

# *Acid Rain Research*

REPORT 27/1992

**Reversibility of acidification:**  
fish responses in experiments  
at Risdalsheia, Norway



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**Abstract:**

The RAIN-project started in 1984, with the purpose to study effects on and changes in water quality, after excluding acid rain. Based on water chemistry, the runoff from the clean rain catchment has developed in a "positive" direction, responding to decreased input of SO<sub>4</sub> and NO<sub>x</sub>. Toxicity of the runoff from the catchments at the RAIN-project, was tested with 5 strains of brown trout.

The chemical composition of runoff water from the clean site has proved to be more toxic than water from the untreated, neighbouring site. The higher toxicity levels after excluding acid rain is assumed to be caused by reduced calcium concentrations in the runoff, while the pH and aluminium levels still were toxic. There proved to be significant difference in tolerance among the five strains tested.

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**Reversibility of acidification:  
fish responses in experiments  
at Risdalsheia, Norway**

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## 1. ABSTRACT

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Toxicity of runoff from the experimental catchments of the RAIN-project, was tested with five strains of brown trout. The RAIN-project (Reversing Acidification in Norway) started in 1984, with the purpose to study effects on and changes in water quality, after excluding acid rain. The restoration experiments at Risdalsheia are conducted at three small headwater catchments. Two of the catchments, KIM and EGIL, are enclosed by roofs. At KIM the rain is cleaned of all acid compounds before being applied as rain under the roof. EGIL receives recycled, untreated rain, and acts as a control. A third catchment, ROLF, is an untreated reference.

Based on water chemistry composition, the runoff from KIM has developed in a “positive” direction. After 7 1/2 years of acid exclusion, sulphate and nitrate concentrations in the runoff have decreased and are compensated by decreased concentrations of base cations and increased alkalinity. Runoff from all catchments continue to be strongly acidic and has high aluminium concentrations because of buffering by high concentrations of organic acids. The improvements in water quality, however, should entail positive biological changes (towards reduced mortality). Three batch experiments with brown trout fry of five different strains were conducted in 1989 using runoff from KIM and EGIL. The chemical composition of water from KIM (roof, clean rain) proved to be more toxic to trout than untreated water from EGIL (roof, acid rain). The higher toxicity at KIM is probably due to the reduction in calcium levels, while pH and aluminium still are at toxic levels. Reduction in sulphate and nitrate concentration in the rain has caused the lower calcium concentration in the runoff at KIM. Rates of chemical weathering are very low in the thin and patchy organic soils. Buffering by natural organic acids causes low pH in runoff despite exclusion of strong acids. Aluminium concentrations remain above toxic levels for trout.

The five strains of brown trout used in the ReFISH programme (**R**estoring **E**ndangered **F**ish in **S**tressed **H**abitats) proved to have different sensitivity to the runoff waters. In the order of decreasing tolerance, the following strain differences were found; Bygland>Genetic>Fossbekk>Bustul=Tunhovd.

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## 2. INTRODUCTION

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By 1986, acidification of surface waters had damaged the fish populations in an area covering 36 000 km<sup>2</sup> in Norway. In a 1000-lake survey conducted by NIVA in 1986, 52% of the lakes had damaged fish populations (Henriksen *et al.* 1989, Rosseland and Henriksen 1990). Efforts to reduce SO<sub>4</sub> and NO<sub>x</sub> to the atmosphere are in part based on the premise that such reduction will restore acidified waters. It has been estimated that a 50% reduction of sulphate in the precipitation would produce a water quality sufficient for fish production in 40% of the damaged lakes (Henriksen *et al.* 1988). Reduced emissions of SO<sub>2</sub> in Sudbury, Canada resulted in recovery of fish populations in several lakes damaged by acid-rain (Gunn and Keller 1990).

The magnitude and rate of response on natural ecosystems to reductions in acid loading is not well known, largely because such effects have been difficult to document in the absence of large scale reductions. A large scale manipulation of a natural head water catchment began in Norway in 1984. The objective of the “RAIN-project” (Reversing Acidification in Norway) is to predict the changes in the biogeochemical processes and the time lag for improvement of the surface water chemistry, in response to reduction in acid deposition (Wright *et al.* 1988). Soil processes are central in understanding catchment-scale response (Reuss *et al.* 1987, Christophersen *et al.* 1990).

The restoration experiment at Risdalsheia are conducted at three small headwater catchments. Two of the catchments, KIM and EGIL, are enclosed by roofs. At KIM the rain is cleaned of all acid compounds before being applied as rain under the roof. EGIL receives recycled, untreated rain, and acts as a control. After 7 years of cleaned precipitation, the annual volume-weighted average SO<sub>4</sub> in runoff from KIM was reduced by about 70%. Nitrate and ammonium in runoff has remained low since the onset in 1984. Base cation concentrations have decreased to some extent, probably in part due to lower concentrations of mobile anions in runoff, again due to the regeneration of the pool of exchangeable base cations in the soil (Wright 1991). In 1989, fish experiments in runoff waters from KIM and EGIL was started to test this changed water quality with respect to fish toxicity. The hypothesis is that the water quality changes reported from Risdalsheia will increase the survival of fish.

The five brown trout strains from the ReFISH programme (Rosseland *et al.* 1990a) have been used as test organisms.

### 3. METHODS

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#### 3.1. DESCRIPTION OF RISDALSHEIA

The de-acidification experiment at Risdalsheia are conducted at three small headwater catchments located close to the Tovdal River and the long-term calibrated catchment area at Birkenes (Figure 1). The vegetation at Risdalsheia is dominated by pine, spruce and birch. The soils are thin, patchy and poorly developed. Soil pH is around pH 3.9-4.5. Bedrock is biotite granite (Wright *et al.* 1988).

Two of the catchments, KIM and EGIL, are enclosed by separate roofs (construction data in Table 1). Rain falling on these is collected in tanks and pumped back under the roof, producing a controlled precipitation. The "rain" at EGIL is pumped untreated and acts as a control, while the "rain" at KIM is first deionized (mixed bed deioniser) then ion adjusted with sea-water to a chemical composition assumed corrected to pre-acidification levels. A third site, ROLF is not covered. Additional data about the RAIN-project are given by Wright *et al.* 1988, Wright 1991. Only runoff from the KIM and EGIL catchments are used in the fish experiments.

The runoff from KIM and EGIL is collected and piped to separate metering houses. In the metering house the water is collected in 500 L tanks (Figure 2) for water quality and volume measurements. This water is then used for the fish experiments. Water flushes from the 500 L tank into a header tank (1 m<sup>3</sup>) before being fed at approx. 20 L/min. to the fish tanks.

Table 1. Physical values for the constructions at KIM and EGIL

	KIM "clean rain"	EGIL "acid rain"
Catchment area	856 m <sup>2</sup>	398 m <sup>2</sup>
Roof area	1165 m <sup>2</sup>	650 m <sup>2</sup>

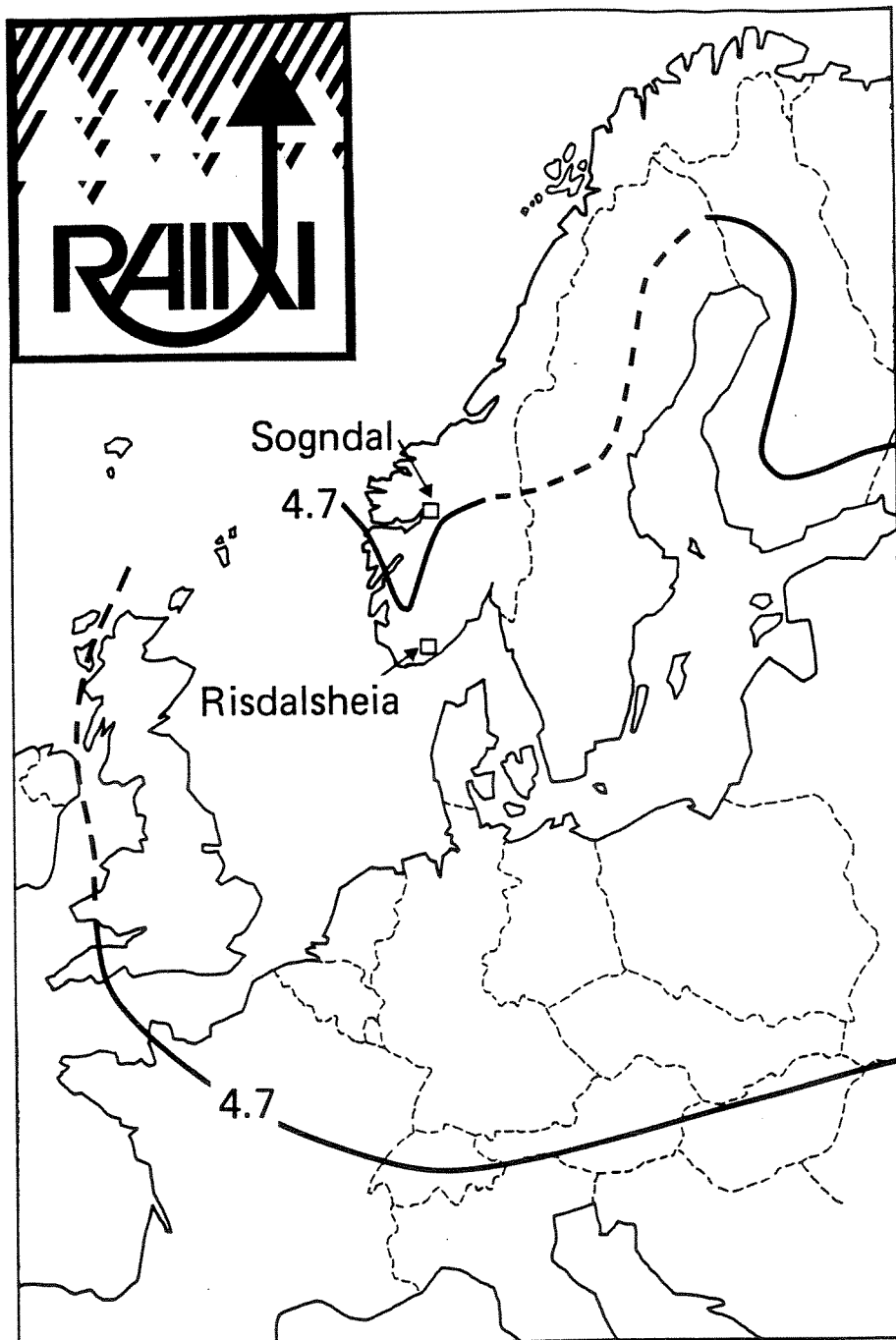


Figure 1. Location of the experimental catchments in RAIN-project. Areas within the pH 4.7 isoline receive precipitation with a yearly weighted-average pH below 4.7.



# Illustration of the Risdalsheia construction and catchment soil depth

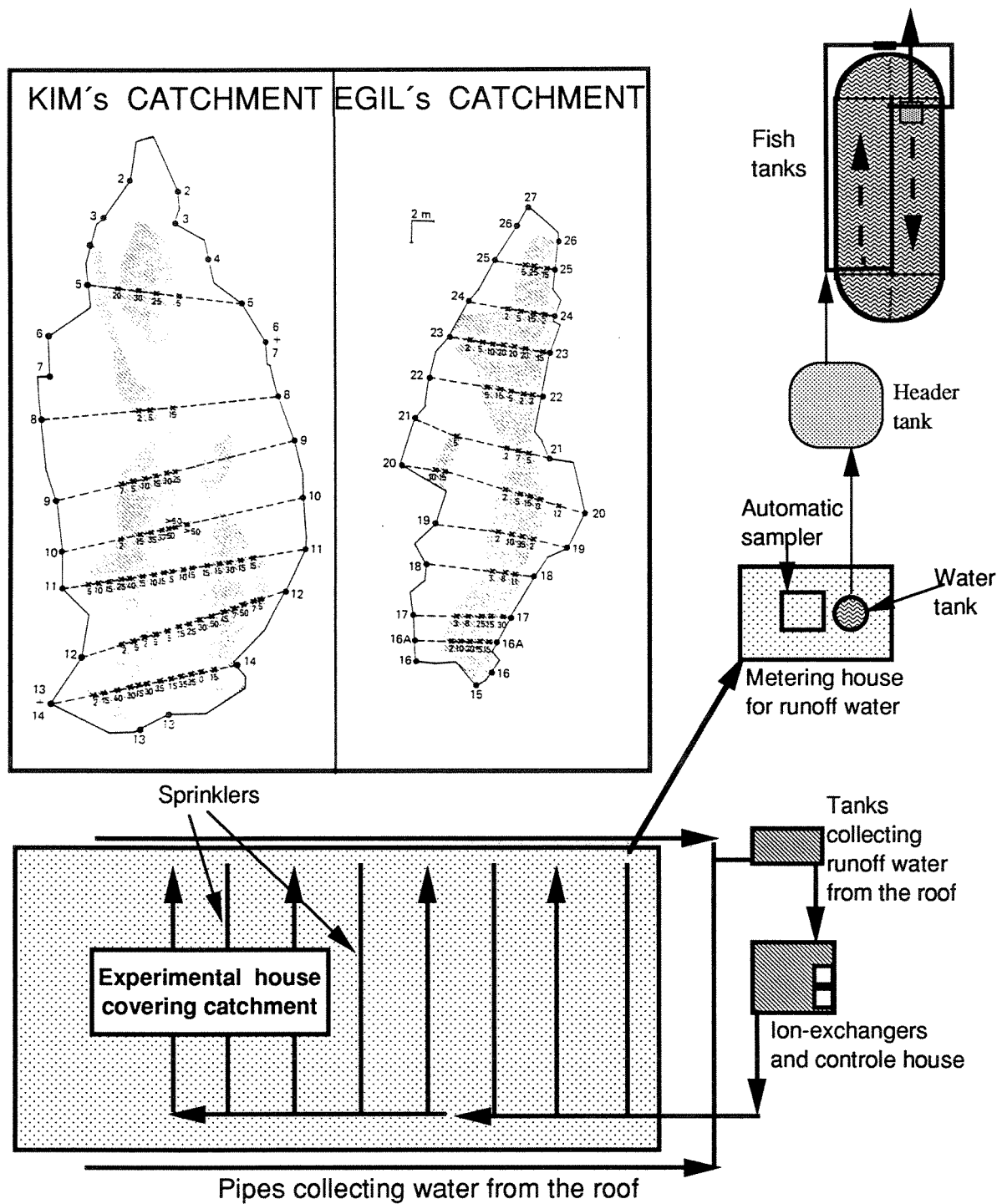


Figure 2. Schematic diagram of the experiment setup of the RAIN-project at Risdalsheia. Arrows indicate flow of water. Runoff water from the catchment is led to the metering house, to the header tank and thereafter to the fish tank. Catchment areas are given for KIM and EGIL together with an outlining of soil (shaded gray) and bare rock (white).

### 3.2. FISH MATERIAL

In the experiment, five strains of brown trout (*Salmo trutta L.*) (Table 2) are exposed to runoff water from KIM (roof, clean rain) and EGIL (roof, acid rain). These are the same fish strains which are being evaluated for acid-tolerance in the REFISH (Restoring Endangered Fish in Stressed Habitats) project (Rosseland *et al.* 1990a).

The water quality at the two hatcheries delivering the test fish differ with respect to pH, and aluminium and calcium concentration, making it difficult to differentiate between life-history, and adaptation to acidic water and genetic characteristics. Fish from Bygland Fish Farm (Fossbekk and Bygland) are hatched in acidic clearwater, pH adjusted with sodium hydroxide. Fish from Oslomarka Fish Farm (OFA) (Tunhovd, Bustul and Genetic) are hatched in natural, near neutral water. The differences in water quality at hatchery of origin does not invalidate the between-catchment comparisons done in this report.

Table 2. Data on origin of fish strains used in the experiments at Risdalsheia.

STRAIN	HATCHERY	REGION OF ORIGIN	ORIGINAL WATER QUALITY
Bygland	Bygland Fish Farm	Aust Agder	Acidic clearwater
Fossbekk	Bygland Fish Farm	Rogaland	Acidic, brookwater
Tunhovd	Oslomarka Fish Farm	Telemark	Pristine clearwater lake
Bustul	Oslomarka Fish Farm	Telemark	Humic lake
Genetic	Oslomarka Fish Farm	250 strains*	Artificial acidic cultured

\* Genetic is an artificial acid cultured strain, cultivated by selecting for pH resistance among 250 supposed acid tolerant strains (Gjedrem 1980)

Water runoff volume from the catchments is dependant both on precipitation and soil hydrology. It was originally planned to conduct experiments in April, May, July, September, October and November 1989. No snow during the winter 1988/89, and little rain during the summer made it impossible to run more than one experiment in May, one in October and one in November 1989.

