure from predators, or competition for and availability of food. Possibly the varying results from different ecological regions and from different authors can also be partly explained by varying amounts of data (field data) and differences in methodology.

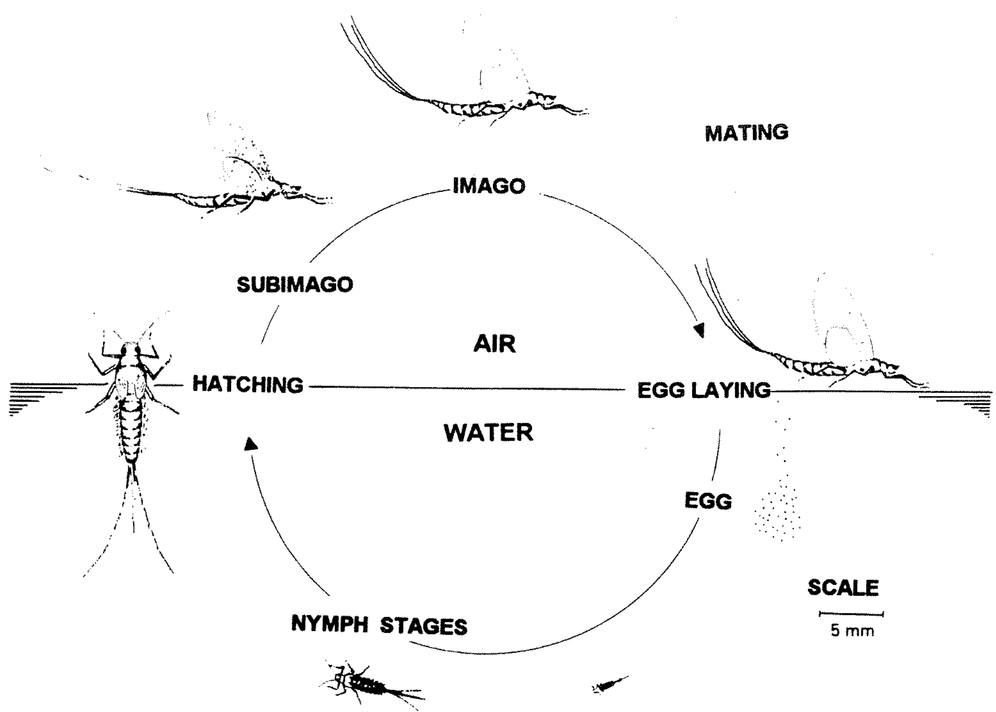


Figure 2. The life cycle of the mayfly *Baetis rhodani*. This organism often has two generations; one with nymphs in winter and one with nymphs in summer. The different stages may show different degrees of sensitivity to acidification.

Raddum limits the validity of his tolerance limits to Southern and Western Norway, and the area of validity to water quality with a low content of calcium and humus and an electrolytic conductance equal to or less than 3.0 mS/m. Engblom/Lingdell have not limited the use of their system to any specific type of water quality, and their data have been collected from most parts of Sweden. According to Engblom/Lingdell's tolerance values, a large number of species show greater tolerance to acid water in Swedish watercourses than in rivers in Southern and Western Norway. Part of the explanation may be that the content of humus and of ions is much higher in Swedish watercourses than in the watercourses in Southern and Western Norway. In Eastern Norway, and parts of the counties of Trøndelag and Finnmark, however, many of watercourses are rich in humus and, compared with the watercourses in Southern and Western Norway, the chemical and biological conditions are more like those of the watercourses in Sweden. Finnish watercourses are also generally rich in humus, and several species seem to show a different (higher) pH-tolerance in these watercourses than in Norwegian rivers and lakes.

The differences in water quality among the different ecological regions may cause a different pH-tolerance among the fauna, and thus different index values and acidification categories. This can be illustrated by taking a closer look at the mayfly *Baetis rhodani* (Fig. 1) in the different Nordic countries. Under the conditions prevailing in Norway, *Baetis rhodani* is regarded as one of the most important indicators of acidification. Firstly, it is found in all parts of the country, in the majority of streams and rivers, and at low and high altitudes. Secondly, Norwegian studies have shown this species to have low tolerance to acid water. It is normally found in water with a pH higher than 6.0, but is probably able to survive episodes with a lower pH (see section 3.1). In Swedish studies, single observations of *B.rhodani* have been reported from rivers with a pH as low as 4.6 (Engblom & Lingdell 1983). In other Swedish studies, *B.rhodani* has been found in rivers with a pH as low as about 5.3 (Otto & Svensson 1983). In an area along the west coast of Finland, *B.rhodani* has been registered in humous and polyhumous rivers (colour values between 100 and 500) with a pH as low as 4.0. Other ordinary macroinvertebrates have also been found at a lower pH in western Finland than in Norway (Nyman et al. 1986). However, in other Finnish studies that included colour values from 5 to 550, *B.rhodani* was only found in water with a high pH and low humus content (Huttunen et al. 1987), in spite of the fact that *B.rhodani* seems to accept acid water and a high content of humus in other Finnish watercourses. It is therefore possible that the presence of *B.rhodani* in these areas may be regulated to a large extent by other chemical, physical or biological factors as well. However, it still appears that many species show greater tolerance to acid water in Swedish and Finnish watercourses than in Norwegian ones.

2.3. Experience from field studies in Eastern Norway and East Finnmark

To obtain more information about the macroinvertebrate fauna in presumably acidified and non-acidified localities in Eastern Norway and in East Finnmark, and in localities with a high content of humus, a number of new localities were investigated.

Buskerud. Seven localities were investigated in the Sokna watercourse in the county of Buskerud (figure 3). Three of these were relatively rich in humus (TOC between 4.0 and 6.2 mgC/l), and two contained little humus (table 1). The ion content was low in all the localities. In general, the pH was between 5.2 and 5.5, but with a maximum of 6.0 and a minimum value of 4.8. The number of species was low, and there were relatively few individuals. The species composition of mayflies, stoneflies and caddis flies indicated marked to severe acidification (table 2). No mayflies were found at all. In general the stonefly fauna consisted of the genuses *Amphinemura* and *Leuctra*. The species *Brachyptera risi* was found in Kolsjøelva, Eidvassbekken and Fjellelva, and *Diura nanseni* in Sandvasselva. These species are given different index values by Raddum and Engblom/Lingdell respectively, which means that the localities are placed in different acidification categories depending on which system is used. The caddis flies consisted mainly of *Rhyacophila nubila*, *Plectrocnemia conspersa* and *Polycentropus flavomaculatus*, species that are very tolerant to acid water. The measured pH-values were somewhat higher than would be expected from the composition of the macroinvertebrate fauna. This applies in particular to the localities Kolsjøelva, Eidvassbekken and Buvasselva using Raddum's tolerance values, and to Sandvasselva and Buvasselva using Engblom/Lingdell's values. It is very probable, however, that the pH-vales during the thaw are much lower than shown in table 1. In two localities in the uppermost reaches of the Aurdal watercourse (Vassfaret), the fauna was more charcteristic of normal, non-acidic conditions. The pH-values in these localities were high; around 7. When Raddum's and Engblom/Lingdell's index values were used to characterize these localities, they indicated that the fauna were only slightly affected, or were not affected, by acidification (figure 3).

Akershus. Three localities were investigated in the county of Akershus (figure 3). Two of these had a relatively low content of humus, while the third had a moderately high content of humus. pH-values were low in all three localities. The fauna was meagre (table 3). Slightly more stonefly species were found at the station with the highest pH. *Leptophlebia vespertina* was the only mayfly recorded, and then only at the locality with the highest content of humus. This species is tolerant to acidification. The fauna indicate severe acidification damage in all three localities.

Table 1. Hydrochemical data and acidification categories (R=Raddum, E/L = Engblom/ Lingdell) for localities in the counties of Buskerud and Akershus (see figure 3).

Name of locality Stream - brook	Date	pH	Conductivity mS/m	Ca mg/l	Alk µequiv/l	labile Al µg/l	TOC mgC/l	Acidification category R	 E/L
Buskerud									
Kolsjøelva	79.03.22	5.2	1.6	1.1	-	-	6.2		
	89.09.28	5.4	1.5	1.1	2.5	66	4.8	0	2
Eidvassbekken	79.03.22	5.4	1.7	1.7	-		5.1		
	89.09.28	5.5	1.6	1.1	3.1	36	4.8	0	2
Sandvasselva	79.03.21	5.4	1.4	0.9	-	-	4.3		
	89.09.28	6.0	1.4	1.2	4.2	21	4.0	0.5	1
Buvasselva	82.05.27	5.2	1.7	1.0	-	-	-		
	83.03.20	5.4	2.1		2.9	-	-		
	86.09.13	5.3	1.3			110	2.7	0.5	1
Fjellelva	82.05.27	4.8	1.6	0.4	0				
	86.09.12	5.2	1.2	0.6	-	136	1.8	0	1
Akershus									
Elvasselva	87.10.03	5.3	2.0	1.2		93	3.9		
	88.11.01	5.2	1.9	1.0		110	3.1		
	89.11.16	5.3	2.0	1.0	0	90	2.1	0	1
Holvassbekken	87.11.10	5.0	3.2	1.5		95	7.5		
	88.11.05	5.0	2.9	1.4		106	7.7		
	89.11.16	5.3	2.0	1.0		89	5.6	0	1
St. Lysernbekken	87.11.10	5.1	2.8	1.2		179	3.3		
	88.11.05	5.1	2.7	1.2		185	3.0		
	89.11.05	5.2	2.8	1.1		169	2.4		

Table 2. Mayflies, stoneflies and caddis flies at different localities in the Sokna watercourse in Buskerud. 28.09. 1989. Symbols as - , + , ++ , +++ denote respectively: No individuals, few-, a moderate number -, and many individuals observed in the sample.

Locality Stream - brook	Kolsjø elva	Eidvass bekken	Sandvass elva	Buvass elva	Fjell elva
Mayflies	-	-	-	-	-
Stoneflies					
Diura nanseni	-	-	+	-	-
Taeniopteryx nebulosa	++	+	+	-	-
Brachyptera risi	+	+	-	-	+
Amphinemura spp.	+	+++	++	+	++
Nemoura spp.	-	++	-	-	++
Protonemura meyeri	-	-	-	-	+
Leuctra sp.	++	++	+	+	++
Caddis flies					
Rhyacophila nubila	+	+	+	-	-
Oxyethira	-	-	-	+	-
Plectrocnemia conspersa	-	+	+	+	+
Polycentropus flavomaculatus	+	-	+	+	-

Table 3. Mayfly, stonefly and caddis fly species observed at three localities in Akershus. 16. 09. and 17. 11. 1989. - , + , ++ , +++ denote respectively : No individuals, few-, a moderate number- and many individuals observed in the sample.