Before entering the fish tanks the runoff water is collected in a 1 m<sup>3</sup> header-tank so that the water-flow entering the fish tanks can be adjusted (Figure 3). The water-flow is set to 20 L/min. The fish tank volume is 2 m<sup>3</sup>. This gives a minimum retention time in the fish tanks of 100 minutes, increasing towards infinity when there is no runoff, due to lack of precipitation. The header tanks and fish tanks were made of glassfibre. All tubing was made of plastic to avoid metal contamination.

The water in the tank was kept under constant circulation using a dry-mounted circulation pump set to 50 L/min. The pump has only plastic components on parts coming in contact with water. The water

flow generated a current that was 2 times body length per second, being an optimal current, sufficiently strong for the fish to swim actively. The oxygen level was measured 1 day after the experiments started (maximum biomass). The oxygen level was never below 95% saturation. There was no nitrogen-gas supersaturation.

The fish were transported to Risdalsheia in plastic bags, filled with water and oxygen to last a minimum of 1 day. Transport time from Oslo was approximately 16 hours, and from Bygland, 6 hours. Upon arriving the fish were acclimated to the ambient water for 5-7 minutes, before release. The general behaviour upon release was normal, with no immediate indication of stress. The tanks were shaded from the sun by use of a plastic cover. The fish were not fed during the experiment.

There was a slight difference in fish size between the five strains (Table 3). The Bustul and Tunhovd strains were significantly smaller ( $p < 0.05$ ) than the other strains, except in the May experiment where Tunhovd fish were larger ( $p < 0.05$ ). The fish were marked by fin-cutting, either of the adipose, or the right or left pectoral or a combination of the two. Fin-markings are given in Table 3. Veterinary examination of the fish prior to the experimental periods did not reveal any diseases or signs of environmental stress.

Table 3. Mean length, standard deviation (SD) and number of fish, for all strains, tested in 1989. The fish were not measured separately at KIM and EGIL in May. The fin cuttings used to identify the strains, are given below the strain name. RP=Right Pectorial, LP=Left Pectorial, A=Adipose, ARP=Adipose+Right Pectorial and ALP=Adipose+Left Pectorial.

PERIOD	VALUE	TUNHOVD	BUSTUL	BYGLAND	FOSSBEKK	GENETIC
SITE		RP	LP	A	ARP	ALP
<b>May</b>	Mean	6.7±0.5	6.0±0.6	6.3±0.6	6.0±0.7	6.3±1.0
KIM+EGIL	No.	26	38	38	26	28
<b>October</b>	Mean	5.4±0.4	5.3±0.6	6.0±0.5	6.2±0.5	6.3±0.8
EGIL	No.	17	20	21	19	20
<b>November</b>	Mean	5.7±0.5	5.2±0.9	6.2±0.8	6.1±0.7	6.0±0.7
EGIL	No.	23	17	43	35	15
<b>October</b>	Mean	5.3±0.6	5.5±0.6	6.4±0.4	6.1±0.4	6.1±0.9
KIM	No.	19	21	20	20	18
<b>November</b>	Mean	5.7±0.4	5.2±0.5	6.0±0.9	6.2±0.6	6.1±0.9
KIM	No.	21	16	44	29	24

Water temperatures at KIM and EGIL varied daily between 8-10 °C in May. In October the temperature varied between 7-10 °C, with a slight higher temperature towards the end of the experimental period. In November the temperature varied between 3-8 °C, with the lowest temperatures towards the end of the experimental period.

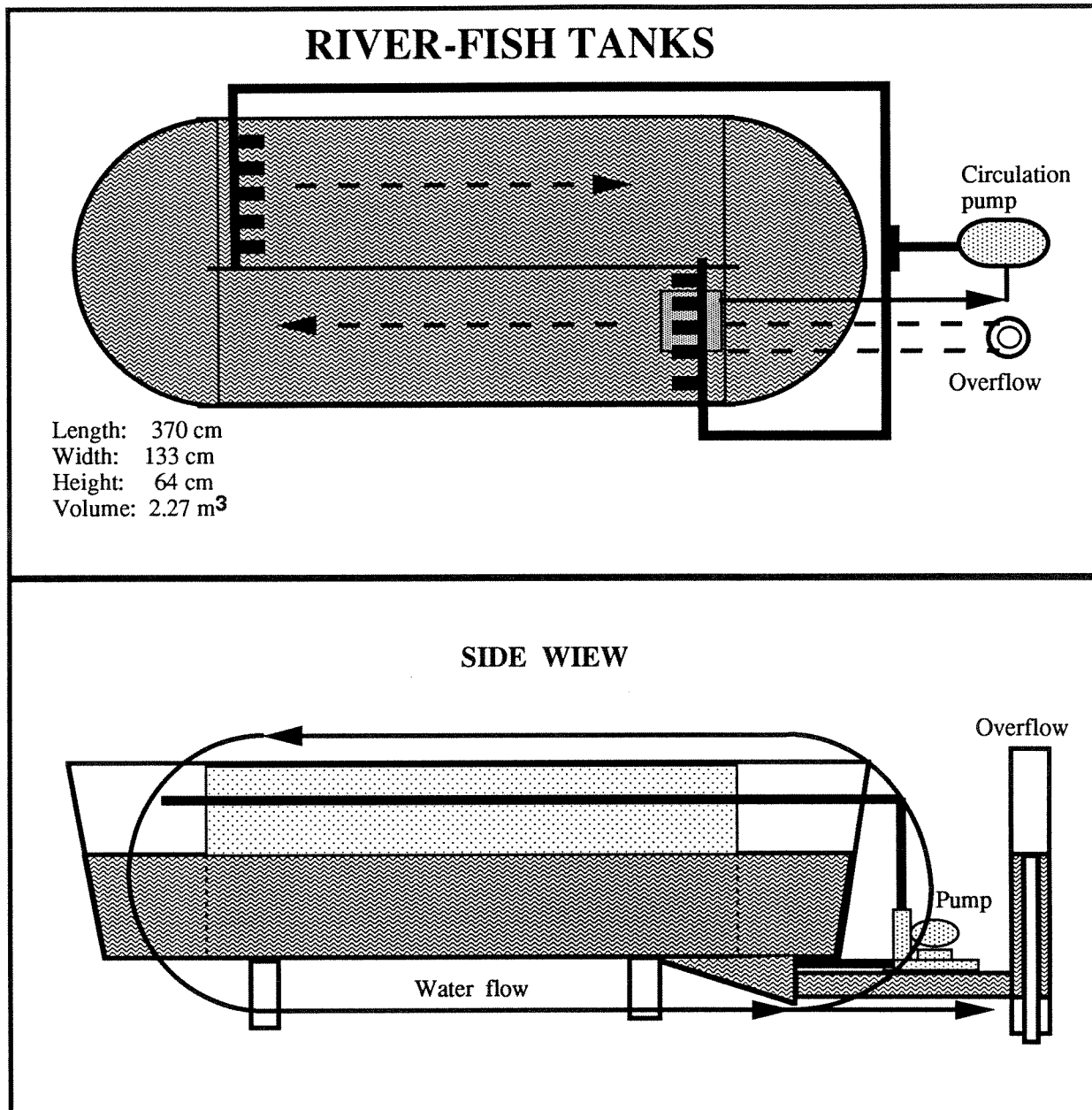


Figure 3. Schematic drawing of the fish tanks. The water is kept under constant circulation using a pump. Excess water is drained through the overflow. In the centre of the tank there is a wall that divides the tank, and produces a "raceway" type of circulation.

### 3.3. STATISTICS

Data were subjected to statistical analysis using Statview SE+Graphics, version 1.03. The following tests were performed:

For differences in fish mean lengths. Anova 2-way test, was used to test for differences in variation in fish lengths.

Friedman's test was used for relative differences in mortality rate, measured as LT<sub>25</sub>, LT<sub>50</sub>, LT<sub>75</sub> and LT<sub>100</sub>. A non-parametric test was used because of high variation in mortality rate for the three exposure periods, and only the rank order of strain dependant mortality was of interest.

Student's paired-t test was used for differences in chemical composition.

## 4. CHEMISTRY RESULTS AND EVALUATION

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### 4.1. METHODS

The water samples were taken both from the fish tanks, and from the main discharge tanks at KIM and EGIL. The results from the fish experiments cover only periods relevant to the experimental periods, while the measurements on runoff water cover the whole year. In previous reports from the RAIN-project, the chemistry is presented as volume-weighted annual means. Data from the RAIN-project are therefore presented here to document the chemical variation in 1989. Additional data on acid exclusion are given by Wright *et al.* (1988) and Wright (1991).

The ANC-value (Acid Nutralising Capacity) is calculated according to Lien *et al.* 1989.

$$\text{ANC} = (\text{Ca} + \text{Mg} + \text{Na} + \text{K}) - (\text{SO}_4 + \text{Cl} + \text{NO}_3)$$

where the units are  $\mu\text{eq/L}$ .

All chemical analyses are done according to standard methods at NIVA. Cations are analysed by atomic adsorption spectrophotometry,  $\text{SO}_4$  and Cl by ion-chromotography,  $\text{NO}_3$  and  $\text{NH}_4$  are analysed colorimetrically. Aluminium species are analysed by ion-exchange colometry (Røgeberg and Henriksen 1985), and TOC by infrared spectroscopy after oxidation to  $\text{CO}_2$ .

## 4.2. pH

The pH was significantly different between KIM and EGIL at the 0.01% level in 1989. The mean pH value (calculated as average  $H^+$  in samples) for 1989 was pH 4.22 at KIM and pH 4.12 at EGIL, with a range from 3.85 to 5.12 (Figure 4 A). The pH was significantly lower at EGIL in May and November, but not in the October experiment (Table 4).

The pH values at KIM and EGIL were equal at the start and at the end of the May experiment, with pH values at EGIL lower than at KIM in the intermediate period (Figure 4 B).

The pH level at EGIL was 0.15 pH higher than at KIM during the first week of the October experiment (Figure 4 C). The pH variation between KIM and EGIL was not significantly different in October.

In the first part of the November experiment the pH increased continuously to a high pH of 5.00 at EGIL and 5.12 at KIM, to return to normal levels by 13 November (Figure 4 C).

To what extent the 0.1 pH difference between KIM and EGIL influences the water toxicity is not clear. The pH is low at both sites, and lower than levels normally reported from natural acidic waters. It is reasonable to assume that the higher  $H^+$ -levels at EGIL would produce a more toxic water quality than at KIM, except for in the first part of the October experiment, where the order was reversed. Likewise the high pH values in the beginning of November would reduce the toxicity level at both experimental sites, with greatest reduction at KIM.

Table 4. Mean  $\pm$ SD, sample numbers (N) and significance levels for pH measurements from May 15-22, October 16-31, November 1-31, all measurements from 1989.

	May	October	November	1989
N=	7	9	14	42
Mean $\pm$ SD. EGIL	4.30 $\pm$ 0.09	4.16 $\pm$ 0.13	4.28 $\pm$ 0.33	4.12 $\pm$ 0.25
Mean $\pm$ SD. KIM	4.35 $\pm$ 0.04	4.13 $\pm$ 0.06	4.43 $\pm$ 0.29	4.22 $\pm$ 0.23
DF =	6	7	14	40
Mean X-Y	-0.056	0.0390	-0.142	-0.066
Paired t-value	-3.099	1.1510	-4.227	-3.397
P=	0.0211	0.2876	0.0008	0.0016

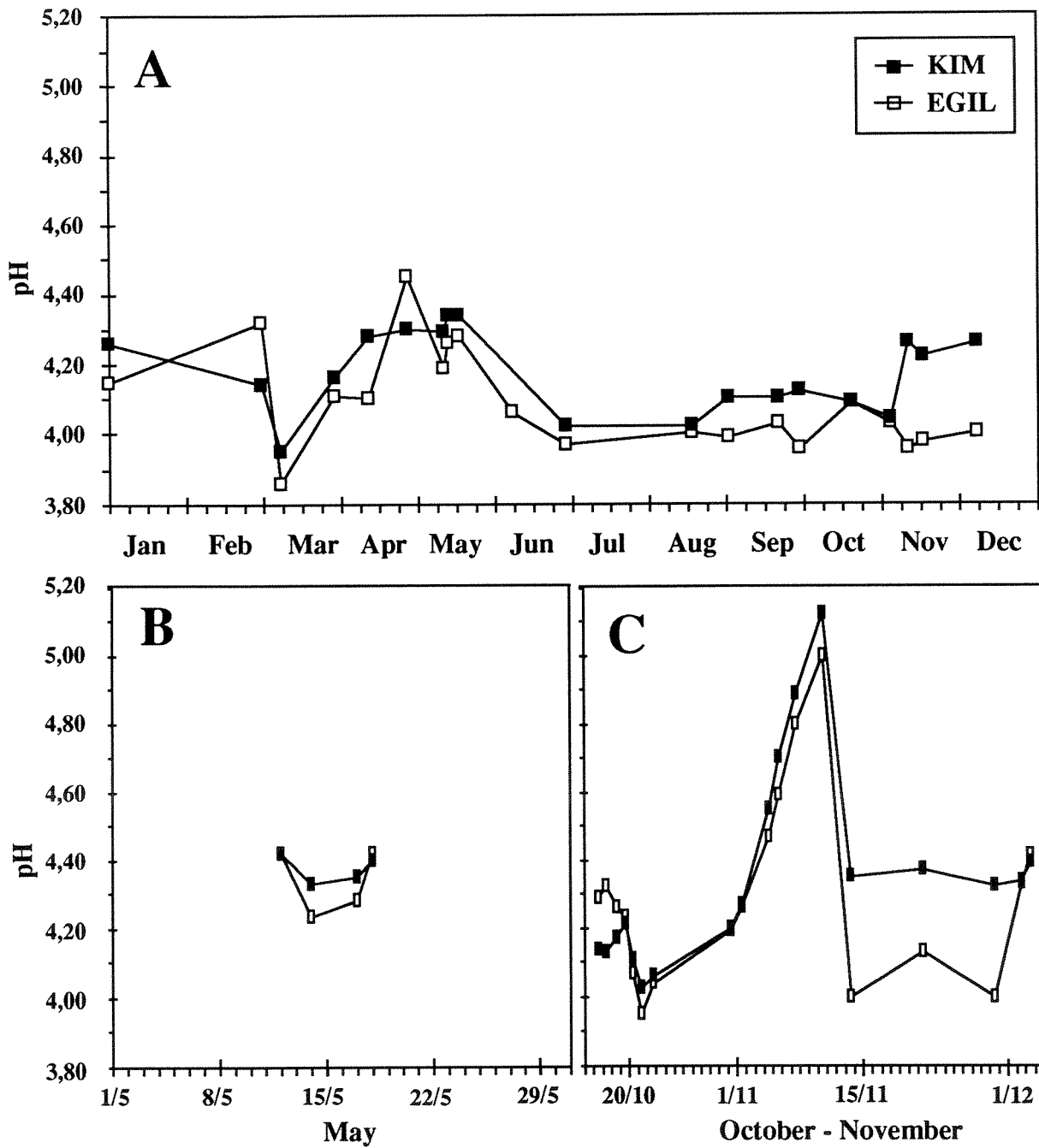


Figure 4. pH variation in runoff from KIM and EGIL in 1989 (A), and measured in the fish tanks in the May-experiment (B), and in the October/November-experiment (C).



### 4.3. CALCIUM

The calcium concentration in 1989 was significantly different ( $p=0.0001$ ) between KIM and EGIL (Table 5). The mean calcium level was 0.48 mg/L at EGIL and 0.32 mg/L at KIM (Figure 5 A).

There were significantly higher calcium concentrations at EGIL in May and November, but not in October. In last part of the October experiment, the calcium concentration was higher at KIM than at EGIL. Likewise the calcium level was 0.2-0.3 mg/L higher than “normal” in the first part of the October experiment.

Calcium is important when determining toxic level of acidic water. Generally 2.5 mg Ca/L is assumed to be at levels protecting fish against toxic effects caused by low/normal concentrations of aluminium, but will not protect at high aluminium concentration (Rosseland 1989). Concentrations as those at KIM and EGIL are only seldom evaluated in terms of protection/reduction of mortality, but the importance of calcium has been shown also at low levels (Leivestad *et al.* 1980; Wood and McDonald 1982; Brown 1983, Sadler and Lynham 1988). Mortality at Risdalsheia is probably modified by the calcium concentration. Thus one can postulate that water from EGIL should be less toxic than water from KIM, and there is a reduced calcium-protection of the fish from the beginning to the end of each experimental period (Figure 5 B and C).