Locality Stream - brook	Elvass elva	Holvass bekken	Store Lysern bekken
Mayflies			
Leptophlebia vespertina	-	+	-
Stoneflies			
Amphinemura spp.	+	-	-
Nemoura spp.	-	+	+
Leuctra sp.	++	-	-
Caddis flies			
Plectrocnemia conspersa	++	++	++
Polycentropus flavomaculatus	++	-	-
Limnephilidae indet.	-	-	+

Hedmark. Six localities, all of them rich in humus, were investigated in the county of Hedmark. The total organic carbon content (TOC) varied from 6.2 to 13.5 mgC/l. In five of the localities the pH values were between 4.5 and 5.0, while the value recorded at the sixth locality (Ulvåa) was 6.6 (table 4). The composition of the fauna was more or less the same in all five acid localities (table 5). The variation between the different stations was greatest for the stoneflies, but the species *Nemoura*, *Amphinemura*, *Taeniopteryx nebulosa* and *Leuctra hippopus* were found in most of them. The largest number of stonefly species was found in Ulvåa. In the acid localities, the mayfly fauna consisted of only one species; *Leptophlebia vespertina*. No mayflies were found in Ulvaa. Only small variations were found for the caddis flies. In general, the species *Rhyacophila nubila*, *Plectrocnemia conspersa* and *Polycentropus flavomaculatus* were found at all the localities. Using Engblom/Lingdell's tolerance limits, the composition of the fauna indicates that all the stations except Ulvåa have suffered severe acidification damage. Ulvåa was characterized as moderately damaged by acidification. Using Raddum's tolerance limits, the composition of the fauna indicates that two of the acid localities and Ulvåa have suffered moderate acidification damage, while the other three are regarded as severely damaged. When the index values are compared with the measurement of pH, Engblom/Lingdell's values seem to give a more correct picture than shown by Raddum's values. The difference is due to the different values given by Engblom/Lingdell and Raddum respectively to the stonefly *Isoperla* (table 9). As regards Ulvåa, Raddum's values seem to give a more correct picture. It must be emphasized, however, that the results are based on a small sample of material, taken once a year. The results would be more reliable if samples were also taken early in the spring.

East Finnmark. It was found that the fauna in East Finnmark consisted to a large degree of the same species as observed further south. Several of the watercourses showed clear signs of acidification damage (figure 4). In most of the localities, however, the fauna was normal (Bækken & Aanes 1990b).

At one locality in Dalelva, NIVA has established a station for continuous monitoring of various chemical parameters, including pH. This provided an opportunity to compare the fauna with the pH during the most acid episodes. By applying Raddum's index values to the macroinvertebrate fauna, the expected pH-range during the acid period was defined as 5.0 - 5.5. It was found that this pH range was recorded for about one month during the thaw. When Engblom/Lingdell's values were applied, a pH-range of 4.5 - 4.9 was indicated for the acid episodes. The measurements show that for a short period lasting for 2 days the pH was between 4.7 and 5.0 at the locality in Daleelva. In this case it is natural to link the index values with different episodes. The question then becomes, what do we want to measure; the pH-range during the most acid of all the episodes, even if this episode may have been of short duration, or an average over an acid episode of longer duration. As yet, we do not know how long an acid episode must last, and how acid the water must be before this has a specific effect on a specific macroinvertebrate community. However, as part of the present project, we have started to investigate these questions in more detail at NIVA's ecotoxicological laboratory, established to study running water (lotic) biotopes.

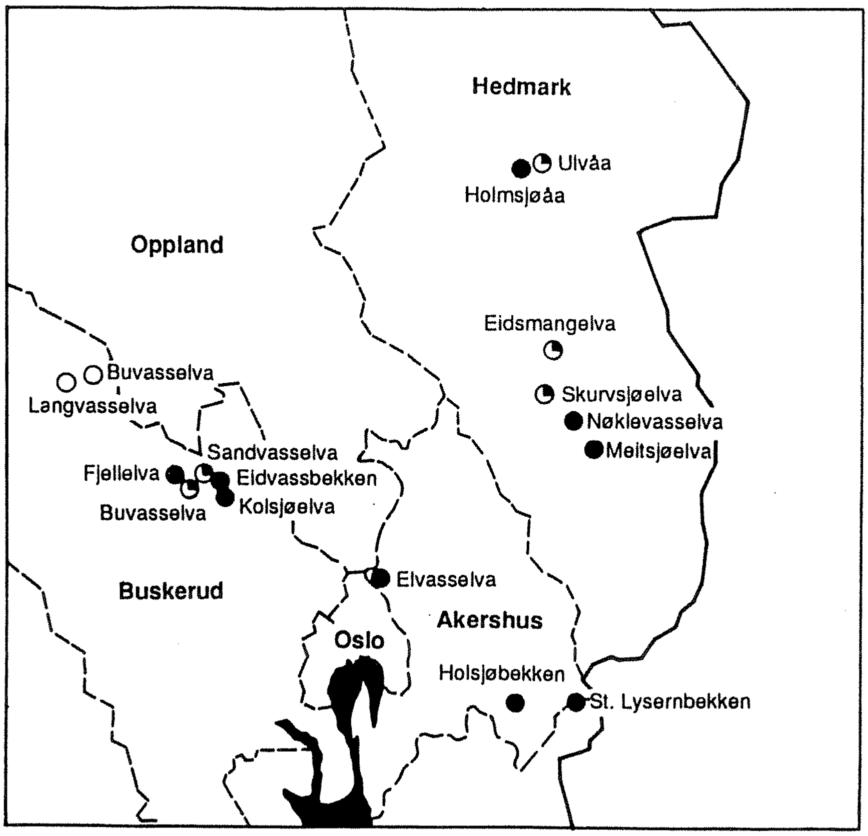
Table 4. Hydrochemical data and acidification categories for localities in Hedmark. (R=Raddum, E/L=Engblom/Lingdell).

Name of locality	Date	pH	Cond. mS/m	Ca mg/l	Alk µequiv/l	Labile Al µg/l	TOC mgC/l	Acidification category R	E/L
Skurvsjøelva	87.10.10	4.5	2.4	0.7	-	107	11.5		
	88.10.05	4.5	2.7	0.7	-	131	12.1		
	89.10.19	4.7	2.3	0.7	0	133	8.5	0.5	1
Eidsmangelva	87.10.10	5.0	1.8	1.0	-	119	9.2		
	88.10.22	4.9	2.0	1.3	-	93	10.7		
	89.10.19	5.5	1.8	1.4	14	44	7.6	0.5	1
Meitsjøelva	87.10.10	4.7	2.4	1.1	-	64	12.4		
	88.10.22	4.7	2.5	1.2	-	86	13.5		
	89.10.19	5.0	2.2	1.1	0	70	8.8	0	1
Nøklevannselva	87.10.10	4.7	2.0	0.6	-	65	7.0		
	88.10.22	4.6	2.1	0.6	-	69	8.0		
	89.10.19	4.8	2.0	0.6	0	73	5.3	0	1
Holmsjøelva	87.10.09	4.7	1.9	1.0	-	24	8.9		
	88.10.20	4.9	1.6	1.0	-	37	6.2		
	89.10.19	5.2	1.4	0.9	0	22	4.0	0	1
Ulvåa	89.10.19	6.6	2.3	2.6	94	10	6.7	0.5	2

The degree of acidification at the above-mentioned localities can be presented by means of index values presented in the forms of a table, but they should also be presented on a map, with the localities dotted in. This can be done in black and white by shading different shares of a circle. If a coloured figure is used, the dots should have the same colour as used by SFT and NIVA to denote degree of pollution; the colours blue, green, yellow and red denote four degrees of pollution, from respectively no visible pollution (no deviation from the natural state) to severe pollution (large deviation from the natural state). If the investigation covers large or small parts of the watercourse, it is informative to present the results by using different colours for areas of the watercourse with different degrees of acidification (figure 4).

Table 5. The mayfly, stonefly and caddis fly fauna at six localities in Hedmark. 19 October 1989. Symbols as - , + , ++ , +++ denote respectively: No individuals, few-, a moderate number-, and many individuals observed in the sample.

Name of locality :	Skurv.	Eidsm.	Meits.	Nøkle.	Holms.	Ulvåa
Mayflies						
Leptophlebia vespertina	++	+	+	+	++	-
Stoneflies						
Diura nanseni	-	-	-	-	-	+
Isoperla sp.	+	++	-	-	-	-
Taeniopteryx nebulosa	-	+	+	+	++	+
Brachyptera risi	-	-	-	-	-	+++
Amphinemura spp.	+++	-	+++	+	-	+++
Nemoura spp.	+	-	++	+	+	+
Protonemura meyeri	-	-	-	-	-	++
Leuctra sp.	++	++	+	++	-	+++
Caddis flies						
Rhyacophila nubila	-	-	+	+	+	+
Oxyethira	+	-	-	-	+	-
Plectrocnemia conspersa	+	+	+	+	+	+
Polycentropus flavomaculatus	-	+	++	++	+	-
Limnephilidae indet.	-	-	+	-	-	-



● **Severe acidification damage**

◑ **Marked acidification damage**

◔ **Moderate acidification damage**

○ **No acidification damage**

Figure 3. The acidification status at localities in the counties of Buskerud, Akershus and Hedmark, stated in terms of acidification damage to the macroinvertebrate community.