Table 5. Mean  $\pm$ SD, sample numbers (N) and significance levels for calcium measurements from May 15-22, October 16-31, November 1-31, all measurements from 1989.

	May	October	November	1989
N=	7	9	14	42
Mean EGIL	0.35 $\pm$ 0.13	0.58 $\pm$ 0.21	0.50 $\pm$ 0.14	0.48 $\pm$ 0.20
Mean KIM	0.17 $\pm$ 0.10	0.51 $\pm$ 0.17	0.34 $\pm$ 0.17	0.32 $\pm$ 0.18
DF=	6	7	14	40
Mean X-Y	0.176	0.07	0.157	0.158
Paired t-value	6.6	1.297	4.733	6.463
P=	0.0006	0.2356	0.0003	0.0001

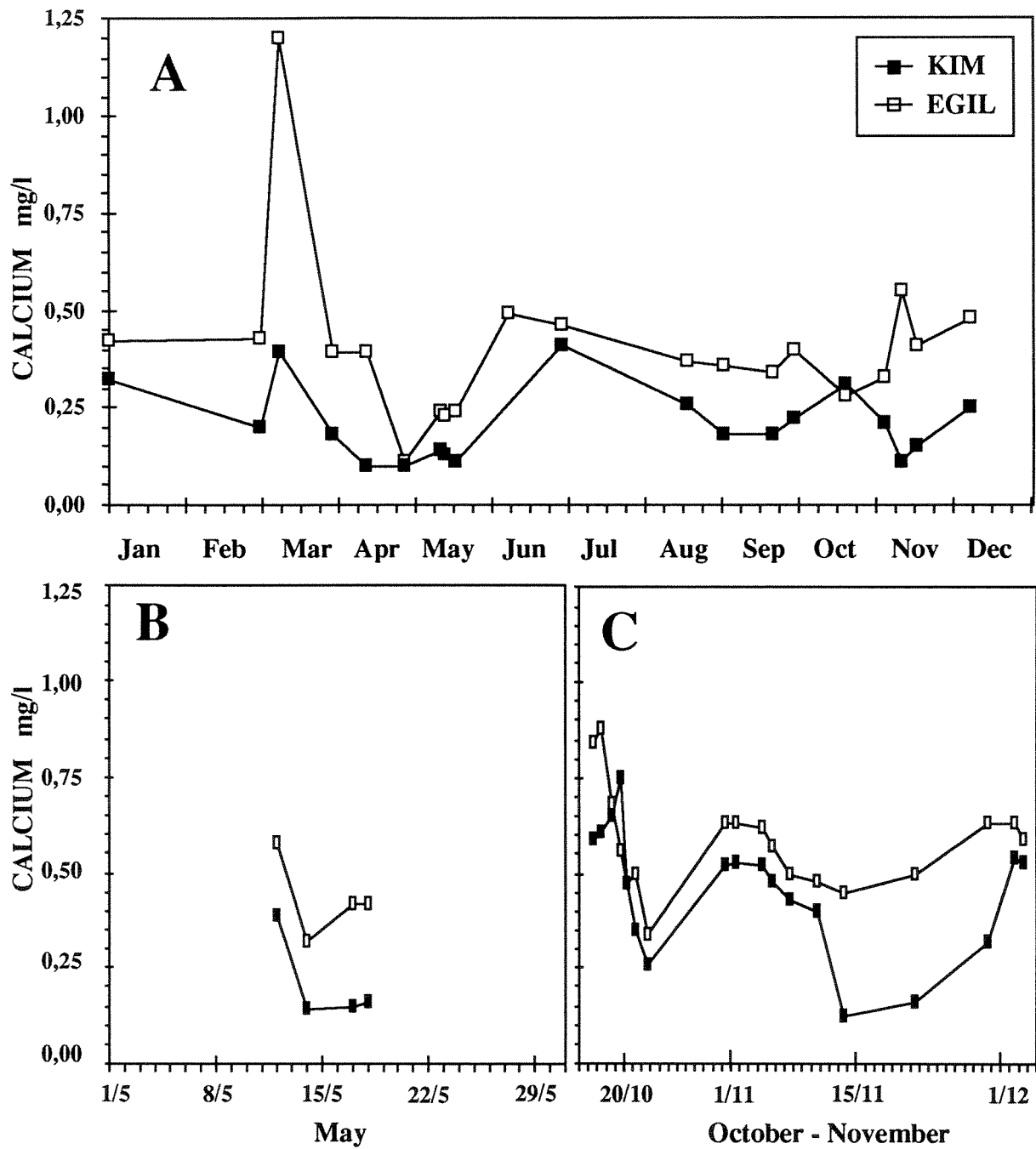


Figure 5. Calcium concentrations in runoff from KIM and EGIL in 1989 (A), and measured in the fish tanks in the May-experiment (B), and in the October/November-experiment (C).

#### 4.4. ALUMINIUM

The reactive (RAI) and inorganic monomeric (labile, LAI) aluminium levels are presented in Figure 6 A-C. The RAI concentration was significantly higher ( $p=0.0001$ ) at KIM than at EGIL, while there was no significant difference for LAI (Table 6). The mean RAI and LAI values for EGIL were 245  $\mu\text{g}$  RAI/L and 128  $\mu\text{g}$  LAI/L whereas KIM had 295  $\mu\text{g}$  RAI/L and 115  $\mu\text{g}$  LAI/L. The slightly lower LAI levels at KIM may be due to the higher pH and TOC levels.

There was no significant difference in RAL concentration between KIM and EGIL in November. The water quality in the May and October experiments was significantly different with respect to RAI. The LAI concentration was significantly higher at KIM in the October experiment, but significantly higher at EGIL in the November experiment. In the first period of the October experiment, the LAI concentration was approximately 50  $\mu\text{g}$  Al/L higher at KIM than at EGIL. In the first period of the November experiment the concentration of LAI at KIM and EGIL were similar, but approximately 100  $\mu\text{g}$  /L higher at EGIL in the last part of the experiment. This difference in LAI concentration should cause the water quality to be more toxic at KIM in the first part of the October experiment, and most toxic at EGIL in the second part of the November experiment.

Table 6. Mean  $\pm$ SD, sample numbers (N) and significance levels for reactive (RAI) and inorganic monomeric (LAI) aluminium measurements from May 15-22, October 16-31, November 1-31, all measurements from 1989.

RAI	May	October	November	1989
N=	7	9	14	42
Mean EGIL	172 $\pm$ 22	286 $\pm$ 48	223 $\pm$ 67	247 $\pm$ 74
Mean KIM	239 $\pm$ 8	386 $\pm$ 37	242 $\pm$ 64	294 $\pm$ 84
DF=	6	7	14	40
Mean X-Y	-67.0	-105.375	-22.2	-49.195
Paired t-value	-6.808	-11.254	-1.015	-4.614
P=	0.0005	0.0001	0.3272	0.0001

LAI	May	October	November	1989
N=	7	9	14	42
Mean EGIL	67 $\pm$ 15	136 $\pm$ 18	125 $\pm$ 54	128 $\pm$ 54
Mean KIM	61 $\pm$ 12	154 $\pm$ 19	83 $\pm$ 28	115 $\pm$ 48
DF=	6	7	14	40
Mean X-Y	-11.429	-23.625	40.8	12.463
Paired t-value	-1.825	-3.459	2.837	1.517
P=	0.1179	0.0106	0.0132	0.1372

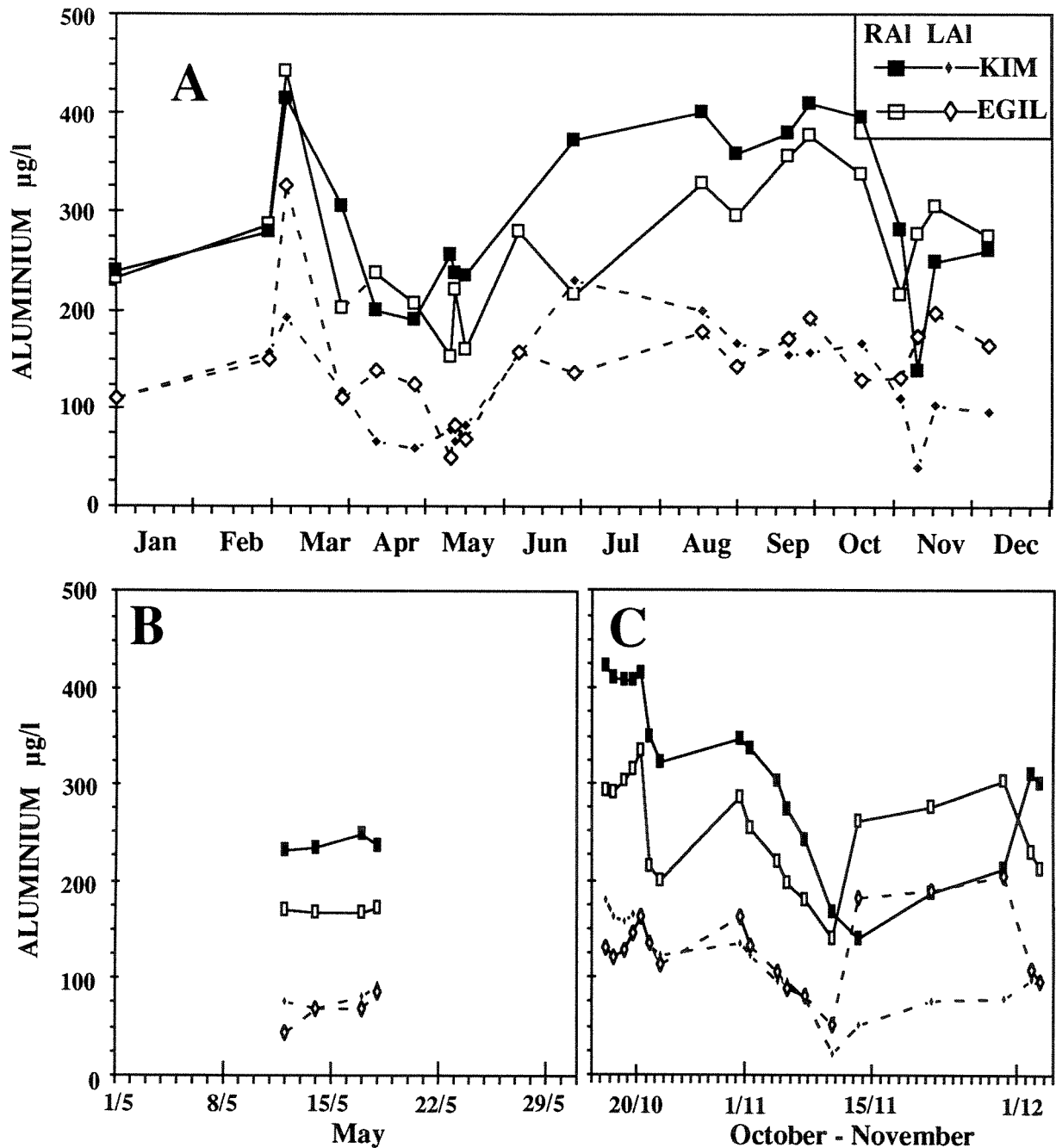


Figure 6. Reactive and inorganic monomeric aluminium concentrations in runoff from KIM and EGIL in 1989 (A), and measured in the fish tanks in the May-experiment (B), and in the October/November-experiment (C).

#### 4.5. MAGNESIUM, SODIUM AND POTASSIUM

The magnesium concentration was significantly lower at KIM than at EGIL in 1989 (Table 7), while the sodium and potassium were not significantly different (Table 7). Mean magnesium level was 0.32 mg/L for EGIL and 0.20 mg/L for KIM. There was no significant difference in the magnesium concentration in November.

Table 7. Mean  $\pm$ SD, sample numbers (N) and significance levels for magnesium sodium and potassium measurements from November 1-31, all measurements from 1989.

<b>Magnesium</b>	<b>May</b>	<b>October</b>	<b>November</b>	<b>1989</b>
N=	*	*	3	17
Mean EGIL	*	*	0.31 $\pm$ 0.05	0.33 $\pm$ 0.20
Mean KIM	*	*	0.18 $\pm$ 0.05	0.20 $\pm$ 0.09
DF=	*	*	2	15
Mean X-Y	*	*	0.137	0.118
Paired t-value	*	*	2.548	3.102
P=	*	*	0.1257	0.0073

\*=No measurements

<b>Sodium</b>	<b>May</b>	<b>October</b>	<b>November</b>	<b>1989</b>
N=	*	*	3	17
Mean EGIL	*	*	2.14 $\pm$ 0.47	1.77 $\pm$ 0.62
Mean KIM	*	*	1.85 $\pm$ 0.05	1.96 $\pm$ 0.46
DF=	*	*	2	15
Mean X-Y	*	*	0.29	-0.17
Paired t-value	*	*	0.742	-1.064
P=	*	*	0.5356	0.304

\*=No measurements

<b>Potassium</b>	<b>May</b>	<b>October</b>	<b>November</b>	<b>1989</b>
N=	*	*	3	17
Mean EGIL	*	*	0.21 $\pm$ 0.07	0.26 $\pm$ 0.27
Mean KIM	*	*	0.28 $\pm$ 0.10	0.25 $\pm$ 0.15
DF=	*	*	2	15
Mean X-Y	*	*	-0.033	0.014
Paired t-value	*	*	-0.376	0.352
P=	*	*	0.7433	0.7294

\*=No measurements

#### 4.6. AMMONIA AND NITRATE

Both ammonia and nitrate levels were significantly higher at EGIL than at KIM (Table 8). Mean ammonia concentration at KIM was 85  $\mu\text{g NH}_4/\text{L}$  in 1989, and 248  $\mu\text{g NH}_3/\text{L}$  at EGIL. Similar calculations for nitrate gave 116  $\mu\text{g NO}_3/\text{L}$  at KIM and 480  $\mu\text{g NO}_3/\text{L}$  at EGIL. The differences in nitrate concentration were high during the whole year, but for  $\text{NH}_4$ , the difference was only slight in October and November.

Table 8. Mean  $\pm$ SD, sample numbers (N) and significance levels for ammonium and nitrate measurements from May 15-22, October 16-31, November 1-31, all measurements from 1989.

Ammonium	May	October	November	1989
N=	7	9	14	42
Mean EGIL	193 $\pm$ 62	82 $\pm$ 56	166 $\pm$ 82	247 $\pm$ 347
Mean KIM	77 $\pm$ 16	40 $\pm$ 28	102 $\pm$ 50	87 $\pm$ 82
DF =	6	7	14	40
Mean X-Y	116.143	43.75	62	155.878
Paired t-value	5.605	2.831	2.761	3.449
P=	0.0014	0.0254	0.0153	0.0013

Nitrate	May	October	November	1989
N=	7	9	14	42
Mean EGIL	376 $\pm$ 102	395 $\pm$ 379	477 $\pm$ 139	481 $\pm$ 379
Mean KIM	52 $\pm$ 34	76 $\pm$ 33	117 $\pm$ 93	116 $\pm$ 175
DF =	6	7	14	40
Mean X-Y	324.143	304.5	364.993	350.854
Paired t-value	8.12	2.297	11.295	6.814
P=	0.0002	0.0553	0.0001	0.0001

## 4.7. SULPHATE AND CHLORIDE

The sulphate concentration (Figure 7) was significantly higher ( $p=0.0001$ ) at EGIL, while chloride concentration was not significantly different (Table 9). Mean sulphate concentration was 4.7 mg/L at EGIL and 2.2 mg/L at KIM. The chloride levels showed more variation, with high levels at KIM during the summer.

Table 9 Mean  $\pm$ SD, sample numbers (N) and significance levels for chloride and sulphate measurements from November 1-31, all measurements from 1989.

Chloride	May	October	November	1989
N=	*	*	3	16
Mean EGIL	*	*	5.13 $\pm$ 1.60	3.38 $\pm$ 2.12
Mean KIM	*	*	3.63 $\pm$ 0.21	3.64 $\pm$ 1.00
DF=	*	*	2	15
Mean X-Y	*	*	1.5	-0.15
Paired t-value	*	*	1.478	-0.33
P=	*	*	0.2775	0.746

Sulphate	May	October	November	1989
N=	*	9	14	35
Mean EGIL	*	4.12 $\pm$ 0.95	4.31 $\pm$ 1.12	4.71 $\pm$ 1.90
Mean KIM	*	2.42 $\pm$ 0.17	2.09 $\pm$ 0.54	2.23 $\pm$ 0.90
DF=	*	7	14	34
Mean X-Y	*	1.688	2.213	2.451
Paired t-value	*	4.615	7.242	8.816
P=	*	0.0024	0.0001	0.0001

\*=No measurements

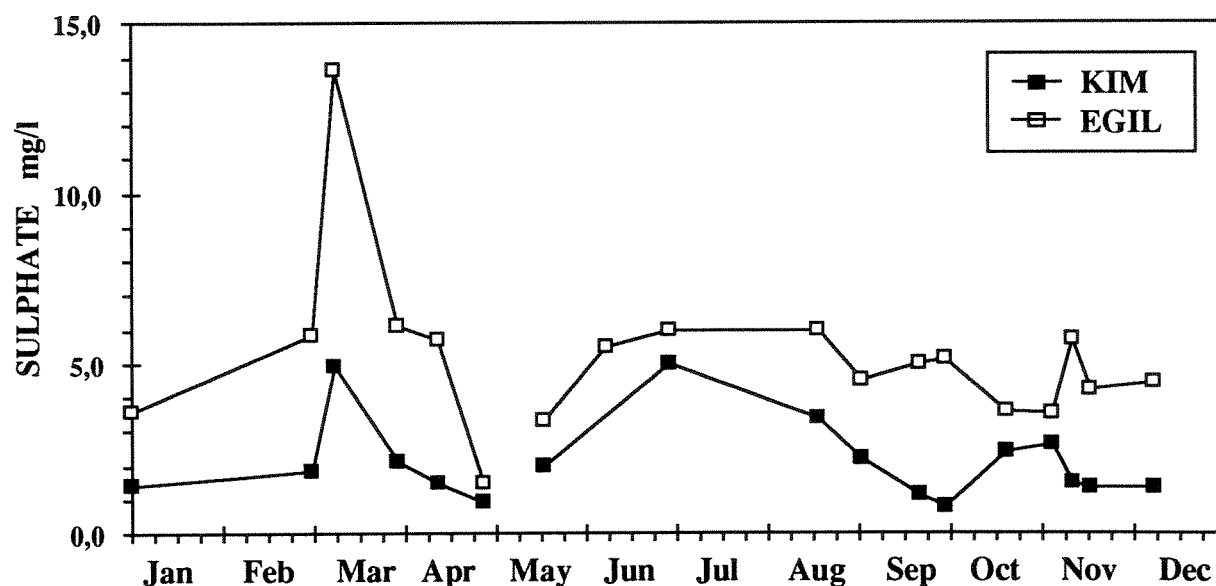


Figure 7. Sulphate concentration in runoff from KIM and EGIL in 1989 .

## 4.8. ACID NEUTRALIZING CAPACITY

The ANC values at EGIL were lower than at KIM (mean values  $-109 \mu\text{ekv/L}$  at EGIL,  $-38 \mu\text{ekv/L}$  at KIM). In spite of the large difference during the year, the difference was only slight during the May and in the first part of the October experiment (Figure 8, Table 10).

Table 10. Mean  $\pm$ SD, sample numbers (N) and significance levels for ANC measurements from 1989.

	May	October	November	1989
N=	*	*	*	16
Mean EGIL	*	*	*	$-100 \pm 56$
Mean KIM	*	*	*	$-38 \pm 23$
DF=	*	*	*	15
Mean X-Y	*	*	*	$-62.438$
Paired t-value	*	*	*	$-5.716$
P=	*	*	*	0.0001

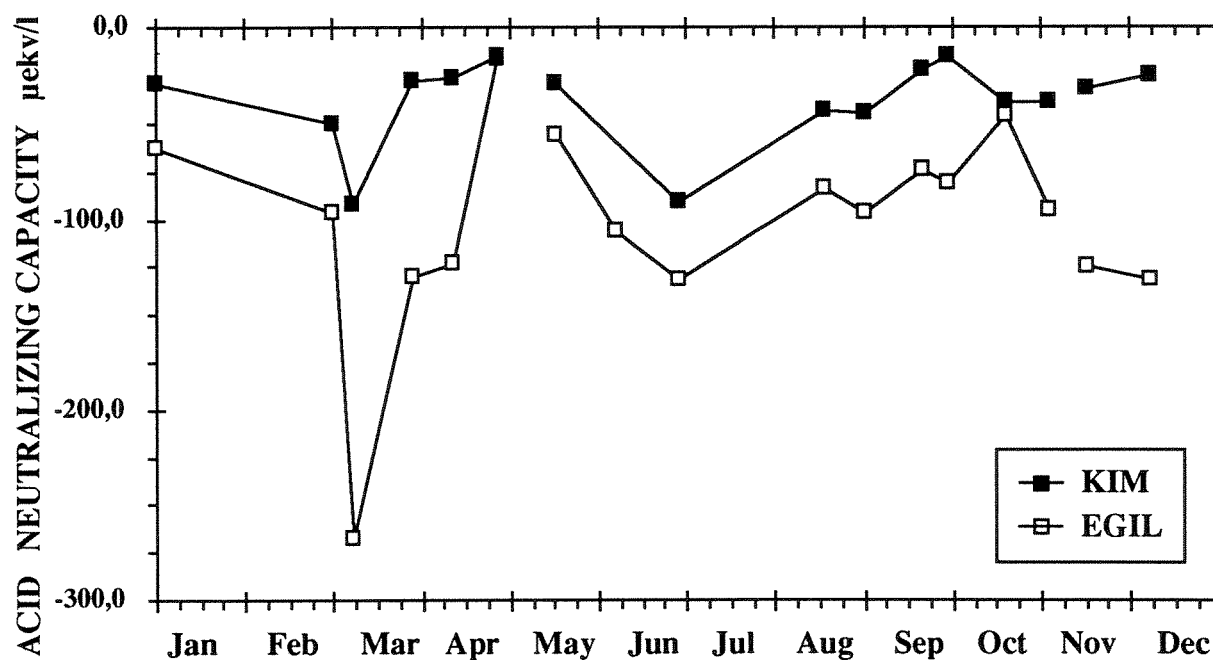


Figure 8. ANC variation in runoff from KIM and EGIL in 1989 .



## 4.9. TOTALE ORGANIC CARBON

The total organic carbon (TOC) concentration was higher at KIM than at EGIL (Figure 9), with mean concentration of 14.96 mg TOC/L at KIM and 10.30 mg TOC/L at EGIL. The difference in concentration was significant for all experimental periods (Table 11).

Table 11. Mean  $\pm$ SD, sample numbers (N) and significance levels for total organic carbon (TOC) measurements from May 15-22, October 16-31, November 1-31, all measurements from 1989.

	May	October	November	1989
N=	7	9	14	42
Mean EGIL	10.8 $\pm$ 1.63	10.4 $\pm$ 2.17	8.3 $\pm$ 1.42	10.3 $\pm$ 2.95
Mean KIM	14.7 $\pm$ 0.75	17.6 $\pm$ 1.82	12.8 $\pm$ 3.20	15.0 $\pm$ 3.73
DF=	6	7	14	40
Mean X-Y	-3.949	-7.064	-4.859	-4.724
Paired t-value	-6.886	-6.885	-5.04	-9.815
P=	0.0005	0.0002	0.0003	0.0001

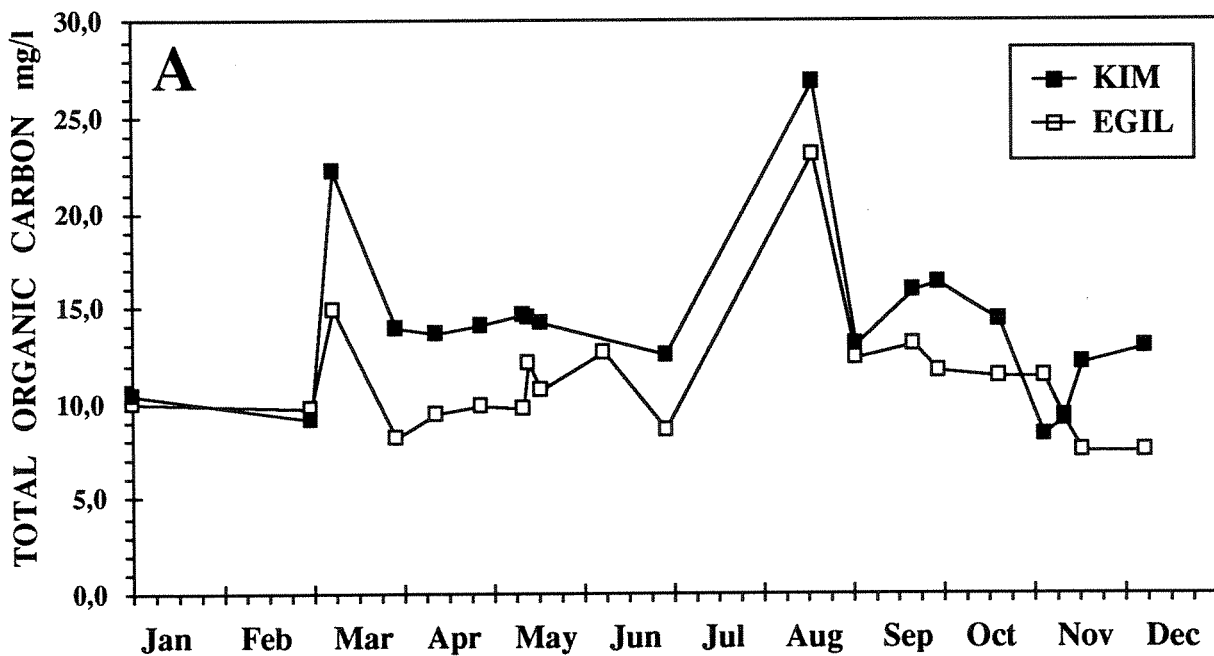


Figure 9. TOC variation in runoff from KIM and EGIL in 1989.

## 5. FISH RESULTS AND DISCUSSION

Because of higher than normal temperatures during the winter 1988/89 there was no snow-fall at KIM and EGIL, resulting in no spring melt (Figure 10, Meteorology station (st. 3656)). To replace winter rain (replacement of snow), water is normally pumped from lake RØrtjern. This might have affected the water quality in the May experiment (due to humic concentration in the lake water), while the experiments in October and November were based on runoff after natural precipitation. Runoff volumes from KIM and EGIL in 1989 are presented in Figure 11. Temperature is measured at the Meteorology station at Herefoss (st. 3845).

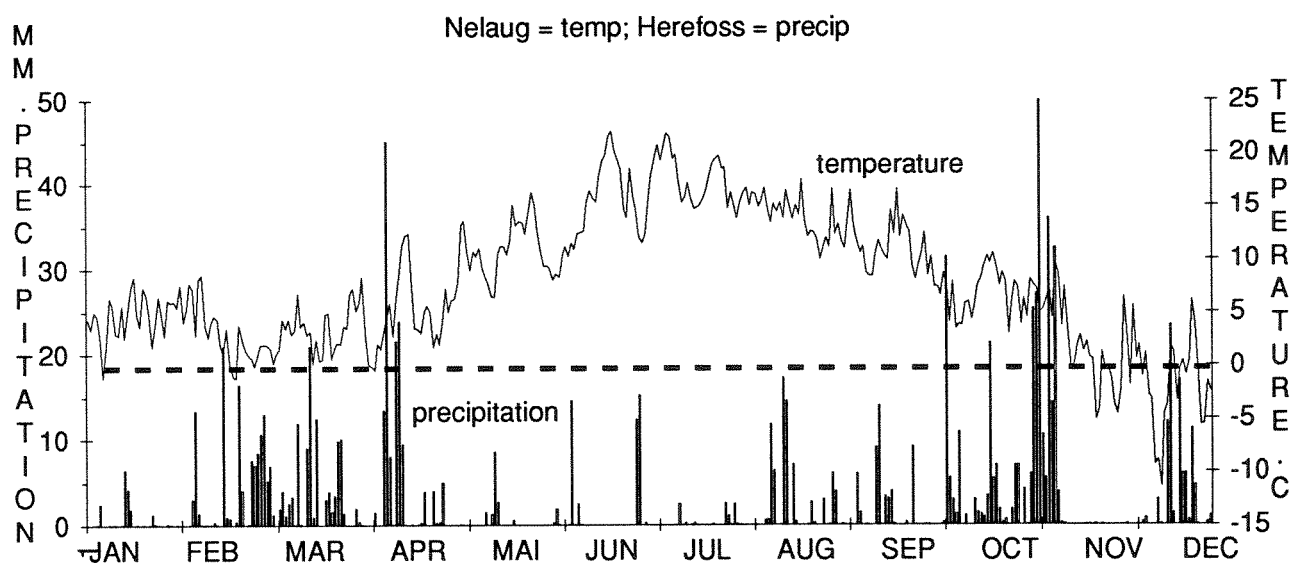


Figure 10. Daily temperature and precipitation in 1989. Temperature is measured at the Meteorological station at Nelaug, st. 3656, and precipitation at Herefoss, st. 3845. Data from Norwegian Meteorological Institute.

It was originally planned to include fish eggs and alevins in the spring experiment, but due to high winter temperatures, neither alevins nor swim-up fry were available in May. New experiments were not possible before October, due to very little precipitation during the summer, producing insufficient runoff (Figure 10).

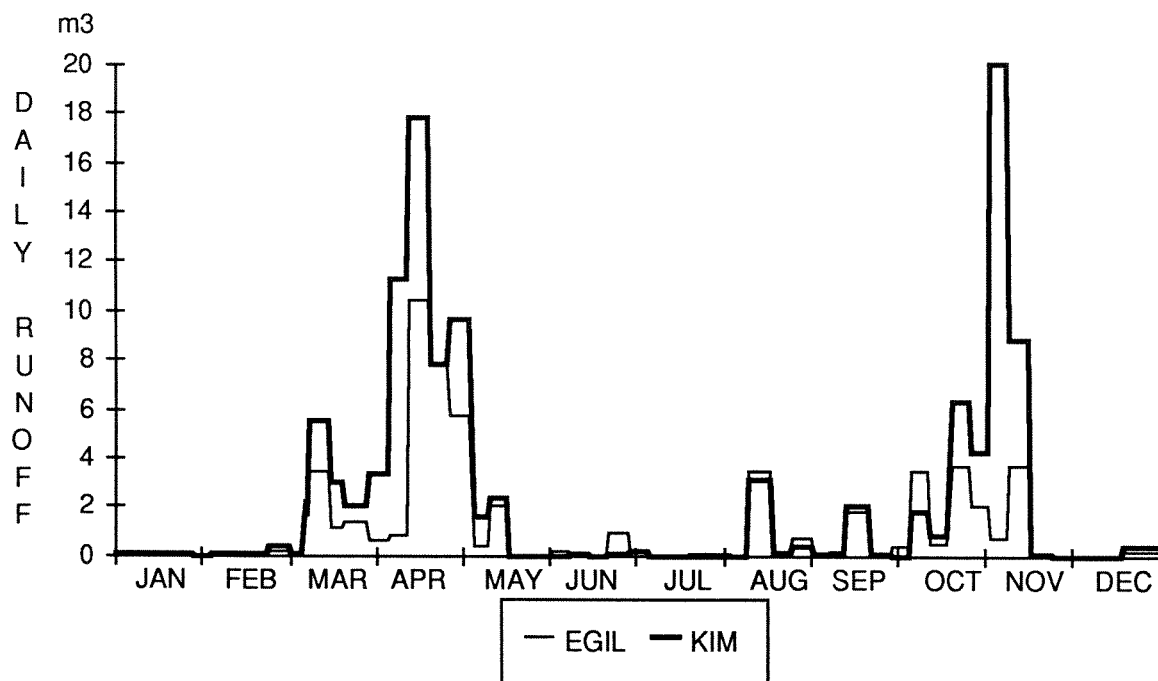


Figure 11. Runoff volumes from KIM and EGIL in 1989.

In the original experimental plan, the aim of was to study differences in mortality on brown trout exposed to runoff water from KIM and EGIL. However, the material makes it possible to study both:

- Comparison of differences in mortality rate at KIM and EGIL.
- Comparison of differences in mortality between the brown trout strains.

Both water quality data from the RAIN-project, and water samples taken from the fish tanks are used to analyse the mortality data.

## 5.1. MORTALITY-RATES AT KIM AND EGIL

### 5.1.1. MAY EXPERIMENT

The experiment started on 15. May 1989. Unfortunately no sampling was done before 48 hours of exposure, causing a loss of valuable information especially among the most sensitive strains. The experiment lasted 6 days, after which very few fish survived.

Cumulative mortality for each strain is presented in Figure 12 A-E. The mortality rate for the Tunhovd, Bustul and the Genetic strain was nearly identical at KIM and EGIL. The Fossbekk strain had a higher mortality rate at KIM (100% within 48 hours) than at EGIL (75% within 48 hours) (Table 12). The Bygland strain had a marked higher mortality at EGIL, with 50% mortality after 66 hours compared to 90 hours at KIM.