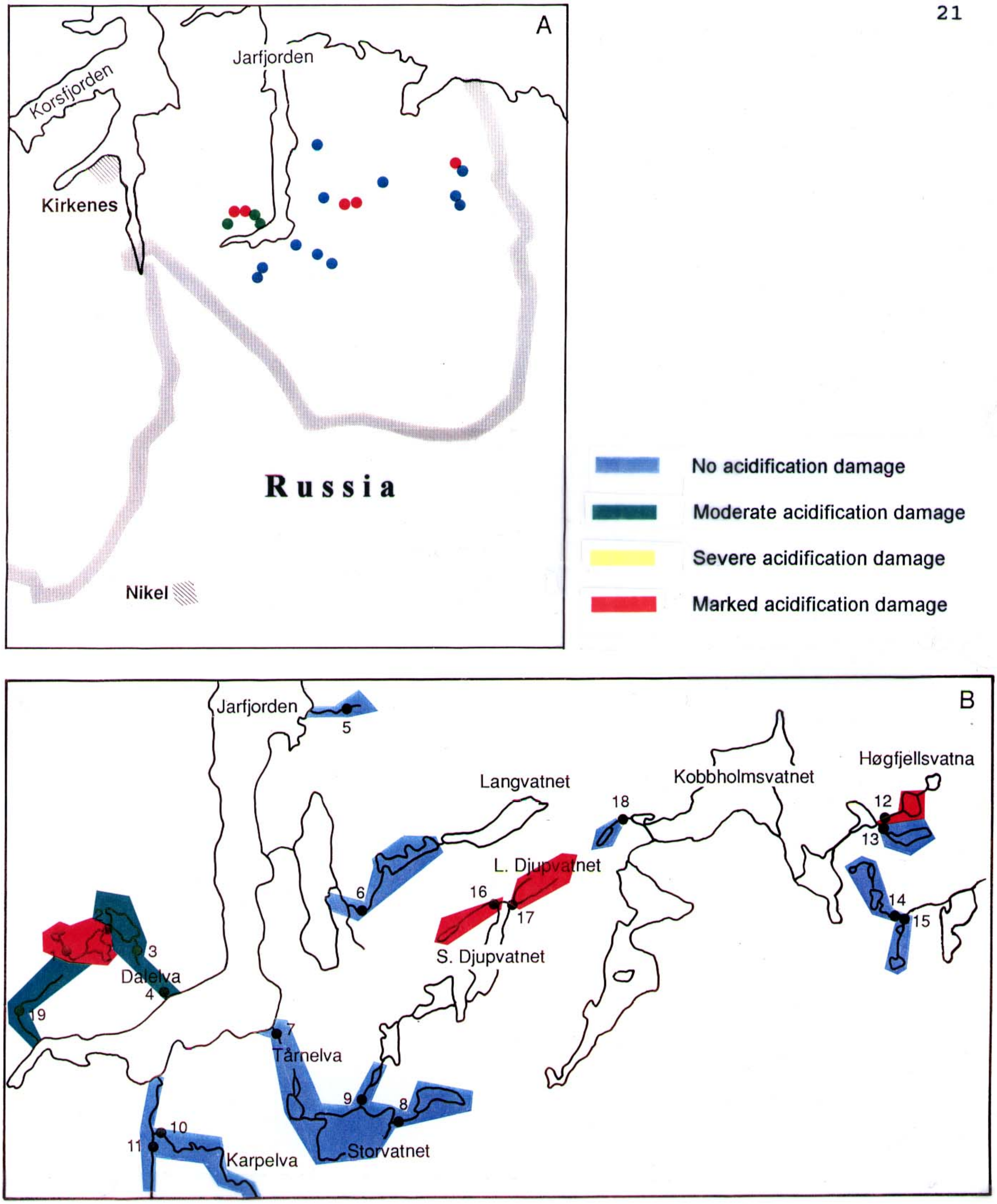


Figure 4. Acidification status in selected watercourses in the municipality of Sør-Varanger, in the county of Finnmark in northern Norway.
A) gives an overall picture and **B)** shows the part of the watercourse that is known to be covered by the investigation. The results are based on knowledge about the pH-tolerance of macroinvertebrates.

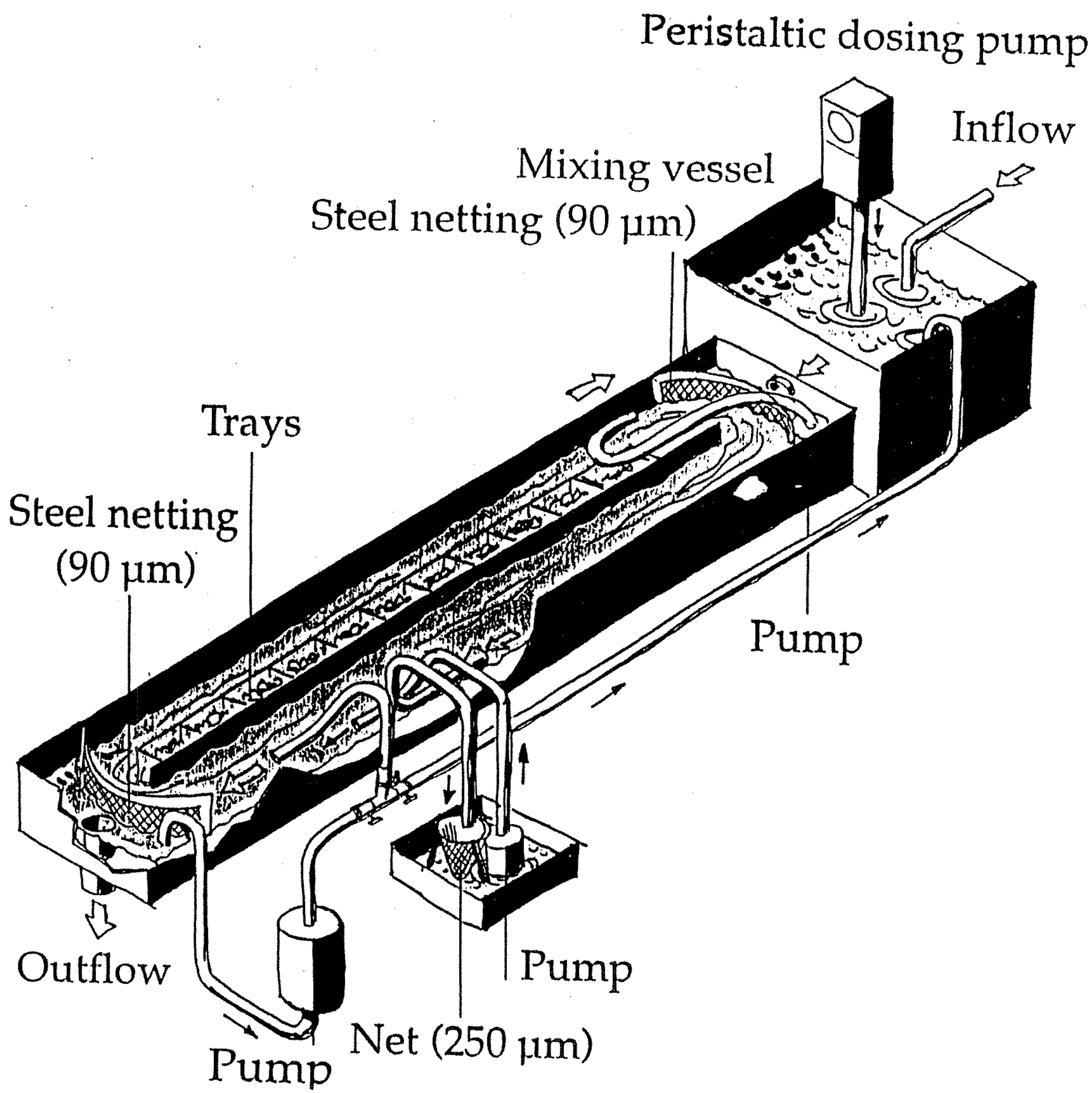


Figure 3.1.1 One of the indoor experimental streams.

3. EXPERIMENTAL STUDIES ON DAMAGE FROM ACIDIFICATION

3.1 Simulation of episodic falls in pH

3.1.1 Background

The acid episodes caused directly by acid rain or by acid water run-off during the thaw are often a decisive factor for the composition of the fauna in the watercourses that become acidified to varying degrees. We do not know with any certainty however, how long the extreme acid episodes must last in order to cause visible reductions in a specific macroinvertebrate community. It is therefore impossible, with our current knowledge, to state the duration of the most acid episodes with an acceptable degree of accuracy based on the macroinvertebrate community.

In an attempt to answer this question, two acidification situations were tested at NIVA's ecotoxicological testing station, (figure 3.1.1) for running water:

Experiment 1. A brief acid episode lasting 10 hours, with a drop in pH from 6.3 to 4.3.

Experiment 2. Two consecutive brief acid episodes with a drop in pH from 6.3 to 4.3 and an intermediate period with a pH around 5.4. This entire episode lasted for one whole day and 10 hours (i.e. 34 hours). This situation reflects a pattern of acidification that has been recorded, for example, in Dalelven in Sør-Varanger, Finnmark (Bækken and Aanes 1990b).

In order to carry out the tests, a macroinvertebrate community representative of the fauna in a slightly acidified river was brought to the laboratory. This was done by placing 30 boxes (15 x 11 x 6 cm) filled with a defined composition of the river substrate in a neighbouring river in order to colonize them with the natural fauna at the site. The boxes remained there for 4 weeks. 10 boxes selected at random were counted at the start of the experiment, to show the composition of the macronivertebrate community before acidification. The other 20 boxes were divided between 2 artificial river systems, 10 being placed in each of them. In addition, 45 individuals of the mayfly species *Baetis rhodani* were added to each river system at two different times (30 + 15). This species was not recorded in the boxes containing river substrate, but its presence was desirable because it is one of the most important indicators of acidification.

One of the artificial rivers was acidified, while the other functioned as a reference river. The water was acidified with continuous doses of diluted sulphuric acid (H_2SO_4).

Many aquatic macroinvertebrate species drift with the water current when the environment becomes too unfavourable. This has been recorded, for example, when the water becomes acidified (Weatherley et al. 1988). Before and after the acid episodes, the drift of organisms was recorded every 12 hours. During the acid episodes, the drift was recorded every 2 hours. At the end of the experiment the composition of the fauna was recorded in both river systems.

3.1.2 Drift of animals after brief acidification episodes

At the start of the experiment, 30 individuals of the mayfly species *Baetis rhodani* were placed in each river. During acidification situation 1), with one brief fall in pH, the total number of animals in the drift was low. In the course of the period before acidification the total drift was between 2.5 and 4.7 individuals per 12 hours in the river that was to be acidified, and between 0 and 1 individuals in the reference river (figure 5). During this period, the pH was around 6.3 in both rivers. During the acidification, the pH dropped from 6.3 to 4.3 in the course of two hours. The drift was 0 individuals per 12 hours in the acidified river and 1.3 individuals per 12 hours in the reference (non-acidified) river. The following night, the drift was 1 animal in the acidified river and 0 in the reference river. The number of *Baetis rhodani* in the drift was larger before the acidification than during or after the acidification. The results do not support the general assertion that the drift of macroinvertebrates increases with acidification. The material was small, however, and does not provide a basis for conclusions concerning drift in relation to acidification.

Before the next acidification episode, an additional 15 individuals of *Baetis rhodani* were added. During the acidification the pH was lowered from 6.3 to 4.3 over a period of 2 hours (figure 5). In the course of the evening, the pH rose again to 5.4 before being lowered again to 4.3 on the following day. The largest drift was again found before the acidification, with 10 macroinvertebrates in the acidified river and 10 in the reference river. In both rivers, 6 of these individuals were *Baetis rhodani*. During the acidification, the total number was between 0 and 4 per 12 hours in the acidified river, and between 2 and 4 in the reference river. In the reference river, a 24-hour variation could be registered, with the largest drift of *B.rhodani* at night. This is a common pattern, but was not observed in the acidified river. The drift of *B.rhodani* increased in the affected river in the course of the acidification period. This may indicate an increasing tendency to drift with a longer period of acidification. The drift material was too small, however, to provide a basis for conclusions concerning drift and acidification. A count of the macroinvertebrate community used in the experiments showed a scarcity of individuals. The number of animals in the drift is partly a function of the potential drifters. Therefore the low density of animals in the community explains the small number of animals in the drift.