The results indicate no systematic difference in the tolerance to the water quality at KIM than at EGIL.

Table 12. LT- values for the five strains exposed to runoff water from KIM and EGIL. The LT-values are uncertain due to few samples.

LT-values	TUNHOVD		BUSTUL		FOSSBEKK		BYGLAND		GENETIC	
	Kim	Egil	Kim	Egil	Kim	Egil	Kim	Egil	Kim	Egil
25%	12	12	12	12	12	15	62	33	15	17
50%	24	24	27	27	24	31	90	67	28	32
75%	36	36	38	38	33	48	120	102	42	48
100%	<120	<120	>144	<120	<48	<120	>144	>144	<120	<120

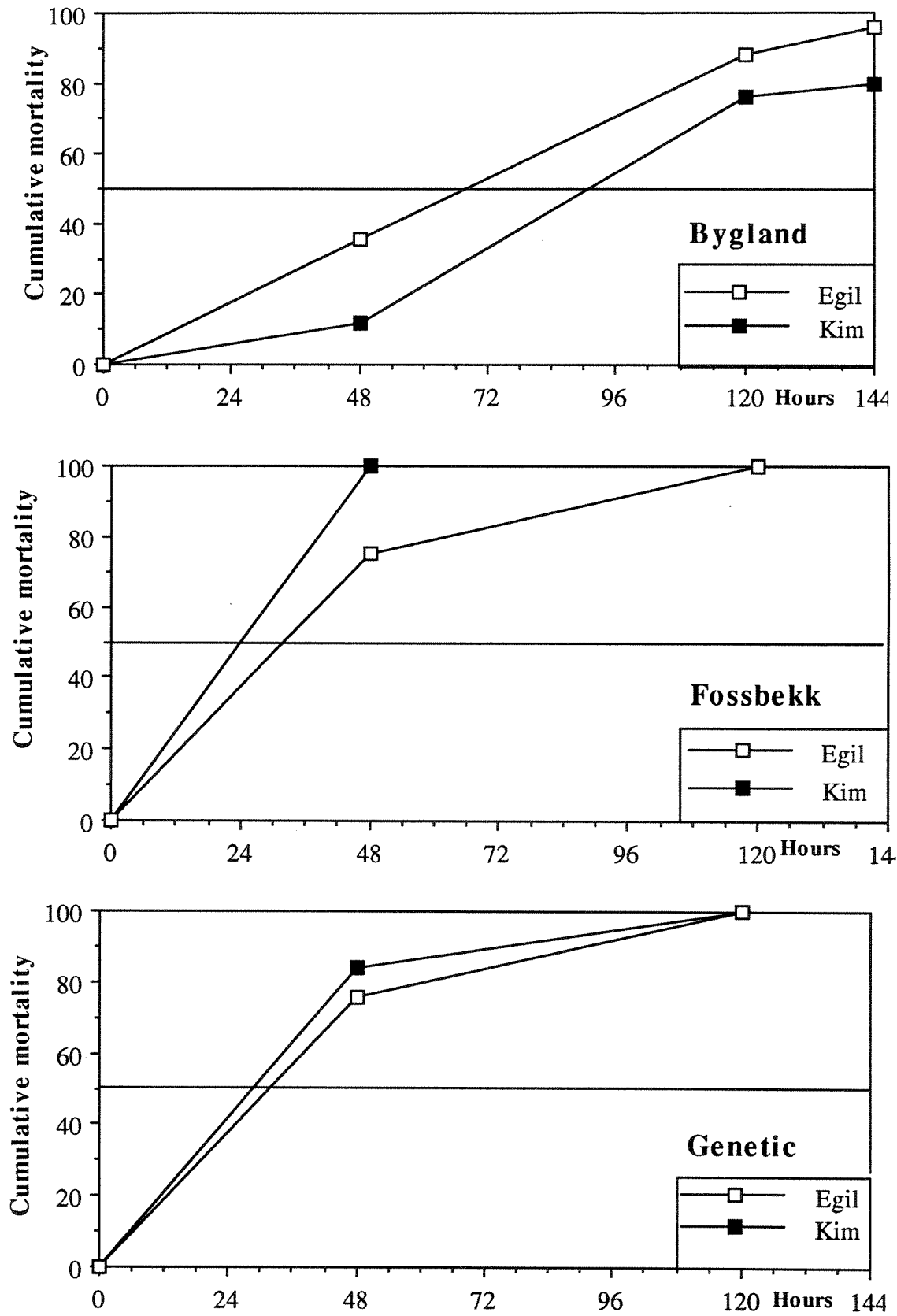


Figure 12. Mortality rates for the fish strains Tunhovd, Bustul, Genetic, Fossbekk and Bygland, exposed to runoff water from KIM (cleaned precipitation) and EGIL (acidic precipitation) in May 1989. (cont.→)

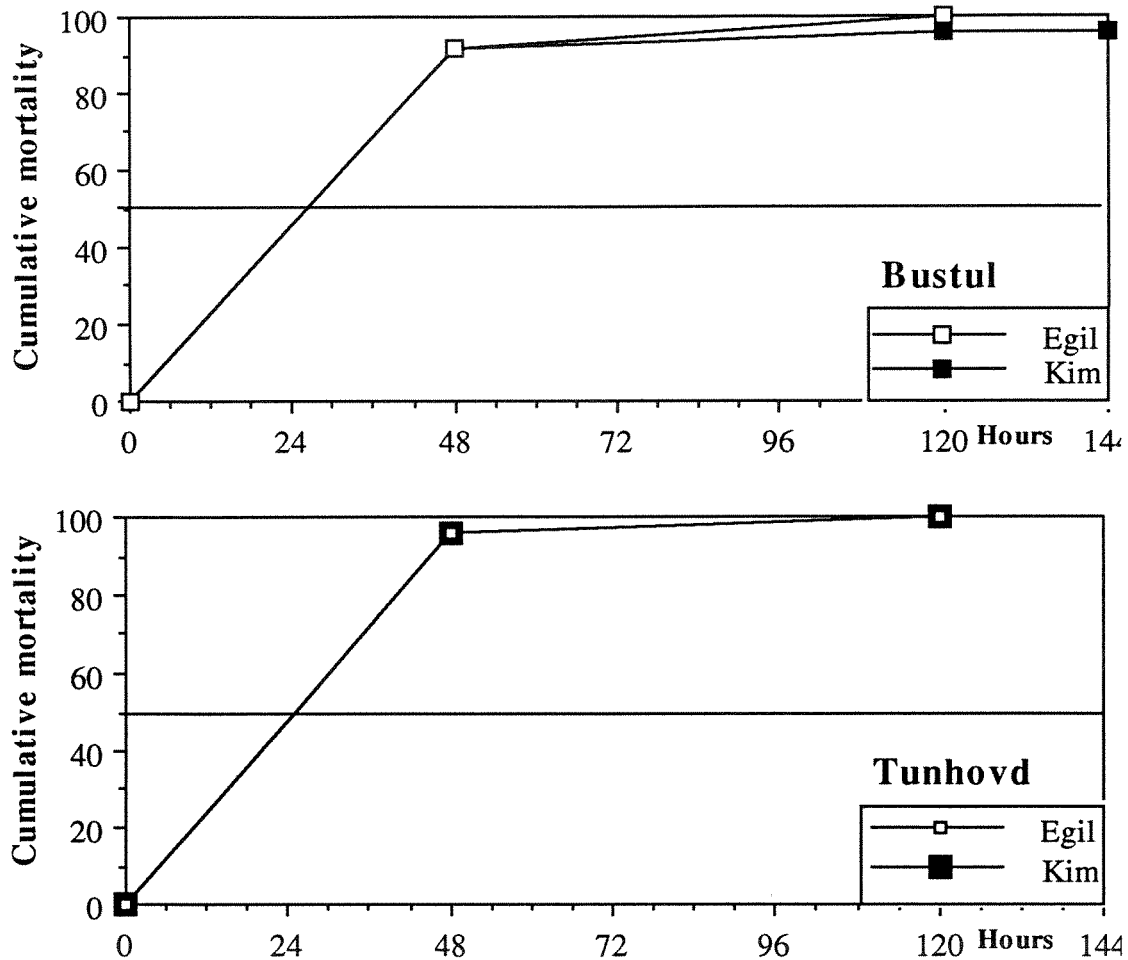


Figure 12. (← cont.). Mortality rates for the fish strains Tunhovd, Bustul, Genetic, Fossbekk and Bygland, exposed to runoff water from KIM (cleaned precipitation) and EGIL (acidic precipitation) in May 1989. Mortality rates for Tunhovd was similar in KIM and EGIL waters.

### 5.1.2. OCTOBER EXPERIMENT

The October experiment started on 16. October 1989 for fish from Bygland Fish Farm. Fish from OFA Fish Farm arrived one day later. All fish were dead within 144 hours (6 days) of exposure (Figure 13, Table 13).

Tunhovd and Bustul had a high mortality rate, reaching more than 50 % mortality within the first 24 hours of exposure. Of the other three strains, only Genetic had a slight mortality at 24 hours of exposure. The Fossbekk and Genetic strain had a high mortality rate at KIM from 24 to 60 hours of exposure, whereupon the mortality rate dropped. This shift in mortality after 60 hours of exposure is not apparent for Tunhovd and Bustul as these strains suffered 100% mortality within 26 hours. The

Bygland strain had a mortality rate significantly different from the other strains, as the mortality was low up to 96 hours.

As for three of the strains in the May results, the different mortality rates at KIM and EGIL indicate a chemical component to the mortality. The shift in mortality rate apparent for Fossbekk and Genetic at KIM might indicate changes in water quality during the experimental period. The mortality rate was clearly higher for all strains at KIM, compared with EGIL.

Table 13. LT- values for the five strains exposed to runoff water from KIM and EGIL in October 1989.

LT-values	TUNHOVD		BUSTUL		FOSSBEKK		BYGLAND		GENETIC	
	Kim	Egil	Kim	Egil	Kim	Egil	Kim	Egil	Kim	Egil
25%	14	22	15	20	46	100	72	102	24	40
50%	18	29	18	24	54	114	102	114	28	54
75%	21	36	21	34	62	130	112	130	39	75
100%	26	72	24	54	<144	<144	144	144	96	84

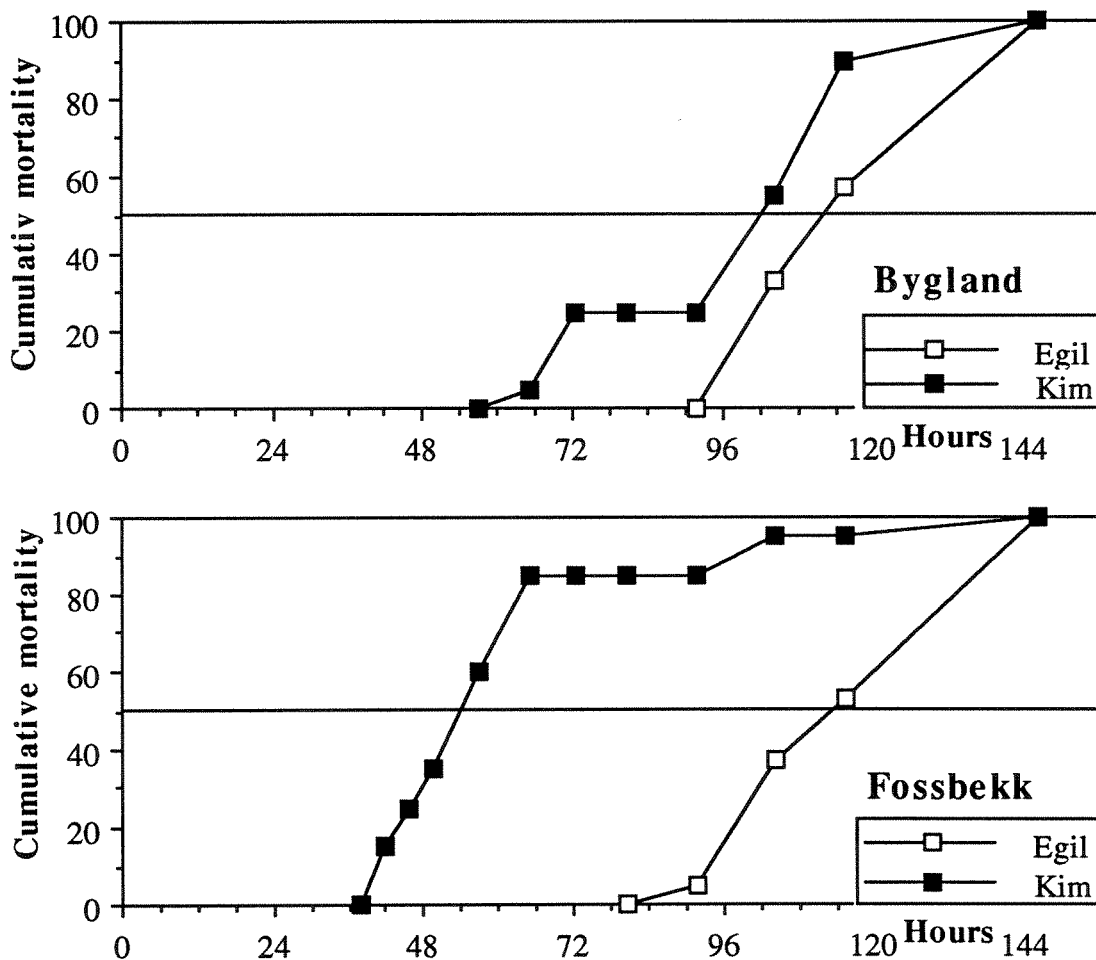


Figure 13. Mortality rates for the fish strains Tunhovd, Bustul, Genetic, Fossbekk and Bygland, exposed to water from KIM (cleaned precipitation) and EGIL (acidic precipitation) in October 1989. (cont. →)

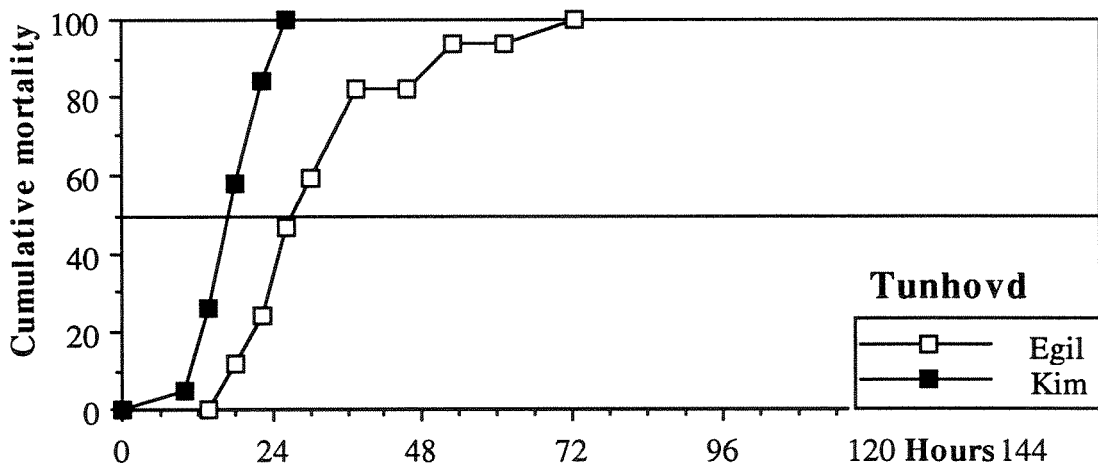
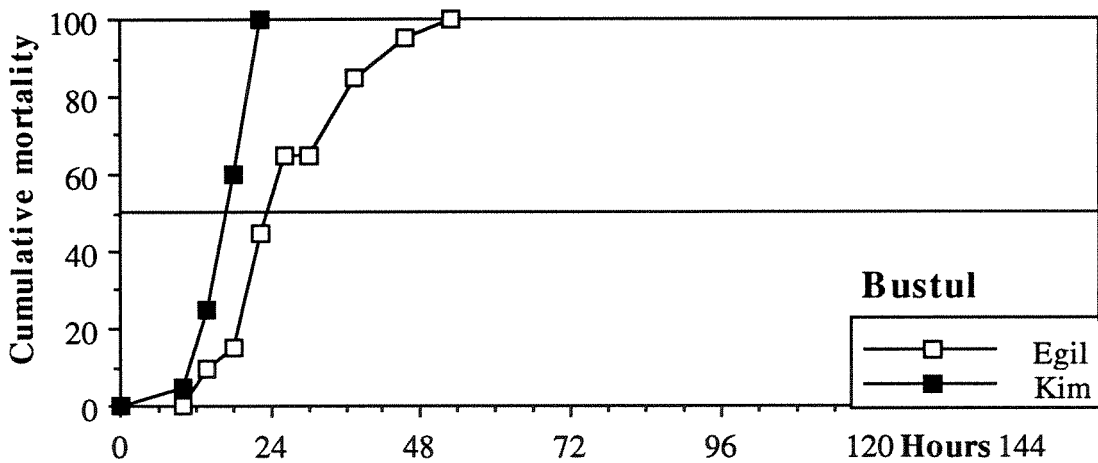
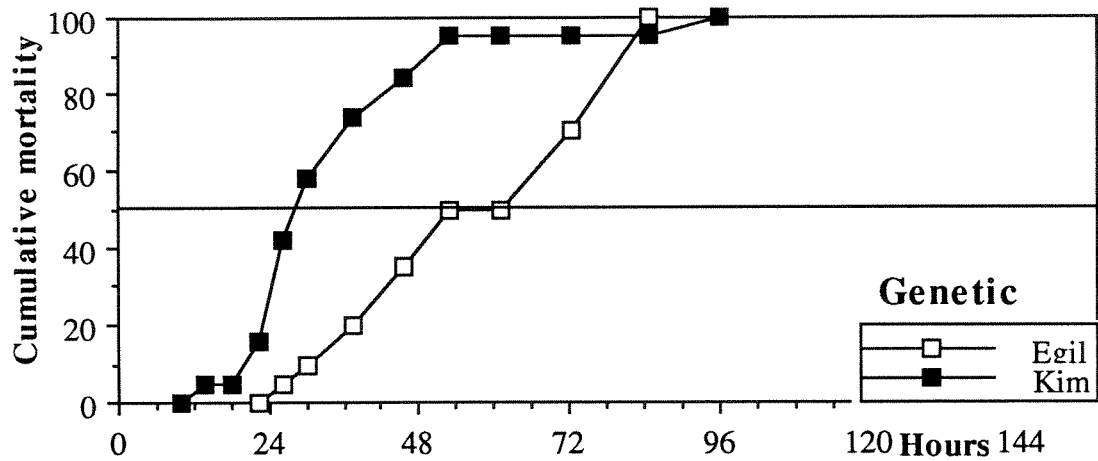


Figure 13. (← cont.) Mortality rates for the fish strains Tunhovd, Bustul, Genetic, Fossbekk and Bygland, exposed to water from KIM (cleaned precipitation) and EGIL (acidic precipitation) in October 1989.



### 5.1.3. NOVEMBER EXPERIMENT

The November experiment started on 31. October 1989. In this exposure the Fossbekk strain was represented by fish hatched and reared both at OFA and Bygland Fish Farm. The mortality rate at KIM and EGIL was identical for both the Tunhovd and the Bustul strain (Figure 14, Table 14). The Fossbekk, Genetic and the Bygland strain had a mortality rate that was fairly similar at KIM and EGIL up to 50% mortality or 48 hours of exposure, whereupon the mortality rate was higher at EGIL than at KIM. Except for the Genetic strain, fish survived 16 days at EGIL, while there were fish surviving 480 hours (24 days) at KIM. The Genetic strain had fish surviving up to 528 hours at EGIL (22 days), at which time only 85% were dead at KIM.

The mortality data from November indicate higher mortality rates at KIM, but they also indicate a shift in the water toxicity after 48 hours at both KIM and EGIL. The change in toxicity to a less toxic water quality appears to be greater at KIM than at EGIL, as the mortality rate for Genetic and Fossbekk strains were higher at KIM initially.

There was a slightly higher, but not significant, mortality rate for Fossbekk fish arriving from OFA Fish Farm than for fish arriving from Bygland Fish Farm. The difference, all though not significant, could be caused by the different water qualities at the two hatcheries and environmental stress factors (transport time and means of transport). The difference is a component that should be taken into consideration when restocking acidic lakes.

Table 14. LT- values for the five strains exposed to runoff water from KIM and EGIL in November 1989.

LT-values	TUNHOVD		BUSTUL		FOSSBEKK		BYGLAND		GENETIC	
	Kim	Egil	Kim	Egil	Kim	Egil	Kim	Egil	Kim	Egil
25%	12	22	12	6	29	33	22	19	24	60
50%	18	26	22	12	40	42	42	42	35	114
75%	30	31	30	24	55	60	96	222	180	276
100%	96	60	50	54	>500	<300	>500	384	>500	>500

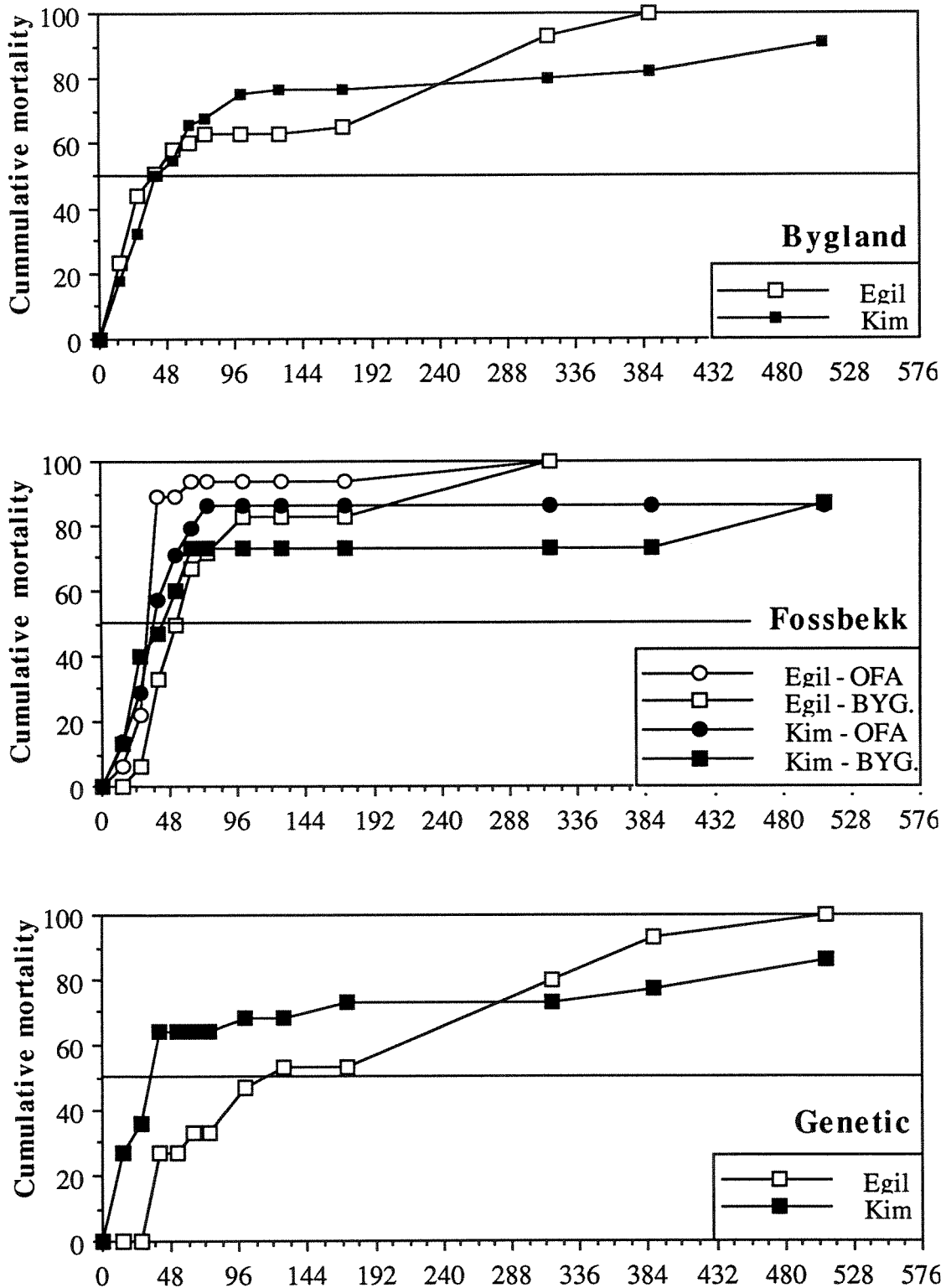


Figure 14. Mortality rates for the fish strains Tunhovd, Bustul, Genetic, Fossbekk and Bygland, exposed to water from KIM (cleaned precipitation) and EGIL (acidic precipitation) in November 1989. Fish of Fossbekk strain from both OFA and Bygland Fish Farms were used. (cont. →)

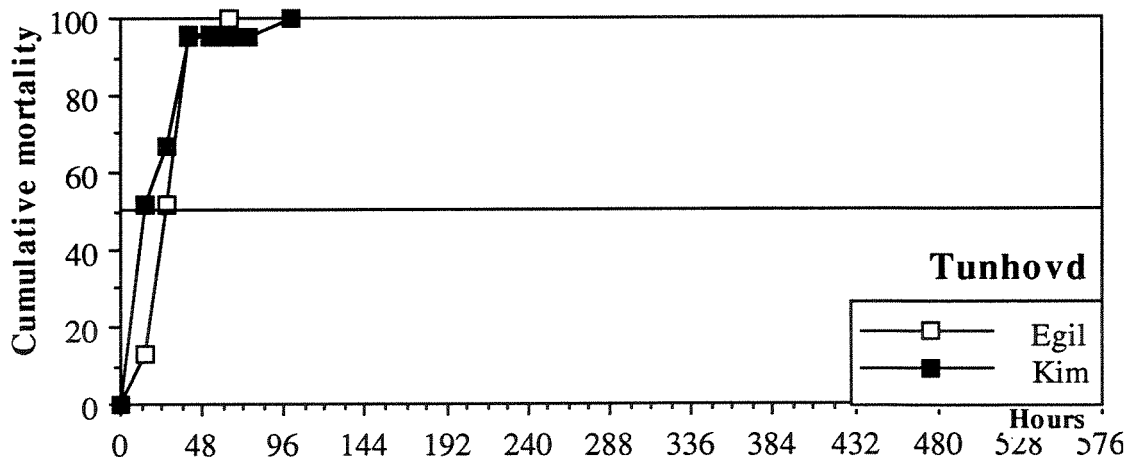
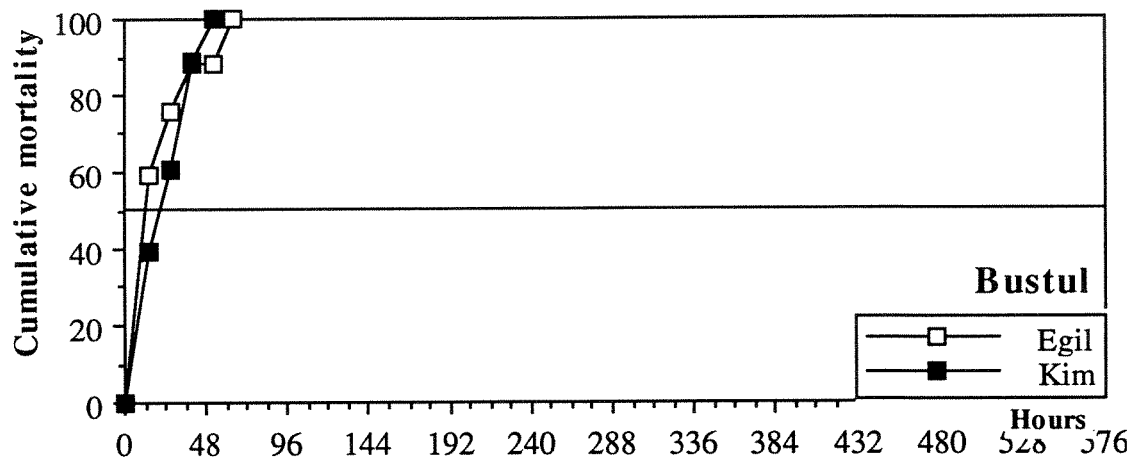


Figure 14. (← cont. ). Mortality rates for the fish strains Tunhovd, Bustul, Genetic, Fossbekk and Bygland, exposed to water from KIM (cleaned precipitation) and EGIL (acidic precipitation) in November 1989.

## 5.2. STRAIN DEPENDANT MORTALITY

### 5.2.1. MAY EXPERIMENT

In the May experiment, the Bygland strain suffered the lowest mortality, with fish surviving for 6 days at both KIM and EGIL (Figure 15).

The Tunhovd and the Bustul strain suffered higher mortality rate than the Genetic and the Fossbekk strain at EGIL. The difference between the four strains is only slight at KIM. This can, however, be caused by the lack of sampling during the first 48 hours. The mortality rates indicate strong strain dependant mortality.

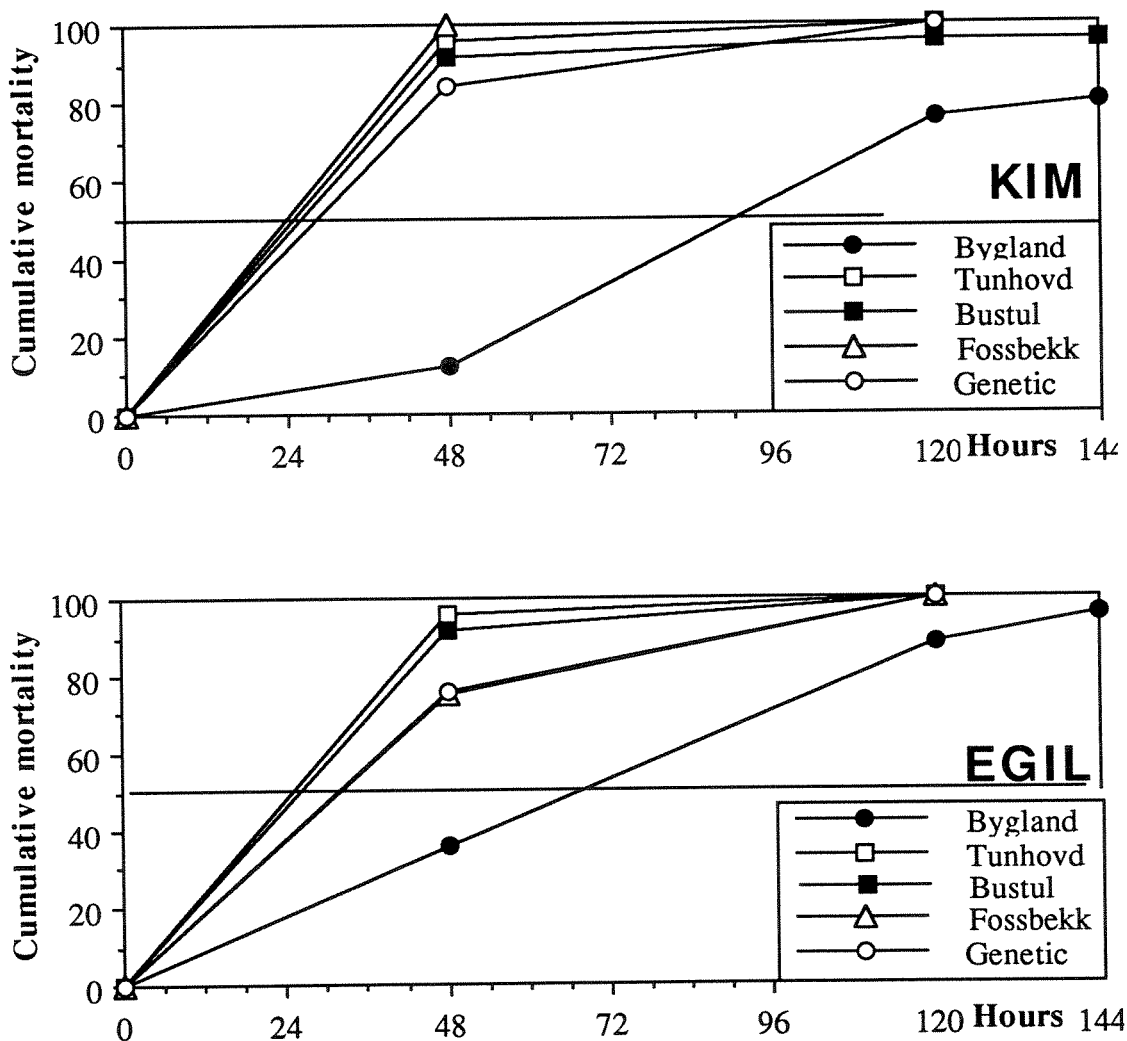


Figure 15. Cumulative mortality for the fish strains, Tunhovd, Bustul, Genetic, Fossbekk and Bygland, exposed to runoff water from KIM (clean precipitation) and EGIL (acid precipitation) in May 1989.