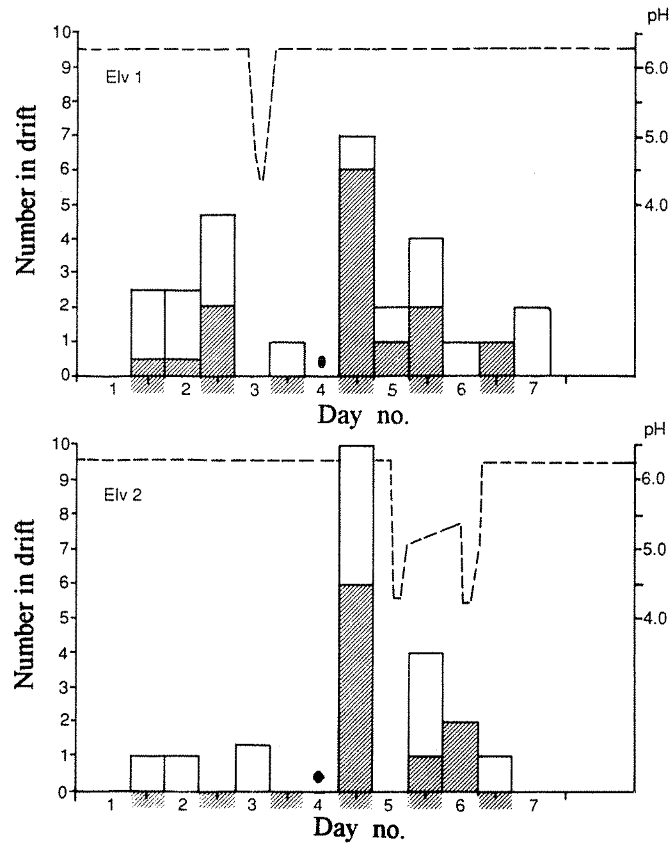


Figure 5. pH-variations and drift of macroinvertebrates with two types of acidification situations. The columns show the number of the mayfly species *Baetis rhodani* (shaded) in relation to the total number of animals in the drift per 12 hours. (Day 4 : ● Drift sampler not functioning).

3.1.3 Changes in a macroinvertebrate community after brief episodes of acidification

The number of individuals and the composition of the macroinvertebrate community before acidification showed a scarcity of individuals and, except for the extra individuals of *B.rhodani* added to the water, was typical of a slightly acidified river. The community was dominated by chironomid larvae, followed by a large number of stoneflies. There were very few mayflies originally; in addition to the 45 *B.rhodani* that were added, the recorded mayflies were 3 *Ameletus inopinatus* and 2 *Heptagenia sulphurea*. Both of these last two species are usually said to be more tolerant than *B. rhodani* to acid water.

After the acidification, the number of individuals in the community in river no. 1 (one brief episode) was reduced considerably; from a total of 527 to 369 individuals (tables 6 and 7). Here too, most of the reduction can be explained by a decrease in the number of chironomids. A marked decrease was also observed in the number of mayflies and blackfly larvae. Only small changes were recorded for the other groups. In the case of the mayflies, the number of *B.rhodani* was reduced by 31 individuals. Seen in relation to the 45 individuals at the start of the experiment, this is a reduction of 69%. Of these 31 individuals, 14 were found in the drift. None of the mayfly nymphs had reached the winged stage, so the remaining 38% had died in the river in the course of the experiment.

Table 6. Composition of the macroinvertebrate community before and after acidification of two artificial rivers with different patterns of acidification. River no. 1 was subjected to one brief acidification episode early in the experiment. River no.2 was subjected to two consecutive acidification episodes late in the experiment. The figures give the number of animals in each river. For a desription of the methodology see page 23.

	Before acidification	**After acidification**	
		River 1	River 2
Nematoda	3	1	0
Oligochaeta	7	8	2
Snails	0	1	0
Water mites	3	5	8
Mayflies	50	17	29
Stoneflies	92	92	68
Caddis flies	5	4	5
Chironomides	300	202	143
Simuliides	47	26	27
Other Dipterans	20	10	17
Beetles	0	3	6
Sum	527	369	299

In river no. 2 (two brief consecutive episodes) a considerable reduction in the total number of individuals was found after the acidification. Here too, the largest reduction was due to a decrease in the number of chironomids, but large reductions also occurred in the number of blackfly larvae, mayflies and stoneflies. Only slight changes were found in the other groups. For *B.rhodani*, the total reduction was 18 individuals, or 40%. Of these 18 individuals, 10 were found in the drift. The remaining 18% had died in the river.

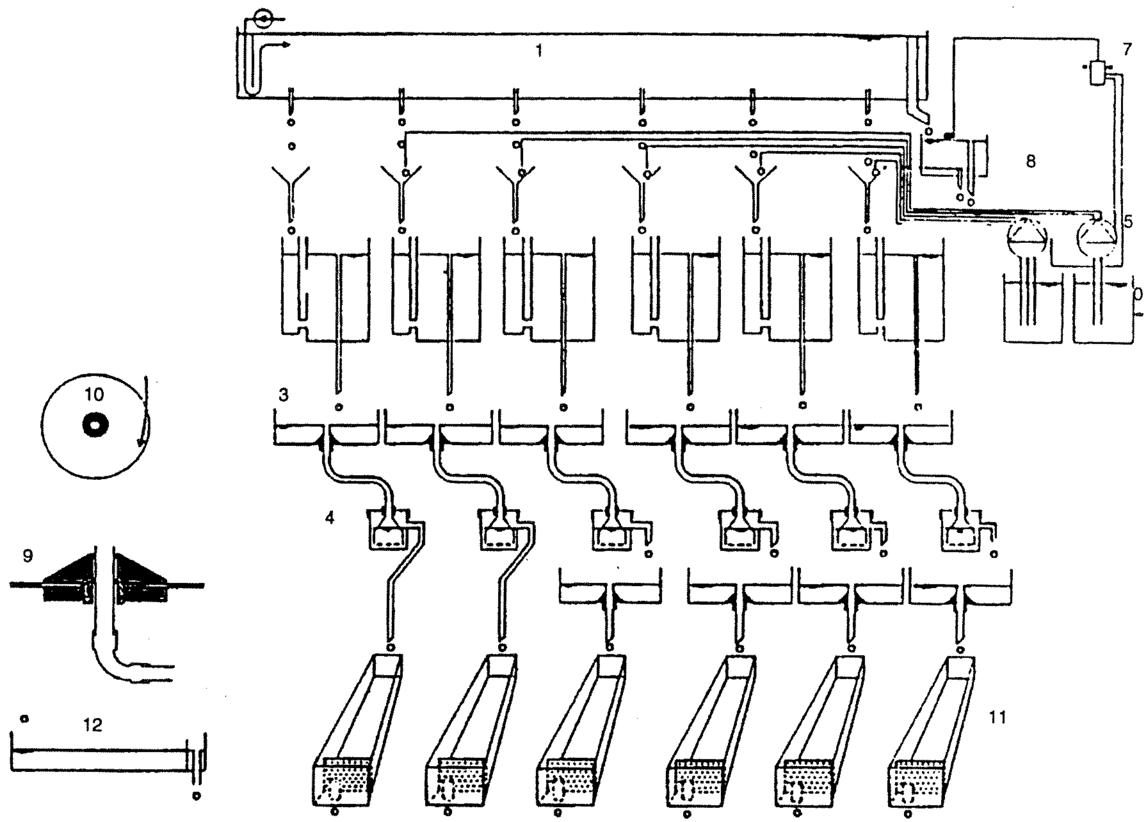
The most probable cause of the reduction in the number of macroinvertebrates in the rivers is the extreme environment they had to withstand during the acidification. The community contained a large proportion of relatively sensitive chironomids and blackfly species originally. These probably died during the actual acidification or immediately afterwards, because the mortality in river no. 2 was registered as early as 18 hours after the acidification had ceased. It was also in this river, with the longest period of acidification, that the highest mortality was recorded (table 6).

The picture was somewhat different for the mayfly *Baetis rhodani*. For this species, mortality was far lower in river no. 2 than in river no. 1, in spite of the fact that the period of acidification lasted much longer in river no. 2. A possible explanation is that the organisms die over a long period of time after the actual acidification has occurred, and the experimental period was thus too short to register mortality in river no. 2.

The experiment has shown that brief acidification episodes may cause large reductions in the macroinvertebrate community. It could not be demonstrated that relatively large individuals of the important indicator *Baetis rhodani* disappear after this kind of acidification episode, but the population was considerably reduced. We cannot ignore the possibility that even short periods of acidification may have long-term effects on eggs, small nymphs and various moulting phases of the life cycle.

Table 7. Mayflies, stoneflies and caddis flies before and after acidification of two artificial rivers. River no. 1: one episode of short duration. River no. 2: to consecutive episodes of short duration. Number of animals per river. For a description of the methodology, see page 23.

	Before acidification	**After acidification**	
		River 1	River 2
Mayflies			
Ameletus inopinatus	3	0	1
Baetis rhodani	45	14	27
Leptophlebia vespertina	2	3	1
Stoneflies			
Isoperla sp.	4	3	6
Siphonoperla burmeisteri	2	3	6
Brachyptera risi	3	0	0
Amphinemura borealis	13	8	6
Amphinemura sulcicollis	43	33	26
Protonemura meyeri	1	0	1
Leuctra hippopus	3	13	5
Leuctra nigra	2	0	2
Leuctra sp.	26	31	18
Caddis flies			
Rhyacophila nubila	0	0	1
Ithytrichia lamellaris	1	0	1
Oxyethira sp.	0	1	0
Polycentropus flavomaculatus	1	0	1
Hydropsyche siltalai	1	0	0
Lepidostoma hirtum	0	1	0
Limnephilidae indet.	2	1	3



1. Reservoir for test water
2. Mixing vessel for test solutions
3. Test aquaria
4. Sieve and test aquaria
5. Peristalic pumps with separate channels to each mixing vessel
6. Tank for chemicals to be tested
7. An automatic swith to stop the peristalic pumps if the water flow changes
8. Thank to control the flow of test water
9. Detail of the outlet from the circular aquaria
10. Test aquaria for lotic macroinvertebrates
11. Test vessel for fish and bigger evertebrates as crayfish and molluscs
12. Details of the test vessel no 11

Figure 3.2.1 The through - flow test system used for the 96 h - LC_{50} tests. (From Aanes, 1989).

3.2 Tolerance tests. The sensitivity of macroinvertebrates to acid water, and to acid water combined with aluminium and humus.

3.2.1 Background

For the large majority of the species included in the index systems for acidification, the tolerance limits have been fixed on the basis of field data. For certain localities, frequent measurements of pH and of other hydrochemical parameters are available, making it possible to relate the composition of the fauna to the chemical condition of the water. In an acidification context, it is of particular importance to take measurements during periods of the year when the acidification is highest, for example, during the thaw. It is also necessary to have a large quantity of hydrochemical and biological data. Determination of the macroinvertebrate's tolerance to acid water, based on these methods, will be reasonably good enough for rough estimations. However, tolerance limits worked out in this way can never be more than approximate. In addition, it is difficult to determine with certainty how the tolerance to acid is affected by other hydrochemical parameters. In order to obtain a more exact picture of the tolerance of a specific species to acid water, and to acid water combined with other hydrochemical or physical parameters, it is necessary to carry out tests in a laboratory.

3.2.2 pH-tolerance

Seven macroinvertebrate species were selected for testing. These were two mayfly species; *Baetis rhodani* and *Heptagenia sulphurea*, three stonefly species; *Protonemura meyeri*, *Brachyptera risi* and *Leuctra hippopus* and the two caddis fly species *Polycentropus flavomaculatus* and *Hydropsyche siltalai*. The species were tested in solutions with pH 3.0, 3.5, 4.0, 4.5, 5.5 and 6.3 over a period of 96 hours (4 days). The experiments were carried out in aquaria (figure 3.2.1) containing filtered, natural surface water. The water flow through the aquaria was continuous, at a rate of 30 l/t. A dose of diluted sulphuric acid (H_2SO_4) was added to the water continuously by means of peristaltic pumps, in order to maintain a stable pH.

The highest mortality was recorded for *Baetis rhodani*. All the individuals died in the course of 24 hours in the most acid solution (figures 6 and 7). Mortality also reached 100% in the course of the experiment at pH 3.5 and 4.0. While 50% mortality was recorded at pH 4.5, all the individuals survived at pH 5.5 and 6.3. These results gave a 96 hour LC_{50}- value of pH 4.5 for *B. rhodani* (table 8). The 96 hour LC_{50}-value is the concentration in the experimental medium at which half of the tested organisms die in the course of 96 hours, and is a common way of presenting results in an ecotoxicological context. The other mayfly, *Heptagenia sulphurea*, was more tolerant. Mortality was 100% at pH 3 and at pH 3.5, and was 70% at pH 4.0, while all the individuals survived at pH 4.5, 5.5 and 6.3. This gave an LC_{50}-value of pH 4.0 for *H. sulphurea* (table 8).

In the case of the stonefly *Protonemura meyeri*, no mortality was recorded in any of the solutions. This shows that the species is very tolerant to acid water. Of the other two stonefly species, *Leuctra hippopus* was the more tolerant (figures 6 and 7). Eighty per cent mortality was recorded at pH 3.0, but little or no mortality at the other pH-values. The results did not provide a basis for calculating LC_{50}-values. *Brachyptera risi* was the most sensitive of the selected stonefly species, with 100% mortality at pH 3.0, 85% at pH 3.5, 20% at pH 4.5, and complete survival at pH 4.0, 5.5 and 6.3. The LC_{50}-value for *B. risi* was pH 3.5.

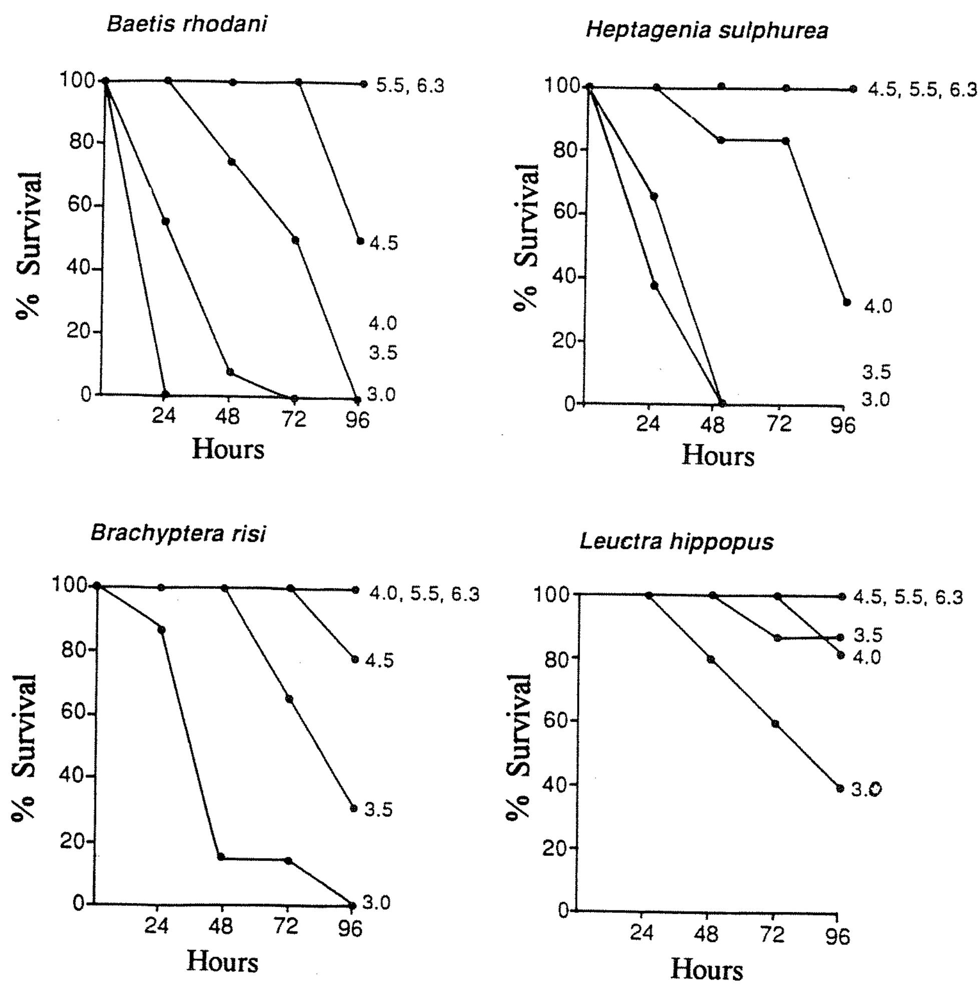


Figure 6. Percentage survival at different pH-values at different times in the course of 96 hours for the two mayfly species: *Baetis rhodani* and *Heptagenia sulphurea* and two stonefly species: *Brachyptera risi* and *Leuctra hippopus*.

Both the tested caddis flies were found to be very tolerant to acid water. For these species, almost no mortality was observed in any of the solutions (figure 7).

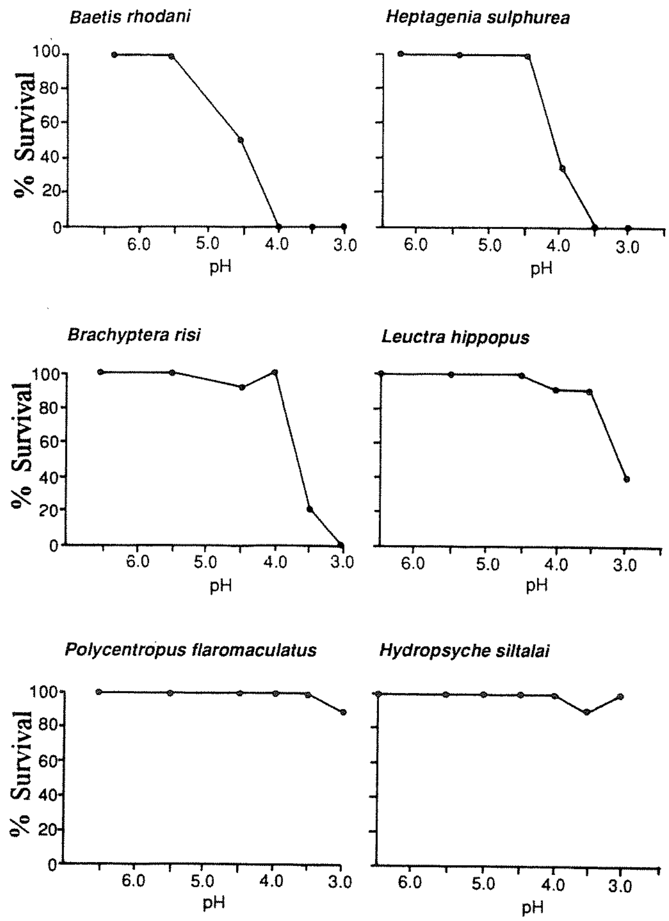


Figure 7. Percentage survival seen in relation to pH for two mayflies: *Baetis rhodani* and *Heptagenia sulphurea*, two stoneflies: *Leuctra hippopus* and *Brachyptera risi* and for two caddis flies: *Polycentropus flavomaculatus* and *Hydropsyche siltalai*.

Table 8. 96 hours LC_{50}-values for the mayflies *Baetis rhodani* and *Heptagenia sulphurea*, and the stonefly *Brachyptera risi* in relation to acid water (pH).

	pH
Baetis rhodani	**4.5**
Heptagenia sulphurea	**4.0**
Brachyptera risi	**3.5**

The LC_{50}-values show the relative difference in pH-tolerance between the macroinvertebrates. The tests also show the highest acid concentration they can survive, at least for short periods. The differences between *B.rhodani* and *H.sulphurea* indicate that these two species have been placed in the right group in Raddum's proposed index, even though the tests were carried out in water somewhat richer in ions than recommended by Raddum with regard to the validity of his system. This indicates that the index values also apply to water with a higher ion content than has been recommended up to now (maximum 3.0 mS/m). Raddum places *B.risi* in the group of species with the highest tolerance to acid (pH < 4.7). Engblom/Lingdell place *B.risi* in the group with the second highest tolerance (4.5 ≤ < 5.0) (see table 9). The above laboratory results support Engblom/Lingdell's proposal. The other three species that were tested were all very tolerant to acid. This applied especially to the caddis flies. Both Engblom/Lingdell and Raddum place *L.hippopus* and *P.flavomaculatus* in the group with the highest tolerance to acid. Engblom/Lingdell place *H.siltalai* in the most tolerant group, while Raddum places this species in the group with second highest tolerance to acid (5.0 ≤ pH ≤ 5.5). Our results support Engblom/Lingdell's proposal.