### 5.2.2. OCTOBER EXPERIMENT

In the October experiment, Tunhovd and Bustul strains suffered the highest mortality at both KIM and EGIL, while the Bygland strain had the lowest (Figure 16). The Fossbekk strain had a mortality rate similar to the Bygland strain at EGIL, while at KIM the mortality rate was intermediate between Tunhovd and Bygland. The Genetic strain suffered an intermediate mortality at both sites.

The mortality data show a strong strain dependant mortality rate.

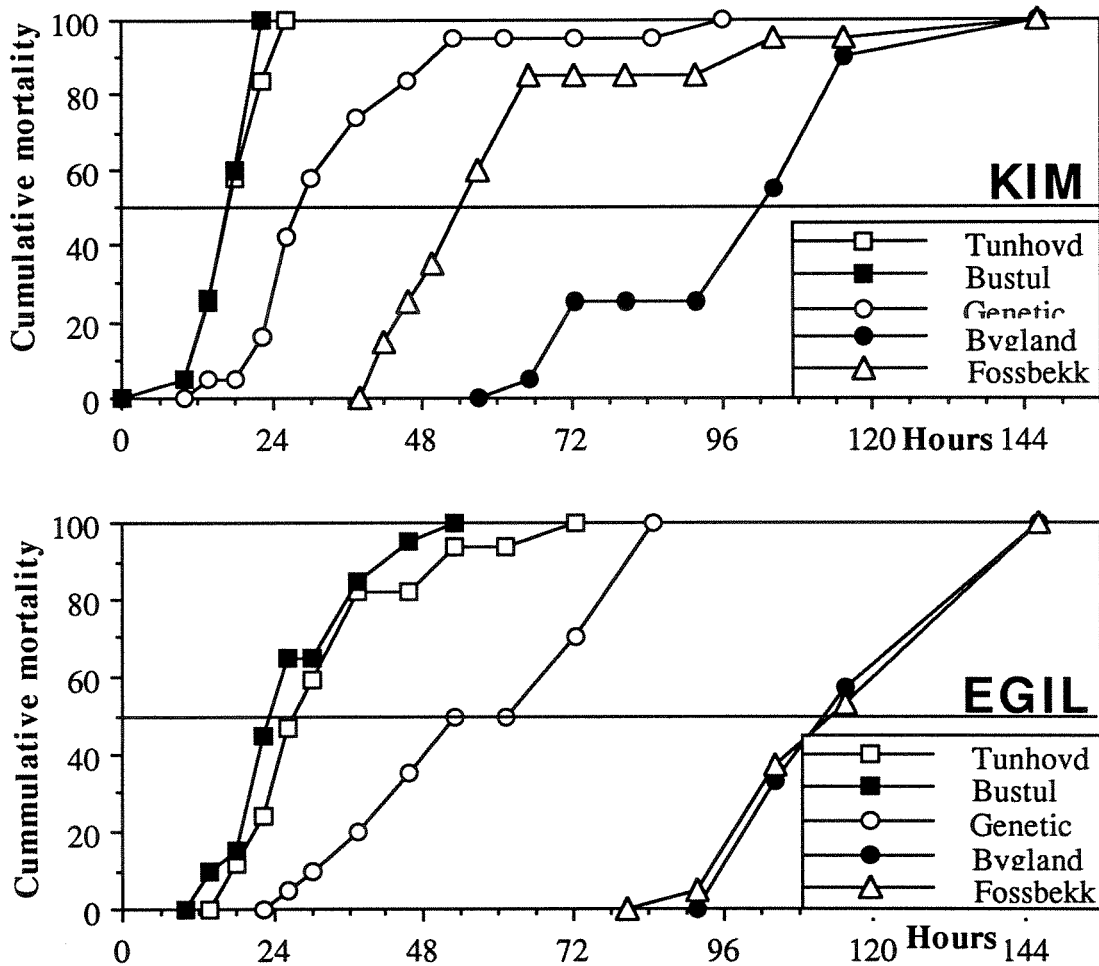


Figure 16. Cumulative mortality for the fish strains Tunhovd, Bustul, Genetic, Fossbekk and Bygland, exposed to runoff water from KIM (clean precipitation) and EGIL (acid precipitation) in October 1989.

### 5.2.3. NOVEMBER EXPERIMENT

In the November experiment, Tunhovd and Bustul strains suffered the highest mortality at both sites (Figure 17). There was only a slight difference in the mortality rate for the three other strains at KIM. At EGIL the mortality rates were more varied, with Genetic showing the lowest mortality rate.

The mortality data show a strong strain dependant mortality rate.

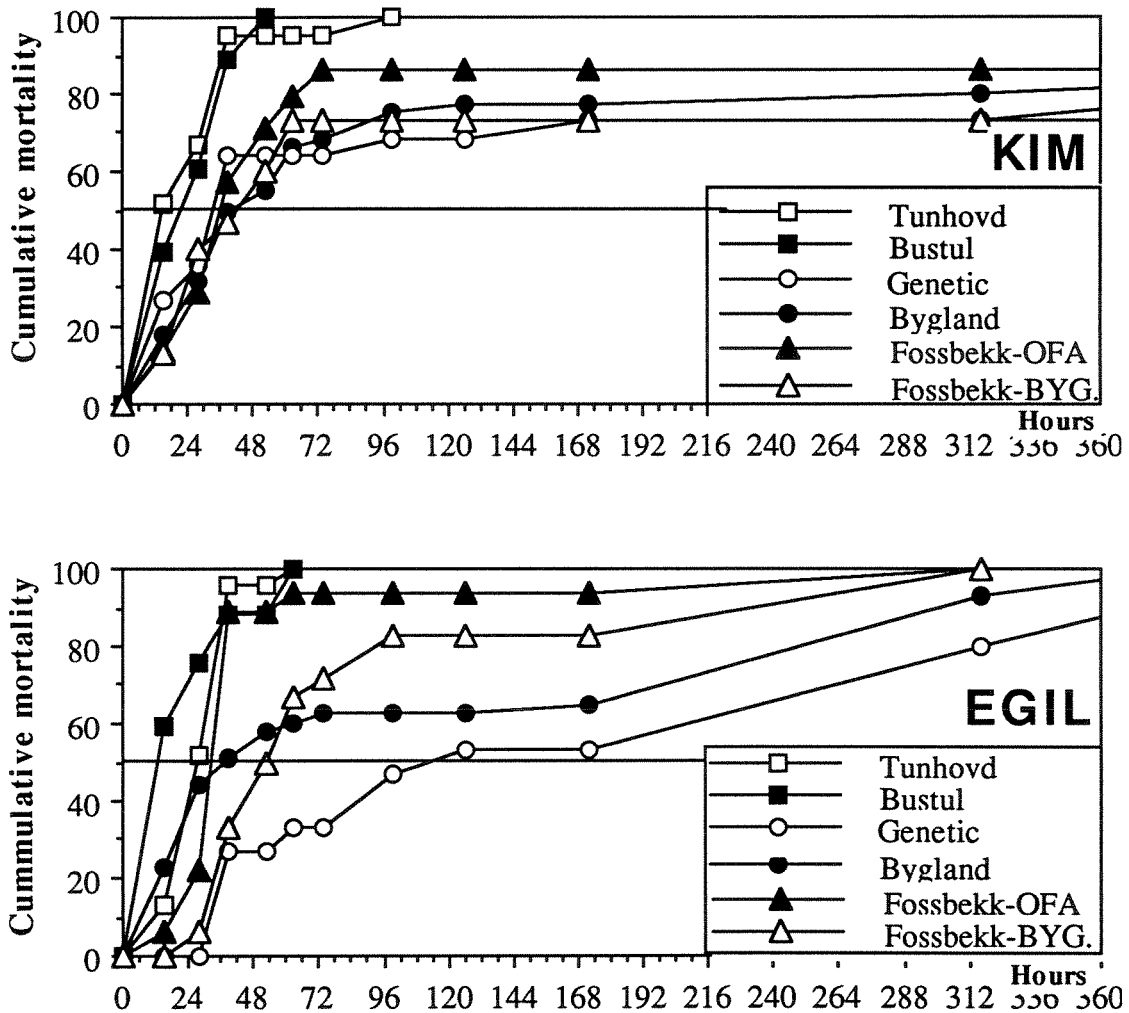


Figure 17. Cumulative mortality for the fish strains Tunhovd, Bustul, Genetic, Fossbekk and Bygland, exposed to runoff water from KIM (clean precipitation) and EGIL (acid precipitation) in November 1989.

### 5.3. VARIATION IN MORTALITY RATES IN 1989

In Figure 18 the mortality rates in 1989 are presented for each strain. Both the Tunhovd and the Bustul strains reached 50% mortality between 12 and 24 hours. The other strains had lower mortality rate, ranked as follows, with respect to exposure period and catchment (Table 15).

Table 15. Ranking of the exposure periods at both KIM and EGIL, based on the LT<sub>50</sub> values for the Fossbekk, Genetic and Bygland strain.

	Highest toxicity				Lowest toxicity	
<b>Fossbekk</b>	KIM-May>	KIM-Nov=	EGIL-May>	EGIL-Nov=	KIM-Oct>	EGIL-Oct
<b>Bygland</b>	KIM-Nov=	EGIL-Nov>	EGIL-May>	KIM-May>	KIM-Oct>	EGIL-Oct
<b>Genetic</b>	KIM-May=	EGIL-May=	KIM-Oct=	KIM-Nov>	EGIL-Oct>	EGIL-Nov

There is no absolute trend in the ranking of the exposure periods, indicating that the ranking cannot be based only on water quality effects on strain, but must also include strains and seasonal dependent interactions. The variation in the toxicity levels of the different exposure periods indicate differences in both water toxicity and strain tolerance.

Exposure time until the first dead fish were observed varied both between the strains, and between the different exposures (Figure 18). The exposure time at which the first dead fish was observed was always less than 30 hours for Tunhovd, Bustul and Genetic. This was the case in May and November for the two other strains, but in October the first dead fish at KIM were observed after 36 hours (Fossbekk) and after 58 hours (Bygland), while no mortality occurred at EGIL before 80 hours (Fossbekk) and 91 hours (Bygland). The low mortality rate, especially for Fossbekk and Bygland, in the beginning of the October exposure is probably caused jointly by these two strains being relatively acid tolerant, and experiencing a water quality of relatively low toxicity.

Because of the measured variation in mortality rates for each strain, the mean mortality rate was calculated for all strains (pooled samples, i.e. "brown trout"), for each experimental period at KIM and EGIL (Figure 19). The water quality and strain dependant mortality rates described previously are obscured, giving a fairly identical mortality development at both KIM and EGIL regardless of season. The only exception to the result was EGIL in October, showing higher mean survival at the LT<sub>50</sub> % level, but the LT<sub>100</sub> % level was similar to the other groups (Table 16). The result indicates that the water quality at EGIL in October was the least toxic.

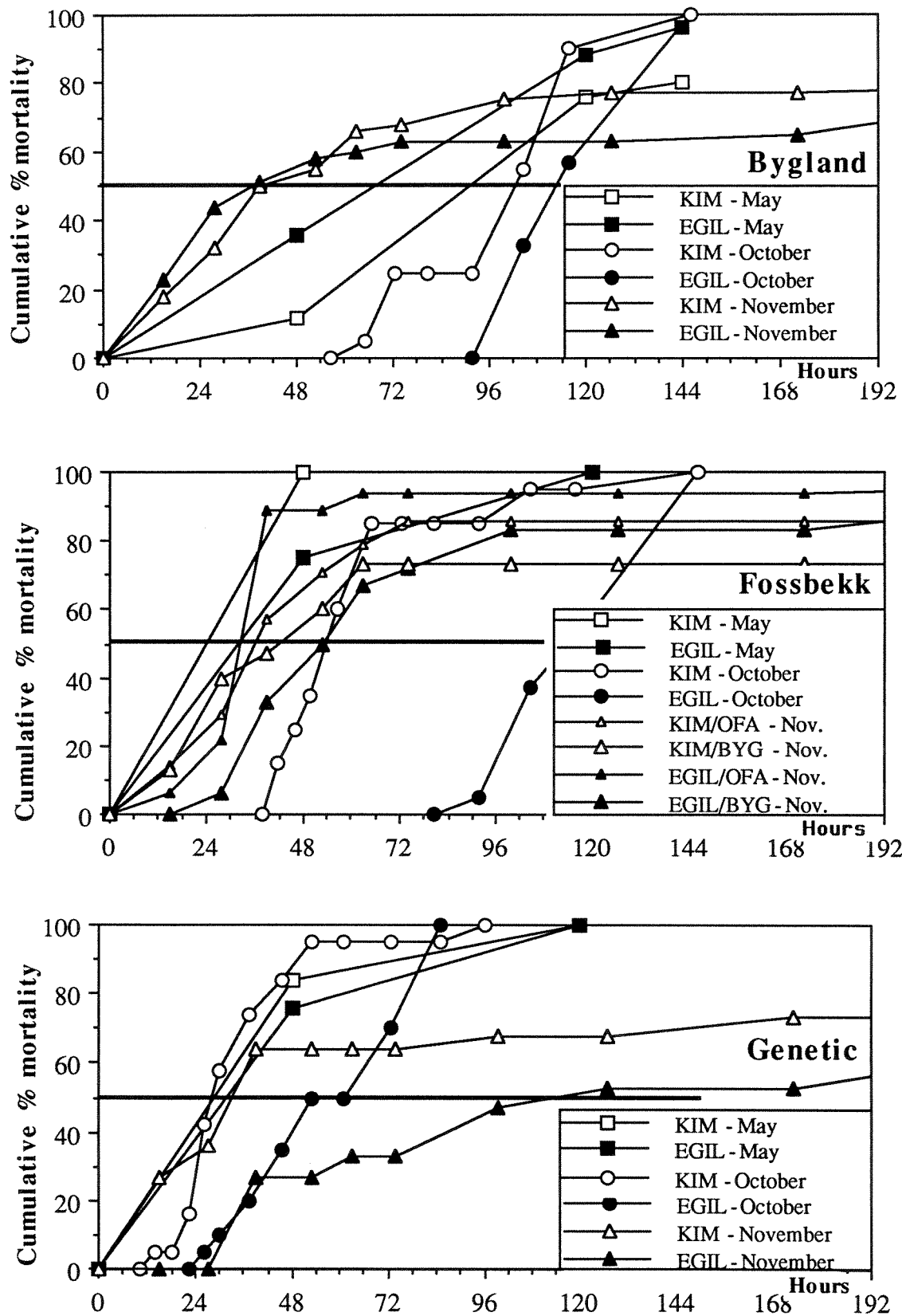


Figure 18. Mortality rates observed in 1989 for the fish strains Tunhovd, Bustul, Genetic, Fossbekk and Bygland. (cont. →).