3.2.3 pH-Al-tolerance

The aluminium content in our rivers and lakes, and especially the labile (the toxic) part of the aluminium fraction increases with increasing acidity. It has been shown in fish that aluminium is an important cause of mortality in acidified water (Rosseland et al. 1990). Because the quantity of aluminium and the pH co-vary, controlled tests have to be carried out in order to distinguish the effects of the one from those of the other. Very little research has been done on the effects of aluminium on macroinvertebrates. Some results indicate that aluminium is toxic to these organisms too (Burton & Allan 1986, Herrmann & Andersson 1986, McCahon & Pascoe 1989, Ormerod et al. 1987, Weatherley et al. 1988). Other results seem to show the opposite; a higher aluminium content can lead to higher survival of macroinvertebrates in acid water (Herrmann 1987, Palawski et al. 1989). In an attempt to clarify this issue, 96-hour tests were carried out at pH values 4.7 and 4.5 against an aluminium gradient. The tests were performed at NIVA's biotesting laboratory, in aquaria with a through flow of water (30 l/h) to which doses of aluminium chloride ($AlCL_3$) and diluted sulphuric acid (H_2SO_4) were added continuously. The nominal values of the doses of total aluminium were 1.1, 0.6, 0.3, 0.2 and 0.1 mgAl/l. A substantial part of the total aluminium was labile aluminium (L. Al) (figures 8 and 9). The temperature was approximately 4°C.

The following species were tested: The mayflies *Baetis rhodani* and *Heptagenia sulphurea*, the stoneflies *Isoperla grammatica, Amphinemura sulcicollis, Protonemura meyeri, Brachyptera risi* and *Leuctra hippopus*, and the caddis fly *Hydropsyche siltalai.*

At pH 4.7, no mortality was recorded for any species other than *Baetis rhodani*. The highest mortality was recorded with the lowest concentration of aluminium. While only 50% survived at this concentration, between 80 and 100% survived at the higher concentrations (figure 8).

At pH 4.5, except for one individual of *H.sulphurea*, mortality was recorded only among *B.rhodani*. On this occasion, two groups of *B.rhodani* were tested; one consisting of small and the other of large individuals. Mortality was clearly highest with the lowest aluminium content. In this case mortality was 100% in both groups. In the aquaria that were dosed with aluminium, survival increased to between 60 and 80% for the large individuals and to between 10 and 50 per cent for the small ones.

In these experiments on pH and Al, the degree of survival did not change very much for the large individuals after the Al-concentration had reached a certain level (approx. 140 μg L. Al/l). For the small individuals, however, a larger number of individuals survived with increasing L. Al-concentration. However, other results, though uncertain, tend to indicate that in these tests, the mortality of small individuals of *Baetis rhodani* was highest at the highest concentrations of aluminium.

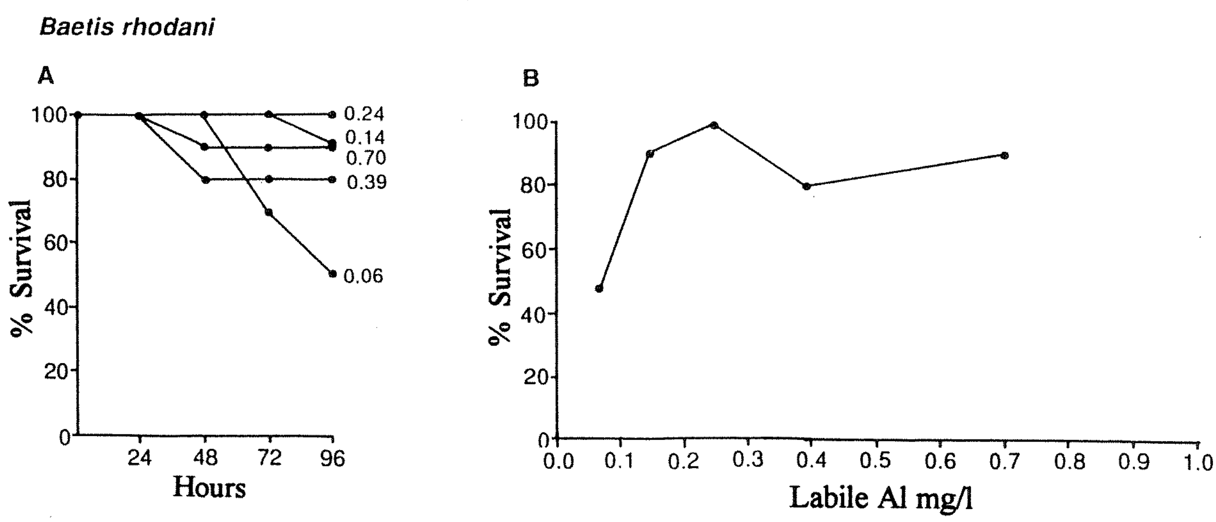


Figure 8. **A.** Percentage survival at different times in the course of 96 hours of the mayfly *B.rhodani* at pH 4.7 and with varying concentrations of labile aluminium.
B. Percentage survival in relation to concentration of labile aluminium.

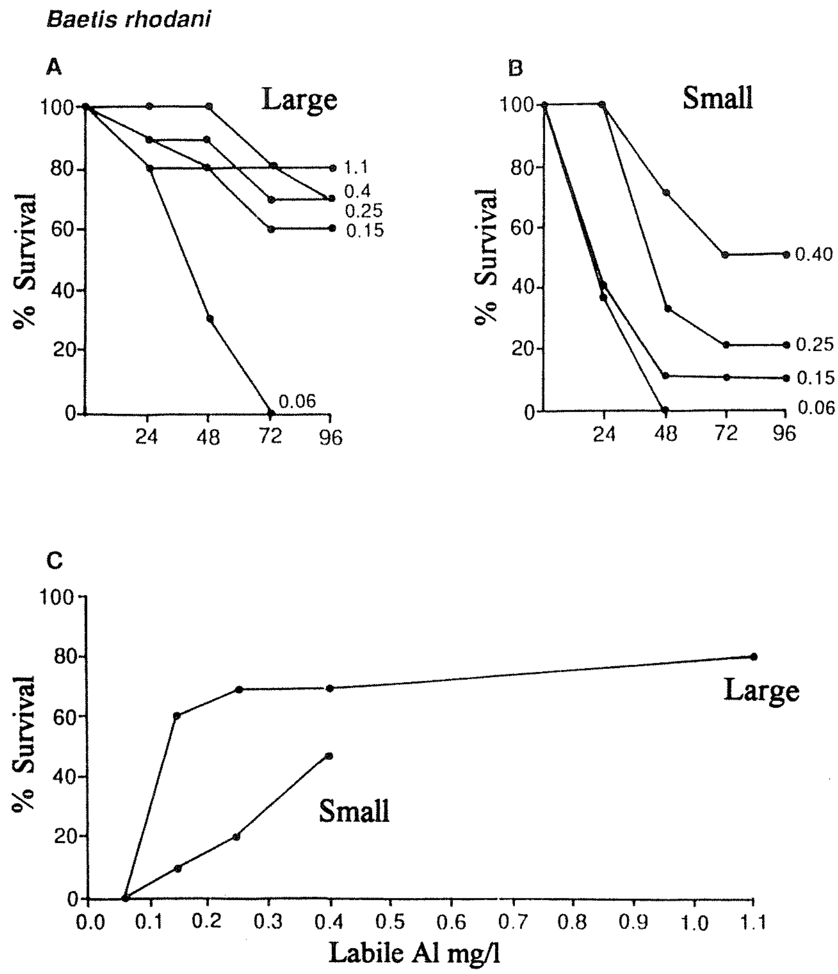


Figure 9. Percentage survival at different times in the course of 96 hours at pH 4.5 and varying concentrations of labile aluminium of **A.** large *Baetis rhodani* and **B.** small *B.rhodani*. **C.** shows the survival of large and small *B.rhodani* in relation to labile aluminium.

3.2.4 pH-humus tolerance

The fact that different authors have found different pH-tolerances for the same macroinvertebrate species has led to speculations about the effect of humus on tolerance to acid. In general, the field data tend to indicate that, in the case of several macroinvertebrate species, a higher humus content increases tolerance to acid. Hargeby & Petersen (1988) suggest that low humus concentrations may have a favourable effect on organisms in acidified rivers. Few reports have been published on experimental studies in this connection, and the question has by no means been clarified.

In an attempt to find out whether humus may alter the pH-tolerance, two species were selected for testing in the laboratory: the mayfly *Baetis rhodani* and the crustacean *Gammarus lacustris*. Both species are regarded as being sensitive to acidification under Norwegian conditions. *B.rhodani*, however, has been also found in very acid water with a high content of humus in localities in Finland.

The tests were performed in two pH-ranges:

A: pH around 4.5, with a maximum value of 5.2 and a minimum value of 4.4.

B: pH around 5.0, with a maximum value of 5.8 and a minimum value of 4.8.

The nominal values of the humus concentrations (TOC) in experiment A were 3, 5, 7, 15 and 20 mgC/l. In experiment B, the humus concentrations (TOC) were 3, 5, 10, 15 and 20 mgC/l. The tests were carried out in small aquaria with no through flow of water. The pH was measured and adjusted at regular intervals. The temperature varied between 10 and 15°C. The experiments lasted for 8 days.

<u>**A:**</u> Around a pH of 4.5, low mortality was recorded for *Baetis rhodani* after 4 days. The reason was that during the period from day 2 to day 4 the pH had risen to 5.2. After adjusting the pH down to 4.4, high mortality was recorded on the following day (figure 10). At this point in time, there was a marked difference in mortality between the animals placed in solutions with a high humus content and those in solutions with little humus. While mortality was 50% in the solution containing 20 mgC/l, it was 90% in the solution with 5 mgC/l and 100% in the solution with 3 mgC/l. After a further three days, mortality was 90 or 100% in all the solutions.

This experiment shows that a higher content of humus (TOC) leads to a higher percentage survival for the mayfly *Baetis rhodani* during short periods with low pH (4.5). During longer periods with low pH, a high humus content does not reduce mortality to any great extent.

<u>**B:**</u> At a pH around 5.0, mortality was low for *B.rhodani* during the first few days. This was expected, especially after the pH rose to a maximum value of 5.8 from day 2 to day 4. After the pH was adjusted to 4.9. higher mortality was recorded for the solution with a TOC-value of 3 mgC/l, while no changes were registered for the other test solutions. At the end of the experiment, mortality was 80% in the solutions containing respectively 3 and 5 mgC/l, but only 20% in the solutions with respectively 10 and 15 mgC/l.

The experiment shows that, with a pH around 5, a higher content of humus (TOC) leads to higher percentage survival for the mayfly *Baetis rhodani*. This applies for longer periods than when the pH is 4.5, and at least during a period of 8 days, which was the time these experiment lasted.

In the case of the crustacean *Gammarus lacustris*, mortality after 8 days at pH 4.5 was clearly dependent on humus content. Mortality at the end of the experiment was 80% both in the solution containing 3 mgC/l and in the solution containing 5 mgC/l, but was 30% in the solution containing 20 mgC/l. With a pH of around 5.0, the total mortality was much lower. The highest mortality, 40%, was recorded in the solution with the lowest humus content (3 mgC/l). Mortality gradually decreased with an increasing content of humus. In the solution with 20 mgC/l, mortality was only 10% after 8 days (figure 11). Both these experiments clearly show that, in *Gammarus lacustris*, pH-tolerance increases with increasing humus content.

It can be concluded that humus reduces the harmful effect of low pH on the macro-invertebrates *Baetis rhodani* and *Gammarus lacustris*. The experiments support the hypothesis that acidified localities with a high humus content are more capable than corresponding localities with little humus are of maintaining a macroinvertebrate community sensitive to acidification. It is clear, however, that the effect of the humus depends on how long the acidification episode lasts, and how acidified the water becomes.

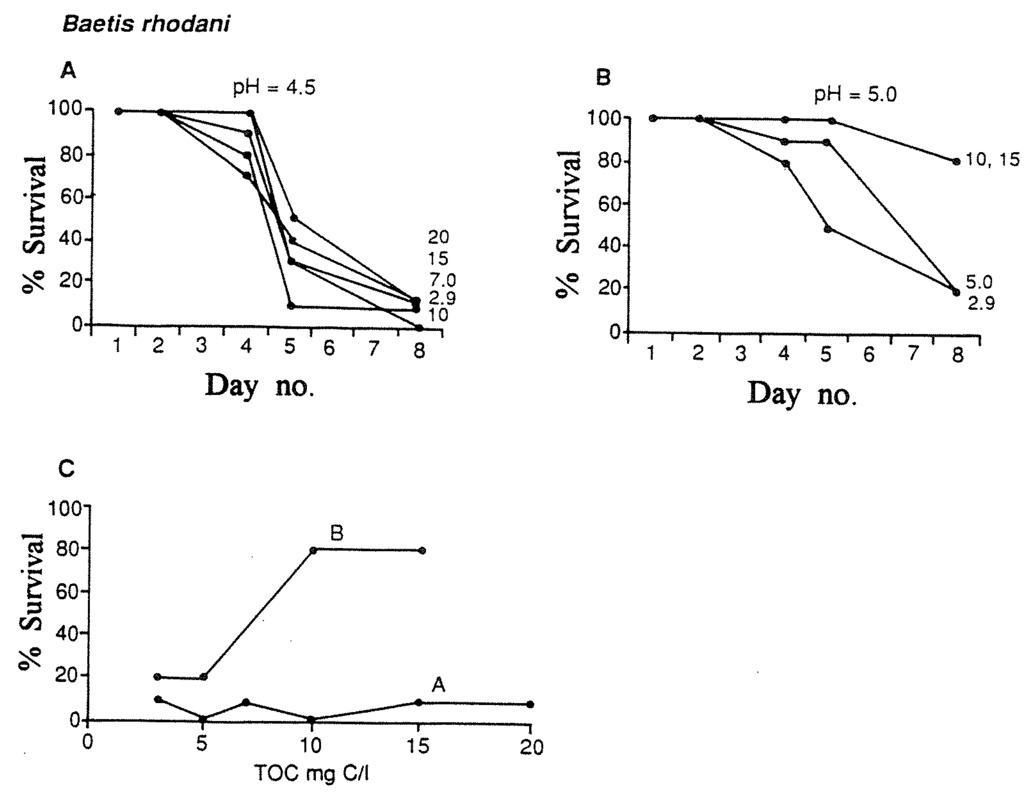


Figure 10. Percent survival in the course of 8 days for *Baetis rhodani* with varying humus content (Total Organic Carbon, TOC). **A.** pH around 4.5. **B.** pH around 5.0. Figure **C.** shows survival in relation to humus content, graph A at pH 4.5, and graph B at pH 5.0.

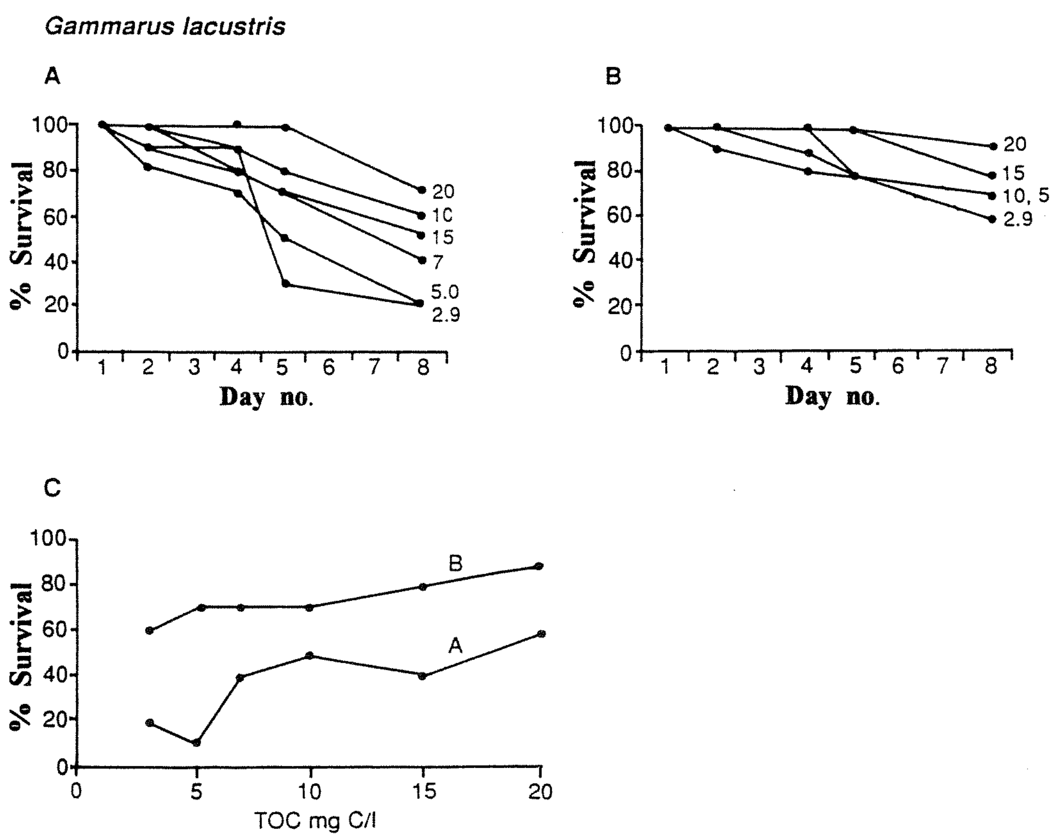


Figure 11. Percentage survival at different times in the course of 8 days for *Gammarus lacustris* with varying contents of humus. **A.** pH around 4.5. **B**, pH around 5.0. Figure **C.** shows survival seen in relation to humus content, graph A at pH 4.5 and graph B at pH 5.0.

4. ADJUSTMENT AND USE OF ACIDIFICATION INDICES BASED ON MACROINVERTEBRATES.

4.1 The tolerance of macroinvertebrates to acidification

Although many macroinvertebrates are placed in the same acidification class in the systems proposed by Raddum and by Engblom/Lingdell respectively, in many other cases the classifications differ (table 9). If an index system is intended to apply to the whole of Norway, some adjustments will have to be made in index values which, in principle, have been calculated for Southern and Western Norway only. Nor is it possible, without reservation, to use index values based on conditions in Sweden. It can be useful, however, to compare these sets of index values and to evaluate them in relation to other information on the tolerance of the different species to acid water.

In this connection, it must be emphasized that tolerance to various types of acid water, that is to say, a high content of H^+ ions (low pH) with the accompanying chemical variables, is not necessarily the same as tolerance to acidification. This is because acidification, in addition to its direct chemical effects on the animals, can also affect them indirectly through changed conditions with regard to food supply, competition and predators. In the following pages, a number of common macroinvertebrates and important indicator species will be discussed, and their position in the index system evaluated.

Snails are found mainly in localities with a high content of calcium and a pH higher than 6.0. According to Økland, they are not found in localities with a pH lower than 5.2, and only a few species are found with a pH between 5.2 and 6.0 (Økland, J. 1969). Økland's values refer to lakes and surface water in summer. *Lymnaea peregra* and *Gyraulus acronicus* are two of the most common freshwater snail species in Norway, and are widespread in most parts of the country. They are found mainly in lakes, but are also common in many rivers. Of the 684 lakes where *L.peregra* was recorded, Økland found only one with a pH lower than 5.5 (Økland, J. 1983). *G.acronicus* seems to show the same pH tolerance as *L.peregra*. It is therefore likely that localities where these species are found, and where snails in general are found, have not been damaged to any noticeable degree by acidification.

The crustacean amphipod, *Gammarus lacustris*, is regarded as a species that is sensitive to acidification. It lives mainly in lakes, but is also found in rivers, especially downstream of lakes with amphipod populations. According to Økland, K.A. (1980), it is not found in localities with a pH lower than 6.0. Since this refers to lakes under summer conditions, it is possible that the species can tolerate lower pH values at times. Laboratory experiments (page 36) have shown that *Gammarus lacustris* can survive for minimum one week at pH 5.0 in water that is relatively lacking in humus (TOC=3.0 mgC/l) and that the pH-tolerance increases considerably with increasing humus content. It is unlikely that localities containing *G. lacustris* have been damaged by acidification.

Table 9. Selected macroinvertebrates and the acidification classes proposed by
(R) Raddum and
(E/L) Engblom/Lingdell respectively.

pH-classes R E/L	pH < 4.7 4.5	4.7 ≤pH < 5.0 4.5 < 5.0	5.0≤ pH ≤ 5.5 5.0 5.4	pH > 5.5 5.4
Index values R E/L	0 1	0.25 2	0.5 3	1 4
Snails				
Lymnaea peregra			E/L	R
Crustaceans				
Gammarus lacustris				E/L R
Mayflies				
Ameletus inopinatus	E/L		R	
Baetis rhodani		E/L		R
Baetis muticus				E/L R
Baetis niger		E/L		R
Heptagenia sulphurea		E/L	R	
Leptophlebia vespertina		E/L R		
Ephemerella aurivillii		E/L		R
Stoneflies				
Diura nanseni	E/L		R	
Isoperla grammatica	E/L		R	
Brachyptera risi	R	E/L		
Amphinemura spp.	E/L R			
Nemoura spp.	E/L R			
Capnia atra		E/L	R	
Capnia pygmaea			R	
Leuctra hippopus	E/L R			
Caddis flies				
Rhyacophila nubila	E/L R			
Ithytrichia lamellaris			E/L R	
Plectrocnemia conspersa		E/L R		
Polycentropus flavomaculatus	E/L R			
Hydropsyche siltalai	E/L		R	

Mayflies in general are regarded as sensitive to acidification. There are large differences, however, between species. The most common of the mayflies, *Baetis rhodani*, is often associated with non-acidified water. The laboratory experiments described above (pages 29, 32, 34) show that, of all the species tested, *B.rhodani* is the most sensitive to acidification. Under the experimental conditions it was more sensitive to acidification than *Gammarus lacustris*. Even though *Baetis niger* and *B. rhodani* have sometimes been recorded in acid water in Swedish and Finnish studies, it must be assumed that both *B.rhodani* and the genus *Baetis* as a group are sensitive to acidification under Norwegian conditions.