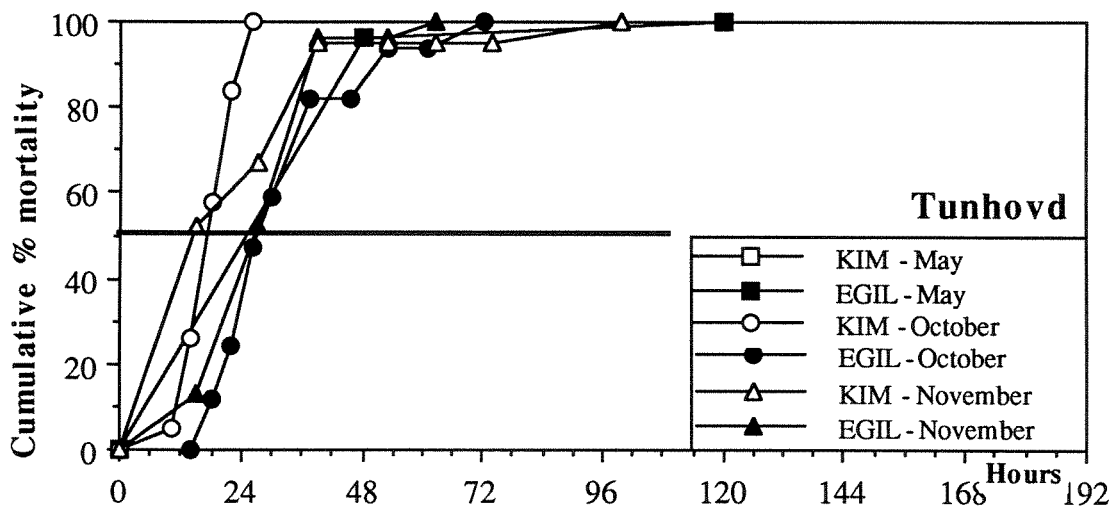
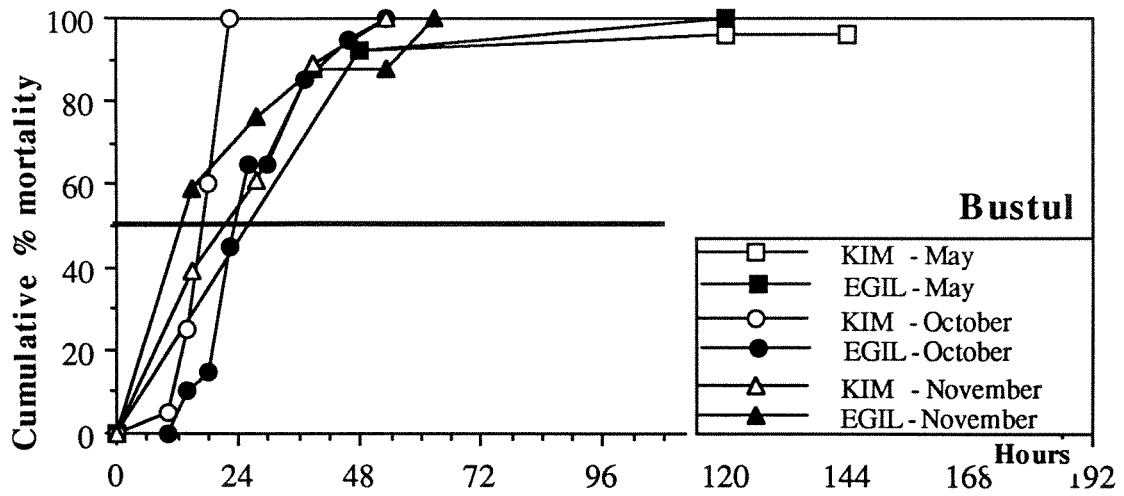


Figure 18. (← cont.). Mortality rates observed in 1989 for the fish strains Tunhovd, Bustul, Genetic, Fossbekk and Bygland.

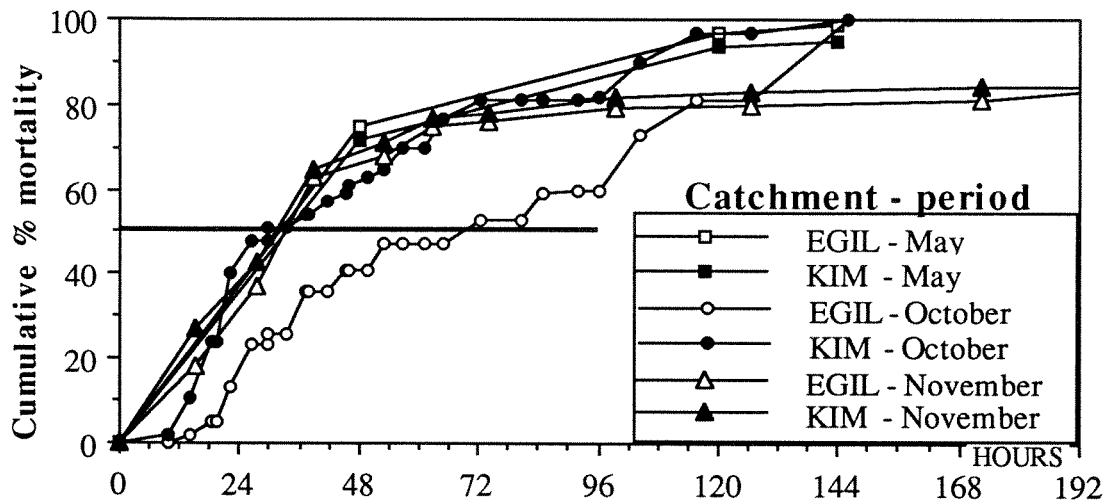


Figure 19. Mean mortality rate calculated for the Tunhovd, Bustul, Genetic, Fossbekk and Bygland strains (pooled samples), for each of the experimental periods at KIM and EGIL in 1989.

Table 16. Mean exposure time until 25, 50 and 75 % mortality, calculated as the average mortality rate of all strains exposed to runoff water from KIM or EGIL.

SITE	PERIOD	MORTALITY			
		25%	50%	75%	100%
KIM	May	20	30	60	>144
EGIL	May	17	33	48	144
KIM	Oct	18	30	63	144
EGIL	Oct	27	69	110	144
KIM	Nov	14	31	63	>200
EGIL	Nov	18	36	60	>200
Mean rate	KIM	17±3.0	30±0.6	62±1.7	>144±
Mean rate	EGIL	21±5.5	46±20.0	73±33.0	>144±

#### 5.4. MORTALITY RATE EVALUATION.

The results indicate a strong strain dependant mortality rate, but the order of mortality was not consistent for the different exposure periods (Figure 12-14). The LT 25, 50 and 75 mortality levels (refer to Table 12-14) were therefore examined for significance in difference in mortality rate (Friedman two-way analysis of variance, Table 17). The probability that the mortality rates for the

strains could derive from individuals from the same population is low, supporting the assumption that there are strain dependent mortality rates (Chi-squared = 17.9, p=0.0013). Similar analysis for fish kept at KIM gave Chi-squared = 9.5, p=0.051, and for EGIL; Chi-squared = 9.7, p=0.047. The significance level was lower than the 5% level at KIM, but the material from both catchments show the same trend, indicating that there is a significant difference in survival between the strains. Analysis on mean ranks at 25 % and 75% mortality level (Table 15) indicate the same result, although the significance levels at 25% mortality are poor.

Ranking the mortality rates show the same rank order for Fossbekk, Genetic and Bygland strains at both KIM and EGIL. Tunhovd and Bustul have a consistent and high mortality rate, different from the other strains. The variation in mortality rates observed in the different exposure periods cannot be explained as only being strain dependent, as the mortality rate normally was higher at KIM than at EGIL, but with significant variations. The main differences between the exposures at KIM and EGIL was the water quality.

Table 17. Friedman two-way anova analysis testing for differences in mortality rates between the strains. The material was tested for KIM and EGIL separate, and of both fields combined. LD values are obtained from Table 13, 14 and 15. Mean ranks, Chi-squares and significance levels are presented.

STRAIN Mortality	Mean Rank KIM				Mean Rank EGIL				Mean Rank KIM+EGIL			
	25%	50%	75%	Σ	25%	50%	75%	Σ	25%	50%	75%	Σ
Tunhovd	1.5	1.3	1.7	4.5	2.2	1.7	1.7	5.6	1.8	1.5	1.7	5.0
Bustul	1.8	2.2	2.0	6.0	1.2	1.3	1.3	3.8	1.8	1.5	1.7	5.0
Fossbekk	3.7	3.2	2.7	9.6	3.7	3.7	3.7	11.1	3.7	3.4	3.2	10.3
Bygland	4.3	5.0	4.7	14.0	4.0	4.3	4.5	12.8	4.1	4.7	4.6	13.4
Genetic	3.7	3.3	4.0	11.0	4.0	4.0	3.8	11.8	3.8	3.7	3.9	11.4
CATCHMENT	25% mortality				50% mortality				75% mortality			
EGIL	Chi =7.90. p=0.0940				Chi=9.66. p=0.0467				Chi=9.86. p=0.0043			
KIM	Chi=8.22. p=0.0840				Chi=9.45. p=0.0508				Chi=8.30. p=0.0820			
KIM+EGIL	Chi=15.4. p=0.0039				Chi=17.9. p=0.0013				Chi=17.2. p=0.0018			

Ranking according to Table 17 gives an order of mortality as:

	High mortality	Low mortality
KIM	Tunhovd>Bustul>Fossbekk>Genetic>Bygland	
EGIL	Bustul>Tunhovd>Fossbekk>Genetic>Bygland	
KIM+EGIL	Bustul=Tunhovd>Fossbekk>Genetic>Bygland	

The high mortality rates observed for Tunhovd and Bustul masks the responses to the variation and differences in the chemical composition of the water quality at KIM and EGIL. In the evaluation of the toxicity levels of the runoff water from KIM and EGIL, it is therefore appropriate to eliminate the two most sensitive strains from further analysis, as the mortality data for the three other strains express more of the variation in water quality.

## 6. DISCUSSION

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The runoff water quality at KIM (roof, clean rain) has changed relative to EGIL (roof, acid rain) after receiving clean precipitation for 7 years. The greatest changes are the reduced sulphate, ammonium and nitrate concentrations in the runoff water, together with a slight increase in the pH, alkalinity and ANC level (Wright 1991). It is therefore reasonable to assume that an improved water quality for fish, would result in a reduced mortality. The fish-experiments, however, do not indicate an improved survival of fish at KIM; at the contrary, the mortality rate was higher (Figure 12-14). Considering the three experimental periods overall, the general trend is that fish from all strains have a higher mortality rate at KIM. The mortality rate is both strain dependent and dependent on variation in the water quality. As such, the results are consistent, and reflect the actual water quality differences at KIM and EGIL.

Fish mortality in acidic water is associated with pH, aluminium and calcium (Rosseland *et al.* 1990b; Leivestad 1982; Baker and Schofield 1980; Munitz and Leivestad 1980). Calculated as mean values for 1989, the pH level is slightly higher at KIM (0.1 pH) than at EGIL. The aluminium concentration at Risdalsheia is normal for acidic waters in the region (Henriksen *et al.* 1988), but the mean RAl concentration was higher (50 µg Al/L) at KIM than at EGIL. The labile concentration was reasonably similar (115 µg Al/L at KIM and 128 µg Al/L at EGIL) at both sites. The slightly lower LAI values at KIM in spite of higher RAl are probably due to the higher TOC and pH levels. The water quality at KIM and EGIL are significantly different for pH, calcium, magnesium, reactive aluminium, TOC and ANC in 1989, but not significant at the 0.01 level for labile aluminium, chloride, and potassium (Table 4-11). The chemical composition of the runoff from the two fields varies from day-to-day, but the variation is not consistent between the fields (Figure 4-11, B and C). This complicates the determination of the toxic element/elements, especially for strains having a low mortality rate as these are exposed to large variation in chemical composition and thus the toxic components during the experiment. There is no supporting evidence in the literature that the difference in RAl concentration can explain the increased mortality rate at KIM. The LAI concentration varies during the three experimental periods. In May the LAI concentration was similar at both KIM and EGIL. In October the the experimental period started with LAI levels 50 µg Al/L higher at KIM than at EGIL, but during the first days of exposure, the concentration became similar. The LAI concentration in the November experiment started with fairly similar values for KIM and EGIL, but the concentration was reduced at KIM while it increased at EGIL. The variation in, and development of the mortality over time, does not easily support LAI being the major component determining the differences in mortality between KIM and EGIL, as higher mortality would be expected at EGIL, due to a generally higher LAI.

Calcium levels higher than 2 - 2.5 mg Ca/L are generally regarded as a level protecting salmonid fish (Leivestad *et al.* 1980; Wood and McDonald 1982; Brown 1983; Wood and McDonald 1987,

Rosseland 1989). Calcium ameliorates the physiological stresses to fish in acidified aluminium-rich water (Rosseland *et al.* 1990b) by protecting the fish against disturbances in the ion-regulating mechanisms (Rosseland *et al.* 1990b). As the relationship between calcium and aluminium in determining toxicity is not entirely clear for all levels of calcium and aluminium, the chemistry data from this experiment do not lend themselves to easy analysis of toxicity. The calcium levels were lower at KIM in 1989 than at EGIL ( $Ca_{\text{mean}} 1989 = 0.32 \text{ mg/L}$  at KIM and  $0.48 \text{ mg/L}$  at EGIL), and very low compared with the assumed protection level. The lower levels of calcium at KIM might explain the higher mortality rate, in spite of the lower concentration of LAI. Calcium concentrations in Norwegian lakes rarely exceed  $1 \text{ mg Ca/L}$ , and are as such below levels assumed to give protection in severely acidified regions. The ameliorating effects of calcium have been questioned by Muniz and Walløe (1990). In their analysis of survey data of brown trout populations in Norway, they found no correlation between calcium concentration and fish status. A likely cause for lack of correlation in their dataset, is however, the fact that low calcium values are of little importance to the fish when both  $H^+$  and aluminium levels are low (Lien *et al.* 1990).

The strain dependant mortality rate indicated in the two water qualities at Risdalsheia show similar differences in tolerance to acid water as obtained from other experiments in Norway (Rosseland and Skogheim 1987, Rosseland *et al.* 1990a) and in England (Sadler and Lynam 1988 and 1989). The Bygland and Fossbekk strains were the most tolerant, with Tunhovd and Bustul being the least. Acclimation to the water quality of origin at the hatchery cannot explain the strain dependant results, as the fish strains (Fossbekk and Bygland) from the Bygland Fish Farm were reared at the “best” water quality (pH), and the same time showed best results in the acid water at Risdalsheia. Likewise, the Fossbekk (OFA Fish Farm), Genetic, Bustul and Tunhovd have all been raised from eyed eggs at OFA Fish Farm and still demonstrated the same strain difference in sensitivity.

The overall results thus indicate a strong strain dependent mortality rate, a result stressing the importance of choosing “correct” strains with which to restock acidic waters. At the same time, the variation in relative mortality rate between the strains in the three experimental periods, suggest that there is no simple relation between water chemistry and mortality.

Brown trout cannot be expected to survive in runoff from KIM as the pH is low (pH 4.2), calcium is low ( $0.3 \text{ mg/L}$ ) and labile (monomeric) aluminium is high ( $115 \mu\text{g Al/L}$ ). The sulphate levels at KIM will, given a 100% reduction in acid rain, never decrease to levels providing a sufficient high pH and calcium level, with the associated improvements of the water quality to ensure complete survival of fish (Richard Wright pers. medd., MAGIC model prediction). No estimates can be made of the changes in composition of groundwater following reductions in emissions, and hence it is impossible to predict the likely effect on Norwegian waters. The water used in the experiments is runoff from extremely small catchments with very thin organic soils, and is not a mixture of ground water and surface runoff

water as would be the case in a more “natural” watershed. Larger catchments with permanent streams and lakes suitable for fish habitats have thicker mineral soils horizons, higher rates of chemical weathering and higher calcium levels at higher aluminium concentrations in runoff. Ground-water addition to a stream would in most cases increase the alkalinity and calcium concentration, and at the same time decrease the aluminium concentration.

Although the exclusion of acid rain at KIM has resulted in dramatic reduction in concentration of sulphate and nitrate in runoff, pH and aluminium concentrations remained too high in 1989 to allow fish survival. The weathering rate is simply too low to provide sufficient levels of base cations and alkalinity. The fish-results from Risdalsheia must therefore be treated with caution concerning the universality of the results, and before the results are extrapolated to larger catchments.

The observations of improved fish populations following reductions in acid deposition near Sudbury, Ontario, Canada, came from fish habitats with large catchments at calcium concentrations  $> 3$  mg/L (Gunn and Keller 1990) and where pH ( $\approx 5.5$ ) levels are higher and LAI levels lower (total aluminium  $\approx 70$   $\mu\text{g Al/L}$ ) than at Risdalsheia. Assuming that lower calcium levels is the explanation for the increased mortality at KIM, increased knowledge on calcium mobilisation from soil and bed-rock is imperative to the understanding of biological effects of reduced acidification in marginal ecosystems.

In this regard the large acidified region of southernmost Norway is intermediate between Risdalsheia and Sudbury. Here calcium concentrations in acidified fishless lakes are typically 0.5-1.5 mg Ca/L with pH 4.5-5.0 and LAI 50-200  $\mu\text{g/L}$ . In such lakes a major reduction in sulphate concentration should result in an increase of pH to above 5, and a decrease in LAI concentration as well as a decrease in the calcium concentration. With respect to toxicity to fish, the increased pH and decreased aluminium should enhance fish survival, but the decreased calcium might offset some of the improvement at least in the first face of recovery.

The water used in the fish experiments was primarily derived from surface runoff, while water in a natural watershed consists of both ground- and surface water runoff. The results are therefore not necessarily correct in predicting the changes in toxicity levels of water after reduction of acid-rain impact. Nevertheless the results from RAIN (Norway) and Sudbury (Canada) indicate that reducing acid-rain will cause a reduction in the calcium concentration of the surface runoff. In marginal and calcium poor areas, with water quality chronically acid but sub-lethal to fish, a transient period of increased toxicity of the runoff water might be speculated. The associated reduction in calcium concentrations in the runoff, do imply a reduction of calcium protection of the fish from acid stress. This will probably only be the case where the calcium concentration in the runoff is low ( $\approx 1$  mg Ca/L as in Norway), and not so much the case where the calcium concentration is high ( $> 3$  mg Ca/L as in Sudbury, Canada).

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