B.rhodani is particularly important because of its widespread distribution, and the fact that it is found in the large majority of unpolluted watercourses in Norway. *Heptagenia dalecarlica* and *Ameletus inopinatus* have also been found in slightly acidified watercourses. The tolerance experiments described above (pages 29, 32) indicate that *H.sulphurea* should be placed in a group that is more tolerant than *B.rhodani* is to acidification. *Heptagenia fuscogrisea* is usually found in slowly flowing stretches of rivers. This species is tolerant. *Ephemerella aurivillii* seems to inhabit localities with pH-conditions corresponding to those of localities inhabited by *B.rhodani*. *Leptophlebia vespertina* is one of the few mayfly species that is very tolerant to acidification. Normally this species is found in lakes, but is also often present in strongly acidified rivers (page 19). *Ephemera* and *Caenis* species are mayflies found primarily in slowly flowing stretches of the river. Both these groups are sensitive to acidification.

In general, stoneflies are more tolerant to acidification than mayflies are. Therefore, a large number of stonefly species may be found in localities moderately damaged by acidification, but no, or very few mayfly species. The genuses *Amphinemura* and *Nemoura* are very tolerant groups, and are often found in acidified localities. The predatory forms *Diura nanseni* and *Isoperla grammatica* are both relatively tolerant, but seem to disappear in the most acidified licalities. *Brachyptera risi* is also a tolerant species, but according to the laboratory experiments described above, is not one of the most tolerant. *Capnia* species seem to be among the most sensitive species, and are found only in moderately acidified rivers. *Leuctra hippopus* is often found in strongly acidified localities, along with the other stoneflies that are most tolerant to acidification. The tolerance tests described above confirmed its high degree of tolerance.

Much remains to be learned about caddis flies and their sensitivity to acidification, but it is the general impression that this group is relatively tolerant to acidification. *Rhyacophila nubila*, *Plectrocnemia conspersa* and *Polycentropus flavomaculatus* are among the few macroinvertebrates found in localities strongly damaged by acidification. The pH-tolerance of *Polycentropus flavomaculatus* and *Hydropsyche siltalai* was tested in the experiment described above, and these species were found to be very tolerant. In spite of this, *H.siltalai* does not seem to be found in the most acid rivers. *Agapetus ochripes* can be found in waters with the same pH-range as waters containing *H.siltalai*. The species sensitive to acidification include the *Glossosoma* species, *Ithytrichia lamellaris* and the *Micrasema* species.

A large number of other species of macroinvertebrates are included in the different index systems. Their index values are associated with varying degress of uncertainty but are being updated continuously.

4.2 Adjustment of the indices

Table 11 shows NIVA's (Bækken & Aanes 1990a) proposal for index values in conformity with the study described above. It is suggested that the division into pH-intervals should be the same as Raddum's. The numerical values are adjusted in relation to Raddum's and Engblom/Lingdell's values, so that 1 designates an unacidified state and 4 the most acidified state. This coincides better with the national system of evaluation described in "Water quality criteria for freshwater" (SFT 1989a). It must be emphasized, however, that the parameter classes used for pH-assessment in "Water quality criteria for fresh water" are not appropriate to describe the acidification situation for the fauna in our watercourses (table 10). Classes 1, 2 and 3 in "Water quality criteria for fresh water" cover the range pH > 5.5, while class 4 refers to pH < 5.5. This means that classes 1, 2 and 3 would be included in macroinvertebrate group 1, while class 4 would be split into three macroinvertebrate groups: 2, 3 and 4.

Table 10. Proposed index values for characterizing acidification damage to lotic macroinvertebrate communities, seen in relation to SFT's system of classification for water quality.

pH values	>5.5	<5.5
Parameter class (SFT 1989a)	1, 2, 3	4
Macroinvertebrate index (Bækken & Aanes, NIVA 1990a)	1	2, 3, 4

4.3 Use of an acidification index:

By using the index values in table 11 it will be possible in most cases to establish to what degree a watercourse is acidified. Because the tolerance to acidification appears to increase with increasing content of humus, other index values should be used in localities with a high content of humus than in localities with a low content. The composition of the fauna may vary somewhat among the different regions of Norway, but there will usually be some macroinvertebrate species present which make it possible to use table 11. Figure 12 presents an example of how different species of mayflies, stoneflies and caddis flies can disappear during a process of acidification.

Before asserting that the composition of the fauna in an acidified watercourse reflects acidification, it must be possible to make comparisons with a non-acidified or slightly acidified past and the typical fauna at that time, that is to say, the natural state. When investigating the effects of acidification on the fauna, unaffected reference localities are often used as a basis for comparison. This can lead to problems if large areas are affected, as they often are by acidification. As a rule, the previous state can be estimated from earlier investigations carried out at the same site or in the same area. In areas rich in humus, with little calcium, the water may be naturally acid due to humic acids. Faced with watercourses of this kind, there is a risk of overevaluating the degree of acidification if the present (acid-tolerant) macroinvertebrate community is compared with a community that has not been exposed to acid water. However, if the relatively few naturally acid watercourses are ignored, it can be assumed that the previous state is a non-acid locality where the fauna contains the groups and species of animals that are typical for such a locality or region. In this case, an index system can be used to classify the biological damage caused by acidification of the watercourse, and to estimate the acidification in terms of expected pH-range in the most acid periods.

Table 11. Proposal for placing macroinvertebrates in acidification classes.
- • denotes ordinary classification and
- (•) alternative classification when the localities contain a high content of humus. From Bækken and Aanes 1990a.

pH-classes	pH<4.7	4.7≤ pH>5.0	5.0≤ pH≤ 5.5	pH>5.5
Index values	4	3	2	1
Snails				
Gyraulus acronicus			(•)	•
Lymnaea peregra			(•)	•
Crustaceans				
Gammarus lacustris				•
Mayflies				
Ameletus inopinatus		(•)	•	
Baetis rhodani			(•)	•
Baetis muticus				•
Baetis niger			(•)	•
Baetis lapponicus				•
Heptagenia sulphurea		(•)	•	
Heptagenia dalecarlica			•	
Leptophlebia vespertina	•			
Ephemerella aurivillii			(•)	•
Ephemera vulgata/danica			(•)	•
Caenis spp.			(•)	•
Stoneflies				
Diura nanseni			•	
Isoperla spp.		•		
Brachyptera risi		•		
Amphinemura spp.	•			
Nemoura spp.	•			
Capnia atra			•	
Capnia pygmaea			•	
Leuctra hippopus	•			
Caddis flies				
Rhyacophila nubila	•			
Glossosoma spp.				•
Agapetus ochripes		•		
Ithytrichia lamellaris			•	
Plectrocnemia conspersa	•			
Polycentopus flavomaculaus	•			
Micrasema spp.				•
Hydropsyche siltalai		•		

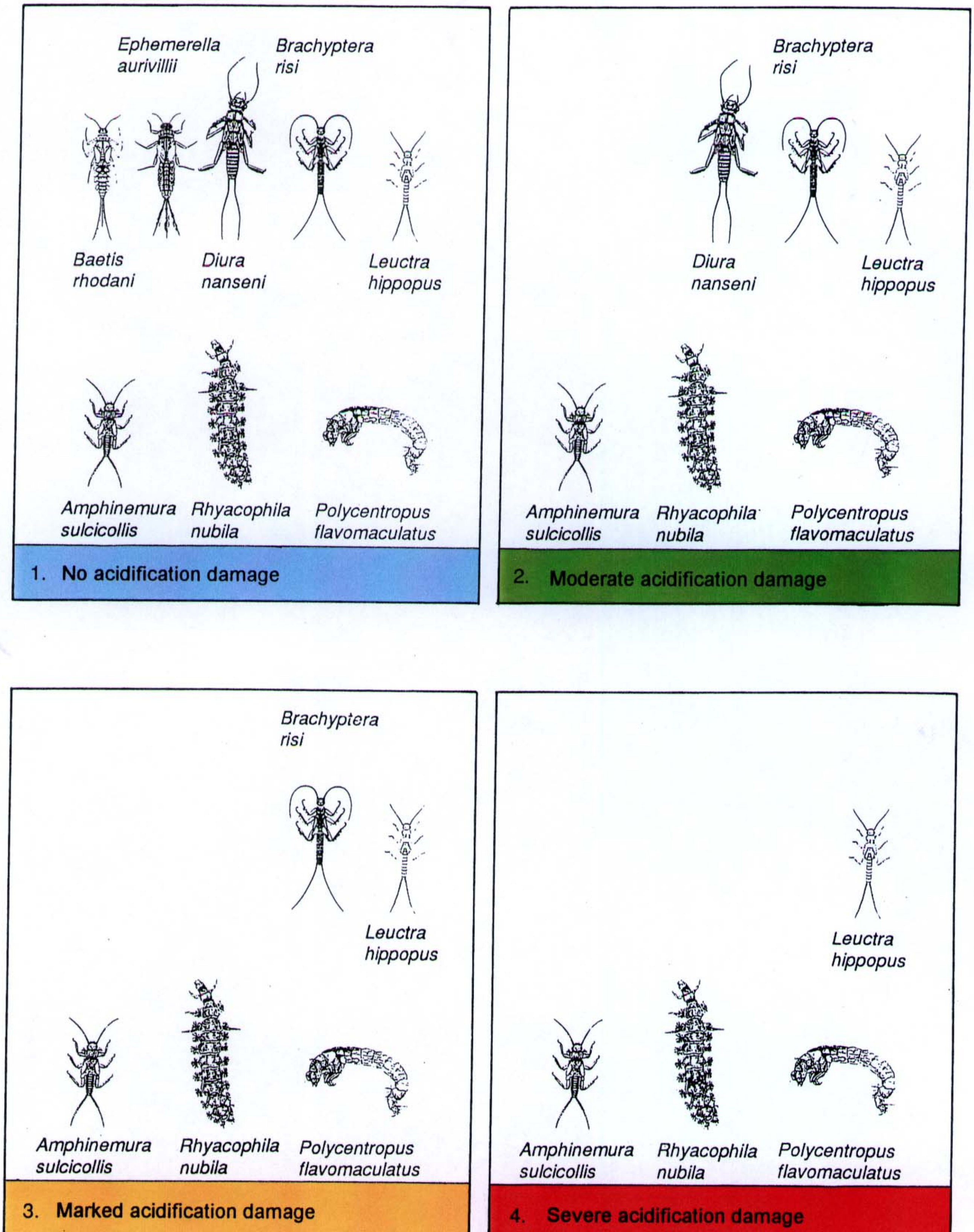


Figure 12. An example of some macroinvertebrates that may be found in different acidification classes. Species that are sensitive to acidification disappear as the acidification increases.

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Classification of Environmental Quality in Fresh Water